EVOLUTIONARY PROCESSES RESPONSIBLE FOR COMPLEXITY IN AQUATIC VASCULAR PLANTS

PH.D. THESIS

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Prohlášení autora / Author’s declaration

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I hereby declare that I made this thesis independently, using only the mentioned references. I did not submit this thesis nor its part for any other degree or diploma.

V Praze, dne 20. července 2020

Jan Prančl

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Abstract

Aquatic plants are a heterogeneous assemblage of species that, although surviving in similar habitats, have evolved from very different genetic and ecological backgrounds. However, many aquatics share a number of anatomical, morphological, metabolic and reproductive adaptations, which have arisen independently in remarkable similarities (through convergence and parallelisms) in many unrelated groups. Despite their evolutionary uniqueness, aquatic plants are markedly underrepresented in contemporary biosystematic studies. Moreover, the taxonomic evaluation of numerous aquatic plant groups is intricate due to the strong morphological reduction and a high degree of phenotypic plasticity. This thesis focuses on two notoriously challenging aquatic plant groups, *Callitriche* and *Ranunculus* sect. *Batrachium*. The combination of several approaches (genome size estimation, chromosome counting, sequencing of nrDNA ITS and plastid *trn*T–*trn*L regions, examination of herbarium collections) was applied in order to improve our knowledge on principal evolutionary processes such as hybridization, polyploidization and cryptic variation and demonstrate their role on the shaping of overall aquatic plant diversity. The distribution of particular species in the Czech Republic was mapped for the first time. For both groups, cytometric genome size estimation proved to be an effective method for distinguishing taxa, even homoploid ones and most hybrids. In the studied range (a large part of Europe for *Callitriche*, central Europe for *Batrachium*), both model groups include five ploidy levels. Intraspecific ploidy level variation was revealed in *Ranunculus fluitans* (2x + 3x), *R. penicillatus* (4x + 6x), *R. peltatus* (4x and rarely 5x, 6x) and *Callitriche stagnalis* (2x and rarely 3x). The phylogenetic relationships of *Callitriche* taxa were reconstructed with particular emphasis on the origin of polyploids *C. platycarpa* and *C. hamulata*. Flow cytometric and molecular analyses also helped to detect multiple cryptic taxa, particularly within *C. stagnalis*, *C. truncata*, *C. heterophylla*, *R. trichophyllus* and *R. penicillatus*. The frequency and manifestations of hybridization differ significantly among both model systems. Four different hybrid combinations were detected in *Callitriche*, two of which were newly described and validated as *C.* ×*nyrensis* and *C. brutia* nothosubsp. *neglecta*. However, hybridization is generally rare in the genus, mainly as a result of the different pollination biology of particular taxa and high proportion of selfing. In contrast, hybridization is a frequent phenomenon in *Ranunculus* sect. *Batrachium*. In total, 16 cytotypes of hybrid origin were revealed in this group, which accounted for ca 15% of the individuals studied. Introgressive hybridization takes place particularly between *R. peltatus* and *R. trichophyllus*. The gene flow tend to be biased towards the big-flowered *R. peltatus*, which is probably more often a pollen donor than the small-flowered *R. trichophyllus*. In the *R. penicillatus* group, the occurrence of a large hybrid swarm was documented, occupying a 150 km long stretch of the Ohře river. Due to the prolific clonal growth, *Callitriche* and *Ranunculus* hybrids often persist in nature without their parental species. The mineral richness of the substrate is the key factor influencing the distribution of *Batrachium* taxa in the Czech Republic, while distribution ranges of *Callitriche* species correlate with the climatic oceanity and continentality.

Abstrakt

Vodní rostliny představují evolučně heterogenní skupinu organismů, které se adaptovaly na život ve velmi specifickém prostředí. Navzdory velké evoluční vzdálenosti mezi jednotlivými příbuzenskými liniemi sdílejí vodní makrofyty mnoho společných anatomických, morfologických, fyziologických a reprodukčních adaptací, které vznikly nezávisle díky konvergentnímu a paralelnímu vývoji znaků. Vodní rostliny představují evolučně unikátní jednotku, které však v současné systematické botanice není věnována odpovídající pozornost. Navíc se vesměs jedná o taxonomicky obtížné skupiny vzhledem k silné redukci tělní stavby a vysoké míře fenotypové plasticity. Tato dizertační práce se zaměřuje na výzkum dvou taxonomicky složitých skupin, hvězdošů (rod *Callitriche*) a lakušníků (*Ranunculus* sect. *Batrachium*). Za pomoci různých metodických přístupů (stanovení velikosti genomu, počítání chromozomů, sekvenace jaderných (*ITS*) i plastidových (*trn*T–*trn*L) úseků DNA, studium herbářových sbírek) jsme zkoumali nejdůležitější evoluční procesy, jako je hybridizace, polyploidizace a kryptická diverzita, a hodnotili jejich vliv na evoluční komplexitu vodních rostlin. V rámci České republiky jsme také poprvé zmapovali rozšíření jednotlivých druhů ze studovaných skupin. Průtoková cytometrie byla potvrzena jako velmi účinná metoda pro studium obou skupin, umožňující rozlišit i homoploidní taxony. Ve studovaném areálu, zahrnujícím velkou část Evropy v případě rodu *Callitriche* a střední Evropu v případě sekce *Batrachium*, bylo zjištěno celkem pět ploidních úrovní u obou modelových skupin. Vnitrodruhová ploidní variabilita byla odhalena u druhů *Ranunculus fluitans* (2x + 3x), *R. penicillatus* (4x + 6x), *R. peltatus* (4x, vzácně i 5x a 6x) a *Callitriche stagnalis* (2x a vzácně 3x). Byly analyzovány fylogenetické vztahy mezi jednotlivými taxony rodu *Callitriche* se zvláštním zřetelem na zjištění evolučního původu polyploidních druhů *C. platycarpa* a *C. hamulata*. Průtoková cytometrie a molekulární analýzy rovněž přispěly k odhalení kryptických taxonů v rámci druhů *C. stagnalis*, *C. truncata*, *C. heterophylla*, *R. trichophyllus* a *R. penicillatus*. Četnost hybridizace a její projevy se výrazně liší mezi oběma zkoumanými skupinami. V rodu *Callitriche* byly odhaleny čtyři hybridní kombinace, z nich dvě byly nově popsány jako *C.* ×*nyrensis* a *C. brutia* nothosubsp. *neglecta*. Z výsledků nicméně vyplývá, že hybridizace je v rámci rodu spíše vzácným jevem, zejména z důvodu existence různých opylovacích systémů napříč jednotlivými druhy a kvůli vysoké míře samoopylení. Naproti tomu u sekce *Batrachium* je hybridizace běžným fenoménem. Celkem bylo zjištěno 16 hybridních cytotypů, což představovalo zhruba 15 % všech studovaných jedinců. Introgresivní hybridizace se odehrává zejména mezi druhy *R. peltatus* a *R. trichophyllus*. Hybridizace mezi těmito druhy má tendenci se odehrávat převážně jedním směrem, od malokvětého *R. trichophyllus* k velkokvětému *R. peltatus*, který je zřejmě častěji otcovským, opylujícím druhem. V rámci agregátu *R. penicillatus* jsme odhalili rozsáhlý hybridní roj, rozšířený na 150 km dlouhém úseku řeky Ohře. Díky své značné schopnosti vegetativního šíření jsou hybridy hvězdošů a lakušníků schopné přetrvávat v přírodě i za nepřítomnosti rodičovských druhů. Jako zásadní faktor určující rozšíření jednotlivých druhů lakušníků v České republice se ukázala být minerální bohatost podkladu, zatímco rozšíření jednotlivých druhů hvězdošů se zdá být ovlivněno spíše oceanitou a kontinentalitou klimatu.

List of contents

Author contribution statement

I declare that I have substantially contributed to all papers included in the thesis. My contributions to particular papers are as follows:

I. **Prančl J.**, Kaplan Z., Trávníček P. & Jarolímová V. (2014): Genome size as a key to evolutionary complex aquatic plants: polyploidy and hybridization in *Callitriche* (Plantaginaceae). – PLoS ONE 9(9): e105997. doi:10.1371/journal.pone.0105997
(**PAPER I,** referred to as **P-I** in the text)

Field work, performing and analysing of flow cytometric data, cultivation of plants for chromosome counting, manuscript preparation. ***Total contribution 80%***

II. **Prančl J.**, Koutecký P., Trávníček P., Jarolímová V., Lučanová M., Koutecká E. & Kaplan Z. (2018): Cytotype variation, cryptic diversity and hybridization in *Ranunculus* sect. *Batrachium* revealed by flow cytometry and chromosome numbers. – Preslia 90: 195–223.
(**PAPER II, P-II**)

Field work, performing of flow cytometric data, cultivation of plants for chromosome counting, manuscript preparation. ***Total contribution 50%***

III. **Prančl J.**, Fehrer J., Caklová P., Bambasová V., Lučanová M. & Kaplan Z.: Intricate evolutionary history of *Callitriche* (Plantaginaceae) taxa elucidated by a combination of DNA sequencing and flow cytometry. – Accepted manuscript.
(**PAPER III, P-III**)

Field work, performing and analysing of flow cytometric and molecular data, cultivation of plants for chromosome counting, manuscript preparation. ***Total contribution 80%***

In addition to the above-mentioned publications, I have also been involved in the series of papers concerning the distribution of vascular plants in the Czech Republic (within the PLADIAS project, published by a broader team of authors). In this thesis, two of these papers are included, mapping the distribution of studied model groups (*Callitriche*, *Ranunculus* sect. *Batrachium*), with my contribution 5–10% to both papers. I compiled herbarium and field data, georeferenced distribution records and write comments for each of included maps.

I. Kaplan Z., Danihelka J., Chrtek J. Jr., **Prančl J.**, Ducháček M., Ekrt L., Kirschner J., Brabec J., Zázvorka J., Trávníček B., Dřevojan P., Šumberová K., Kocián P., Wild J. & Petřík P. (2018): Distributions of vascular plants in the Czech Republic. Part 7. – Preslia 90: 425–531.
(**DISTRIBUTION PAPER I, DP-I**)

*Mapping of 12 taxa, seven maps included in the thesis (*Callitriche*).*

II. Kaplan Z., Danihelka J., Chrtek J. Jr., Zázvorka J., Koutecký P., Ekrt L., Řepka R., Jelínek B., Grulich V., **Prančl J.** & Wild J. (2019): Distributions of vascular plants in the Czech Republic. Part 8. – Preslia 91: 257–368.
(**DISTRIBUTION PAPER II, DP-II**)

*Mapping of nine taxa (*Ranunculus *sect.* Batrachium)

PART A – GENERAL CHAPTERS

1. AQUATIC PLANTS: A POLYPHYLETIC GROUP WITH LOTS OF COMMON FEATURES

It is challenging, if not impossible, to precisely define the term "aquatic vascular plants", "hydrophytes", "aquatic macrophytes" etc. By broadest possible definition, aquatic plants include all species that are adapted to living in aquatic environments (freshwater and saltwater), but it is obvious there is a continuous transition between aquatic and terrestrial habitats. Raunkiaer (1934) in his famous classification of life forms considered hydrophytes as plants that have vegetative parts submerged or floating, but not emerging into the air, and surviving unfavourable seasons as submerged buds. Actually, this concept excludes many true aquatic plants, such as numerous graminoids and annual macrophytes surviving unfavourable seasons as seeds. According to the widely used definition from Cook et al. (1974), aquatics are plants whose photosynthetically active parts are submerged in water of floating on the water surface permanently or, at least, for several months each year. Later, Cook (1990) refined his own definition and divided aquatic plants into two basic groups: *hydrophytes*, which are physiologically bound to water, at least part of the generative cycle takes place in or on the surface of water, and *helophytes*, including essentially terrestrial plants whose photosynthetically active parts tolerate long periods submerged or floating. However, both definitions do not fully take in account that many aquatic species are amphibious, capable of forming both submerged and fully emerged (subterrestrial) ecomorphoses. Another delimitation by Chambers et al. (2008) is probably most accurate, but at the same time quite general: *aquatic macrophytes are plants that actively grow permanently or periodically submerged below, floating on, or up through the water surface.* Following this definition, aquatic vascular plants currently include 3457 species from 456 genera and 93 families (Murphy et al. 2019), representing ca 1.1% of the overall world diversity of tracheophytes (cf. Christenhusz & Byng 2016).

Aquatic vascular plants are derived from their terrestrial ancestors. The evolutionary invasion of aquatic environments by terrestrial vascular plants is estimated to represent more than 200 independent events (Cook 1999). Aquatics are present in very old as well as in the youngest main clades of vascular plants (e.g., *Isoëtes* in lycopods, Salviniales in ferns, Nymphaeales and *Ceratophyllum* in ancient angiosperms versus Menyanthaceae and many species of Apiaceae in some of most recently diverged clades of dicots, Asterales and Apiales; APG 2016). On the other hand, aquatic plants are very unevenly distributed across the major groups of tracheophytes: for example, about half of the macrophyte species is gathered in monocots, while no aquatics are known in gymnosperms (Murphy et al. 2019). Aquatic plants are indeed an extremely heterogeneous assemblage of species that, although surviving in similar habitats, have evolved from very different genetic and ecological backgrounds. Nevertheless, many macrophytes share a number of traits and evolutionary tendencies, which have arisen independently in remarkable similarities (through convergence and parallelisms) in many unrelated groups of plants surviving in aquatic environment. As a result, aquatic plants (especially those that survive underwater of floating) are highly specialized organisms – often to such an extent that it is no longer possible for these highly adapted lineages to return back to terrestrial conditions (Sculthorpe 1967).

The primary goal of the following chapters is to summarize the main common trends shaping the evolution of aquatics, leading to their peculiar morphological, physiological and reproductive adaptations and affecting the speciation and genetic diversity of individual aquatic groups. I focus mainly on fully submerged or floating angiosperms but also on amphibious plants that have, under certain conditions, submerged or floating vegetative organs.

2. CONVERGENT TRENDS CHARACTERISTIC OF AQUATIC PLANTS

The key factors affecting plants in aquatic environments are: a) difficult exchange of gases, b) reduced light availability, c) the need of buoyancy, d) the necessity of anchoring in soft bottom sediments, e) effects of disturbance factors, e.g. water flow, waves and surf. On the other side, temperature fluctuations in water are far less pronounced than in terrestrial environments (Sculthorpe 1967, Bornette & Puijalon 2011). The synergic effect of the above mentioned factors exerts selective pressure on all plant lineages, which have ventured back to the water. As a consequence, aquatic plants show multiple evidences of convergent evolution (i.e., the independent evolution of similar features in lineages arising in different periods or epochs in time; Du & Wang 2014, Stayton 2015). This chapter discusses the most striking anatomical, morphological, metabolic and reproductive adaptations that arose as the results of similar selective pressure, and characterizing aquatic plants as a whole.

2.1 Specialized structure of vegetative organs

All rooting macrophytes have to cope with anaerobic conditions in the substrate. Two fundamental ways of surviving anoxia are common: hydrophytes feed gases to the inundated tissues through an aerenchyma or lacunar system (Jackson 1985, Rascio 2002, Jung et al. 2008), and the roots are also usually able to tolerate anaerobic respiration (glycolysis coupled to alcoholic fermentation; Drew 1997, Summers et al. 2000). The roots, rhizomes and other underground parts often represent a significant proportion of biomass, which is particularly true for emerging species colonizing littoral zones with a muddy substrate (e.g. up to 80–90 % of the fresh weight biomass in *Schoenoplectus lacustris*, *Equisetum fluviatile* and *Phragmites australis*; Sculthorpe 1967). Macrophytes with less developed root system are often bound to quieter waters. However, many species are able to withstand large drag forces through other adaptations, such as the creation of prostrate forms in environments affected by waves (see below) or forming the dense patches with sharp outer margins in streams (Sand-Jensen 2003, Sand-Jensen & Pedersen 2008, Bornette & Puijalon 2011).

The anatomy and shape of leaves show surprising uniformities across various groups of quite unrelated hydrophytes. Floating leaves of rooting macrophytes (*nymphaeids*) exhibit the utmost conservatism of form and structure. The leaves on the water surface must occupy as large area as possible and at the same time be resistant to tearing and sinking, e.g. due to rainfall. Therefore, many floating leaves possess firm, leathery, ovate or elliptic blades with entire margins. The greatest physical advantages accrue to more or less circular leaves with a petiole attached in the middle of the leaf surface (e.g. deeply notched leaves with prominent basal lobes in Nymphaeaceae, *Nymphoides* and *Sagittaria guayanensis* or even peltate leaves in Cabombaceae). The stomata are usually present only in the upper epidermis, and they are often permanently open (Sculthorpe 1967, Shtein et al. 2017).

Numerous morphological similarities of submerged leaves have evolved more like the reaction to the absence of excessive irradiance than the adaptation to an aquatic life; however, these adaptations also have advantages in nutrient intake (Sculthorpe 1967). The submerged organs usually lack stomata (Raven 2002, Li et al. 2019) and the transpiration is absent, but the acropetal water transport is maintained in many truly submerged plants, especially through the hydathodes on the young, actively growing leaves (Pedersen & Sand-Jensen 1993, Pedersen et al. 1997). The cuticle is extremely thin or even absent from submerged organs (Arber 1920, Li et al. 2019), also the pubescence is strongly reduced or absent, chloroplasts are present even in the epidermis (Ronzhina & P'yankov 2001, Galati et al. 2015). Although most aquatic plants are able to absorb nutrients over the entire plant surface (Denny 1980), rooted aquatic plants usually receive the bulk of nutrients (nitrogen and phosphorus) from the sediment (Carignan & Kalff 1980, Chambers et al. 1989, Rattray et al. 1991). On the other side, many ions (K^+, Ca^{2+}, Mg^{2+}, Na^+, Cl^-, SO_4^{2-}...) are preferentially absorbed by shoots and leaves from the water (Barko et al. 1991).

The submerged leaves have a high ratio of surface area to volume, which increases their efficiency in absorbing dissolved gases and nutrients (Sher-Kaul et al. 1995). The great majority of entire leaves are conspicuously elongated, filiform or setaceous (narrow-leaved Potamogetonaceae, *Zannichellia*, *Ruppia*), linear or linear-lanceolate (*Elodea*, *Egeria*, *Hydrilla*, *Lagarosiphon*, submerged ecomorphoses of *Hippuris* and *Rotala*, some species of *Callitriche*) or ribbon-like, arranged in rosette (*Vallisneri*a, *Sagittaria*, marine monocots). If the leaf blade is broad, it usually has a very thin membranous structure (*Potamogeton lucens*), often with undulate margins (*Aponogeton*, *Potamogeton crispus*, *Ottelia alismoides*, submerged leaves of

Nuphar) or even being fenestrated (two Madagascan species of *Aponogeton*, Les et al. 2005). As an analogy to these perforated leaves, submerged leaves deeply dissected into linear or thread-like segments are present in numerous (mostly dicotyledonous) lineages with pinnate or palmate venation (*Cabomba*, *Ceratophyllum*, *Ranunculus* sect. *Batrachium*, *R. flabellaris* and *R. gmelinii*, *Haloragis brownii*, *Myriophyllum*, *Hottonia*, *Limnophila*, *Bidens beckii*; Cook 1990). This structural feature, like the ribbon-like leaves, probably offers little mechanical resistance to the current. Dissected leaves may thereby have contributed to the ability of some species to colonise streams as well as standing water (Sculthorpe 1967).

Certain physiological problems confronting terrestrial vascular plants are minimised or eliminated in the aquatic environment, which leads to a strong morphological reduction of numerous vegetative organs. The need for mechanical strength and rigidity is alleviated, therefore submerged tissues contain little or no lignin, and submerged hydrophytes generally do not possess a dominant erect axis (Sculthorpe 1967). The conducting tissues are strongly reduced, especially the xylem part, the vascular strands are mostly condensed into a single central cylinder (Arber 1920, Wetzel 1983). The vascular bundles lack true vessels in many aquatic genera (e.g. *Callitriche*, Hegelmaier 1864; *Potamogeton*, Cheadle 1942) or even being almost totally devoid of any vascular elements (e.g. *Egeria*, *Najas*, *Ruppia*, *Zannichellia* and the marine monocots, Cheadle 1942; *Ceratophyllum*, Schneider & Carlquist 1996). However, the view that the xylem has no functional role in these genera can be partly an artifact because the tracheary elements, if not well lignified, are poorly visible using old microscopic techniques (Pedersen et al. 1997).

The free-floating habit is morphologically most specialized and shows the most glaring structural reductions. The leaves of the least modified free-floating hydrophytes are produced in rosettes on the strongly shortened stem, with richly developed adventitious roots in the basal part (monocotyledonous *Eichhornia*, *Hydrocharis*, *Limnobium*, *Stratiotes* and *Pistia*, dicotyledonous *Trapa*). The genera *Salvinia*, *Ceratophyllum* and *Aldrovanda* lack roots; in the case of the pteridophyte *Salvinia*, the roots are functionally substituted by the highly specialized finely dissected leaves (Sculthorpe 1967, Cook 1990). The carnivorous genus *Utricularia* also lost roots and, moreover, possesses a highly reduced "fuzzy" vegetative organs blending the developmental programmes of leaves and shoots (Rutishauser 2016). The duckweeds

(*Lemnoideae*, Araceae) are the smallest vascular plants whose body is reduced to a minute thallus (“frond”); the genera *Wolffia* and *Wolffiella* even lack roots and vascular system (White & Wise 1998). Some ecological analogy to *Lemnoideae* is evident also in the strongly reduced pteridophyte *Azolla*.

There is surely no stranger case of morphological reduction in angiosperms than the Podostemaceae (Malpighiales), the most diversified strictly aquatic family (Murphy et al. 2019). Representatives of this family grow submerged on naked rocks in rapidly flowing sections and waterfalls of clean rivers, mostly in tropical and subtropical regions. Various Podostemaceae have dorsoventrally flattened thalloid bodies closely resembling algae, bryophytes or lichens, which are interpreted as creeping roots or creeping stems. The members of the largest subfamily, *Podostemoideae*, lack obvious shoot apical meristem and almost all cells of the thallus retain the potential meristematic capacity (Hammond 1936, Katayama et al. 2011, Rutishauser 2016).

2.2 Phenotypic plasticity

Aquatic habitats rarely show stable conditions. Like their terrestrial counterparts, water macrophytes encounter mechanical stress, such as wind blowing, waving and flowing of the water. Moreover, most of aquatic plants must deal with strong environmental gradients, sudden water-level fluctuations and often with a complete drying of the water bodies. These forces maintain many aquatic habitats in the early succession stages (Lake 2000, Prach et al. 2014). As an adaptive response to a heterogeneous and dynamically changing environment, numerous hydrophytes show classic manifestations of phenotypic plasticity, whose extent in some aquatic groups is exceptional even in the context of whole tracheophytes (Arber 1920, Sculthorpe 1967, Cook 1968, Dorken & Barrett 2004).

During the transition from terrestrial to aquatic environment, most aquatics retained the ability to grow in subterrestrial conditions. On the other side, the backward transition of any obligatory aquatic lineage to the land has never been clearly demonstrated. Some cases of obligatory terrestrial species are known in aquatic genera *Callitriche* (Philbrick & Les 2000, Ito et al. 2017a) and *Isoëtes* (Larsén & Rydin 2016). Nevertheless, in these genera there are also many amphibious species, even in their ancestral clades.

Some aquatics (especially nymphaeids with big floating leaves growing from rhizomes, or minute free-floating hydrophytes such as *Lemna* or *Azolla*) survive desiccation, though successfully, rather as a “virtue of necessity”. On the other side, many aquatic plants are truly amphibious, thriving equally well under submerged and subterrestrial conditions. When growing on a wet substrate, they usually form prostrate ecomorphoses rooting at the nodes, with shortened internodes and thicker leaves possessing normally developed stomata (e.g. *Callitriche*, *Ranunculus* sect. *Batrachium*, *Elatine*; Schotsman 1954, Cook 1966, Sculthorpe 1967, Molnár et al. 2015). In contrast, the vegetative parts of *isoetids* (small tufted plants with short rigid and hollow leaves, e.g. *Isoëtes*, *Littorella*, *Subularia aquatica*, *Lobelia dortmanna*, some species of *Eriocaulon*; Raven et al. 1988, Madsen et al. 2002, Smolders et al. 2002) show a little extent of visible phenotypic plasticity, no matter whether growing submerged of emerged. Some aquatics are also capable to form prostrate submerged forms in reaction to waving (*Zannichellia palustris*, Kaplan 2010b) or as a hibernating ecomorphoses during winter (*Ranunculus fluitans*, *R. circinatus*; Cook 1966).

Heterophylly, i.e. the leaf form alteration in response to environmental conditions (Goliber & Feldman 1990, Li et al. 2019), is the most prominent manifestation of phenotypic plasticity in aquatic plants. In the strictest sense, the change from one leaf type to another is abrupt and easily recognized (e.g. representatives of Nymphaeaceae, Cabombaceae, broad-leaved species of *Potamogeton*, *Luronium*, *Myriophyllum*, *Persicaria amphibia*, *Hippuris*, *Bidens beckii*, submerged versus emerged leaves of *Sagittaria sagittifolia*). However, the transition between leaf types can be continuous (e.g. amphibious species of *Callitriche*, floating and emerged leaves of *Sagittaria sagittifolia*; Jones 1955, Hroudová et al. 1988). In many cases, there is no clear boundary between heterophylly and heteroblasty (i.e. changes in leaf shape during growth development; Zotz et al. 2011, Li et al. 2019). The development of specific types of leaves is often bound to flowering (e.g. entire leaves of heterophyllous species of *Ranunculus* sect. *Batrachium*; Cook 1966, 1969). Certain types of leaves are associated with a juvenile stage of development, e.g. the submerged ribbon-like leaves of various aquatic monocots. The ability of flowering and fruiting in the phase of ribbon leaves is sometimes explained as a neoteny (e.g. *Alisma gramineum*, *Sagittaria pygmaea*, *Sparganium natans*; Sculthorpe 1967).

The heterophylly often has different manifestation even among the closely related species within some genera. While the floating leaves are (almost) always present in adult plants of *Potamogeton natans*, *P. polygonifolius* and

Ranunculus peltatus, the same type of leaves is produced only under certain conditions in other congeners (*P. gramineus*, *R. aquatilis*, *R. baudotii*; Cook 1966, Wiegleb & Kaplan 1998). The factors influencing the formation of different leaf types are complex (light intensity, photoperiod, temperature, depth, water flow, osmotic stress...) and multiple phytohormons are involved in the regulation of heterophylly (Li et al. 2019).

2.3 Photosyntetic pathways

The photosynthesis in water presents a serious challenge to submerged plants. The light availability is severely limited by depth, turbidity and also by the own density of the aquatic plant community (Kirk 1994, Colmer et al. 2011, Pedersen et al. 2013). An even more significant problem is the availability of the carbon dioxide, whose diffusion in stagnant water is about four orders (10^4-fold) slower than in the air (Madsen & Sand-Jensen 1991, Maberly & Madsen 2002a). The dissolved inorganic carbon in water consists

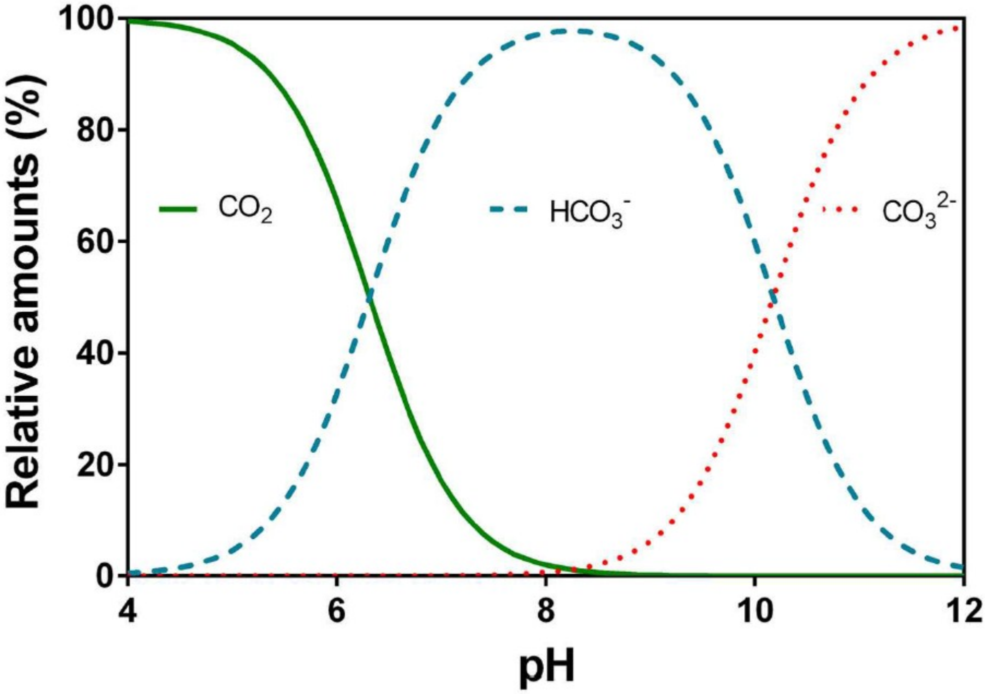

FIG. 1. Relative speciation of CO_2 constituents in water as a function of pH (under temperature 20°C and conductivity of 250 µS.cm^{-1}). Adopted from Pedersen et al. (2013).

of three constituents (free CO_2, bicarbonate ions HCO_3^-, carbonate ions CO_3^{2-}), whose proportional changes depending on ionic strength, temperature, and primarily pH (Mackereth et al. 1978). Below pH 6, the most of inorganic carbon is dissolved as CO_2, between pH 7–10 HCO_3^- dominates; at pH higher than 10, a significant proportion of the dissolved carbon is in the form of CO_3^{2-} (reviewed in Pedersen et al. 2013, Fig. 1). Hence, the inorganic carbon availability is a much more limiting factor for aquatic than for terrestrial leaves. In many alkaline freshwaters (including the seawater), the pool of HCO_3^- is much higher than CO_2. As a consequence, many macrophytes are capable of using bicarbonates as a supplementary source of inorganic carbon. The use of HCO_3^- is prominent in seagrasses and it was detected in ca 50% of tested submerged and freely floating freshwater angiosperms (Maberly & Madsen 2002a, Iversen et al. 2019), but this ability is absent among aquatic pteridophytes (Pedersen et al. 2013). In amphibious aquatics, the uptake of bicarbonates is rarer, reported in all six studied species of *Ranunculus* sect. *Batrachium*, two species of *Schoenoplectus* and two species of *Hygrophila* (Iversen et al. 2019, Horiguchi et al. 2019). In contrast, all seven examined amphibious species of *Callitriche* are restricted to free CO_2, and the only two known HCO_3^--using taxa in the genus are the obligatory submerged *C. hermaphroditica* and *C. truncata* (Maberly & Madsen 2002b, Iversen et al. 2019).

During the day, pH of water bodies may fluctuate strongly. When sunlight, photosynthetic organisms consume CO_2 (and consequently even HCO_3^-) and shifting the pH of the water to alkaline values. At night, when the respiration prevails, the concentration of CO_2 and pH returns to the initial values (Pedersen et al. 2013). Carbonates and bicarbonates can react with both acids and bases and buffer pH changes. Therefore, the pH fluctuation is highest in waters of low alkalinity, especially if they are also rich in nutrients (Wurts & Durborow 1992, Tucker & D'Abramo 2008). Especially in vegetation-rich water bodies of high pH, bicarbonate users can eventually outcompete all obligate CO_2 users (Pedersen et al. 2013).

Metabolization of HCO_3^- to CO_2 results in OH^- anions release, which causes alkalization of environment and reduces the efficiency of bicarbonate use. Therefore some aquatic plants (*Potamogeton*, *Elodea*) developed *polar leaves* with HCO_3^- uptake confined to the lower (abaxial) surface of the leaves. This side is actively acidified, which leads to a formation of an electric potential difference across the leaf. The OH^- anions are released on the upper (adaxial) side of leaves, and the highly alkaline pH on this side results in a precipitation and gradual accumulation of $CaCO_3$ (visible as whitish flakes and films on the leaves, e.g. in *Potamogeton lucens*; Prins et al. 1980, Raven & Hurd 2012).

An interesting way of the carbon uptake is known for isoetids (see above). These small, slow growing, usually evergreen perennials with short leaves in a rosette, often dominate in soft-water lakes. The leaves of isoetids possess very low permeability and the plants receive most of inorganic carbon from the sediment by roots, which represent a substantial part of biomass (Raven et al. 1988, Madsen et al. 2002, Kolář 2014). Moreover, the isoetids oxidate the sediment which reduces the availability of nutrients and iron and helps to create and maintain oligotrophic conditions (Smolders et al. 2002). Some isoetids (many aquatic species of *Isoëtes*, *Littorella*) are endowed with CAM metabolism, which enables them to uptake CO_2 not merely in the daytime but also in the dark when the CO_2 concentration is highest (Raven et al. 1988).

The CAM metabolism in aquatics is not limited to isoetids, but it was also recorded in a few species of *Sagittaria*, *Vallisneria* (facultatively), *Deinostema*, *Lilaeopsis* and two annual aquatic *Crassula* species (syn. *Tillaea*; Webb et al. 1988, Keeley 1998a,b). The "classical" C_4 metabolism occasionally occurs in emerged graminoids (*Spartina*, *Orcuttia*, *Cyperus*, some *Eleocharis* taxa; Keeley et Rundel 2003) but it is rare and facultative in submerged aquatics, being recorded in some species of Hydrocharitaceae (*Hydrilla verticillata*, *Elodea canadensis*, *Egeria densa*, *Ottelia alismoides*; Holaday & Bowes 1980, Casati et al. 2000, van Ginkel et al. 2000, Zhang et al. 2014). The record holder in this respect is *Ottelia alismoides*, operating C_4, bicarbonate use and also CAM metabolic pathway when the CO_2 is deficient (Shao et al. 2017).

2.4 Vegetative propagation and perennation

Vegetative parts of aquatic plants are much more easily dispersed than are clonal fragments of terrestrial plants. Clonal reproduction also provides several other evolutionary benefits, such as reduction of the costs associated with generative reproduction and a rapid replication of adaptive genotypes after colonization of new environments or after surviving the unfavourable periods. As a result, the high ratio of vegetative spread to sexual propagation is characteristic of most aquatic plants (Barrett et al. 1993, Grace 1993, Philbrick & Les 1996, Silvertone 2008, Barrett 2015). The preference for clonality is most glaring in ± stable aquatic habitats, such as larger water bodies and perennial streams (Sculthorpe 1967), and also in rivers, where the propagules have a high capacity to disperse over long distances (Nilsson et al. 2010, Sarneel 2013). The production of seeds is very rare for several aquatic lineages (e.g. Lemnoideae, *Stratiotes*, some seagrasses, *Aldrovanda*, *Rorippa aquatica*, central-European populations of *Ranunculus fluitans*; Sculthorpe 1967, Turała-Szybowska 1977, Cook & Urmi-König 1983, Les 1988, Cross et al. 2016), some species are not able to form viable seeds (or spores) at all (*Salvinia molesta*, *Myriophyllum latifolium*, *Utricularia australis*, *U. ochroleuca*, *U. stygia*, *U. bremii*; Loyal & Crewal 1966, Orchard 1985, Taylor 1989, Les et al. 1995, Gabel & Les 2000). Also some well-known dioecious invasive aquatic species spread exclusively vegetatively within their secondary ranges, due to the fact that only plants of one sex have been introduced (*Elodea canadensis*, *Egeria densa*, *Lagarosiphon major*, *Myriophyllum aquaticum*; Cook & Urmi-König 1984a, 1985, Lambertini et al. 2010, Scribailo & Alix 2014). Many macrophytes are able to stay completely submerged for many growing seasons, but never flower under such conditions and spread exclusively vegetatively (e.g. *Juncus bulbosus*, *Butomus umbellatus*, *Ranunculus circinatus*, *Littorella uniflora*, several species of *Callitriche*; Cook 1966, Sculthorpe 1967, Prančl 2013, Kolář 2014).

The vegetative organs of almost all submerged macrophytes are very brittle and any violent disturbance of the water shatters the plant body into myriad fragments. Almost any detached fragment, if it includes a bud or at least a node, can regenerate a new individual. The same goes for reduced free-floating plants (Sculthorpe 1967, Umetsu et al. 2012, Li et al. 2015, Heidbüchel & Hussner 2019). Mechanically more resistant aquatics developed a number of other vegetative propagules: long rhizomes (nymphaeids, broad-leaved *Potamogeton*, *Cryptocoryne*, *Calla*, *Menyanthes*, most of emergent aquatics in littoral zones…), stolons (*Marsilea*, *Hydrocleys*, *Luronium*, *Echinodorum*, *Eichhornia*, *Littorella*, *Limosella*…), tubers (*Stuckenia*, *Sagittaria*, *Hydrilla*), adventitive buds (i.e. gemmipary; *Ceratopteris*, *Nymphaea micrantha*, *Rorippa aquatica*) or new individuals arising from plantlets or turions in place of flowers (pseudovivipary; *Posidonia oceanica*, numerous Alismataceae – *Caldesia*, *Baldellia*, *Ranalisma*, *Luronium*, *Echinodorus*; Sculthorpe 1967, Ballesteros et al. 2005). The whole plant bodies of minute free-floating aquatics (*Lemna*, *Azolla*) effectively spread epizoochorically over short distances by waterfowl (Coughlan et al. 2016).

Furthermore, the whole individuals of *Wolffia* can survive gut passage through birds and thus also have a potential for endozoochorous dispersal (Silva et al. 2018).

Strictly annual aquatic species persisting the unfavourable period exclusively as seeds are usually confined to habitats affected by regular drying (especially the specialized species growing on exposed bottoms of dried water bodies). Annuals are rare also among floating (*Trapa*), amphibious (e.g. *Elatine*, *Callitriche palustris*; but most of these species are probably capable of being shortly perennial) and submerged macrophytes (most species of *Najas*, some species of *Ruppia* and *Lepilaena*; Vollebergh & Congdon 1986, Triest 1988, Les et al. 2010).

Many aquatic species with annual shoots perennate via *turions* (winter buds, hibernacula). Turions are modified shoot apices consisting of modified, short leaves crowded on extremely shortened stems (Sculthorpe 1967, Adamec 2018). They are formed in at least 14 genera from 9 families, growing in subtropical to polar zones (*Brasenia*, *Ceratophyllum*, *Caldesia*, *Potamogeton*, *Hydrocharis*, *Elodea*, *Hydrilla*, *Stratiotes*, *Spirodela*, *Lemna*, *Wolffia*, *Myriophyllum*, *Aldrovanda*, *Utricularia*; Adamec 2018). Typical turions are morphologically distinct organs capable of entering the true dormancy, detaching from the parent plant and germinating only when it is separated (Adamec 2008). However, there is a continuum between less specialized types with dense clusters of little-modified apical leaves (e.g. *Ceratophyllum*, *Elodea*) and typical, strongly condensed turions (e.g. *Utricularia*, *Aldrovanda*, *Hydrocharis*; Sculthorpe 1967). The definition of the term is thus at least partly arbitrary. Turions usually overwinter at the bottom and germinate in spring, although there are some exceptions (e.g., a large fraction of the turions of *Potamogeton crispus* sprouts in autumn and grow during the winter; Sastroumoto 1981, Heuschele & Gleason 2014).

In the time of germination, the new stems from turions usually grow much faster and are competitively stronger than seedlings of the same individual. For example in narrow-leaved *Potamogeton* species, the seedling recruitment is rare in established populations, whereas propagation by turions is very effective (Kaplan & Štěpánek 2003). On the other side, the longevity of turions is limited. Unlike seeds, turions under natural conditions usually only survive from one season to the next and no longer (Adamec 2018).

Perennial taxa unable to form turions overwinter most often as rhizomes, eventually tubers (see above) or in the stage of small dense green shoots at the bottom (*Ranunculus* sect. *Batrachium*, *Callitriche*; Schotsman 1954, Cook 1966). Other strategies include the presence of dormant shoot buds near the bottom (e.g. *Myriophyllum spicatum*, *Persicaria amphibia*), the sinking of non-dormant rootless shoots to the bottom (*Lemna trisulca*, several species of *Utricularia*; Bartley & Spence 1987) or perennating at the bottom in virtually unchanged morphological state (isoetids; Sculthorpe 1967).

2.5 Sexual reproduction and pollination systems

Sexuality in the aquatic environment is costly and often does not play a major role. However, generative reproduction is essential for survival in certain circumstances (especially in cases of desiccation or other severe stress; Brock et al. 2003, Pieterse 2013) as well as for colonization of newly established sites. Despite the overall preference of vegetative reproduction, aquatic plants have evolved an array of remarkable adaptations of pollination systems (Sculthorpe 1967, Philbrick & Les 1996).

Most of aquatics have aerial flowers, including a number of entirely submerged macrophytes. This brings specific problems: a peduncle must be able to withstand the strains from wind, waves and current. Therefore the flowers are often supported by floating leaf (broad-leaved *Potamogeton*, *Ranunculus* sect. *Batrachium*, *Nymphoides*), aggregation of floating or submerged leaves (*Callitriche*, *Ludwigia*, *Hottonia palustris*) or keep afloat through a special aerenchymatic device (inflated peduncles of *Hottonia inflata*, inflated petioles of *Trapa*, whorl of shoot floats in *Utricularia inflata*). The hydrophytes with aerial flowers often have to make great efforts to reach the water surface: e.g., *Hippuris vulgaris* has been seen to flower from a depth of 3 m (Grainger 1947), and *Phragmites australis* even from a depth of 12 m (Lake Baikal; Z. Kaplan, unpubl.).

For numerous aquatics with emerged flowers, the morphology of the flowers is the best evidence of their evolutionary relationships, because since there are no significant modifications on them. The flowers of some groups (e.g. *Utricularia*, *Hottonia*, *Lobelia dortmanna*, *Bidens beckii* or some species of *Ranunculus* sect. *Batrachium*) are very similar to their terrestrial entomogamous relatives, in contrast to their highly specialized vegetative organs (Sculthorpe 1967). The flowers of aquatic plants are disproportionally biased towards white colour (ca 43% of all aquatic genera), which may probably enhance fitness by making flowers more conspicuous to pollinators (Philbrick & Les 1996). However, relatively small number of aquatics is distinctively entomophilous. Most aquatic insects do not feed on nectar or pollen, but use flowers for mating, shelter and protection from predators (Philbrick & Les 1996). Therefore the nectar- and pollen-offering flowers are not quite typical in aquatic habitats, being mostly visited by unspecialized insects (e.g. day-blooming *Nymphaea*, *Nuphar*, *Butomus*, some species of *Utricularia*; Prance & Anderson 1976, Taylor 1989, Fernando & Cass 1997, Lippok & Renner 1997). The special case of insect-mediated pollen transport includes pollination by beetles attracted to floral scents and floral thermogenesis in two unrelated groups possessing remarkably similar flowers (night-blooming *Nymphaea* subg. *Hydrocallis* and *Victoria*, Ervik & Knudsen 2003; *Nelumbo*, Li & Huang 2009).

About a third of all aquatic plants are wind pollinated (Cook 1988). The vast majority of them have obvious terrestrial relatives which are also anemophilous (Poales) or belong to (almost) exclusively anemophilous families (Hydatellaceae, Haloragaceae) or lineages (the clade containing *Hippuris* and *Callitriche* within Plantaginaceae). Only in two genera, *Brasenia* (Cabombaceae) and *Limnobium* (Hydrocharitaceae), and in the anemophilous families of Alismatales (e.g. Scheuchzeriaceae, Juncaginaceae, Potamogetonaceae) it is likely that the evolution from entomophily to anemophily has taken place in the aquatic environment (Cook 1988, Du & Wang 2014, Albach et al. 2005, Ross et al. 2016). The shift towards anemogamy is connected with a strong reduction of floral morphology and with a frequent occurrence of unisexual flowers (*Typha*, *Sparganium*, *Limnobium*, some species of *Myriophyllum*, *Littorella*, *Callitriche*; Cook 1988).

Most of macrophytes with aerial flowers are capable of selfing (Philbrick & Les 1996). In contrast, mechanisms to prevent autogamy (or geitonogamy) are rare. The most extreme form of sexual specialization is dioecy, known in several anemophilous species of *Myriophyllum* (Orchard 1985, Scribailo & Alix 2014) and four entomophilous species of *Nymphoides*; moreover, one another species of this genus is gynodioecious (Ornduff 1966, Tippery & Les 2011). A unique reproductive system is present in *Sagittaria latifolia*: populations of this species are usually either monoecious or dioecious, and the androdioecic or gynodioecic populations occur on the northern portion of its geographical range. While dioecious populations of *S. latifolia* are characteristic of stable habitats, monoecious populations typically occur in habitats affecting by frequent disturbance. Moreover, the female flower production in monoecious populations varies positively with plant size

(Sarkissian et al. 2001, Dorken et al. 2002, Yakimowski & Barrett 2016). In *Hydrilla verticillata*, two "biotypes" with different floral biology are widely recognized: a monoecious biotype is highly invasive and rapidly spreading in North America, with shoots senescing over winter and repopulation occurring through prolific turions. Dioecious biotype has more persistent shoots but produces fewer turions and, although it has also been introduced to the United States, does not currently show significant range expansion (True-Meadows et al. 2016). Both biotypes have been reported to be triploid in their introduced ranges (Benoit et al. 2019).

The presence of heterostyly is another way to favor allogamy (Barrett 2019). In aquatic plants, distyly has been reported for *Hottonia* (Vermeesch & Triest 2006) and all but one genera of Menyanthaceae (Ornduff 1966, 1988, Olesen 1986), whereas tristyly is known in two genera of Pontederiaceae (*Eichhornia* and *Pontederia*; Barrett 1977, 1989) and both tristyly and distyly take place in several genera of Lythraceae (*Lythrum*, *Decodon*, *Nesaea*, *Rotala*; Ganders 1979, Ornduff 1979, Eckert & Barrett 1993). However, virtually all heterostylous (and also dioecious) aquatics are clonal. The formation of large clones of these species may result in the spatial isolation of flowers from compatible pollen sources, with consequences for fertility (Barrett 2015). For example, the Chinese population of distylous *Nymphoides peltata* are self-incompatible and highly clonal, which leads to biased morph ratio (anisoplethy) and strongly reduced seed set (Wang et al. 2005). In tristylous species, the mid-styled floral morph (M-morph) is often self-compatible (e.g. in *Pontederia*, Puentes et al. 2013). In *Eichhornia*, breeding systems range from outcrossing tristylous populations to predominantly self-fertilized, semi-homostylous populations (Barrett 1989). For example, the rooting macrophyte *E. azurea* is predominantly self-incompatible, being pollinated by specialized nectar-feeding insects (da Cunha & Fischer 2009), whereas the invasive free-floating *E. crassipes* is self-compatible, producing seeds abundantly in populations monomorphic for style morph (Barrett 1977). The populations of *E. crassipes* are largely monomorphic, even in the native range, with dominating mid-styled morph (Zhang et al. 2010). The breakdown of heterostyly is also reported for distylous species: in *Hottonia palustris*, the high self-compatibility has been confirmed (Vermeesch & Triest 2006). Self-incompatibility has rarely been reported in homostylous aquatics, being expected e.g. for the invasive *Ludwigia grandiflora* (Dandelot et al. 2005), exclusively unfertile *Rorippa aquatica* (Les et al. 1995), homostylous member of the Menyanthaceae family *Villarsia albiflora* (Ornduff 1988), and also for certain populations of *Ranunculus penicillatus* (Turała-Szybowska 1978).

Hydrophily (or hydrogamy), i.e. water-mediated pollination, represents the final stage of adaptation to underwater life and entails dramatic modifications of the floral systems. Two general forms are recognized: epihydrophily (ephydrophily), two-dimensional pollination via pollen transport at the water surface, and hypohydrophily (hyphydrophily), three-dimensional pollination below the water surface (Les 1988, Philbrick 1991). Hydrophily is a rare phenomenon, obligatorily occurring in ca 130 species of 18 genera (Les et al. 1997). The overwhelming majority of the hydrophilous species are placed in Alismatales, elsewhere the incidence of hydrophily is known only in *Ceratophyllum* and *Callitriche* (Les 1988, Philbrick & Les 1996). Epihydrophily can be interpreted as an initial stage on the way from anemophily towards hypohydrophily. The obligate epihydrophily has been reported in six genera of Hydrocharitaceae and in *Althenia* and *Lepilaena cylindrocarpa* from the former Zannichelliaceae, it is also the prevailing pollination mode in *Ruppia* (Cook 1982, Cox 1988, Cox & Knox 1989, Cook & Guo 1990, Les 1988, Philbrick 1988, Ackerman 2000). It also takes place in several predominantly anemogamous genera (*Potamogeton*, Kaplan & Štěpánek 2003, Zhang et al. 2009a,b*; Callitriche*, Schotsman 1954, 1982). In five of six epihydrogamous genera of Hydrocharitaceae (freshwater genera *Vallisneria*, *Lagarosiphon*, *Nechamandra*, *Appertiella* and monotypic seagrass genus *Enhalus*), the pollination is mediated through the collision of floating staminate flowers ("pollen boats") that have detached from submerged male inflorescences, with buoyant, long-stalked pistillate flowers (Cook 1982). This peculiar mechanism ("maleflower epihydrophily") seems to have evolved at least twice independently in the family (Chen et al. 2012, Du & Wang 2014). In another genus of the Hydrocharitaceae, monotypic *Hydrilla*, the similar reproduction mode has been reported, which cannot be simply classified neither as anemophily nor the epihydrogamy: the male flowers are liberated from mother plant as buds, which then open explosively at the water surface, the pollen grains are shooted in the air and fall down on the stigmas (Cook & Lüönd 1982). In the remaining genera (*Elodea*, *Lepilaena*, *Ruppia*), the pollen grains *per se* are dispersed on the water surface to emergent or slightly submerged female flowers (Cook & Urmi-König 1985, Cox & Knox 1988).

In the case of hypohydrophily, the perfectly wettable pollen lacking an exine (or possessing the rudimentary exine) is liberated underwater. Hypohydrophily is polyphyletic, representing a notable example of convergent evolution. It evolved at least nine times, including six separate origins in Alismatidae (*Zannichellia*, *Pseudalthenia* and *Lepilaena bilocularis* from the former Zannichelliaceae, Zosteraceae, *Posidonia*, Cymodoceaceae and two independent lineages in Hydrocharitaceae: *Najas* and marine genera *Halophila* + *Thallasia*), two separate origins in *Callitriche* (about eight of ca 75 species) and a single origin in *Ceratophyllum* (all six species; Les 1989, Cook & Guo 1990, Les et al. 1997, Philbrick & Les 2000, Lansdown 2008, 2009, Chen et al. 2012, Du & Wang 2014). A significant number of hypohydrophilous species is accumulated in an informal group of seagrasses, i.e. the exclusively marine monocots including 11–13 genera from four families (Les et al. 1997, Ackerman 2006). However, the most diversified hypohydrophilous genus *Najas* with 30–40 recognized species is restricted to fresh and brackish waters (Triest 1988, Ito et al. 2017b). In marine genera *Halodule*, *Amphibolis* (both Cymodoceaceae) and *Halophila* (Hydrocharitaceae), the pollen spreads predominantly two-dimensionally at the water surface. Although possessing wettable exineless pollen, they are sometimes interpreted to be epihydrophilous (Cox & Knox 1988, 1989).

Hydrophily is strongly connected with the prevalence of dicliny: from 18 hydrophilous genera, 11 include dioecious species, 7 have monoecious species and only two genera possess hermaphroditic flowers (*Ruppia*, *Posidonia*; Les 1988, Du & Wang 2014). Unisexuality can be both ancestral condition and derived state in hydrophiles: it is a primitive rather than derived state in the Hydrocharitaceae, but appears to evolve underwater in the case of the Cymodoceaceae (Du & Wang 2014). The flowers are strongly reduced, particularly in hypohydrophilous species. With the exception of *Enhalus* and *Thalassia* (Hydrocharitaceae), the flowers of hypohydrophilous plants lack perianth parts or these organs are bract-like and minucious in appearance (Ackerman 2006). The pollen is usually released in large amounts and exhibits structural modifications that apparently maximize transport efficiency. In most seagrasses, the pollen grains are extremely long, filiform, spreading in clusters; in other genera, globular pollen grains form filiform chains, or germinate precociously and disperse as a tangled mass of pollen tubes (Sehgal & Ram 1970, Les 1988, Philbrick & Anderson 1992, Ackerman 2006).

The separation of carpellate and staminate reproductive structures suggests a potential for facilitating generative reproduction and limiting self-pollination, especially in dioecious taxa. Indeed, the seed set is abundant in many species of seagrasses (Les 1988), and the genus *Najas* is even almost exclusively annual. The single dioecious annual species of *Najas*, *N. marina* s.l., shows obligate outcrossing and high pollination effectiveness (Huang et al. 2001). The self-incompatibility combined with a high seed set has also been recorded in some populations of hermaphroditic seagrass *Posidonia oceanica* (Sinclair et al. 2014). Surprisingly, the biotic underwater polination mediated by marine invertebrates has been confirmed for dioecious seagrass *Thalassia testudinum* (van Tussenbroek et al. 2016). On the other side, numerous species of seagrasses, especially the dioecious, show rare flowering, biased proportion of sexes, and often grow in unisexual clonal beds (Les 1988). The same applies for epihydrogamous *Elodea*, which typically exists in unisexual colonies with plants of both sexes rarely coexisting (Cook & Urmi-König 1985). Hypohydrogamous monoecious, predominantly annual species *Zannichellia palustris* has both male and female flowers in close proximity, which leads into the prevalence of geitonogamous pollination accompanied by highly efficient seed formation (Guo et al. 1990).

Many species of aquatic plants are capable of submerged pollination through various ways of selfing, despite possessing pollen of aerial type (with an exine) which is not adapted for underwater spread. Cleistogamy (the presence of non-opening flowers) or the opening of flowers coming after the early pollination in closed bud (i.e. the analogy to cleistogamy) are the most common mechanisms of autogamy in submerged conditions. Such mechanisms occur frequently in habitats where the water-level fluctuates, e.g. in submerged ecomorphoses of *Ottelia*, *Ranunculus* sect. *Batrachium*, *Elatine*, *Podostemum*, *Subularia*, *Limosella* or *Lobelia dortmanna* (Philbrick & Anderson 1987, Culley et Klooster 2007). Cleistogamy is almost an exclusive way of reproduction in *Ranunculus trichophyllus* subsp. *eradicatus* (Cook 1966) and it is assumed to be an exclusive way of pollination in obligatory submerged *Myriophyllum farwellii* (Philbrick & Anderson 1987). It is also known for the aerial flowers of some species of *Utricularia* (Rutishauser 2016). Another mechanism of autogamous underwater pollination is *hydroautogamy*: the pollen is borne from anther to stigma by gas bubble released from the dehisced anther (Philbrick & Les 1987). Hydroautogamy is sometimes considered a transitional stage between aerial pollination and hydrophily (Philbrick 1988). It has been reported in three genera of Potamogetonaceae (*Potamogeton*, *Stuckenia*, *Groenlandia*; Philbrick & Anderson 1987, Guo & Cook 1989, 1990, Zhang et al. 2009b), *Ruppia* and *Althenia* (Cook & Guo 1990, Martínez-Garrido et al. 2015).

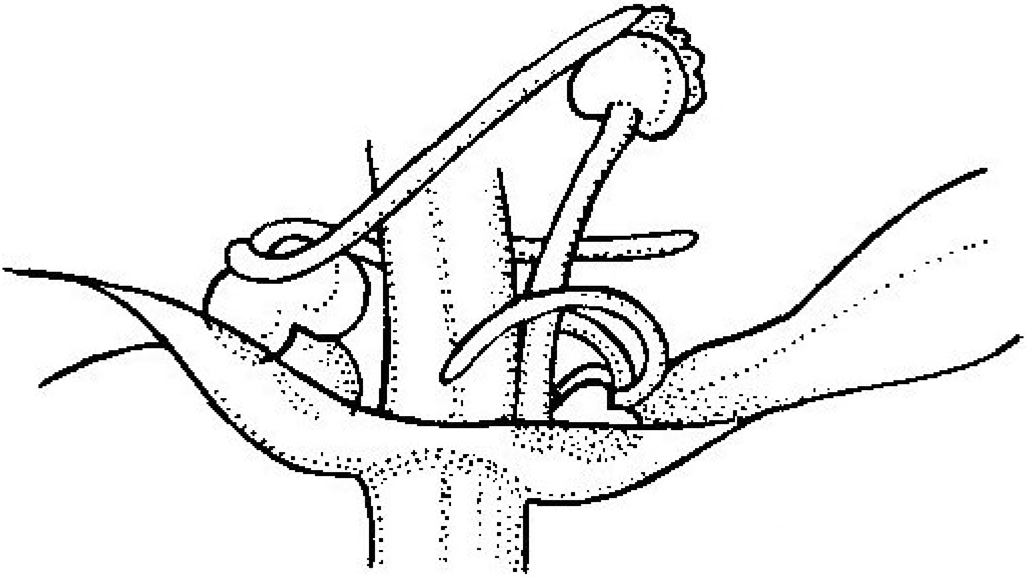

FIG. 2. Geitonogamous self-pollination in *Callitriche regis-jubae*, mediated by bending of the stigma to the anther of the male flower in the opposite leaf axil. Adopted from Schotsman (1982).

Remarkable ways of geitonogamous selfing between unisexual achlamydous flowers are present in *Callitriche*. In some species, the pollination is mediated by bending the stamen to the adjacent stigmas or vice versa ("contacters", Schotsman 1982; Fig. 2). Geitonogamy through the contact of male and female flower is the prevailing way of pollination for some taxa with the pollen of aerial type, which are therefore able to set fruit completely submerged (e.g. *C. lusitanica*, *C. lenisulca*), but it is present also in some hypohydrophilous species. The couple of *C. hamulata* and *C. brutia* possesses submerged flowers and pollen with lacking or strongly reduced exine, adapted to spread underwater. Such contact pollination allows successful fruit formation even in rapidly flowing water. Moreover, both species are amphibious and form subterrestrial ecomorphoses. On the land, the minucious flowers in the leaf axils are often flood with little drops (usually due to dew or rain), in which the pollen grains have germinated (Schotsman 1954, Schotsman 1982). In seven species (holarctic *C. palustris* and six New World taxa), the unique mechanism of internal geitonogamy has evolved: the pollen tubes germinate directly inside anther that does not open at all, and grow through the vegetative tissue of filament and node to achieve the adjacent ovaries (Philbrick 1984, Philbrick & Bernardello 1992). This pollination mode, like cleistogamy, is very effective in environments where strong and unpredictable water level fluctuations take place.

Apomixis has not yet been unequivocally confirmed for any "true" aquatic plant (cf. Carman 1997); however, it has been reported for a few wetland species (e.g. *Calamagrostis purpurea*, Nygren 1949). Indirect evidence of apomixis has been observed in *Trithuria* (Hydatellaceae, Nymphaeales), but more detailed research is still needed in this case (Rudall et al. 2008, Hörandl & Hojsgaard 2012).

2.6 Seed dispersal and seed bank

Aquatic plants are often reported to have relatively high capacity of long-distance dispersal of sexual propagules (Santamaría 2002, Les et al. 2003). For example, the seagrass *Heterozostera nigricaulis* dispersed twice independently from Australia to South America (ca 14,000 km; Smith et al. 2017). Hydrochory, the ubiquitous phenomenon among aquatics, is considered more successful in long-distance propagule dispersal than other dispersal modes (if we omit anthropochory), being of particular importance in marine habitats and river systems. Nevertheless, it is not always easy to determine if the propagation over long distances is realized by seeds or by vegetative fragments, as both are well buoyant in many aquatics (Nilsson et al. 2010).

Endozoochory mediated by migrating waterfowl (especially Anatideae) is also reported as the frequent way of the long-distance dispersal of seeds (Figuerola & Green 2002, Brochet et al. 2009), but there is still controversy over its importance over very long distances. Aquatic birds have relatively fast digestion and a majority of the ingested seeds are ejected within 100–300 km. Moreover, seed transport from north to south (on autumn migrations) probably outweighs the opposite direction (Clausen et al. 2002). The passage through guts is less lethal for smaller seeds than for larger one (Figuerola et al. 2010, van Leeuwen et al. 2012). Aquatic birds are also ectozoochorous vectors, but the importance of ectozoochory in the spread over long distances has not been studied in as much detail (Coughlan et al. 2017). In marine environments, also dugongs and sea turtles are vectors for long-distance seed dispersal (up to 650 km, Tol et al. 2017).

Contrary, anemochory is very lossy and does not ensure efficient transport of seeds to island-like aquatic habitats, thus it is not frequent among aquatics. However, some robust littoral species with large seed production may be good colonizers of new localities (e.g. *Typha*, *Phragmites australis*; Soomers et al. 2013).

Seed dormancy is an effective mechanism that allows aquatic communities to survive unfavorable conditions (Brock et al. 2003). Numerous aquatic habitats are subject to periodical drought (such as temporary ponds in regions with a Mediterranean climate; Aponte et al. 2010, Brock 2011) or episodic draining (central-European fishponds; Šumberová et al. 2011, Poschlod & Rosbakh 2018). The existence of long-term soil seed bank is necessary not only to survive dry periods. The exposed bottoms of water bodies host specialized plant communities of short-lived wetland species, which are often not able to survive underwater (Šumberová 2006). Especially the intensively managed fishponds are for the most of the time eutrophic, with strongly turbid water and a thick layer of anaerobic sapropel on the bottom. The presence of aquatic vegetation in such fishponds is often restricted to short periods after occasional draining, when the water is clear and at the same time rich in released nutrients (Kaplan 2010a).

The seed longevity of some aquatic species is fascinating. The seeds of *Nelumbo nuccifera* that germinated after being preserved for about 1300 years in a dry lakebed in northeastern China are the second longest-lived example of a viable seed of known age (Shen-Miller et al. 2002, Porteous et al. 2019). In central-European fishponds, the seeds of mudflat species may survive 50–100 years between particular events of draining (Poschlod & Rosbakh 2018). Similarly, the seeds of some aquatic species could survive 50–150 years lasting burial under agricultural fields in the sediments of former ponds (‘ghost ponds’, Alderton et al. 2017). Due to this extreme dormancy, the viable seeds of some threatened species, although appearing to be almost of completely vanished, may be still present in a seed bank (Šumberová et al. 2011, Poschlod & Rosbakh 2018). This can be successfully utilized for the restoration of some endangered aquatic species: a good example is *Potamogeton coloratus*, the formerly extinct species of the Czech

Republic. It has recently been re-established from the seed bank on the locality from which it had been absent for 32 years (Kaplan et al. 2014).

3. GENERAL EVOLUTIONARY PROCESSES AND OTHER PHENOMENA SHAPING THE PLANT EVOLUTION AND THEIR MANIFESTATIONS IN AQUATICS

Aquatic macrophytes are regarded as being among the taxonomically most intricate angiosperms. The morphological expression of aquatic plants is strongly determined by two opposing forces – a considerable reduction of the plant body (multiple taxa can share a similar phenotype), and at the same time an extensive phenotypic plasticity (one taxon can exhibit multiple morphologies). Only genetically fixed variation is eligible for formal classification in such cases. However, its identification is difficult because the most prominent morphological differences may arise due to the environmentally induced plasticity while the true genetic variation often remains cryptic. Moreover, the aquatic plants, despite their evolutionary uniqueness and economic importance, are markedly underrepresented among biosystematic studies (Barrett et al. 1993). Some attention was given perhaps only to carnivorous and invasive aquatic plants, whereas the evolution of many other aquatic groups has not yet been studied thoroughly using current genetic approaches. For example, the broadly distributed genus *Trapa* (Lythraceae) has been classified into 1–70 species according to different concepts; however, a comprehensive taxonomic evaluation of the genus employing molecular markers is still missing (Cook 1990, Zhang et al. 2019).

In this chapter I focus on the main evolutionary trends that are responsible for the creation of genetic variation, play a role in establishing of new taxa and have significance in taxonomic treatments of challenging aquatic plant groups.

3.1 Polyploidization

Polyploidization, the process of genome multiplication, is considered one of the crucial forces in plant evolution, fundamentally affecting the diversification and speciation (Soltis et al. 2009, Weiss-Schneeweis et al. 2013, Alix et al. 2017). All angiosperms undergone at least one polyploidization event in their evolutionary history (Jiao et al. 2011, Alix et al. 2017). The proportion of recent (genetically non-diploidized) polyploids is estimated between 30–70% in angiosperms, with most estimates near 50% (Soltis et al. 2015). Polyploidization brings genetic and phenotypic novelties and often leads to instant reproductive isolation, which makes it a ubiquitous mechanism of sympatric speciation (Coyne & Orr 2004, Adams & Wendel

2005, Flagel & Wendel 2009, Hegarty et al. 2013, Tayalé & Parisod 2013, Kolář et al. 2017). In addition, a number of changes arising as a direct result of polyploidization has adaptive significance and increases the evolutionary potential of polyploid lineages. New polyploids can be able to colonize new habitats, show wider ecological amplitude, or be more competitive in comparison with diploids (Parisod et al. 2010, Husband et al. 2013, Segraves 2017, Baduel et al. 2018, Baniaga et al. 2020). Consequently, polyploidy can be an important factor in species invasion success (Schierenbeck & Ellstrand 2009, Pandit et al. 2011, 2014, te Beest et al. 2012).

Polyploidy has a significant impact on geographic and environmental distribution of vascular plants on a global scale (Ramsey & Ramsey 2014, Rice et al. 2019). Particularly, clonal reproduction is thought to facilitate polyploid establishment and polyploidy is strongly associated with vegetative reproduction (Herben et al. 2017, Van Drunen & Husband 2019). Because the overwhelming majority of macrophytes are highly clonal, it can be generally assumed that the proportion of polyploids in aquatic plants is rather high. Indeed, polyploidy has been reported in 85% of aquatic plant genera, for that the chromosome counts were known (Les & Philbrick 1993); however, no estimation of its frequency at species level is yet available.

The highest ploidy levels in aquatic plants are known for *Rumex hydrolapathum* (2n=20x=200), *Nymphaea gigantea* (2n=16x=~224, i.e. the highest chromosome number among aquatics), *Ranunculus lingua* (2n=16x=128), *Elatine hexandra* (2n=12x=108), some populations of *Ottelia alismoides* (2n=12x=132) and *Lemna minor* (2n=ca12x=126), *Isoëtes lacustris* (2n=10x=110), some populations of *Aponogeton distachyos* (2n=10x=80) and *Najas guadalupensis* (2n=10x=60; Pellicer et al. 2013, Kalinka et al. 2015, Rice et al. 2015). Paradoxically, several species of *Equisetum*, which possess the second highest chromosome number among aquatics (2n=216), are genetically diploid (Soltis 1986). By comparison, the smallest chromosome number among aquatic plants has been reported for three species of *Callitriche* (2n=6; reviewed in the case study **P-I**, see below).

Two main types of recent polyploidy are recognized: autopolyploidy (genome duplication within a species) and allopolyploidy (polyploidy formed through the combined process of interspecific hybridization and genome doubling; Soltis et al. 2015, Alix et al. 2017). Actually, polyploidy is rather a continuum from the doubling of identical genomes to doubling of highly differentiated genomes (Parisod et al. 2010). The relative abundance of autoand allopolyploids is still the subject of questions. According to some sources, autopolyploids arise much more often than allopolyploids; on the other side, allopolyploids likely have a significant evolutionary advantage, because they possess greater niche differentiation compared to progenitor and also have more genetic resources to cope with environmental stress (Barker et al. 2015). In the study of Barker et al. (2015) analysing 4,003 plant species, the number of auto- and allopolyploid taxa were at approximate parity. However, we should not forget that allopolyploids are morphologically and molecularly more conspicuous and the actual number of autopolyploids may therefore be underestimated (Soltis et al. 2007, Parisod et al. 2010). In the dataset of Barker et al. (2015), more than 87% of the allopolyploids were recognized as named species, while the same is true only for ca 12% of autopolyploids. In aquatic plants there are only a few cases in which the origin of polyploids has been determined using molecular markers and it is mostly an evidence of allopolyploidy. For example, *Nymphaea candida* is an allopolyploid that originated at least twice by hybridization between mainly European *N. alba* and mainly Asian *N. tetragona* (Volkova et al. 2010). In *Ranunculus* sect. *Batrachium*, the river species *R. penicillatus* s.l. is considered an allopolyploid complex of taxa emerging from the hybridization of *R. fluitans* with several other species (Cook 1966). For some of Polish populations of *R. penicillatus*, the recurrent origin from the parental species *R. fluitans* and *R. peltatus* has been confirmed (Zalewska-Gałosz et al. 2015). In the complex of *Ruppia maritima*, cryptic triploid and tetraploid lineages of putative allopolyploid origin have been discerned (Ito et al. 2010). From other examples, *Callitriche platycarpa* has been confirmed as an allotetraploid arising through the hybridization of diploid species *C. cophocarpa* and *C. stagnalis* (Bączkiewicz & al., 2007; Schwarzacher & al., 2017) and *Najas canadensis* is an allotetraploid derivative of *N. flexilis* and *N. guadalupensis* (Les et al. 2015a). Allopolyploid origin has also been revealed for three octoploid species of *Potamogeton* (Kaplan et al. 2013), two hexaploid species of *Myriophyllum* (Lü et al. 2017) and octoploid *Trapa japonica* (Takano & Kadono 2005). In the case of octoploid *Nasturtium microphyllum*, both auto- and allopolyploid origin from the now-extinct tetraploid predecessor(s) is possible (Mandáková & Lysák 2019). Until present, apparently no direct evidence of autopolyploid origin has been reported for any aquatic plant species.

In recent decades, along with the rapid development of molecular and cytogenetic techniques, great emphasis has been placed on the study of mixed-ploidy species, i.e. taxa containing at least two ploidy levels. Mixed-ploidy species offer unique insights into the processes governing polyploid evolution and speciation, enable the study of cytotype frequencies and distributions, and their phenotypic, ecological, and evolutionary consequences. About 16% of all plant species contain ploidy variation (Kolář et al. 2017). In aquatic plants, two or more ploidy levels have been reported at least for 112 species (Les & Philbrick 1993; no recently reviewed data are available). Some aquatics can be regarded as an excellent model cases of mixed-ploidy species: for example, eight ploidy levels has been reported for *Phragmites australis* (3x, 4x, 6x, 7x, 8x, 10x, 11x, 12x), seven ploidy levels for *Aponogeton distachyos* (2x, 3x, 4x, 5x, 6x, 7x, 10x), six ploidy levels and multiple aneuploid chromosome counts for *Ottelia alismoides* (2x, 4x, 5x, 6x, 10x, 12x), six ploidies for *Najas guadalupensis* (2x, 6x, 7x, 8x, 9x, 10x; Bolkhovskikh et al. 1969, Cook & Urmi-König 1984b, Van Bruggen 1985, Les & Philbrick 1993, Lambertini et al. 2006). The subfamily of duckweeds (Lemnoideae, Araceae) is the extreme case of the plant group with ploidy level variation: the genera *Lemna*, *Spirodela*, *Wolffia* and *Wolffiella* all contain species with counts ranging from 2n=20–80 (or even up to 2n=126 in *Lemna minor*) and eight species are reported to possess four or more ploidy levels (Urbanska-Worytkiewicz 1975, Les & Philbrick 1993, Les et al. 2002). In *Ranunculus* sect. *Batrachium*, two or more ploidy levels have been reported for 16 of 30 recognized species (Wiegleb et al. 2017). Also the genus *Cryptocoryne* is rich in mixed-ploidy species, including nine aquatic species possessing more than one ploidy level (Petersen 1989). However, almost all of our knowledge about mixed-ploidy aquatic plants species is based merely on chromosome counting. There are still very few multidisciplinary studies studying the ploidy level variation and its manifestations within aquatic species on the broader geographic scale and/or on a larger number of individuals.

One of the few species, in which the ploidy level variation and its consequences have been thoroughly studied, is *Butomus umbellatus*: two cytotypes (diploids and triploids) have been reported, differing in their distribution, ways of reproduction and also morphology. In central Europe, diploids are self-compatible, produce a big number of seeds and grow mainly in fishponds, whereas the more common triploids are self-incompatible, rarely form seeds, reproduce almost exclusively vegetatively and prevail in river floodplains (Krahulcová & Jarolímová 1993, Hroudová & Zákravský

1993a,b). However, the study focused on mixed populations of both cytotypes in eastern Slovakia did not reveal any differences in habitat requirements of diploid and triploid plants (Rydlo 2016). In the British Isles, triploids are also more common and show spatial separation from diploid populations (Bailey & Preston 2001). Both cytotypes have been introduced to North America, but contrary to European populations, diploids are much more common there, being also more proficient in clonal spread (Eckert et al. 2003, Lui et al. 2005).

Acorus calamus is the other case of repeatedly examined mixed-ploidy species. Four geographically partially distinct cytotypes are known (2x, 3x, 4x, 6x; Kaplan 2016), from which only triploids became naturalized in the large secondary areas, although they are sterile and can only spread vegetatively (Röst 1978). All four cytotypes exist in natural populations in Indian Himalayan region, but they are geographically more or less isolated from each other (Mittal et al. 2015).

In karyologically and genetically highly variable *Phragmites australis* (see above), tetraploids prevail in Europe, whereas octoploids dominate in the Southern Hemisphere. Octoploids have arisen many times independently and in different areas of the species distribution range (Lambertini et al. 2006). In the area of the Danube Delta (Romania), four ploidy levels have been found to grow sympatrically (4x, 6x, 8x, 12x; Hansen et al. 2007). However, it is impossible to identify the particular cytotypes of *P. australis* morphologically (Hansen et al. 2007, Achenbach et al. 2012). The North American invaders of European origin are able to outcompete native North American clones of *P. australis*; however, there was no effect of ploidy on competition (Pyšek et al. 2020).

The remarkable case of invasive dynamics has been revealed among South American invasive species of *Ludwigia*. Two closely related taxa have been introduced to Europe, diploid self-compatible *L. peploides* and decaploid self-incompatible *L. hexapetala*. In France, decaploid has been spread in the first wave of invasion in the 19th century, but it has been outperformed by diploid in the 20th century, which now dominates there (Dandelot et al. 2005). In a cultivation experiment, the diploid species had much higher growth rate and biomass accumulation than the polyploid if the substrate was nutrient-rich (Grewell et al. 2016). This finding is contradictory to the general view that polyploids are the better invaders than diploids (see above).

3.2 Interspecific hybridization

Over time, the perception of hybridization has undergone considerable changes. Although initially regarded to be an evolutionary noise or dead end, hybridization is now considered one of the most important processes of plant speciation (Mallet 2007, Soltis & Soltis 2009). Two main ways how the new species can arise through hybridization are recognized: allopolyploidization and homoploid hybrid speciation. Allopolyploidization has long been recognized and thoroughly studied and it is confirmed to be relatively frequent (see the Chapter 3.1). On the other side, homoploid hybrid speciation (the origin of reproductively isolated species without change in chromosome number) is considered to be rare, but our knowledge about the emergence of new species in this way is still very sketchy (Abbott et al. 2010, Schumer et al. 2014, Yakimowski & Riesenberg 2014). Anyway, an increasing number of homoploid hybrid species have been reported for plants and animals in recent years (Ru et al. 2018).

Around 25% of plant species hybridize with at least one other species (Mallet 2005). In aquatics, the single available review found hybridization to be reported in 20% of plant genera (Les & Philbrick 1993). However, hybridization between aquatic plants is very probably more abundant but largely overlooked due to simplified morphology and extensive phenotypic plasticity of many macrophytes. Moreover, some hybrids can mimic another unrelated species. For example, hybridization between two very dissimilar species of *Potamogeton*, *P. natans* (lacking submerged leaves) and *P. lucens* (possessing only submerged leaves) leads to the formation of the hybrid *P. ×fluitans*, which is morphologically very similar to the another unrelated species *P. nodosus* (Kaplan 2001, 2005).

Among aquatics, the genus *Potamogeton* represents a "classical" example of the group with extreme number of hybrids, with at least 99 confirmed hybrid combinations (Kaplan et al. 2018). Almost all *Potamogeton* hybrids are sterile. However, the single hybrid *P. ×angustifolius* (*P. gramineus* × *P. lucens*) is capable of producing fruits and even participated in a formation of natural primary triple hybrid *P. ×torssanderi* (*P. gramineus* × *P. lucens* × *P. perfoliatus*; Kaplan & Fehrer 2007). In the genus *Nymphaea*, several thousands of artificial hybrid cultivars have been developed during the more than 100 years of horticultural targeted breeding, including many different parent species from different continents. Garden cultivars were repeatedly introduced into natural habitats where they survive successfully for long periods. Their fertility is usually very low because of the high pollen sterility (Les & Philbrick 1993, Slocum 2005, Kabátová et al. 2014). On the other side, much less information is available for natural *Nymphaea* hybrids (e.g., Les & Philbrick 1993, Kabátová et al. 2014, Dąbrowska et al. 2015). Another but less explored genus with frequent hybridization is *Aponogeton*, including several hybrids being cultivated by aquarists (Les & Philbrick 1993). Hybrids have even been repeatedly revealed among hypohydrophilous, underwater-pollinated species, namely in freshwater *Najas* (Les et al. 2010) and seagrasses *Ruppia*, *Halodule* and *Posidonia* (Ito et al. 2010, Ito & Tanaka 2011, Sinclair et al. 2019).

Even if the interspecific hybrids are largely sterile, they may still have a great evolutionary potential. Firstly, primary hybrids (if they are heteroploid or arising with a participation of an unreduced gamete) often represent an intermediate step (“triploid bridge”) in the process of formation of new allopolyploids that can arise via backcrossing with parental species. Moreover, the lower degrees of fertility often correlate with the greater rates of unreduced gamete formation and consequently with the potential to arising of allopolyploids (Ramsey & Schemske 1998, Levin 2012, Mason & Pires 2015). Secondly, sterile hybrids often show heterosis and can be very competitive due to the prolific clonal growth. In more permanent habitats, such as streams and large water bodies, hybrid colonies can persist for a considerable period. Several remarkable examples have been confirmed: sterile hybrid *Stuckenia ×bottnica* (*S. pectinata* × *S. vaginata*) has been found in several rivers in England, although the recent European distribution of one of the parental species, *S. vaginata*, is now restricted to Scandinavia. In England, *S. vaginata* occurred in the past, but the latest fossil fruits are dated ca 30,000 years before present; thus, the hybrid can be considered a glacial relict (King et al. 2001). Later, the same hybrid combination has also been found in Denmark, where *S. vaginata* does not occur either (Kaplan et al. 2013). Another *Stuckenia* hybrid, S. ×*fennica* (*S. filiformis* × *S. vaginata*), also occurs in Eastern Europe outside the current distribution of *S. vaginata* (Bobrov, 2007). Similar case is the Swedish hybrid *Potamogeton ×lanceolatifolius*, which was widely recognized to be a cross between *P. gramineus* and *P. polygonifolius*. However, the molecular analyses showed that the second parent of that hybrid is not *P. polygonifolius* but *P. nodosus*, which currently does not occur in the whole of Scandinavia (Kaplan & Fehrer 2011). Finally, *Potamogeton floridanus* was previously classified as an endemic species of Florida. Actually, the targeted research revealed that this taxon is a hybrid of *P. pulcher* and *P. oakesianus*; the second parental species currently does not occur in Florida and its nearest sites are ca 1,100 km away (Kaplan et al. 2018).

Even widespread aquatic taxa, traditionally recognized as species, can actually be sterile hybrids. Probably the best case would be the widely distributed *Utricularia australis*, which has been confirmed to be an almost completely sterile diploid F_1 hybrid of *U. tenuicaulis* (only occurring in Japan) and *U. macrorhiza* (Kameyama et al. 2005, Kameyama & Ohara 2006). *Utricularia australis* indeed show sterility throughout its distribution range, however, the conclusions about its origin are based solely on the study of Japanese populations. Whether *U. australis* is indeed a diploid hybrid throughout its broad range (and should thus be best classified as *U.* × *australis*), or also contains polyploid populations remains unclear yet (Fleischmann 2012). Another case of sterile hybrid "species" in *Utricularia* includes the couple of *U. ochroleuca* and *U. stygia*. Both taxa have been considered polymorphic hybrids between *U. intermedia* and *U. minor*, originated from repeated hybridization events: *U. intermedia* was the pollen donor for *U. ochroleuca* and mother species for *U. stygia*. Moreover, some populations of *U. ochroleuca* underwent backcrossing with one of the parental species (reviewed in Adamec 2020).

If the hybrids are at least partly fertile, the contact with parental species may lead to extensive introgression or to the formation of the homoploid hybrid derivatives (Soltis & Soltis 2009, Levin 2012). Introgression means the incorporation of genetic material from one entity (typically species) to another through hybridization and backcrossing. This process could obscure the boundaries between species, which are consequently characterized as being "porous" or "semipermeable". Some introgressed alleles can be maintained by natural selection ("adaptive introgression"); thus, introgression may have positive fitness consequences in the recipient species and can lead into local adaptation and speciation (Harrison & Larson 2014, Suarez-Gonzales et al. 2018). On the other side, hybridization and introgression of introduced taxa can lead to a "genetic extinction" of native species (Rhymer & Simberloff 1996).

In aquatic plants, several cases of introgression have been recorded. Introgressive hybridization among three *Rorippa* species has been confirmed in the area of northern Germany. In periodically disturbed habitats at the river Elbe, bidirectional introgression between tetraploid species *R. amphibia* and *R. sylvestris* takes place. The polymorphic hybrid *R.* ×*anceps* favours temporally open sites with muddy sediments; however, the hybrid is perennial and self-incompatible and usually does not survive permanently in such disturbed habitats. Thus the natural dynamic of the river system has a positive effect on the formation of hybrid zones but a negative effect on the persistence of the hybrids. In the more anthropogenic landscape in northern Germany, hybrid zones between *R. amphibia* and *R. sylvestris* have been not found. In contrast, man-made habitats are suitable for arising of contact zones between allogamic *R. amphibia* and autogamic *R. palustris*. The introgression is unidirectional from *R. palustris* into *R. amphibia* (Bleeker & Hurka 2001). The experimental crossing showed that tetraploid *R. amphibia*, if hybridized with another species, diploid *R. austriaca*, generates primary triploid hybrids. These hybrids were capable of forming a small amount of seeds in the cases they were pollinated by pollen from parental species. Subsequently, the F2 generation arising from the hybrid seeds consisted of vital tetraploid plants with high quality pollen. This demonstrates the way that can lead to a formation of tetraploid hybrid swarms from heteroploid parents (Jarolímová 2005).

In *Ranunculus* sect. *Batrachium*, experimental crossing revealed that probably all *Batrachium* species are potentially capable of crossing. While hybrids of diploid species tend to be sterile, hybrids of higher ploidies are mostly at least partially fertile and capable of forming a viable F_2 generation. In the second filial generations, morphologically stable segregants can arise (Cook 1966, 1970, Hong 1991). Although the introgressive hybridization of water-crowfoots has not yet been unequivocally confirmed in nature, several particular cases show strong suggestion that introgression can play an important role in this group (Wiegleb & Herr 1983, Dahlgren 1991, Hong 1991).

Introgressive hybridization can have a significant impact on rare and relict species. A good example is *Nuphar pumila*, a climate relict having few isolated localities in Central Europe. In the Alps, bidirectional introgression between *N. pumila* and recently spreading (but native) *N. lutea* has been confirmed. Hybrids were found in over 60% of studied populations and more than 40% of investigated *N. pumila* individuals were admixed (Arrigo et al. 2016). Contrary, these two species appear to only rarely hybridize throughout their Russian distribution range (Volkova et al. 2018).

An extensive introgression between native and introduced aquatic species has been revealed in the genus *Myriophyllum*. Eurasian *M. spicatum* is naturalized throughout North America and considered to be the most noxious aquatic weed there. Hybrids between introduced *M. spicatum* and its sister species, native *M. sibiricum*, have been repeatedly detected in northern parts of USA, where ranges of both species overlap (Moody & Les 2002, 2007, Sturtevant et al. 2009, Zuellig & Thum 2012, LaRue et al. 2013a). The continuous morphological overlap between *M. spicatum* and *M. sibiricum* has been observed in large regions, especially in the area of Great Lakes (Moody and Les 2007), the fertility of the hybrid and the occurrence of introgressive hybridization in natural populations have been experimentally demonstrated (LaRue et al. 2013a). Moreover, the hybrid appears to evince similar or even worse invasive behaviour than *M. spicatum* (LaRue et al. 2013b).

An excellent model system for studying of homoploid hybrid speciation has recently been found in the genus *Bolboschoenus*. In Europe, *B. laticarpus* has been described as a stable hybrid species, morphologically and genetically intermediate between *B. planiculmis* and *B. yagara*, exceeded both taxa by inhabiting a wider range of ecological niches (Hroudová et al. 2014, Píšová et al. 2017). Later, plants from eastern China, formerly assigned to *B. yagara*, were revealed to be hybridogenous taxon, genetically equivalent to European *B. laticarpus*, but independently arised and apparently younger. Finally, the Australian *B. medianus* has been confirmed to have a similar genetic backgroud, being a hybrid between *B. yagara* and *B. caldwellii*, an Australian vicariant of *B. planiculmis*. Also the local endemic of the eastern United States, *B. novae-angliae*, has been confirmed to be a homoploid hybrid (Píšová 2018). Moreover, the recent hybridization and perhaps introgression of *B. planiculmis* has been revealed in some inland populations of *B. maritimus*, whereas samples of *B. maritimus* from habitats with a high salinity (sea coasts) were genetically pure (Píšová et al. 2017).

The evidence of homoploid hybrid speciation has also been detected within *Sparganium erectum*. The partially fertile *S. erectum* subsp. *oocarpum* has been identified to be a stable hybrid between *S. e.* subsp. *erectum* and *S. e.* subsp. *neglectum* (Píšová & Fér 2020).

3.3 Evolutionary importance of genome size

Genome size (the amount of DNA contained in the cell nucleus) is a parameter extremely variable among plant species but typically constant within an individual and little variable within species. Currently, genome size is known for about 11,500 vascular plant species, ranging ca 2,440-fold among tracheophytes (Pellicer et al. 2018, Pellicer & Leitch 2019). The smallest plant genomes are known for *Genlisea tuberosa* (1C ≈ 61 Mbp ≈ 0.062 pg DNA), *G. aurea* (some strains with 64 Mbp ≈ 0.065 pg), *G. oxycentron* (75 Mbp ≈ 0.077 pg) and *Selaginella selaginoides* (78 Mbp ≈ 0.080 pg). On the other side, the largest genome size has been revealed in *Paris japonica* (1C 148.9 Gbp ≈ 152.2 pg DNA), *Tmesipteris obliqua* (147.3 Gbp ≈ 150.6 pg), *Trilium ×hagae* (125.5 Gbp ≈ 128.3 pg) and *Trilium rhombifolium* (109.0 Gbp ≈ 111.5 pg; Fleischmann et al. 2014, Hidalgo et al. 2017, Pellicer et al. 2018). However, the range of genome size among angiosperms is distinctly skewed towards small genomes, with a mean genome size of 1C = 5.0 Gbp = 5.13 pg of DNA (Pellicer & Leitch 2019).

Genome size has multiple ecological and evolutionary consequences. Because nucleic acids are large molecules rich in nitrogen and phosphorus, a general prediction can be made that species with large genomes would be less competitive in nutrient-limited environment. Indeed, robust evidence of this view has repeatedly been provided (Šmarda et al. 2013, Guignard et al. 2016). Additionally, small genomes have faster cell cycle and therefore faster growth and short generation time (Bennett 1972). Consequently, plants with smaller genomes are more common in extreme and time-limited environments (Bennett 1987, Knight et al. 2005) while endangered taxa have larges genomes than common species (Vinogradov 2003). Genome size is also negatively correlated with weediness and invasiveness (Bennett 1987, Kubešová et al. 2010, Pandit et al. 2014, Suda et al. 2015). On the other side, invasiveness is positively correlated with chromosome number and ploidy level, hence polyploid species with small genomes have the best potential to be invasive (Pandit et al. 2014).

A potential of genome size in influencing the rate of plant molecular evolution and hence speciation still remains not satisfactorily answered. According Bromham et al. (2015), genome size is negatively correlated with rates of molecular evolution, i.e. the plants with smaller genomes generally have a broader evolvability spectrum. In contrast, Puttick et al. (2015) demonstrated that it is no link between overall genome size and diversification, but the diversification rates of plants are positively correlated with rates of genome size evolution (including both increasing and decreasing of the genome size via multiple processes).

For aquatic plants, comprehensive analyses of the genome size and its effects are still lacking. Moreover, aquatics are very unevenly explored; for example, the genome size has not yet been determined for any species of the largest aquatic group, Podostemaceae (Pellicer & Leitch 2019) and more or less comprehensive data are only available for the groups of Nymphaeaceae (Pellicer et al. 2013), Lentibulariaceae (Fleischmann et al. 2014, Veleba et al. 2014) and Lemnoideae (Wang et al. 2011, Hoang et al. 2019).

Because a large number of aquatics occur in frequently disturbed or periodic habitats, we can expect rather small genome sizes among them. Indeed, some aquatics belong among plants with smallest known genomes. The family of Lentibulariaceae includes a large number of aquatic species with extremely small genomes, with smallest values estimated for *Genlisea aurea* (see above; 1C=0.065 pg DNA) and *Utricularia purpurea* (1C=0.077 pg; Fleischmann et al. 2014, Veleba et al. 2014; note that *G. tuberosa* is not considered an aquatic plant sensu Murphy et al. 2019, see above). Interestingly, the sister genera *Genlisea* and *Utricularia* show the highest DNA mutation rates known among all angiosperms (Rutishauser 2015). Many other aquatics fall into the category of plants with very small genome sizes (1C<1.4 pg sensu Leitch et al. 2005), such as *Groenlandia densa* (1C=0.16 pg), *Spirodela polyrhiza* (1C=0.18 pg) or *Myriophyllum humile* (1C=0.21 pg; Šmarda et al. 2019, Prančl et al., unpubl.). In the very thoroughly explored Czech flora, small genomes prevail in hydrophytes, having in general slightly smaller genome sizes than in hemicryptophytes and significantly smaller than in geophytes. The distribution of their genome sizes is not significantly different from terophytes (that also include some aquatics) and woody plants (Šmarda et al. 2019). On the other side, several aquatic plants also possess large genomes, such as *Equisetum laevigatum* (1C=25.70 pg, diploid), *Bolbitis heudeloti* (1C=25.37 pg, diploid), *Damasonium alisma* (1C=23.62 pg, tetraploid), *Ranunculus lingua* (1C=21.84 pg, hexadecaploid) and *Crinum natans* var. *calmistratum* (1C=20.95 pg, diploid). From ten aquatics with largest known genome sizes, five belong to the family of Alismataceae (*Damasonium alisma*, *Alisma lanceolatum*, *Luronium natans*, *Sagittaria sagittifolia*, *S. latifolia*; Pellicer & Leitch 2019, Šmarda et al. 2019). Among invasive aquatics, most examined species possess small or very small genomes (*Myriophyllum heterophyllum* 1C=0.26 pg, *M. aquaticum* 0.66 pg, *M. spicatum* 0.85 pg, *Eichhornia crassipes* 1.28 pg, *Lagarosiphon major* 1.65 pg, *Cabomba caroliniana* 1.78 pg), but there are also cases of invasive species with more or less average genome sizes (*Elodea canadensis* 4.83 pg, *Egeria densa* 7.85 pg; Pellicer & Leitch 2019, Šmarda et al. 2019, Prančl et al., unpubl.).

Recently, estimating of genome size using flow cytometry is frequently used to help resolve taxonomic issues. The main advantage of flow cytometry for that purposes is the ability to rapidly analyse large numbers of individuals. In many cases, flow cytometry is sufficiently sensitive to separate even closely related species, their hybrids and homoploid taxa, often uncovering taxonomic novelties (Doležel et al. 2007, Loureiro et al. 2010, Bennett & Leitch 2011). Despite the undeniable suitability of the method, very few systematic studies utilizing flow cytometry are available for aquatic plants. In *Nymphaea*, flow cytometric screening facilitated the reliable determination of closely related species, their hybrids (involving both reduced and unreduced gametes) and also enabled the distinguishing between native species and numerous garden cultivars (Volkova et al. 2010, Kabátová et al. 2014, Dąbrowska et al. 2015). The genome size analyses supported the recent allopolyploid origin of *N. candida*, as the genome size of this species was almost identical to the sum of the values estimated for the putative parent species, *N. alba* and *N. tetragona* (Volkova et al. 2010; see above). Flow cytometry also helped to the identification of closely related species *Nasturtium officinale*, *N. microphyllum* and their hybrid *N.* ×*sterile* (Morozowska et al. 2015) and enabled to identify multiple morphologically indistinguishable cytotypes of *Phragmites australis* (Meyerson et al. 2016, Pyšek et al. 2020). The study of North American populations of *Cabomba caroliniana* revealed remarkably high intraspecific genome size variation on the population level (23.6%). While the native populations of *C. caroliniana* from southern U.S. generally possessed the higher genome sizes, the introduced and spreading populations from northeastern U.S. and northeastern Canada had both high and low DNA content. This results support the hypothesis that the introduced distribution range of *C. caroliniana* is the result of multiple introductions (McCracken et al. 2013).

3.4 Intraspecific genetic variation

The intraspecific genetic diversity ascertained by molecular methods on different levels and geographic scales can uncover a range of hidden but principal information about various aspects of aquatic plant life, e.g. the proportion of vegetative and generative reproduction, gene flow efficiency, or evolutionary histories and temporal geographical changes of particular genotypes (Barret et al. 1993, Fér 2008). Aquatic plants possess a number of peculiar characteristics fundamentally affecting the genetic variation, such as the discrete and patchy nature of many aquatic habitats, a strong preference for vegetative against sexual reproduction, an effective vegetative spread within a population, and ubiquitous self-compatibility (see the previous chapters). Based on these premises, a "typical" aquatic plant species should theoretically exhibit low infra-population diversity but high genetic differentiation among populations. Of course, such extremely simplified view is far from reality. The similar patterns have indeed been observed in numerous highly clonal and/or autogamous aquatics, e.g. in *Ceratophyllum* and *Zannichellia* species, *Potamogeton pusillus* agg., *Ottelia alismoides* or *Callitriche cophocarpa* (Les 1991, Triest & Vanhecke 1991, Kaplan & Štěpánek 2003, Buczkowska et al. 2008, Chen et al. 2008). On the other side, many aquatic plants have mixed modes of reproduction and different models of dispersal. Several studies showed that populations of some clonal aquatic species often had high clonal diversity, nearly similar to non-clonal plants, suggesting a great importance of sexual reproduction (e.g. *Phragmites australis*, *Stuckenia pectinata*, *Ruppia cirrhosa*, *Myriophyllum alterniflorum*, *Vallisneria spinulosa* and several *Sagittaria* species; Chen et al. 2007, Lambertini et al. 2008, Abbasi et al. 2016, Lyu & Wang 2016, Martínez-Garrido et al. 2017, García-Girón et al. 2019). Surprisingly, the high genetic variation and no pattern of large-scale geographic variation have been repeatedly observed in rarely flowering species of *Lemna*, apparently as a result of frequent somatic mutations and effective zoochorous disperal of duckweed fronds (Vasseur et al. 1993, Crawford et al. 2001). In anemophilous *Phragmites australis*, the majority of populations are polyclonal and all populations in Europe could be considered members of a single meta-population (Lambertini et al. 2008). Indirect evidences of the long-distance dispersal by waterbirds has been detected for a number of species, based of the findings that geographic distance is unrelated to the genetic distance between populations (Figuerola & Green 2002). For example, levels of population differentiation of *Stuckenia pectinata* are lower on the southeastern Swedish coast than elsewhere on the Baltic Sea and the Gulf of Bothnia, in correlation with the massive occurrence of migrating waterfowl in that particular area (King et al. 2002).

The manifestation of genetic diversity can vary dramatically within a single species if it grows in ecologically different habitats. Pollux et al. (2007) studied genetic diversity of *Sparganium emersum* in two nearby rivers in the Ruhr Area. In the slowly flowing river Ruhr, *Sparganium* individuals are emerged and sexually reproducing, hence the gene flow among populations mediated by buoyant seeds is effectively ongoing. In contrast, only sterile submerged plants with ribbon-like leaves are present in the rapidly flowing river Swalm, and those populations are monoclonal or oligoclonal and genetically isolated. In *Stuckenia pectinata*, the preference of clonality increases with latitude and wave action (King et al. 2002), whereas sexual propagation play a major role on sites where its tubers are heavily foraged by swans (Hangelbroek et al. 2002).

River corridors are unique linear, continuous aquatic habitats, enabling long-distance hydrochorous dispersal. It is often predicted that the dispersal of aquatics in streams is more or less unidirectional, leading to the accumulation of genotypes in downstream parts. However, numerous studies on river plant species have shown that the genetic diversity in upper parts of rivers is not significantly lower than in the downstream areas (‘drift paradox’; Honnay et al. 2010). Upstream dispersal has been detected in ca 75% of studied stream and riparian plant species; it is probably mediated mainly by waterfowl, but also by other animals and wind (Wubs et al. 2016). The manifestations of the drift paradox can substantially vary even among the nearby rivers of the same catchments. In the Ruhr area, the strong unidirectional genetic diversity pattern of *Sparganium emersum* was observed in the Niers River, but notably, no such unidirectional pattern has been detected in nearby Swalm and Ruhr Rivers (Pollux et al. 2007, 2009). The complex pattern of genetic diversity can also be demonstrated on an example of *Hydrilla verticillata* in the Kako River system, Japan. Nine distinct genotypes of this almost exclusively vegetatively reproducing species have been detected in that single river system, including monoecious triploids, dioecious triploids and dioecious diploid. All genotypes probably invaded the river system independently, have established themselves and occupy specific sections of the river, with no apparent accumulation of genotypes in downstream parts (Nakamura & Kadono 2000).

In aquatic plants, genetic variation has been often used to study the dynamics of invasive species. The analysis of patterns of genetic diversity among native and introduced populations can give insight into historical processes associated with colonization history, including founder events, genetic bottlenecks and the occurrence of multiple introductions (Dlugosch & Parker 2008, Ward et al. 2008, Zhang et al. 2010). The populations of aquatic plants in non-native areas may have a strikingly low genetic variation due to the combination of bottleneck effect and the predominant, or even exclusive, vegetative spread. In most extreme cases, all invasive populations originate from a single individual or clonal lineage. Such pattern of variation has been reported e.g. for *Hygrophila polysperma* in its invasive area (North America, Australia, Germany), while the substantial infra- and interpopulation genetic variation is maintained in the native range (India, Banghladesh; Mukherjee et al. 2016). The invasion of a single clone has also been demonstrated for *Salvinia minima* in USA and *Ludwigia grandiflora* in California (Okada et al. 2009, Rowe et al. 2018). In the extensive introduced distribution range of *Eichhornia crassipes*, 80% of the populations sampled contained a single clone, with one particular clone native to Peru dominating in 74.5% of the populations sampled (Zhang et al. 2010). In other cases, the patterns of genetic diversity are more complex. In *Elodea canadensis* and *E. nuttallii*, a majority of introduced populations consist of a single haplotype; however, some intrapopulation genetic variation has repeatedly been revealed in both species in several non-native areas, despite their exclusively vegetative reproduction. This variation probably represents a result of rapid post-dispersal somatic mutations (Lambertini et al. 2010, Huotari et al. 2011, Huotari & Korpelainen 2013). Multiple introduction has been confirmed for *Myriophyllum spicatum*, whose invasive populations in North America consist of two independently introduced haplotypes native in China and Korea (Moody et al. 2016), and *Myriophyllum heterophyllum*, whose invasive populations from northeastern and western USA showing the complex genetic pattern (Thum et al. 2010). North-American population of *Najas minor* have been introduced twice independently from China, and the additional hybrid genotype has been revealed there (either independently introduced or formed in the introduced area; Les et al. 2015b). *Hydrilla verticillata* is highly variable in its native range, and both widely naturalized forms (monoecious and dioecious biotypes, see the Chapter 2.5) are apparently of hybrid origin (Benoit et al. 2019). Finally, diploid and triploid plants of *Butomus umbellatus* have independently been introduced into North America, triploids were not found to have arisen *de novo* in the non-native range. Surprisingly, diploid populations, although producing abundant seeds, do not show significantly higher genetic variation than sexually sterile triploids and reproduce mainly vegetatively, possibly because seeds are outcompeted by bulbils (Eckert et al. 2003).

3.5 Cryptic variation

Cryptic variation is genetic variation that is not reflected in morphology but may be an essential source of physiological and evolutionary potential of the living organisms. Accumulation of this type of variation may result into the formation of cryptic species, i.e. two or more genetically distinct lineages that are (at least superficially) morphologically uniform but reproductively isolated from each other (Bickford et al. 2007, Chenuil et al. 2014, Paaby & Rockman 2014). Four main evolutionary pathways can lead to a formation of cryptic species: i) recent divergence (sister taxa of recently evolved members of a species complex), ii) parallelism – an independent evolution of a derived character state in different taxa from a similar and shared ancestral trait, iii) convergence – an independent evolution of a character state between taxa from different ancestral traits, and iv) stasis, i.e. retention of the same ancestral character state over an extended period (Struck et al. 2018).

In aquatic plants, the level of morphological disparity is often in blatant contradiction to the true evolutionary relationships between taxa. For example, *Ranunculus rionii* and some lineages of *R. trichophyllus* are one of the evolutionarily most distant taxa within *Ranunculus* sect. *Batrachium* (Bobrov et al. 2015); however, they are morphologically very similar and often confused with each other. On the other side, *Potamogeton natans* and *P. praelongus* are relatively closely related but morphologically completely dissimilar (Kaplan et al. 2013). Due to strong morphological reduction and phenotypic plasticity, we can expect frequent occurrence of cryptic species in aquatics. However, there are only a few molecularly confirmed cases. The cryptic, molecularly clearly separated taxon has been revealed within *Ottelia alismoides* in Thailand, which, however, does not show any morphological features that could serve to description of a new species (Ito et al. 2019).

The seagrass *Halophila ovalis* includes several cryptic lineages, molecularly divergent enough that they could be considered species (Waycott et al. 2002). Multiple genetically distinct but morphologically undistinguishable lineages are also known in widespread *Stuckenia pectinata* (Kaplan & Fehrer, unpubl.). In *Ranunculus trichophyllus*, five genetically distinct lineages have been recently revealed (Zalewska-Gałosz et al. 2015, Bobrov et al. 2015). In another cases, the discovery of the cryptic genetic lineage led to the subsequent discovery of distinguishing characters and description of the new species (*Myriophyllum jacobsii*, Moody 2011; *Ruppia bicarpa*, Ito et al. 2016; *Trithuria fitzgeraldii*, Sokoloff et al. 2019). The molecular and morphometric study on German populations of *Najas marina* s.l. confirmed the division of this polymorphic species into two morphologically partially different taxa, *N. marina* and *N. major*. The recognizing of both species is further complicated by the formation of intermediate hybrids, which are, however, sterile (Rüegg et al. 2017, 2019).

Cryptic species generate numerous taxonomic ambiguities. In the case of cryptic diversity, more than anywhere else, it is difficult or even impossible to define the species‘ boundaries. Considering the example of two traditionally recognized species of *Potamogeton*, Eurasian *P. compressus* and North American *P. zosteriformis*: these two taxa are sufficiently different in molecular markers but usually indistinguishable by means of morphology, as confirmed by morphometric analysis. Both species are vicariant but it cannot be entirely excluded that their distribution ranges may partially overlap, e.g. in the easternmost Asia (Kaplan & Marhold 2012). The classification of such cases will probably always be somewhat controversial, whatever taxonomic solution is adopted.

Furthermore, it is worth noting that intraspecific taxa (particularly the rank of subspecies) are rarely recognized in macrophytes, compared to terrestrial plants. The main reason of such discrepancy is a usually insufficient number of stable morphological characters even at species level, hampering the morphological delimitation of genetically defined lineages within species. Although there are some cases of widely recognized subspecies (e.g. within some species *Najas* and *Callitriche*; Triest 1988, Lansdown 2008), molecular support for such an assessment has probably not yet been clearly demonstrated for any aquatic taxon recognized at the subspecies level.

Cryptic variation is important not only at species (or subspecies) level. A large amount of unrecognized diversity is certainly related to recent polyploids (in particular autopolyploids) and hybrids (see the Chapters 3.1, 3.2). The frequency of such unrecognized taxa can be high even in well-studied areas; e.g. a single two-week field work in New England, USA resulted in a description of four new hybrids of *Potamogeton* (Kaplan et al. 2009). Recently, also an issue of cryptic invasions is extensively investigated, i.e. the introduction and spread of non-native lineages within the species' native range, and their impact on indigenous genotypes. *Phragmites australis* is by far the most widely investigated taxon for intraspecific cryptic invasions (Morais & Reichard 2018). Several European haplotypes of the common reed have been introduced to North America, displace the native genotypes and cause genetic depletion through hybridization with them (Saltonstall 2002, Lambertini et al. 2012, Meyerson & Cronin 2013). Similarly, an Eurasian haplotype of *Ceratophyllum demersum* has recently spread to America, Australia and South Africa, where also native genotypes has been revealed (Hyldgaard et al. 2017).

3.6 Distributional trends

Until recently, it has been generally accepted that aquatic plants tend to have broader distribution ranges and rarer occurrence of local endemism than their terrestrial counterparts. This is explained by a combination of selection for specific stress-tolerant taxa, the selective advantages provided by clonal growth, broad plastic responses to environmental factors, as well as by relatively low heterogeneity of aquatic environments at large scales and effective long-distance dispersal of sexual or even vegetative propagules (Sculthorpe 1967, Hutchinson 1975, Cook 1983, Santamaría 2002, Les et al. 2003). Aquatic plants also show relatively little taxonomic differentiation compared with terrestrial groups. Of the 33 strictly aquatic families, 30 include fewer than 10 genera, 17 contain only one genus and three consist of a single species (Santamaría 2002).

Indeed, 60% of aquatic plant species occur in more than one continent (Sculthorpe 1967). Aquatic and wetland taxa represent 41% of all plants species regarded to be widespread (e.g. *Lemna minor*, *Phragmites comunnis*, *Ceratophyllum demersum*, *Potamogeton crispus* and *Stuckenia pectinata*, which is apparently the most widespread aquatic plant species; Les et al. 2003, Murphy et al. 2019). Compared to terrestrial taxa, a disproportionally large number of aquatics manifests intercontinental disjunctions, even at the species level (e.g. *Ranunculus hederaceus*, *Eriocaulon septangulare*, *Potamogeton polygonifolius* and *P. epihydrus* show transatlantic disjunction, *Ceratopteris pteridoides* and *Heterozostera nigricaulis* possess transpacific disjunction, *Nymphaea lotus*, *Tenagocharis latifolia* and *Vossia cuspidata* have disjunct ranges including Africa and South Asia, and *Callitriche antarctica* is distributed through the subantarctic islands around the entire Antarctic; Mason 1959, Cook 1966, Sculthorpe 1967, Wiegleb & Kaplan 1996, Smith et al. 2017). Long-distance dispersal by birds is regarded to be an explanation for most of the widely disjunct aquatic plant distributions (Les et al. 2003), although direct evidence is not available. However, the recent world-wide study investigating distribution patterns of all aquatic plants (Murphy et al. 2019) showed that most aquatics have rather small distribution ranges, like the majority of terrestrial species. Only 42 aquatic species can be considered truly widespread, whereas at least 207 species have its distribution area of less than 20,000 km^2. The largest number of local endemics are known for the groups of Podostemaceae, Araceae (particularly the genus *Cryptocoryne*) and *Isoëtes* (Murphy et al. 2019); South African *Isoëtes eludens* is probably the rarest aquatic plant in the world, being known from a single temporary rock pool that is 2 m wide and 10–15 cm deep (Roux et al. 2009). The highest α-diversity of aquatics is located in tropical to subtropical areas of the Neotropics, especially the cerrado area of Brazil; this area is also the richest in local endemics (Chambers et al. 2008, Murphy et al. 2019). Other global hotspots of aquatic species diversity are situated in the east of North America, Europe (especially France) and East Asia (Murphy et al. 2019).

In Europe, the highest diversity of aquatic plants is reported from the Atlantic and Mediterranean regions. There are several explanations for such pattern of diversity, however, an unequivocal answer to this question is still missing. No clear latitudinal richness trends for European aquatic plants have been found; however, the species richness in Europe positively (although relatively weakly) correlates with a rate of evapotranspiration, i.e. the variable closely associated with rainfall and temperature (Chappuis et al. 2012). The species diversity further depends on a number of other factors, such as alkalinity, nutrient availability, salinity, small-scale heterogeneity of aquatic habitats etc. (Vestergaard & Sand-Jensen 2000, Santamaría 2002).

The distributions of plant taxa are markedly influenced by historic migrations, particularly by post-glacial colonizations after the last Ice Age (ca 12,000 years BP; Taberlet et al. 1998, Provan & Bennett 2008, Médail & Diadema 2009). Tremendous amounts of water bodies emerging during glacial recession in a warm period of early Holocen conduced to the rapid spread of aquatic macrophytes along the melting ice-sheet margin (Dieffenbacher-Krall & Jacobson 2001, Sawada et al. 2003, Väliranta et al. 2015). However, phylogeographical reconstructions of postglacial migrations are strikingly rare among aquatics compared to terrestrial species (Volkova et al. 2018). The greatest attention was paid to this topic in North America. The migration routes of many aquatics from their glacial refugia in southeastern U.S. and ice-free Alaska have been reconstructed using pollen proxy data (Dieffenbacher-Krall & Jacobson 2001, Sawada et al. 2003). Recently, also a few molecular phylogeographic studies are available from this region: in *Podostemum ceratophyllum*, the populations north of the glacial boundary show an order of the magnitude lower genetic diversity than in those to the south (Fehrmann et al. 2012). The phylogeographic study of *Najas gracillima* in North America helped to elucidate the postglacial spread of the species in eastern North America while also revealing that populations from California are not native, being introduced from another continent (Les et al. 2013). In the last years, phylogeographic history has been also studied in several species in eastern Asia. The results of the study on *Nelumbo nuccifera* indicated an occurrence of multiple glacial refugia in North-Eastern China (Li et al. 2010). Also the current distribution of *Hippuris vulgaris* and *Ranunculus bungei* in the high-alpine areas of the Qinghai-Tibetan Plateau suggest the postglacial spread from multiple refugia, but the phylogeographic structure of the populations of that species remained obscure (Chen et al. 2013, 2014). The extensive phylogeographic study of *Nuphar lutea* and *N. pumila* has revealed that both species have the origin in the Russian Far East and later spread westwards to Siberia and further to Europe. *Nuphar pumila* also migrated eastwards to North America. The postglacial colonization of Europe by *N. lutea* has taken place in two ways, from south and from the Siberian refugia (Volkova et al. 2018). Unfortunately, there is a virtually complete lack of studies focusing on postglacial migrations of European aquatics from their Mediterranean (or another) refugia. The historical geographic patterns of European aquatic flora remain to be resolved in the future.

4. OBJECTIVES AND PLANT MODEL SYSTEMS

As summarized in preceding chapters, aquatic plants are characterized by numerous specific adaptations that predetermine them to be taxonomically challenging but evolutionarily extraordinarily remarkable organisms. Although a wealth of knowledge is available about various aspects of their life, including ecophysiology, anatomy, reproductive systems and pathways of propagation, aquatics are markedly underrepresented in contemporary biosystematic studies. Our knowledge on principal evolutionary processes such as hybridization, polyploidization and genesis of cryptic variation in aquatics is low, especially in comparison with the growing body of these data on terrestrial plants. To fill this gap, my research was focused on two model systems, *Callitriche* and *Ranunculus* sect. *Batrachium*. These groups were selected because they are unique in sharing a remarkable combination of (1) high diversity, (2) strong morphological reduction combined with extensive phenotypic plasticity, (3) occurrence of hybridization and (4) ploidy level variation. On the other hand, both groups apparently differ in the extent to which the individual evolutionary processes contributed to their outstanding complexity. The main objectives of presented studies are to unveil the intrinsic genetic variation of aquatic plants and demonstrate the role of particular evolutionary processes on the shaping of overall aquatic plant diversity.

General objectives of the thesis are as follows:

i) Is flow cytometry a suitable basic method for determination of particular aquatic taxa?

Aquatic plants are notoriously known as taxonomically troublesome. Although some molecular studies have been published, the capacity of such methods to determine a larger number of samples and to reveal the basic taxonomic structure at the population level is limited. However, the frequent occurrence of polyploidy in both model groups gives good hope for the applicability of flow cytometry.

ii) What is the actual extent of cryptic variation in aquatics and which evolutionary processes are involved in the process of cryptic taxa formation?

Aquatic plants are undoubtedly among the groups of organisms in which we can expect frequent occurrence of cryptic genetic diversity. In the studied model systems, special attention should be paid to the role of polyploidy and interspecific hybridization. Each of these processes is obviously involved in both groups. However, the extent of variation that can be linked to each of these processes remains unknown. Specifically, the following questions are addressed:

- How important is the role of polyploidization and hybridization in the shaping of particular lineages?
- What is the pattern and frequency of hybridization among individual taxa? Does introgressive hybridization shape the evolution of studied groups?
- Are different ploidy levels acting at hybridization dynamics in different ways?

iii) What is the impact of particular reproductive ways on the overall plant diversity of aquatic habitats?

Aquatic plants show a remarkably large diversity of reproductive systems. However, little is known about how particular reproductive ways can affect speciation and taxonomic identity of individual aquatic species. The presence of different pollination systems even in closely related species suggests that the ways of reproduction can have a fundamental impact on interactions among aquatic taxa. The case studies focus on answering the following questions:

- Can different pollination patterns affect the frequency and direction of hybridization?
- Is clonal growth an efficient way to maintain non-fertile taxa (such as primary hybrids) in aquatic habitats?

iv) Which factors affect the distribution patterns of aquatic plant species in the Czech Republic?

Geographic distribution is one of the basic characteristics of plant species, reflecting their evolutionary history, habitat preferences and interactions with other organisms. Aquatics are often characterized to have large distribution ranges due to the effective long-distance dispersal and the relative uniformity of aquatic habitats. However, the distribution data of macrophytes at the regional scale are often incomplete or even erroneous and therefore cannot faithfully reflect the actual behaviour of particular taxa in nature. The mapping of aquatic taxa from the studied groups is intended to answer the following questions:

- What are the main variables determing the distribution of aquatic plants in the Czech Republic?
- Can the distribution of particular species affect the frequency of hybridization among them?
- Do some species show recent spread or decline in Central Europe?

4.1 Model genus Callitriche

The almost cosmopolitan genus *Callitriche* (water-starwort, Plantaginaceae; Fig. 3) comprises of about 75 amphibious, submerged or subterrestrial species (Hassemer & Lansdown 2018). In Europe, 14–15 native and four introduced species have been reported, comprising widely distributed species

FIG. 3. *Callitriche cophocarpa*, floating leaf rossetes with visible male flowers. Photo J. Prančl, “Modřanské laguny” pools, Prague, Czech Republic.

FIG. 4. *Callitriche palustris*, fruits. Photo J. Prančl (cultivated plants).

as well as taxa believed to be local endemics, particularly in the Mediterranean area (Lansdown 2008, Lansdown et al. 2017). *Callitriche* stands occur in almost all types of standing and running waters, even including small puddles on forest paths.

Callitriche taxa are extremely difficult to identify, particularly because of a high degree of phenotypic plasticity, overall reduction of the plant body and frequent occurrence of solely vegetative plants lacking important characters on fruits (Schotsman 1954, 1967, Lansdown 2008; Fig. 4). The genus is variable karyologically, possessing eight different chromosome numbers in Europe, ranging from diploids to octoploids (Schotsman 1967, Philbrick 1994, Philbrick & Les 2000). Hybridization in *Callitriche* is little known although at least one species is confirmed to be allopolyploid (*C. platycarpa*) and one primary sterile hybrid is recognized (Schotsman 1967, Martinsson 1991, Lansdown 2008, Bączkiewicz et al. 2007, Schwarzacher et al. 2017).

Despite the fact that most of the species have a similar appearance, an extraordinarily scale of reproductive modes occurs throughout the genus. *Callitriche* is the only genus of angiosperms that exhibits both aerial pollination and true hypohydrophily. Both reproductive strategies are linked with various ways of self-pollination (geitonogamy), which are realized via contact between male and female flowers or through unique 'internal geitonogamy' (pollen tube growth through non-floral vegetative tissues; Philbrick 1984). The reproductive mode is often a species-specific feature (Schotsman 1982, Martinsson 1996).

My research into the genus *Callitriche* focused primarily on European species, investigating the genome size and genetic variation between and within individual taxa, specifically focusing on hybridization processes, phylogenetic relationships and evolutionary origins of polyploids.

FIG. 5. *Ranunculus peltatus*, a heterophyllous, big-flowered water crowfoot. Photo P. Koutecký, "Choryňský mokřad" protected area, Czech Republic.

4.2 Model group Ranunculus sect. Batrachium

Ranunculus sect. *Batrachium* (water crowfoots, Ranunculaceae; Fig. 5) consists of 30 recognized species, occurring predominantly in the Northern Hemisphere. The world diversity centre is believed to be in Europe, where 19 species are known, with the largest number of taxa concentrated in the Atlantic and Mediterranean regions (Wiegleb et al. 2017). *Batrachium* taxa colonize a wide range of wetland habitats, which is reflected in their extensive phenotypic plasticity, especially in vegetative parts. The group is well known for occurrence of heterophylly, corresponding to different life strategies, i.e. possessing of entire (mainly floating) and/or divided (mainly submerged) leaves (e.g. Cook 1966, Hong 1991). Flower size differs among species and is associated with the mode of reproduction: small flowers (Fig. 6) indicate a higher degree of autogamy (or even cleistogamy), while large-flowered plants (Fig. 5) tend to be self-incompatible (Hong 1991).

FIG. 6. *Ranunculus rionii*, a homophyllous, small-flowered water crowfoot. Photo P. Koutecký, “Vypálenky” protected area, Czech Republic.

Polyploidy is frequent within *Batrachium*. To date, five ploidy levels (ranging from diploids to hexaploids) in various lineages are known. Moreover, *Batrachium* species exhibit a remarkable intraspecific variation. Differentiation at the ploidy level is recorded for more than half of the species, often with more than two ploidy levels per species (e.g. Cook 1962, Turała 1969, Dahlgren 1991, 1993). This is particularly known for *R. penicillatus* agg. (2x, 3x, 4x, 6x), which is considered a complex of several polytopic allopolyploid taxa (Zalewska-Gałosz et al. 2015). Nevertheless, large variation in the chromosome number has been detected also for several other species that are not assumed to be of hybrid origin, such as *R. peltatus* (2x, 4x, 6x) and *R. trichophyllus* (2x, 4x, 5x, 6x). In general, the reticulate evolution of water crowfoot makes the research of its phylogeny very difficult.

Despite its evolutionary attractiveness and taxonomic complexity, molecular and cytogenetic markers have been insufficiently employed to unravel the intraspecific variation of European *Batrachium*. Only three molecular studies are available for European species. Albeit being of a limited geographical scope, all indicate the presence of cryptic taxa, particularly within *R. trichophyllus* (Telford et al. 2011 on British plants, Zalewska-Gałosz et al. 2015 on Polish plants and Bobrov et al. 2015 in North Europe, all combining ITS and cpDNA markers).

All water-crowfoot species are probably capable to hybridize with other members of the subgenus (Cook 1966, 1970, Dahlgren 1991, Hong 1991). A high degree of clonal growth and autogamy is considered to be an important factor leading to frequent fixation and stabilization of hybrids and new polyploids in nature (Cook 1966).

My research into the *Ranunculus* sect. *Batrachium* focused primarily on recent hybridization events, occurrence of polyploidy and cryptic variation in central Europe and adjacent areas.

5. METHODS

The combination of several approaches was applied to accomplish the set goals. Three case studies (**P-I, P-II, P-III**) were based of the thorough sampling and subsequent genome size estimation using propidium iodide flow cytometry. To a lesser extent, also DAPI flow cytometry was used for analysing of bulked samples. In all papers, cytometric results were supplemented by conventional chromosome counting made from tips of adventive roots collected on cultivated plants (**P-I, P-II, P-III**) or from the roots of seedlings (**P-II**). For the genus *Callitriche*, direct sequencing of nuclear ribosomal (ITS) and plastid (*trn*T–*trn*L) DNA regions was applied in order to unravel the phylogenetic relationships among particular taxa. The sequencing was combined with cloning and RFLPs of ITS to clarify the origin of polyploid species and hybrids. The phylogenetic patterns were estimating using Maximum likelihood, Bayesian and NeighborNet analyses (**P-III**). Environmental parameters based on climatic data from the WorldClim database were assessed to elucidate the degree of ecological divergence among morphologically similar but ecologically different taxa of *Ranunculus* sect. *Batrachium* (**P-II**).

To reconstruct the distribution of particular species in the Czech Republic, specimens from more than 30 herbaria were revised, georeferenced and used for mapping along with our field data (**DP-I, DP-II**).

6. KEY RESULTS AND CONCLUSIONS

The research conducted on the selected model groups has resulted into important taxonomic and evolutionary implications and has significantly expanded our knowledge about processes shaping the aquatic plant evolution. The conclusions of case studies presented can also serve as a solid basis for future taxonomic revisions of study groups on a wider scale.

A summary of the most important results:

Genome size

Flow cytometric genome size estimation is a very efficient method for taxonomic delimitation, determination and investigation of aquatic plant taxa from both studied groups. Mean 2C-values varied significantly in both groups, spanning 7.36-fold in *Callitriche* and 3.59-fold range in *Ranunculus* sect. *Batrachium*. Mean monoploid genome sizes (1Cx-values) were also highly variable, spanning 3.16-fold in *Callitriche* and 1.74-fold range in *Batrachium* (**P-I,II,III**). Consequently, flow cytometric analyses are able to distinguish even morphologically similar homoploid taxa (e.g. diploid *Callitriche stagnalis*, *C. cophocarpa*, *C. lenisulca* and *C. obtusangula*, **P-I,III**; tetraploid *Ranunculus baudotii*, *R. peltatus* and *R. penicillatus*, **P-II**).

Polyploidy and origin of polyploids

Five ploidy levels were revealed for both *Callitriche* (2x, 3x, 4x, 6x, 8x) and *Ranunculus* sect. *Batrachium* (2x, 3x, 4x, 5x, 6x). Chromosome counting confirmed the numbers quoted for particular taxa in published sources. Chromosome numbers are for the first time provided for the poorly known taxon *C. brutia* var. *naftolskyi* (**P-III**) and for several hybrids from both groups (**P-I, P-II**).

Intraspecific ploidy level variation was detected in *Ranunculus* sect. *Batrachium*, but it was found to be less common that previously reported. Two ploidy levels were revealed in *R. fluitans* (2x, 3x) and *R. penicillatus* (4x, 6x). Additionally, putative pentaploids and hexaploids were rarely found among ordinary tetraploids of *R. peltatus* (**P-II**). In contrast, *Callitriche* species generally exhibit a constant ploidy level. The only exception is a single triploid plant found for the ordinarily diploid *C. stagnalis* (**P-I**); the results of molecular analyses suggest its autopolyploid origin (**P-III**).

The origin of selected polyploid *Callitriche* species was investigated in detail (**P-III**). Diploid *C. cophocarpa* was confirmed to be the parental species of tetraploid *C. platycarpa*, but we did not find direct evidence for the putative allopolyploid origin of this species. However, this can be an artifact caused by ongoing concerted evolution of the ITS region. Allopolyploid origin was confirmed for *C. hamulata*, which is probably an allooctoploid derivative of *C. brutia* var. *brutia* and *C. cophocarpa*/ *C. platycarpa*. In *Ranunculus* sect. *Batrachium*, indirect evidence of autopolyploid origin was ascertained for a single hexaploid population of *R. penicillatus*: we found to grow this taxon together with one of its putative parental species (*R. trichophyllus* cytotype B) and with a primary hybrid of both putative species (*R. fluitans* × *R. trichophyllus* B). We also assume an autopolyploid origin of the triploid *R. fluitans*, whose monoploid genome size is almost identical to that of the diploid cytotype of this species (**P-II**).

Cryptic taxa

Cryptic variation was detected in both groups. In *Callitriche*, previously unknown cryptic taxa were detected within *C. truncata*, *C. stagnalis* and *C. heterophylla* (**P-III**). In *Ranunculus* sect. *Batrachium*, cryptic variation was revealed within *R. trichophyllus*, in which three distinct tetraploid cytotypes were detected ('cytotype A', 'cytotype B' and 'subsp. *eradicatus*' from high alpine lakes), differing in their ecology and distribution. The putative allopolyploid aggregate of running-water taxa referred to as *R. penicillatus* is even more complex, including six different cytotypes (A-F; **P-II**).

Phylogeny

The phylogenetic relationships of *Callitriche* taxa were reconstructed and compared with previously published studies (**P-III**). Based on the combined results of all analyses, *C. brutia* var. *naftolskyi* was newly reclassified at the subspecies level. The other cryptic taxa in *Callitriche* require further study.

Hybridization and the impact of different reproductive strategies

The frequency and manifestations of hybridization differ significantly among both model systems. Hybridization appears to be rare in *Callitriche*, despite the frequent co-occurrence of water-starwort taxa. Four *Callitriche* hybrids were detected, of which only one (triploid *C. cophocarpa* × *C. platycarpa*) is relatively common (**P-I,III**). In two cases, hybridization between *Callitriche* species was confirmed to be reciprocal (**P-III**). Two hybrids are newly described and validated as *C.* ×*nyrensis* and *C. brutia* nothosubsp. *neglecta* (**P-III**). The different pollination biology of particular taxa, high proportions of selfing (geitonogamy) and in some cases also ecological differences between species are presumably the main reasons why water-starworts rarely hybridize. Surprisingly, a putative hybrid *C. cophocarpa* × *C. hamulata* arose from the crossing between a hypohydrogamous species with another species possessing aerial pollination (**P-I**).

In contrast, hybridization is frequent in *Ranunculus* sect. *Batrachium*. We revealed 16 cytotypes of hybrid origin, which accounted for ca 15% of the individuals studied. Seven of the hybrid cytotypes were identified as F1 hybrids and the rest are of unknown or uncertain origin. Because most of the hybrids are partially fertile, an introgression can take place. In the *R. penicillatus* group, the occurrence of a large hybrid swarm (*R. penicillatus* cytotype A × *R. peltatus*) was documented, occurring in a 150 km stretch of the Ohře river, Czech Republic. Hybrids with variable genome sizes frequently arise also from hybridization of the tetraploids *R. peltatus* and *R. trichophyllus* in which the gene flow tends to be largely unidirectional towards *R. peltatus*. Such biased gene flow might be driven by a large difference in flower size: the big-flowered *R. peltatus*, possessing flowers with a large amount of pollen, is more likely to be a pollen donor than the small-flowered *R. trichophyllus*. Subsequently, the hybrids are more likely to be pollinated by *R. peltatus* than by *R. trichophyllus*. We have even repeatedly encountered populations of plants (particularly in rivers) of obvious hybrid appearance, which have, however, a genome size within the range of "pure" *R. peltatus*. These plants might be multiple backcrosses towards *R. peltatus* (**P-II**).

Clonal reproduction is crucial for hybrid survival. We revealed the abundant occurrence of the hybrid *Callitriche* ×*vigens* in the Otava river, Czech Republic, although one of its parents, *C. platycarpa*, has never been found throughout that river basin (**P-I, DP-I**). Another case is a unique hexaploid *Batrachium* hybrid found in the upper Vltava river, Czech Republic; it has the lowest monoploid genome size so far recorded in *Batrachium*, that is lower than in any of its potential parental species. This completely sterile hybrid occupies an approximately 25 km long section of the river, without the presence of any other species. Clonal propagation is also suggested as virtually the only way of reproduction in *R. fluitans*, as both diploid and triploid populations were almost consistently unfertile (**P-II**).

Distribution

The case studies contributed to the better understanding of the distribution of some taxa in Europe. During the fieldwork, *Callitriche* ×*vigens* was found for the first time in the Czech Republic and Austria, *C. hamulata* and *C. brutia* subsp. *brutia* in Hungary, *C. obtusangula* in Denmark and Slovakia, *C. brutia* subsp. *naftolskyi* in Spain, *C. platycarpa* in southern Italy and Sicily, and *C. lusitanica* in continental Greece (**P-I, P-III**).

The distribution of the studied aquatic species in the Czech Republic display specific geographic patterns even on a local scale. In *Ranunculus* sect. *Batrachium*, mineral richness is the key factor determining the distribution of particular taxa. Whereas *R. baudotii*, *R. circinatus*, *R. rionii* and *R. trichophyllus* (cytotype B) are found in mineral-rich waters, other species (*R. peltatus*, *R. trichophyllus* cytotype A) prefer acidic substrates (**DP-II**). Although the WorldClim analyses show the correlation of distribution of some taxa with temperature-related variables (**P-II**), this is probably a false correlation due to the fact that in the Czech Republic the mineral-rich sediments are mostly situated in the warmer areas. The preferences for waters of different pH fundamentally influences the frequency of hybridization among particular taxa. For example, the couple of ecologically similar species *R. peltatus* and *R. trichophyllus* (cytotype A) often grow together and also often hybridize. In contrast, *R. aquatilis* and *R. peltatus*, that rarely grows together, also rarely hybridize, although the genetic barrier between these species is weak (**P-II, DP-II**).

For *Callitriche*, there is no clear preference for different substrates in the Czech Republic. However, several species have sharply delimited distribution ranges, correlated with the climatic oceanity and continentality (a clearly sub-Atlantic distribution of *C. platycarpa* and absence or rarity of *C. stagnalis* and *C. hamulata* in the most continental Pannonian region versus slight continental tendence of *C. cophocarpa*). This distributional pattern is rather surprising for such abundant species with more or less broad ecological amplitudes (**DP-I**).

General conclusions

i) Thorough understanding of biodiversity is a fundamental prerequisite for providing a convenient taxonomic framework, which is indispensable for every field of biology. My research provided an in-depth insight into the evolution of aquatic plants and brought new evidence of cytogenetic variation, hybridization and introgression within studied groups. The results corroborate that both selected groups (*Callitriche* and *Ranunculus* sect. *Batrachium*) are suitable models for investigating of polyploidization and hybridization, comparable with more thoroughly surveyed model cases among terrestrial plants.

ii) Until now, aquatic plant species have mostly been reported to show relatively little taxonomic differentiation. However, our case studies provide evidence that numerous macrophyte species possess remarkable intraspecific variation. The results have shown that the classification on the level of subspecies, although not yet widely used in aquatics, is legitimate and meaningful in some well-founded cases.

iii) The occurrence of cryptic genetic variation is indeed a frequent phenomenon among aquatics. Their occurrence is due to morphological reduction, phenotypic plasticity and parallel evolution of traits. Consequently, the actual diversity of aquatic plants is presumably significantly higher than previously thought.

iv) As expected, primary hybrids of aquatic plant species, regardless of their sterility, often successfully survive and spread through clonal growth. The assumption can be made that such hybrids represent substantial but neglected part of overall aquatic plant diversity.

v) Aquatics show a remarkable diversity of pollination modes. The results suggest that differences in reproductive modes (particularly the different rate of autogamy and geitonogamy) fundamentally determine the frequency and direction of hybridization among aquatic plant taxa.

vi) Aquatic plants are generally described to possess broad distribution areas and higher environmental similarity at large scales than in the terrestrial habitats. Despite expectations, the thorough examination revealed that the distribution of some species from the studied groups show geographic pattern on a local scale. Such patterns clearly reflect the sensitive response to environmental factors such as climatic oceanity and mineral richness, which are often considered more important for determining the distribution of terrestrial plants.

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PART B – CASE STUDIES

PAPER I

Prančl J., Kaplan Z., Trávníček P. & Jarolímová V. (2014): Genome size as a key to evolutionary complex aquatic plants: polyploidy and hybridization in *Callitriche* (Plantaginaceae). – PLoS ONE 9(9): e105997.

***Callitriche obtusangula*, a water starwort species found for the first time in Denmark in this study.** Photo J. C. Schou, Varming, Denmark. Adopted from Prančl & Schou (2014).

Genome Size as a Key to Evolutionary Complex Aquatic Plants: Polyploidy and Hybridization in *Callitriche* (Plantaginaceae)

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ABSTRACT

Despite their complex evolutionary histories, aquatic plants are highly underrepresented in contemporary biosystematic studies. Of them, the genus *Callitriche* is particularly interesting because of such evolutionary features as wide variation in chromosome numbers and pollination systems. However, taxonomic difficulties have prevented broader investigation of this genus. In this study we applied flow cytometry to *Callitriche* for the first time in order to gain an insight into evolutionary processes and genome size differentiation in the genus. Flow cytometry complemented by confirmation of chromosome counts was applied to an extensive dataset of 1077 *Callitriche* individuals from 495 localities in 11 European countries and the USA. Genome size was determined for 12 taxa. The results suggest that many important processes have interacted in the evolution of the genus, including polyploidization and hybridization. Incongruence between genome size and ploidy level, intraspecific variation in genome size, formation of autotriploid and hybridization between species with different pollination systems were also detected. Hybridization takes place particularly in the diploid – tetraploid complex *C. cophocarpa* – *C. platycarpa*, for which the triploid hybrids were frequently recorded in the area of co-occurrence of its parents. A hitherto unknown hybrid (probably *C. hamulata* × *C. cophocarpa*) with a unique chromosome number was discovered in the Czech Republic. However, hybridization occurs very rarely among most of the studied species. The main ecological preferences were also compared among the taxa collected. Although *Callitriche* taxa often grow in mixed populations, the ecological preferences of individual species are distinctly different in some cases. Anyway, flow cytometry is a very efficient method for taxonomic delimitation, determination and investigation of *Callitriche* species, and is even able to distinguish homoploid taxa and identify introduced species.

INTRODUCTION

Aquatic plants are characterized by several specific adaptations to the water environment, including considerable morphological reduction, prolific clonal propagation and extensive phenotypic plasticity (e.g. [1–10]). These characteristics can make classification of these plants particularly difficult, with this difficulty increased by the frequent parallel evolution of traits in unrelated taxa [11–16]. Indeed, aquatic plants are regarded as being among the most taxonomically challenging angiosperms.

Aquatic plants play a key ecological role in aquatic ecosystems and often exhibit complex evolutionary histories. Polyploidy and hybridization have been crucial to the evolution of many aquatic plant groups [7], namely in *Ranunculus* subg. *Batrachium* [11,17–20], *Potamogeton* [15,16,21–26], *Lemnaceae* [27–28], *Nymphaea* [29–30], *Elodea* [31], *Myriophyllum* [32] and many others. Intrageneric chromosome number variation has been reported from 80% and hybridization from 20% of all aquatic plant genera [7]. Newly established polyploids and hybrids can be fixed by frequent clonal growth, with sterile primary hybrid genotypes persisting hundreds or even thousands of years without the presence of the parental species [16,33–37]. However the new taxa that arise through these processes are often morphologically undetectable. Indeed, in general, a substantial part of the variation in aquatic plants is undoubtedly cryptic and detectable only using molecular techniques.

Despite the interesting evolutionary scenarios posed by aquatic plants, they are markedly underrepresented in contemporary biosystematic studies [5] – likely due in large part to the daunting challenges associated with these scenarios, as well as the difficulty in detecting taxonomic differences. Thus, our overall knowledge of the principal processes that have driven evolution of aquatic taxa is limited, especially in comparison with the information developed regarding terrestrial plants. This has limited our potential to understand the wider context of evolution and systematics of these plants.

In the current study, we examine the aquatic genus *Callitriche* (water-starwort) which comprises about 60 species throughout the world. In Europe, about 15 native and 4 rare, introduced species have been reported [38–40]. *Callitriche* is notoriously considered one of the most difficult aquatic plants to identify. Taxonomy of *Callitriche* is based mostly on the generative features, particularly fruits. Unfortunately, these characters are very small, difficult to observe and often not available due to the frequent occurrence (or even prevalence) of solely vegetative plants. Virtually all vegetative characters of water-starworts are extremely variable and mostly unusable without extensive experience. Therefore, although many detailed morphological studies on water-starworts have been published (e.g. [38,40–50]), their reliable determination is still restricted to just a few specialists, with several taxa recognizable only in the case of well-developed, adult individuals.

Water-starworts, among the most common aquatic plants throughout Europe, inhabit almost all types of standing and running waters, even including small puddles on forest paths. Despite this, they are generally overlooked by field biologists, even though individual *Callitriche* species can differ substantially in their ecology and may serve as diagnostic taxa of various phytosociological units [49,50]. Water-starworts may also represent suitable model organisms for the study of phenotypic plasticity [51–54], physiological processes associated with growth [55–57], metabolism in aquatic environments [58–61], plant patch formation in streams [62,63], phytoremediation of polluted watercourses [64], and some taxa may even serve as a potential antioxidant-rich diet supplement [65,66]. Pollination biology of water-starworts is strikingly diversified and among the most remarkable of all the angiosperms [45,67–70]. However, *Callitriche* is still only rarely a subject of scientific research due to taxonomic and methodological difficulties (e.g., observation, scoring, cultivation, and experimentation). Finding a method that allows easy and reliable determination of water-starworts in various developmental stages would therefore promote exploration of many aspects of aquatic and wetland ecosystems.

Evolution of the genus has featured recurrent polyploidization and aneuploid reduction of chromosome numbers in various lineages [70]. Therefore, chromosome counting has often been used for the genus, especially in Europe. To date, chromosome numbers for 35 taxa are available (summarized in Table S1) and 11 different chromosome counts are known, ranging from 2n = 6 to 2n = 40. The most common diploid chromosome number is 2n = 10, but the diploids 2n= 6 and 2n = 8 have also been found in some species.

In Europe, all three diploid chromosome numbers are known, and at least four polyploid species occur (*C. palustris* 2n = 20, *C. platycarpa* 2n = 20, *C. brutia* 2n = 28, *C. hamulata* 2n =38). Callitriche platycarpa is considered to be an allotetraploid that has arisen through the hybridization of the diploid species *C. cophocarpa* and *C. stagnalis* [71,72], confirmed by allozyme analysis of plants from north-western Poland [73]. The origin of other European polyploids is unknown.

Recent hybridization has also been detected in the genus: the triploid (2n= 15) *Callitriche* ×*vigens* (*C. cophocarpa* × *C. platycarpa*), the sole primary hybrid currently known and validly described, has repeatedly been found in areas of co-occurrence of the parental species (e.g. [34,47,74,75]). However, to-date this hybrid has never been confirmed by molecular studies. More generally, the lack of molecular investigation of this genus leaves open the possibility of undiscovered hybrids within it. In particular, the amount of hybridization between taxa with the same ploidy levels would have escaped detection by chromosome counting.

To date, however, chromosome counting has remained the exclusive cytogenetic method used. However, the potential utility of chromosome counting per se for taxa determination is limited, because only two European (*C. brutia*, *C. hamulata*) and a single African (*C. vulcanicola*) species possess unique chromosome numbers. The phylogenetic relationships between most of the taxa remain unclear because molecular techniques have been only sporadically applied to *Callitriche*. These include phylogenetic analysis of European and North American species ([70], unfortunately using rbcL as a marker, which is not sufficiently variable) as well as allozyme and RAPD analyses on a small geographic scale [73,76–78]. The combination of rbcL and ITS applied to northern Italian *Callitriche* [79] was unfortunately not supported by adequate determination of plants, and the data interpretation in this study is largely questionable.

The variation in chromosome numbers in *Callitriche* encourages use of the genome size as a species-specific marker. Flow cytometry (FCM), which has undergone a boom in plant sciences over the last decade, represents an excellent tool for this purpose. FCM is a rapid, easy, statistically robust and relatively cheap method [80,81], frequently and successfully applied to evolutionarily and taxonomically intricate plant groups such as polyploid complexes [82–86]. Due to the high accuracy of the measurements, FCM is often able to distinguish even closely related homoploid taxa (reviewed in [87]), and it is also frequently used for detection of hybrids [87–91]. An indisputable benefit of FCM is its facility to analyze a large number of individuals (e.g., at a population level) rapidly, even allowing detection of rarely occurring cytotypes, hybrids and aneuploids ([92–95], etc.). Finally, flow cytometry permits analyses using a very small amount of plant material. This is extremely useful in the aquatic environment, where researchers often find only small vegetative fragments.

Unfortunately, flow cytometry has so far been only sporadically applied to research on aquatic plants (Nymphaeales [96,97]; *Nymphaea* [98,99]; *Cabomba* [100,101]; *Rorippa* [102]; *Nasturtium* [103]; *Lemnaceae* [104];

Zannichellia [105]). To date, no studies using FCM are available for *Callitriche*. In fact, the genome size has been estimated only once for Western European water-starwort species, using photometric cytometry with the Feulgen staining method [106]. However, this method cannot process large numbers of samples and is unable to reliably distinguish small differences in genome size among taxa with the same ploidy level.

In this study, we applied flow cytometry combined with chromosome counting to improve our understanding and identification of Central European water-starworts. The following aims were addressed: (1) testing flow cytometry as a method for reliable determination of the Central European *Callitriche* species; (2) determining the chromosome numbers based on cytometrically analysed samples; (3) comparing the new counts with previously published records; and (4) estimating the hybridization rate in the studied area.

MATERIAL AND METHODS

Field sampling

Plant samples were collected in Belgium, Czech Republic (majority of samples), Denmark, Germany, Hungary, Italy, Netherlands, Norway, Poland, Slovakia and Sweden in 2007–2014. Our sampling included all seven Central European species, several specimens of hybrid origin (see below) and also the Mediterranean species *C. lenisulca*, which is apparently closely related to the Central European *C. cophocarpa* [107]. In addition, we included seven samples of European species that were collected in the western USA (*C. stagnalis* and *C. hamulata*, both introduced, and *C. palustris*, native to both Europe and North America). In total, 1076 plants from 494 localities were obtained (for locality details, see Table S2). Voucher specimens are preserved in the herbarium of the Charles University in Prague (acronym PRC).

We put an emphasis on visiting the widest possible range of aquatic habitats, including small and commonly neglected biotopes (e.g., puddles on forest paths, eutrophic ditches). At each locality, sampling covered observed morphological variation. The sampling was carried out even in stands comprising only sterile plants. The fresh plant material was placed in plastic bags and transported rapidly to the FCM laboratory. In the cases of longer transport, plants were wrapped in moist paper towels (not too wet, in order to avoid rotting) and then sealed in plastic bags. Aquatic plants preserved in this way stay fresh for 2–3 weeks, enabling analysis of samples even from distant regions.

Flow cytometry

All 1077 plants were analysed using FCM. If multiple samples were collected from a population, these samples were first analysed simultaneously (approximately 5 samples in a single run) using 49,6-diamidino-2-phenylindole (DAPI) fluorochrome to reveal the possible presence of multiple cytotypes. About 0.25 cm^2 of leaf tissue was chopped together with an appropriate volume of the internal standard using a sharp razor blade in a Petri dish containing 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20, [108]). *Bellis perennis* L. was selected as a primary reference standard, as it has a similar, but non-overlapping genome size with the majority of the studied samples (2C = 3.96 pg, [109]; 2C-value was calibrated according to the following internal standard). *Glycine max* (L.) Merr. 'Polanka' (2C = 2.50 pg, [80]) served as a reference standard for *Callitriche palustris* and *C. obtusangula*, because the genome size of these taxa overlapped with *Bellis*. The crude suspension was filtered through a 42-mm nylon mesh and incubated for ca 5 min. at room temperature. After incubation, isolated nuclei were stained with 1 mL of Otto II buffer (0.4 M $Na_2HPO_4.12H_2O$) supplemented with DAPI (4 μg/ml) and b-mercaptoethanol (2 μl/ml). Samples were run on the flow cytometer after about one minute of staining, using a Partec PA II flow cytometer (Partec GmbH, Münster, Germany) equipped with a mercury arc lamp as the UV light excitation source. The fluorescence intensity of 3000 particles was recorded. Histograms were evaluated using FloMax software, ver. 2.4d (Partec GmbH).

Subsequently, all detected cytotypes from all populations were analyzed separately using propidium iodide FCM in order to estimate the variation in genome size. To determine the genome sizes for particular taxa in absolute units (pg of DNA), 178 samples representing all cytotypes were measured at least three times on different days to account for occasional fluctuations. Because genome size generally differed substantially between taxa (see below), a further 416 samples were each measured only once as a single measurement was sufficient for reliable assignment. The staining solution consisted of 1 mL of Otto II buffer supplemented with propidium iodide (50 μg/ml), RNase A, type IIA (50 μg/ml) and b-mercaptoethanol (2 μl/ml). The fluorescence intensity of 5000 particles was recorded using a Partec CyFlow instrument equipped with a green diode-pumped solid-state laser (Cobolt Samba, 532 nm, 150 mW output power). If the range in variation of the three measurements exceeded the 2% threshold, the outlying value was discarded and the sample re-analysed.

Because DAPI FCM was not able to distinguish *C. cophocarpa* and *C. stagnalis* in simultaneous analyses, the samples were measured individually using propidium iodide staining when either of these species was suspected.

Morphological identification

Sufficiently developed plants exhibiting essential morphological characters were identified based on recent morphological studies [40,49,50]. These determinations were then compared with genome sizes obtained from FCM analysis, with discordance suggesting hybrid plants. Identification of juvenile and sterile samples was facilitated using FCM. Species for which overlapping or very similar 2C-values were found even when using propidium iodide staining (see below), were identified by the most relevant morphological characters: *C. palustris* and *C. obtusangula* were distinguished on the basis of conspicuously different fruits, and *C. obtusangula* by its remarkable elongate-ellipsoid and curved pollen grains. Sterile plants with 2C-values within the ranges of variation of these two species were cultivated until they produced characters necessary for unambiguous identification. *Callitriche lenisulca* was distinguished from Italian samples of *C. obtusangula* through its unique flower pattern (nodes with only male or female flowers alternating approximately regularly along the stem), small stamens and anthers, and spherical pollen grains [40,45].

Chromosome counts

The chromosome number was counted in at least one sample of each detected cytotype. Selected plants were cultivated in a tank (depth about 20 cm) until they began to form adventive roots on the stem. These adventive roots were used for chromosome counting.

The root tips were pre-treated in a saturated water solution of p-dichlorbenzene for approximately two hours, then fixed in a 3:1 mixture of 96% ethanol and acetic acid, macerated in a 1:1 mixture of ethanol and hydrochloric acid for 30 s, washed in water and stained with lacto-propionic orcein. The number of chromosomes was determined under a Carl Zeiss Jena

NU microscope equipped with an Olympus Camedia C-2000 Z camera and Olympus E – 510 Digital SRL Camera.

Altogether, chromosome numbers of seven taxa were determined. The remaining three taxa (*C. hermaphroditica*, *C. lenisulca*, autotriploid *C. stagnalis*) failed to grow in cultivation.

Data analysis

Differences in genome sizes between particular taxa were tested by Bonferroni (Dunn) t Test (α = 5%) using the SAS 9.2 statistical package (SAS Institute, Cary, NC, USA) and depicted as boxplots in STATISTICA. Distribution map was created using DMAP for Windows [110].

To compare the ecological preferences and co-occurrence of individual taxa, all localities were divided on the basis of habitat into the following seven categories (see Table S2): ditch (artificial depression/channel with muddy bottom and standing or almost standing eutrophic water), exposed bottom (of drained water body or from coastal zone), fishpond (artificial water body established for fish farming), lake (large natural water body), pool (small water body with standing water, natural or arising spontaneously after human disturbances), puddle (very small and shallow temporary accumulation of water, usually on paths, with substrate remaining wet after surface water disappearance), reservoir (moderate to large artificial water body, not for fish farming), river (flowing watercourse with high flow rates; subdivided into two subcategories: mud – muddy bottom, sand – sandy bottom) and stream (small to moderate flowing watercourse; subdivided the same as the previous category).

The categories ‘‘fishpond’’, ‘‘lake’’ and ‘‘reservoir’’ were subsequently merged into the common category ‘‘reservoir’’, due to the small number of lakes and reservoirs among the studied localities and the poor ecological differentiation between them.

RESULTS

Genome size variation

In total, 12 taxa of *Callitriche* were recorded, the overwhelming majority of these differing clearly and with statistical significance in nuclear DNA content (Table 1, Figs 1, 2, 3). Among the eight generally recognized species, six can be unambiguously defined by means of genome size

(*C. hermaphroditica*, *C. stagnalis*, *C. cophocarpa*, *C. lenisulca*, *C. platycarpa*, *C. hamulata*). The diploid species *C. stagnalis* and *C. cophocarpa* have similar genome sizes (difference between means 7.0%), and simultaneous analyses of these species did not result in double-peaks. However, 2C-values of both these species are non-overlapping and differed significantly in a Bonferroni (Dunn) t Test.

The other taxa analysed from Central and Atlantic Europe, *C. palustris* and *C. obtusangula*, surprisingly exhibit very similar, overlapping genome sizes, although they differ in DNA-ploidy level (see below).

The situation regarding diploid taxa sampled in Italy is more complicated. The Mediterranean species *C. lenisulca* differs significantly in genome size from both *C. cophocarpa* (difference between means 12.2%) and *C. obtusangula* from north-western Europe (difference 7.8%). However, Italian plants, assigned to *C. obtusangula* on the basis of pollen shape and gross morphology, possess a genome size distinct from north-western European plants of this species, with their 2C-value exactly intermediate between *C. lenisulca* and NW *C. obtusangula* (difference from *C. lenisulca* 3.9%, from NW *C. obtusangula* 3.8%; differences are small but statistically significant). On FCM histograms, *C. lenisulca* exhibited a peak clearly distinct from the peak of the internal standard *Bellis perennis* (Fig. 4), whereas both types of *C. obtusangula* always overlapped with *Bellis* and had to be measured with the *Glycine* standard.

TABLE 1. Flow cytometric results for 178 individuals of 12 *Callitriche* taxa, for which the genome size was determined using propidium iodide staining

Taxon	Chromosome number (2n)	DNA ploidy level	No. of samples	Mean 2C-value ± s.e. (pg DNA)	2C-value range (pg DNA)	Variation (max/min, %)	Mean 1Cx-value (pg DNA)	Mean chromosome size (pg)	Internal standard*
C. hermaphroditica	6	2x	5	1.95 ± 0.03	1.92 – 2.00	3.99	0.98	0.33	B
C. stagnalis	10	2x	26	2.99 ± 0.03	2.95 – 3.04	3.40	1.50	0.30	B
C. cophocarpa	10	2x	35	3.20 ± 0.04	3.11 – 3.26	4.96	1.60	0.32	B
C. lenisulca	10	2x	3	3.59 ± 0.02	3.58 – 3.61	0.89	1.80	0.36	B
C. obtusangula (Italy)	probably 10	2x	3	3.73 ± 0.03	3.71 – 3.76	1.35	1.87	0.37	G
C. obtusangula	10	2x	5	3.87 ± 0.02	3.85 – 3.88	0.95	1.94	0.39	G
autotriploid *C. stagnalis*	probably 15	3x	1	4.55	–	–	1.52	0.30	B
C. ×*vigens* [*C. cophocarpa* × *platycarpa*]	15	3x	17	4.66 ± 0.04	4.62 – 4.72	2.26	1.55	0.31	B
C. palustris	20	4x	19	3.90 ± 0.04	3.85 – 3.96	2.86	0.98	0.20	G
C. platycarpa	20	4x	22	6.18 ± 0.05	6.06 – 6.29	3.73	1.55	0.31	B
hybrid from Tichá Orlice River	29	6x	3	7.62 ± 0.02	7.60 – 7.64	0.53	**	0.26	B
*C. hamulata****	38	8x	39	8.90 ± 0.08	8.73 – 9.10	4.95	**	0.23	B

* B = *Bellis perennis* (2C = 3.96 pg); G = *Glycine max* 'Polanka' (2C = 2.50 pg).

** 1Cx-value cannot be meaningfully calculated due to aneuploid chromosome counts of these taxa.

*** *Callitriche hamulata* has recently been assigned to *C. brutia*, as *C. brutia* var. *hamulata* (Kütz. ex W.D.J. Koch) Lansdown [39,40]. Both taxa are closely related and can be distinguished morphologically perhaps only in their fertile terrestrial forms. Nevertheless, a thorough study of the entire *C. brutia* complex on a large geographic scale, supported by statistical and analytical methods, is not yet available. *Callitriche brutia* and *C. hamulata* possess distinct chromosome numbers and apparently have different evolutionary histories (although the histories of both species are completely unknown and perhaps complex). In addition, their distribution and habitat requirements are partially different. For these reasons, we retain separate taxonomic treatments of these species, at least until the complex is subjected to a critical review using appropriate genetic markers and the mechanism of its origin elucidated.

In the Czech Republic, Germany and Denmark, non-fertile plants with aborted, deformed, yellow pollen grains were repeatedly found (26 samples). The genome size of these plants lies between some diploid species (*C. stagnalis*, *C. cophocarpa*) and tetraploid *C. platycarpa*, which suggests a triploid DNA-ploidy level. These plants were assigned to the F1 hybrid *C. cophocarpa* × *C. platycarpa* (*C.* ×*vigens*, see Discussion). The exception was the sole plant (specimen C125-13) from northern Bohemia (Czech Republic), which strongly resembled *C. stagnalis* and was partially fertile (but with most mericarps deformed or poorly developed). This plant possessed a slightly (and not significantly) smaller genome size than other triploids, which would better fit an autotriploid of *C. stagnalis*. This inference was confirmed by means of allozyme analysis (J. Prančl *et al.*, unpublished data).

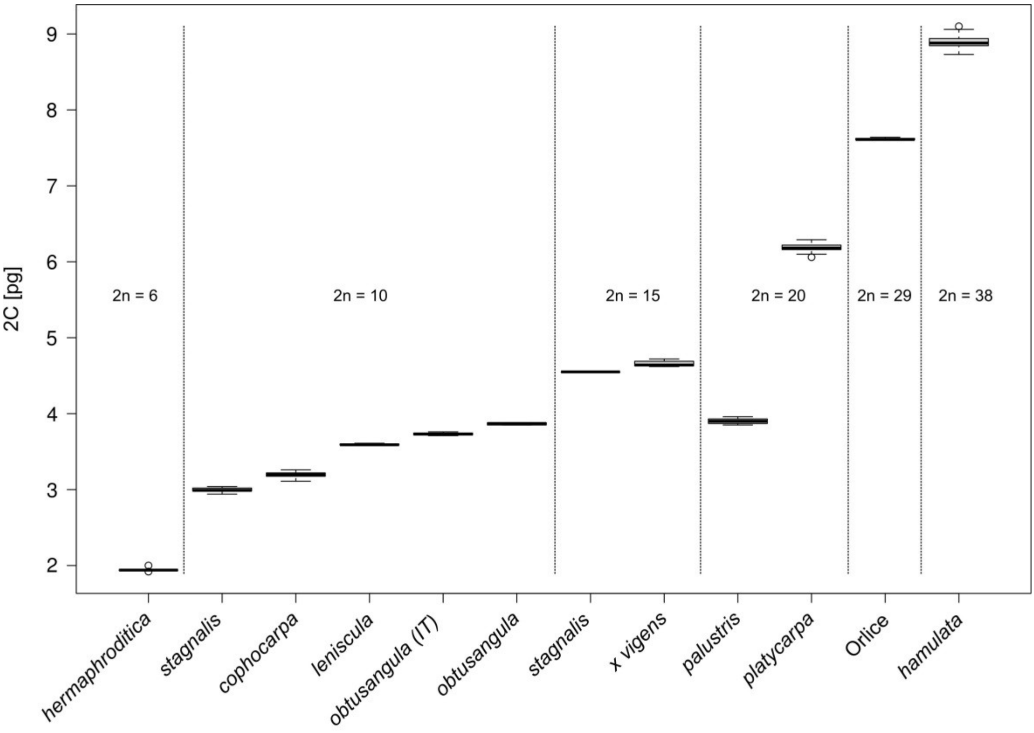

FIG. 1. Box-and-whisker plots showing the holoploid genome sizes (2C-values) for 9 *Callitriche* species and two hybrids: *C. cophocarpa* × *C. platycarpa* (*C.* ×*vigens*) and a hybrid (probably *C. hamulata* × unreduced gamete of *C. cophocarpa*) from the Tichá Orlice River, Czech Republic (Orlice). Taxa with different chromosome numbers are separated by vertical lines. For *C. obtusangula*, values for samples from Italian (IT) and north-western Europe were plotted separately, due to significantly different genome sizes.

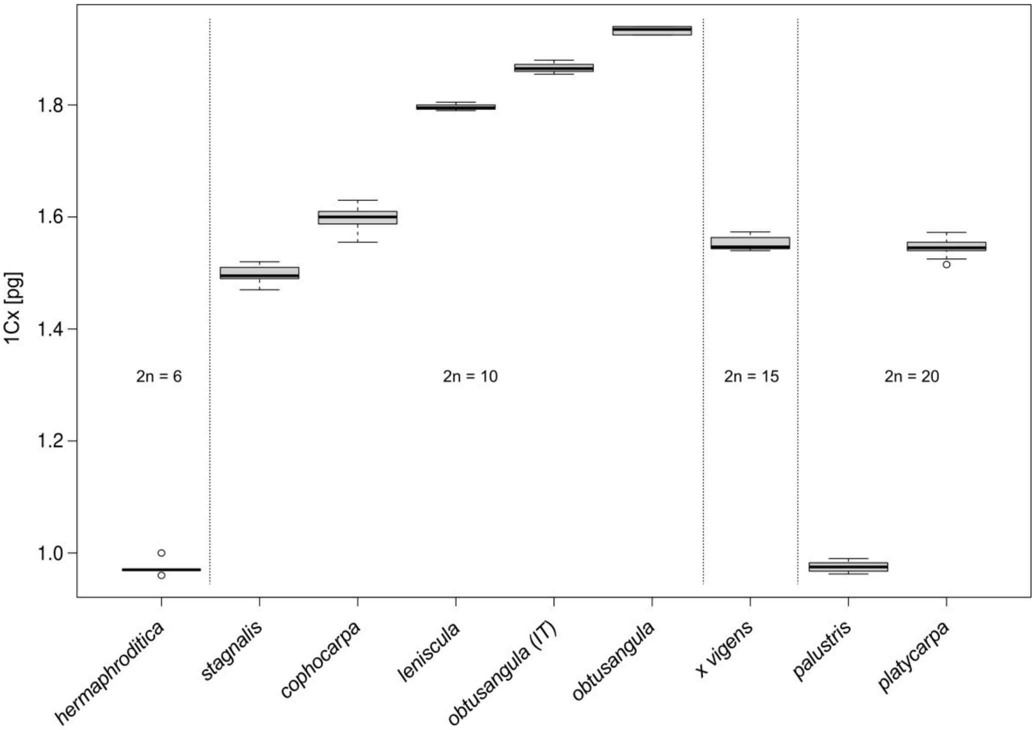

FIG. 2. Box-and-whisker plots showing the monoploid genome sizes (1Cx-values) for 9 *Callitriche* taxa. The species *C. hamulata* (2n=38) and the hybrid from the Tichá Orlice River (2n=29) were not included due to aneuploid chromosome counts.

Other plants most likely representing a product of interspecific hybridization (samples C061-12, C065-12, C066-12) were discovered at three sites in the Tichá Orlice River (eastern Bohemia). This hybrid had submerged aborted flowers and colourless, irregular pollen grains with shrunken protoplasts. *Callitriche hamulata* is considered to be one of the parental species, based on its large genome size. The tetraploid *C. platycarpa* (reduced gamete) and the diploids *C. stagnalis* and *C. cophocarpa* (unreduced gametes) are possible as the second parent.

Regarding the European species occurring in North America, *C. palustris* from USA (specimen C048-13) showed the same genome size as all the conspecific samples from Europe. Also *C. stagnalis*, which is naturalized in North America [111], does not differ in genome size from European conspecifics (C053-13, C054-13). Finally, the occurrence of *C. hamulata* on the Pacific coast of the USA [40] was confirmed by the samples from there having

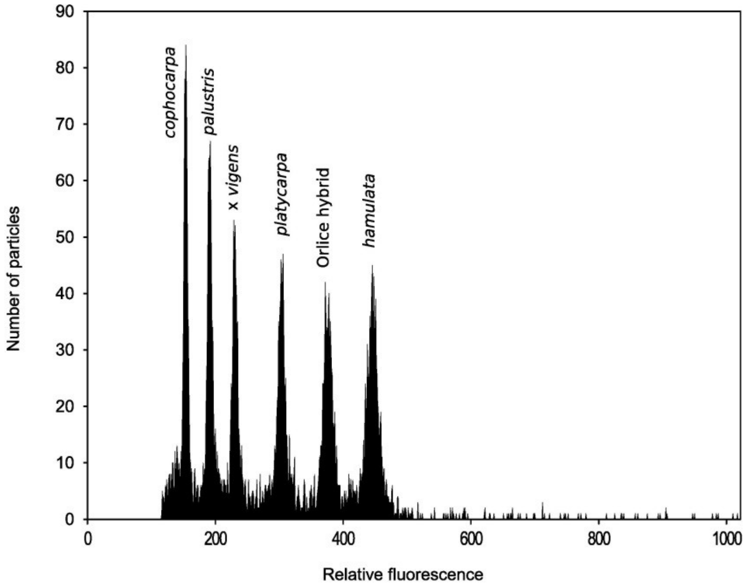

FIG. 3. Flow cytometric histogram showing simultaneous analysis of 6 *Callitriche* taxa: *C. cophocarpa* (2n=10), *C. palustris* (2n=20), hybrid *C. cophocarpa* × *C. platycarpa* (*C.* ×*vigens*, 2n=15), *C. platycarpa* (2n=20), hybrid from the Tichá Orlice River (2n=29) and *C. hamulata* (2n=38). Nuclei of all samples were isolated, stained with propidium iodide and analysed simultaneously.

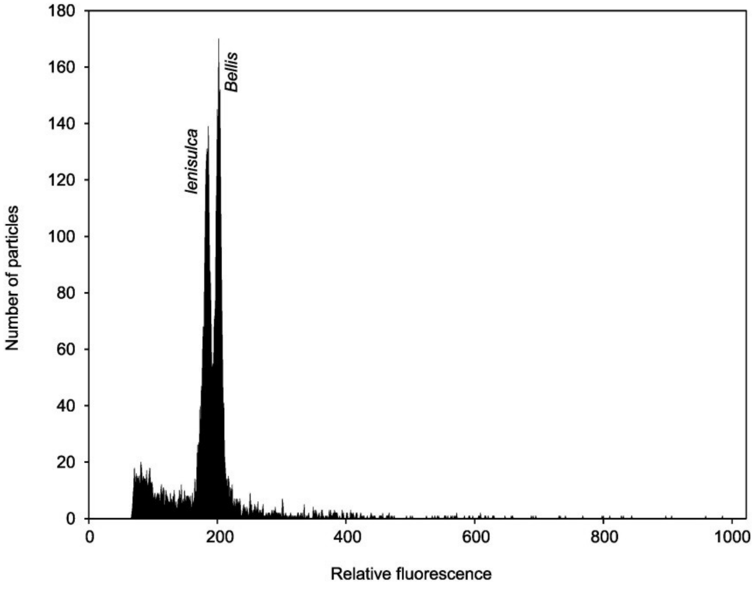

FIG. 4. Flow cytometric analysis of *Callitriche lenisulca* with *Bellis perennis* as an internal standard, using propidium iodide staining.

genome size identical to that of the European samples of this species (C049-13, C050-13, C051-13, C052-13).

Chromosome counts

The chromosome number was determined for 12 individuals of 8 taxa (see Table 2, Fig. 5). Chromosome numbers quoted in published sources were confirmed in all studied species. *Callitriche obtusangula* (2n = 10) and *C. palustris* (2n = 20) differ in DNA ploidy level, although they cannot be distinguished by genome size. Triploid chromosome number (2n = 15) was confirmed in plants assigned to the hybrid *C.* ×*vigens*. Non-fertile plants discovered in the Tichá Orlice River possess an extraordinary chromosome number 2n = 29.

Karyotypes of species with 2n = 10 are different. *Callitriche cophocarpa* has two pairs of slightly bigger acrocentric chromosomes (Fig. 5b). *Callitriche stagnalis* possesses one pair of large metacentric chromosomes; the dimensions of particular chromosomes are the most variable among all the studied diploids (Fig. 5a). *Callitriche obtusangula* has one pair of large acrocentric chromosomes (Fig. 5c).

TABLE 2. Chromosome numbers of 8 *Callitriche* species counted in this study.

Taxon	Ref. no.*	Country	Chromosome number (2n)
C. stagnalis	C013-12	Czech Republic	10
C. cophocarpa	C002-12	Czech Republic	10
C. obtusangula	C052-12	Netherlands	10
C. ×*vigens* [*C. cophocarpa* × *C. platycarpa*]	C059-08	Czech Republic	15
	C021-12	Czech Republic	15
C. palustris	C019-12	Czech Republic	20
C. platycarpa	C011-12	Czech Republic	20
hybrid from Tichá Orlice River	C066-12	Czech Republic	29
C. hamulata	C007-12	Czech Republic	ca 38
	C050-13	USA (introduced)	38
	C084-13	Czech Republic	38
	C094-13	Czech Republic	38

*For samples details, see Table S2.

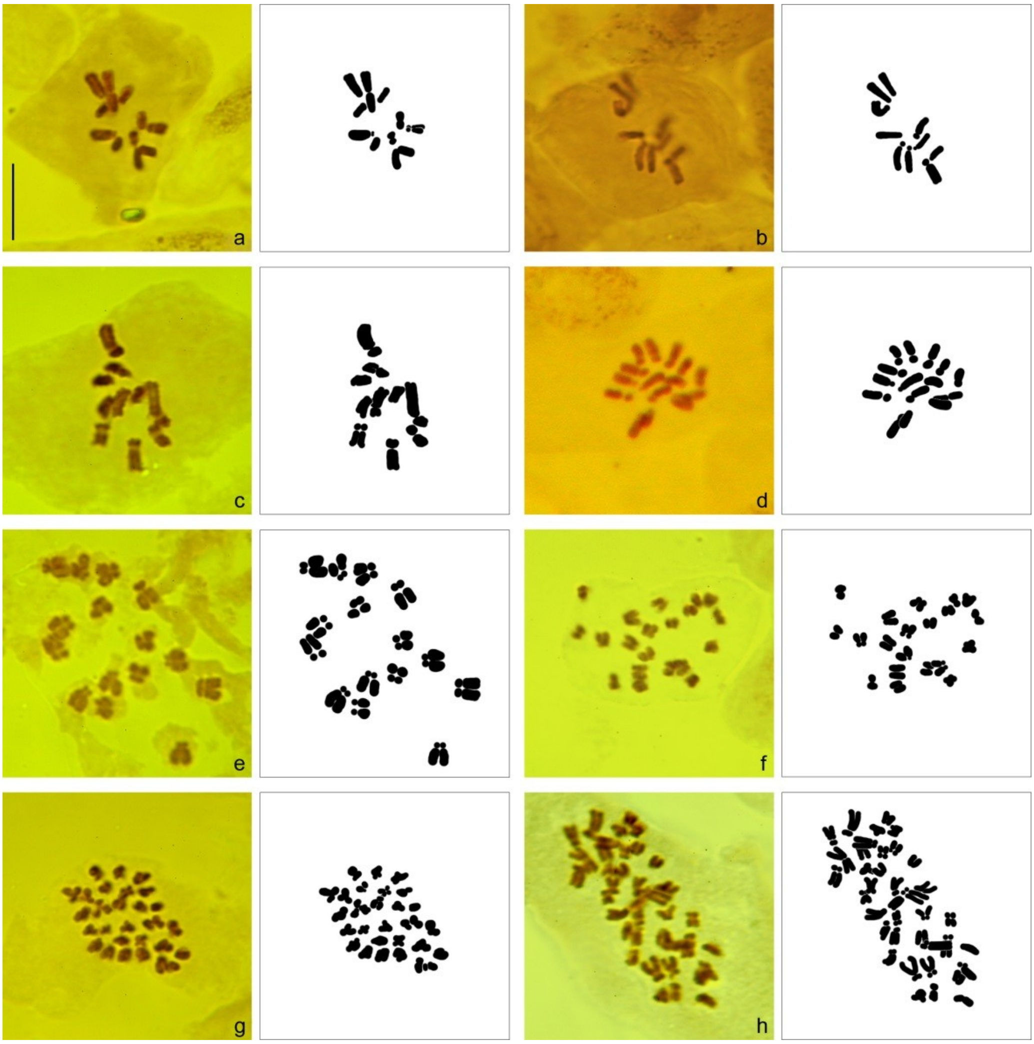

FIG. 5. Chromosomes (photograph of the cytological preparation on the left with its interpretation on the right in each pair) of selected species and hybrids of *Callitriche* at mitotic metaphase in somatic cells, arranged according to increasing chromosome number and genome size: a – *C. stagnalis*, specimen C013-12, 2n = 10; b – *C. cophocarpa*, C002-12, 2n = 10; c – *C. obtusangula*, C052-12, 2n = 10; d – *C.* ×*vigens*, C059-08, 2n = 15; e – *C.* ×*vigens*, C021-12, 2n = 15; f – *C. palustris*, C019-12, 2n = 20; g – probable hybrid *C. hamulata* × *C. cophocarpa*, C066-12, 2n = 29; h – *C. hamulata*, C050-13, 2n = 38. Scale bar identical for all figures = 10 µm.

Geographical distribution

Four species (*Callitriche cophocarpa*, *C. hamulata*, *C. palustris* and *C. stagnalis*) were recorded as common in Central Europe. Detailed screening performed in the Czech Republic revealed the limits of the distribution of the Subatlantic species *C. platycarpa* in the north-western part of Bohemia (see Fig. 6). Where the range of *C. platycarpa* overlaps that of the related but rather continental species *C. cophocarpa*, many populations were shown to be triploids and assigned to *C. ×vigens*. However, this hybrid was also abundant in the Otava River in southern Bohemia, where *C. platycarpa* has never been found (cf. [50]).

The occurrence of *C. platycarpa* in southern Italy (specimen C010-13) is also noteworthy, because this species was until recently known only from the northernmost Italian regions (cf. [40]). The Mediterranean-Atlantic species *C. obtusangula* was for the first time found in Denmark during this study (cf. [112]; specimens C034-12, C048-12) and the Subatlantic species *C. hamulata* was for the first time reliably recorded from Hungary (specimen C14-001).

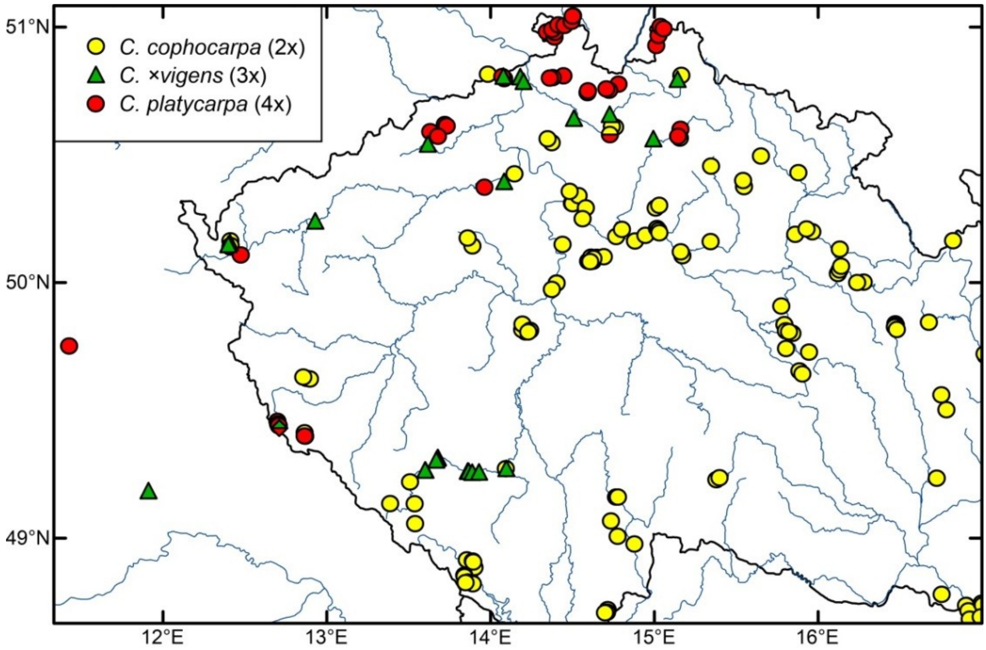

FIG. 6. Map of sampled localities of diploid *Callitriche cophocarpa*, tetraploid *C. platycarpa* and their triploid hybrid *C. ×vigens* in north-western Czech Republic. Two populations with co-occurrence of two taxa are shown as two-colour bisected symbols.

Ecological preferences

The habitat preferences of *Callitriche* taxa from 495 investigated localities are summarized in Table 3. For *C. cophocarpa*, *C. hamulata*, *C. palustris* and *C. stagnalis*, the numbers of localities were sufficiently high (>75) to assess environmental preferences. The strongest relationship with a specific habitat type was recorded for *C. stagnalis*. In total, 78.8% of *C. stagnalis* localities were in puddles. *Callitriche palustris* was found almost exclusively in standing water or on moist, exposed bottoms, with only one locality (1.3%) in running water. In contrast, *C. hamulata* showed the strongest relation to running waters (53.8% of localities). In rivers and streams, bottom substrate can play an important role: streams with *C. hamulata*, predominantly had sandy bottoms (87.0%), whereas those with *C. cophocarpa* had mostly muddy bottoms (75.0%).

Callitriche taxa often occurred in mixed populations. Thus, 88 localities (17.8% of all localities, 31.5% of all samples) supported more than one taxon. Ten localities (2.0%) supported three taxa, and one locality (0.2%) supported four taxa. Generally, the proportion of mixed localities was highest in *C. palustris* (44.7% of all localities, compared to 34.7% for *C. platycarpa*, 33.9% for *C. hamulata*, 31.7% for *C. stagnalis* and 22.0% for *C. cophocarpa*). The most frequent co-occurrences were the pairs *C. platycarpa* and *C. hamulata* (14 populations, i.e. 28.6% of *C. platycarpa* localities) and *C. palustris* and *C. stagnalis* (17 populations, i.e. 22.7% of *C. palustris* localities).

DISCUSSION

This study represents the first application of flow cytometry to the genus *Callitriche* and the most comprehensive application of FCM to aquatic angiosperms (in terms of the number of plants collected as well as the number of populations). It has provided insights into methodological, evolutionary, taxonomic, and ecological issues, discussed below.

Genome size as a tool for identification of Callitriche *species*

Flow cytometry has proven to be a reliable, fast, inexpensive and easy tool to distinguish Central-European *Callitriche* taxa (Table 1, Figs 1, 2, 3). Even homoploid species are recognizable on the basis of genome size. *Callitriche obtusangula* and *C. palustris* are the only two species indistinguishable by this approach. Fortunately, both species differ markedly in fruit shape, size and colour, pollen grain shape, and floating leaf shape [40], and mostly also in the general appearance of the plants. In fact, these two taxa are probably the most distinctive species of *Callitriche* in Europe. Thus, confusion between them is unlikely. Due to the unique mode of self-pollination (internal geitonogamy; [113]), individuals of *C. palustris* are almost always abundantly fertile [49], greatly facilitating determination.

Despite the very similar genome sizes, both species differ in ploidy level (diploid *C. obtusangula* vs. tetraploid *C. palustris*). This striking fact is made possible by the large genetic distance between these species: the widespread *C. palustris* seems to be more closely related to American than European taxa [70], whereas *C. obtusangula* is an exclusively European and North African species [40]. The species also have substantially different life strategies. *Callitriche palustris* is mostly an annual species with a rapid life cycle [42,49]; therefore, evolutionary constraints leading to small genome size may play an important role in this species (cf. [114,115]). On the other hand, *C. obtusangula* is typically perennial, often forming luxuriant vegetative stands. Several other examples of ecologically different congeners are currently known for which genome sizes ratios are incongruent with ploidy levels (e.g. *Chenopodium* [84]; *Anthoxanthum* – Chumová & Trávníček, unpublished data).

The main obstacle to research on water-starworts (and aquatic plants in general) lies in the enormous phenotypic plasticity of these plants and lack of prominent morphological characters. The absence of tools enabling unambiguous determination of particular taxa has resulted in frequent misidentifications and unreliable records. In many such morphologically challenging plant groups, flow cytometry has proven to be the first efficient tool for species and hybrid determination and served as the fundamental method for ensuing studies (e.g. *Chenopodium album* agg. [84]; *Dryopteris carthusiana* agg. [116]; *Fallopia* sect. *Reynoutria* [117]; *Pilosella* [118]). Likewise, easy identification of *Callitriche* species using FCM opens up great opportunities for further interdisciplinary research on this evolutionarily remarkable genus. We highly recommend FCM for taxa delimitation in forthcoming molecular studies on the genus. Genome size can also serve as an independent, species-specific character to define groups in taxonomic research.

TABLE 3. Ecological preferences of 594 *Callitriche* samples of 9 species and 2 hybrids and co-ocurrence of particular taxa in mixed populations

Taxon	Total no. of populations	Mixed with other taxa	Type of habitat*									Average altitude
			ditch	exposed bottom	pool	puddle	reservoir	river (mud)	river (sand)	stream (mud)	stream (sand)	
C. cophocarpa	150	33	23	11	26	28	18	1	1	32	10	398
C. hamulata	172	58	1	13	21	17	27	5	45	7	36	411
C. hermaphroditica	4	–	–	–	–	–	4	–	–	–	–	442
C. lenisulca	3	–	2	–	1	–	–	–	–	–	–	83
C. obtusangula	8	–	3	–	–	–	–	–	1	2	2	169
C. palustris	76	34	–	22	1	37	15	1	–	–	–	449
C. platycarpa	49	17	5	1	4	7	8	2	–	16	6	306
C. stagnalis	104	33	5	1	4	82	5	1	–	4	2	413
C. stagnalis autotriploid	1	–	–	–	–	1	–	–	–	–	–	525
C. ×vigens	25	9	2	1	13	–	5	2	1	–	1	365
hybrid from Tichá Orlice River	3	3	–	–	–	–	–	–	2	–	1	278
Total samples	595	187	41	49	70	172	82	12	50	61	58	398
Total localities	495	88	39	38	58	135	69	9	46	53	48	399
Mixed localities	88	88	2	9	11	32	11	3	3	8	9	390

* For details, see Materials and Methods.

Callitriche truncata subsp. *occidentalis* from sect. *Pseudocallitriche* can perhaps be confused only with *C. hermaphroditica* in this region (both species have 2n = 6). *Callitriche brutia*, which is closely related and often indistinguishable from *C. hamulata* ([39,44]; see comment in Table 1 footnote), has a unique chromosome number (2n = 28, in contrast with 2n = 38 in *C. hamulata*). Because exact genetic delimitation is necessary for further taxonomic assessment of these two problematic taxa, flow cytometry will be able to serve as a basic method for their delimitation.

The situation in the Mediterranean area is more complicated. Additional diploid species (2n = 6–10) are reported from that region, including *C. lusitanica*, *C. pulchra*, *C. truncata* subsp. *truncata* from sect. *Pseudocallitriche* and *C. cribrosa*, *C. lenisulca*, *C. regis-jubae* from sect. *Callitriche*. Our FCM results for *C. lenisulca* provide the foundation for further research on these species. *Callitriche lenisulca*, which is very similar and maybe closely related to *C. cophocarpa* or *C. obtusangula* [107], differs significantly in genome size from both these species. Both *C. lusitanica* and *C. cribrosa* have a different chromosome number (2n = 8) and it is likely that they will be distinct using FCM.

Two similar but significantly different 2C-values were identified in *C. obtusangula*. Italian plants have a smaller genome size than samples from north-western Europe. This differentiation may be associated with the several different karyotypes of this species described by Schotsman [44,119]. Two karyotypes were reported from France [38], one of which occurs in Atlantic region and the other in Mediterranean region and the Rhine Valley. These two karyotypes were described as somewhat different ecologically, although morphologically indistinguishable. Molecular approaches will be necessary to elucidate their evolutionary origins.

The genome size of the Italian *C. obtusangula* was intermediate between that of Subatlantic specimens of this species and *C. lenisulca*. However, pollen of the Italian *C. obtusangula* was normally developed, which makes it less probable that these plants are F1 hybrids. Additionally, hybridization between these taxa is less likely due to the presence of an effective self-pollination system in *C. lenisulca* ([45]).

Evolution of polyploid Callitriche platycarpa

The origin of polyploid species is currently a widely studied phenomenon. In taxonomically difficult groups that include polyploids, repeated origins of polyploid taxa appears to be the rule rather than the exception [120–122]. Especially in aquatic plants, many of which have undergone considerable morphological reduction, the possibility that allopolyploids recognized as single species may actually be polyphyletic cannot be excluded (see, for example, *Ranunculus penicillatus*; [17,123]). In waterstarworts, the tetraploid *C. platycarpa* is believed to be an allotetraploid with the parental species *C. cophocarpa* and *C. stagnalis* (see above). The observed range of genome size for *C. platycarpa* is equal to the sum of these two diploid congeners (see Table 1, Figs 2 and 3), which may support this hypothesis. Anyway, a molecular approach will be necessary to elucidate the evolution of *C. platycarpa*, as autopolyploid origin of some populations cannot be excluded and multiple allopolyploid formation through reciprocal hybridization events is also possible.

Hybridization

Species-rich genera of aquatic plants may produce extremely high numbers of hybrids (e.g. 99 sufficiently recognized hybrids in Potamogeton; [26]). Many aquatic hybrid clones can occupy large areas, produce dominant stands [16,33,123,124], or even exhibit invasive behaviour [125] or extensive introgression [19,20]. However, the results of this study suggest that hybridization between most Central-European *Callitriche* species is not common, despite the frequent co-occurrence of most taxa (see above). The different pollination biology of particular taxa, high proportions of selfing (geitonogamy) and in some cases also ecological differences between species are presumably the main reasons why water-starworts rarely hybridize. For example, *C. hermaphroditica* and *C. hamulata* are hypohydrogamous (pollinated through wettable exine-reduced pollen under the water surface), whereas the rest of the studied species have pollen with an exine, which is not adapted to spread freely underwater [67,126]. Some species have highly geitonogamous pollination, realized via contact between male and female flowers (''contacters'': *C. hamulata* [42], *C. lenisulca* [45]) or growth of pollen tubes through filaments and non-floral vegetative tissues (''internal geitonogamy'': *C. palustris* [113]). Therefore, in some cases, even though the stands of different species intermingle at a locality, transfer of pollen to the stigmas of the second species may be physically hardly possible.

The exception to the rule is the triploid hybrid taxon, which we assigned to the F1 hybrid *C. cophocarpa* × *C. platycarpa* (*C.* ×*vigens*). We consider this parental combination the most probable, because triploids were morphologically intermediate between *C. cophocarpa* and *C. platycarpa* (or indistinguishable from one or the other) and were detected almost exclusively in areas of co-occurrence of both species. The genome size of the triploids also best fits the hybrid combination of these two species. The hybrid was found at 25 localities; in some regions (northern and western Bohemia) it seems to be relatively abundant (see Fig. 6).

The hybrid is perennial, forming lush and highly viable vegetative stands, and was found occurring without the presence of parents in the overwhelming majority of localities. Triploid plants were also detected in the Otava River in southern Bohemia, where *C. platycarpa* is not known from the river basin. A similar case is known from Scandinavia [34] where *Callitriche ×vigens* is frequent in southernmost Sweden, although one of the parental species (*C. cophocarpa*) is fairly rare there. Occurrence of hybrids in different areas very long after the disappearances of their parents is well documented in *Potamogeton* and *Stuckenia* [16,35,37,127–129], and it is probable in *Ranunculus* subg. *Batrachium* [17,33].

We cannot yet, however, entirely rule out that some populations of triploids may be of different origin (including hybridization between *C. platycarpa* and *C. stagnalis*, hybridization of diploids *C. stagnalis* and *C. cophocarpa* involving unreduced gametes, or formation of autotriploids of both species). In any case, the combination *C. cophocarpa* × *C. platycarpa* is the most probable, because (i) unreduced gametes are much rarer than reduced gametes; (ii) the putative parental taxa also share pollination systems and ecological preferences, with both often occurring in permanent water bodies, where the newly established non-fertile hybrids can persist. In contrast, *C. stagnalis* prefers to grow in very shallow water or terrestrially, often remains non-flowering in deeper water, and probably possesses a higher rate of geitonogamous pollination [50]. The single plant identified as autotriploid *C. stagnalis* is the very rare exception. This plant was found in a puddle on a forest path, unlike all the other triploids.

The most notable case of hybridization was detected in the Tichá Orlice River. All populations of the hybrid taxon were located in mixed populations with *C. hamulata*, from which they were morphologically indistinguishable without careful inspection of flowers. On the basis of the observed chromosome number ($2n = 29$), these plants probably represent a cross between *C. hamulata* ($2n = 38$) and a diploid ($2n = 10$) or tetraploid ($2n = 20$)

species. The observed genome size (7.60–7.64 pg DNA) can be explained as the hybrid *C. hamulata* × *C. platycarpa* (expected 2C-value 7.40–7.70 pg), *C. hamulata* × unreduced gamete of *C. cophocarpa* (7.48–7.81 pg) or perhaps *C. hamulata* × unreduced gamete of *C. stagnalis* (7.32–7.59 pg). Thus, we hypothesize that this is a rather surprising hybrid between hypohydrogamous, underwater-flowering *C. hamulata*, and a non-hypohydrogamous species with (predominantly) aerial flowers. To date, an analogous case of hybridization has never been observed in angiosperms. The only other *Callitriche* species that has been observed in the Tichá Orlice River is *C. cophocarpa*. In one locality (C061-12), both *C. hamulata* and *C. cophocarpa* co-occurred with the hybrid. Therefore, the parental combination *C. hamulata* (reduced gamete) × *C. cophocarpa* (unreduced gamete) is most likely, but confirmation of this tentative identification by means of molecular markers would be necessary.

Flow cytometry does not enable us to confidently distinguish potential hybrids between homoploid species with similar genome sizes. In the present study, this limitation mainly involves the species *C. stagnalis* and *C. cophocarpa*, which are broadly sympatric in Central Europe. However, there is a clear (although narrow) gap between the genome sizes of the species, without any intermediate values. Many plants with extreme 2C-values and individuals appearing morphologically intermediate were cultivated but no reduced fertility or other indications of hybrid origin were ever observed. Based on these facts, we can exclude hybridization between these species occurring widely in nature. However, this crossing must have occurred at some point, due to the existence of the allopolyploid *C. platycarpa*, which has the same parental species.

Reliability of published chromosome numbers and genome sizes

Callitriche species have relatively large chromosomes which can be counted relatively easily. Errors arising directly during the counting process are apparently rare in this genus. Mistakes caused by misidentification are much more likely. Fortunately, the monographer and *Callitriche* expert H. D. Schotsman published reliable chromosome counts for most European species in the 1950s and 1960s. These data served as a reference for other researchers. However, confusion can often occur between species with the same chromosome number (in Europe, especially between the species with 2n = 10). Without examining original specimens, it is usually impossible to know whether the published data were based on accurate identification. Chromosome counting combined with genome size determination will allow elimination of most confusion in the future.

The genome sizes obtained by Pijnacker & Schotsman using photometric cytometry with the Feulgen staining method [106] possess somewhat different absolute values and lower accuracy, but the ratio between 2C-values of particular taxa are ± similar to that in our study. The most striking difference is in 2C–values of *C. palustris* and *C. obtusangula*, which appeared to be clearly distinct in the study by Pijnacker & Schotsman but overlapping in our study.

Geographical distribution of Callitriche *taxa*

Flow cytometry can fundamentally refine our understanding of the distribution of particular taxa (cf. [130,131]). For example, the taxonomically difficult species *C. platycarpa* has been previously reported from various areas of the Czech Republic but not from the most northern and western parts of the country [132]. In contrast, our study involving flow cytometry detected *C. platycarpa* only in these areas. Therefore, we conclude that the local limit of the distribution of this species passes through this region. In light of our findings, the occurrence of *C. platycarpa* in more eastern parts of Central Europe (e.g., in Slovakia; [133]) seems to be very unlikely.

We also detected *C. hamulata* for Hungary, *C. ×vigens* to the Czech Republic and recorded *C. obtusangula* in Denmark, which is its northernmost known occurrence in continental Europe.

Callitriche hermaphroditica has been recently referred to as very rare and close to extinction in Central Europe, and probably isolated from its continuous distribution range in Northern Europe [49]. We found this species only in a single pond system in eastern Bohemia (C088-12, C089-12, C090-12). *Callitriche hermaphroditica* is very variable in fruit characters [40], and its intraspecific division needs clarification throughout its extensive distribution range. Based on the fruit size [39], Czech populations clearly belong to subsp. *hermaphroditica*. The additional sample (C127-13) obtained from Sweden was unfortunately sterile and failed to grow in cultivation.

FCM also enables easier identification of introduced taxa. On the west coast of North America, widespread occurrence of plants considered to be *C. hamulata* has recently been reported [40] and this was confirmed using flow cytometry.

Ecological properties and their consequences

Many *Callitriche* species show relatively broad ecological amplitude and often grow in mixed populations. Despite this, we found that the frequency of occurrence in different biotopes can vary substantially between species. Knowledge of the ecology of water-starworts may facilitate their identification in the field because some species have never been recorded in certain types of habitat in spite of an extensive field survey.

During this study, considerable differences were recorded in the frequency of flowering and fruiting among different species. Whereas mature plants of *C. palustris* almost inevitably produced flowers and fruits in each biotope from which it was recorded, *C. cophocarpa* and *C. platycarpa* remained sterile at most localities (other species were also often sterile, but with lower frequency). Although both *C. cophocarpa* and *C. platycarpa* often grow and form large stands in flowing water, they almost never develop fruits under these circumstances. Both taxa also rarely flower in shaded habitats. For these reasons, species that seldom fruit are highly under-recorded in field surveys. Flow cytometry allows estimation of the true abundances of *Callitriche* species in particular biotopes and also enables identification of mixed populations even in habitats where some species have never been observed to produce fruits or fruit very rarely.

CONCLUSIONS

This study represents the first application of flow cytometry to the genus Callitriche. FCM was shown to be the best analytical method for distinguishing Callitriche species. This technique also helped increase our knowledge of variation, hybridization, distribution and ecology of particular taxa.

This genus has been considered extremely difficult taxonomically and therefore has been an unpopular subject for research. Nevertheless, the water-starworts are quite intriguing in terms of the evolution of pollination mechanisms and the frequent incidence of polyploidy in different evolutionary lineages [40]. We validated FCM as a powerful tool not only for determining *Callitriche* taxa, but also as a basic method for future multidisciplinary research on the genus. Moreover, applying this method also to other, similarly complex aquatic plant groups should be very promising.

SUPPORTING INFORMATION

Table S1 Complete list of chromosome numbers published for the genus *Callitriche*.
(DOC)

Table S2 Locality details and genome sizes of 494 *Callitriche* populations from 11 countries, including reference number, number of analysed samples, genome size (three times-measured values are in bold, containing mean and standard deviation), geographic coordinates, altitude, type of habitat (with categories described in Materials and Methods), other *Callitriche* taxa recorded on the same locality, date of collection and collector name(s) (JanR = Jan Rydlo, JarR = Jaroslav Rydlo, JP = Jan Prančl, KK = Klára Kabátová, PT = Pavel Trávníček, ZK = Zdeněk Kaplan).
(DOC)

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: JP ZK. Performed the experiments: JP PT VJ. Analyzed the data: JP PT ZK VJ. Contributed reagents/materials/analysis tools: JP ZK PT VJ. Contributed to the writing of the manuscript: JP ZK.

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SUPPORTING INFORMATION

TABLE S1. Complete list of chromosome numbers published for the genus *Callitriche*

Section	Taxon	Native distribution	2n	References	Notes
Pseudocallitriche	***C. hermaphroditica*** L.	boreal Europe and Asia, boreal and temperate areas of North America	**6**	Canada: [1]; Czechoslovakia (Bohemia): [2,3]; Denmark: [4]; England: [5,6]; Greenland: [7]; Poland: [8]; Russia: [9]; Sweden: [10]; USA: [1,11]; unknown origin: [12] (cultivated plants, Munich Botanical Garden), [13]	most of published counts not distinguish between subsp. *hermaphroditica* and subsp. *macrocarpa*(as decribed in [14]); at least Martinsson [10] referred to both subspecies, Jones [5] to subsp. *macrocarpa* and Černohous [2] to subsp. *hermaphroditica*
	C. truncata subsp. ***occidentalis*** (Rouy) Schotsman	coastal areas of W Europe and Mediterranean	**6**	England: [12]; Great Britain: [6]; Portugal: [15]; unknown origin: [16,17]	
	C. pulchra Schotsman	Greece – island of Gavdos, N Libya	**8**	Libya: [18]	
	C. lusitanica Schotsman	Iberian Peninsula, NW Africa	**8**	Morocco: [19]; Portugal: [12,19]; Spain: [19]	
Callitriche	***C. cribrosa*** Schotsman	Iberian Peninsula, Italy, NW Africa	**8**	Morocco: [20,21]	

Section	Taxon	Native distribution	2n	References	Notes
Callitriche	***C. stagnalis*** Scop.	most of Europe, NW Africa and Macaronesia, probably Middle East	**10**	Belgium: [12]; Canada (naturalized): [22]; Czechoslovakia (Bohemia): [23]; Czech Republic: **this study**; Denmark: [4]; England: [5,24,25,26]; France: [12,24,27]; Germany: [12,24,28,29]; Great Britain: [6,30]; Italy: [12,31,32]; Morocco: [19]; Netherlands: [12,33]; New Zealand (naturalized): [34]; Poland: [35,36]; Portugal: [12,24]; Russia: [37]; Sweden: [38]; Spain: [39] (Baleares), [40]; Switzerland: [12,24]; USA (naturalized): [1]; Wales: [41]	Jørgensen [4] reported also 2n=20, but this number is apparently referred to *C. platycarpa,* which was not recognized by Jørgensen
	C. regis-jubae Schotsman	SW of Iberian Peninsula, NW Africa	**10**	Morocco: [42]	
	C. cophocarpa Sendtn.	central, N and E Europe	**10**	Czechoslovakia: [43,44] (Slovakia), [23] (Bohemia), [45] (Moravia); Czech Republic: **this study**; Denmark: [6]; France: [46]; Germany: [12,28,29,47]; Poland: [35,36]; Slovakia: [48]; Sweden: [38,49]; Switzerland: [12,27,47]; Yugoslavia: [12,47]	other published chromosome numbers are doubtful (2n=12 for Sweden [50]; both 2n=10 and 2n=12 for Slovakia [43])
	C. lenisulca Clavaud	European & Asian Mediterranean	**10**	France: [51]	
	C. mathezii Schotsman	Morocco	**10**	Morocco: [52]	
	C. obtusangula Le Gall	W & S Europe, NW Africa	**10**	England: [5,12,24,26]; France: [12,24]; Germany: [12,24,29]; Great Britain: [6]; Italy: [12]; Morocco: [19]; Netherlands: [12,24,33], **this study**; Portugal: [12,24]; Tunisia: [19]; Wales: [41]	

Section	Taxon	Native distribution	2n	References	Notes
Callitriche	***C. favargeri*** Schotsman	Ethiopia	**10**	Ethiopia: [53]	
	C. hedbergiorum Schotsman	Ethiopia	**10**	Ethiopia: [54]	
			20	Ethiopia: [55]	
	C. deflexa A. Braun	Mexico to Argentina	**10**	Morocco (introduced): [19]; Portugal (introduced): [15,19]	
	C. peploides Nutt.	SE USA, Cuba, Central America	**10**	USA: [1]	
	C. terrestris Raf.	E & SE USA	**10**	USA: [1]	
	C. muelleri Sond.	E Australia, New Zealand, Kermadec Islands	**10**	New Zealand: [34]	
	C. ×vigens K. Martinsson [*C. cophocarpa* × *platycarpa*]	N & central Europe	**15**	Czech Republic: **this study**; Denmark: [4,56]; France: [12,46,47]; Germany: [27,29,57,58]; Sweden: [38,59]; Switzerland: [12,27,47]	Winge [60] erroneously reported 2n=16 from the same locality as Jørgensen [4]
	"C. stagnalis"		**15**	New Zealand: [34]	no additional informations
	C. vulcanicola Schotsman	central tropical Africa	**18**	Kenya: [55]	
	C. platycarpa Kütz	Atlantic Europe	**20**	Belgium: [12]; Czech Republic: **this study**; England: [12,26]; France: [12,27,46]; Germany: [12,27,28,29]; Great Britain: [6,30]; Netherlands: [12,33]; Poland: [36]; Spain: [12,40]; Sweden: [38]; Switzerland: [12,47]; Wales: [41]	

Section	Taxon	Native distribution	2n	References	Notes
Callitriche	***C. palustris*** L.	Europe (predominantly central, N and E), Asia & North America (predominantly boreal and temperate)	**20**	Canada: [1,61]; Czech Republic: **this study**; Denmark: [4]; France: [62]; Germany: [12,29]; Netherlands: [12,33]; Poland: [8]; Russia: [9,37,63,64,65,66], [67] (Chukotka); Spain: [40,62]; Switzerland: [12]; USA: [1,11]	
	C. anceps Fernald	Greenland, Alaska, Canada, USA	**20**	Greenland: [68]; USA: [49]	taxonomically unclear species (cf. [69,70])
	C. subanceps Petrov	E Russia	**20**	Russia (Chukotka): [71]	taxonomically unclear species
	C. heterophylla Pursh var. ***heterophylla***	Nova Scotia, USA, Mexico, Guatemala, Hispaniola	**20**	Canada: [72]; USA: (this variety?)	
			40	USA: [1]	the record 2n=10 for USA ([11], variety not specified) is doubtful
	C. heterophylla var. ***bolanderi*** (Hegelm.) Fassett	British Columbia to S California	**20**	Canada: [1,72]; USA: [1]	
	C. marginata Torr.	British Columbia to Gulf of California	**20**	Canada: [1]; USA: [1]	
	C. nuttallii Torr.	USA – Mexican Gulf area	**20**	USA: [1]	
	C. petriei R.Mason subsp. ***petriei***	New Zealand	**20**	New Zealand: [34]	
			30	New Zealand: [34]	2 records, in Dawson [73] reported as *C.* "aff. *petriei*"
	C. petriei subsp. ***chathamensis*** R. Mason	Chatham Islands	**20**	New Zealand (Chatham Islands): [34]	

Section	Taxon	Native distribution	2n	References	Notes
Callitriche	***C. brutia*** Petagna	W, NW and SW Europe, NW Africa, Middle East (?)	**28**	France: [12,19]; Germany: [29]; Morocco: [19]; Portugal: [15,19]; Spain: [40,74], [39] (Baleares); Wales: [26,41,75]	the number 2n=20 for Iceland [76] is doubtful
	***C. hamulata* × *platycarpa*?**		**29**	Sweden: [59]	the single population; parental species of this hybrid were not reliably proved
	***C. hamulata* × *cophocarpa*?**		**29**	Czech Republic: **this study**	
	C. hamulata Kütz. ex W.D.J. Koch	W, N & central Europe, Greenland	**38**	Czechoslovakia (Bohemia): [45,78]; Czech Republic: **this study**; Denmark: [4]; England: [5,77]; France: [12,27,47,79]; Germany: [12,27,29,47]; Great Britain: [6,26]; Iceland: [76]; Netherlands: [12,33]; Poland: [35]; Spain: [40]; Switzerland: [12,47]; Wales: [12,41,47]	the records of 2n=40 for Iceland [50,76] are doubtful; Wołek [80] states that he counted the material from S Poland, but without indicating the chromosome number
	C. trochlearis Fassett	Oregon, California	**40**	USA: [1]	
	C. antarctica Engelm. ex Hegelm.	Tasmania, Tierra del Fuego, Falklands & other subantarctic islands	**40**	Australia (Macquarie Island): [81]; Falkland Islands: [82]; New Zealand (Campbell Island): [34]	[81] – meiosis (n=20)
	C. aucklandica R. Mason	Auckland Islands	**40**	New Zealand (Auckland Islands): [34]	

Section	Taxon	Native distribution	2n	References	Notes
Callitriche	***C. stagnalis*** Scop.	most of Europe, NW Africa and Macaronesia, probably Middle East	**10**	Belgium: [12]; Canada (naturalized): [22]; Czechoslovakia (Bohemia): [23]; Czech Republic: **this study**; Denmark: [4]; England: [5,24,25,26]; France: [12,24,27]; Germany: [12,24,28,29]; Great Britain: [6,30]; Italy: [12,31,32]; Morocco: [19]; Netherlands: [12,33]; New Zealand (naturalized): [34]; Poland: [35,36]; Portugal: [12,24]; Russia: [37]; Sweden: [38]; Spain: [39] (Baleares), [40]; Switzerland: [12,24]; USA (naturalized): [1]; Wales: [41]	Jørgensen [4] reported also 2n=20, but this number is apparently referred to *C. platycarpa,* which was not recognized by Jørgensen
	C. regis-jubae Schotsman	SW of Iberian Peninsula, NW Africa	**10**	Morocco: [42]	
	C. cophocarpa Sendtn.	central, N and E Europe	**10**	Czechoslovakia: [43,44] (Slovakia), [23] (Bohemia), [45] (Moravia); Czech Republic: **this study**; Denmark: [6]; France: [46]; Germany: [12,28,29,47]; Poland: [35,36]; Slovakia: [48]; Sweden: [38,49]; Switzerland: [12,27,47]; Yugoslavia: [12,47]	other published chromosome numbers are doubtful (2n=12 for Sweden [50]; both 2n=10 and 2n=12 for Slovakia [43])
	C. lenisulca Clavaud	European & Asian Mediterranean	**10**	France: [51]	
	C. mathezii Schotsman	Morocco	**10**	Morocco: [52]	
	C. obtusangula Le Gall	W & S Europe, NW Africa	**10**	England: [5,12,24,26]; France: [12,24]; Germany: [12,24,29]; Great Britain: [6]; Italy: [12]; Morocco: [19]; Netherlands: [12,24,33], **this study**; Portugal: [12,24]; Tunisia: [19]; Wales: [41]	

Section	Taxon	Native distribution	2n	References	Notes
Callitriche	***C. favargeri*** Schotsman	Ethiopia	**10**	Ethiopia: [53]	
	C. hedbergiorum Schotsman	Ethiopia	**10**	Ethiopia: [54]	
			20	Ethiopia: [55]	
	C. deflexa A. Braun	Mexico to Argentina	**10**	Morocco (introduced): [19]; Portugal (introduced): [15,19]	
	C. peploides Nutt.	SE USA, Cuba, Central America	**10**	USA: [1]	
	C. terrestris Raf.	E & SE USA	**10**	USA: [1]	
	C. muelleri Sond.	E Australia, New Zealand, Kermadec Islands	**10**	New Zealand: [34]	
	C. ×vigens K. Martinsson [*C. cophocarpa* × *platycarpa*]	N & central Europe	**15**	Czech Republic: **this study**; Denmark: [4,56]; France: [12,46,47]; Germany: [27,29,57,58]; Sweden: [38,59]; Switzerland: [12,27,47]	Winge [60] erroneously reported 2n=16 from the same locality as Jørgensen [4]
	"C. stagnalis"		**15**	New Zealand: [34]	no additional informations
	C. vulcanicola Schotsman	central tropical Africa	**18**	Kenya: [55]	
	C. platycarpa Kütz	Atlantic Europe	**20**	Belgium: [12]; Czech Republic: **this study**; England: [12,26]; France: [12,27,46]; Germany: [12,27,28,29]; Great Britain: [6,30]; Netherlands: [12,33]; Poland: [36]; Spain: [12,40]; Sweden: [38]; Switzerland: [12,47]; Wales: [41]	

Section	Taxon	Native distribution	2n	References	Notes
Callitriche	***C. palustris*** L.	Europe (predominantly central, N and E), Asia & North America (predominantly boreal and temperate)	**20**	Canada: [1,61]; Czech Republic: **this study**; Denmark: [4]; France: [62]; Germany: [12,29]; Netherlands: [12,33]; Poland: [8]; Russia: [9,37,63,64,65,66], [67] (Chukotka); Spain: [40,62]; Switzerland: [12]; USA: [1,11]	
	C. anceps Fernald	Greenland, Alaska, Canada, USA	**20**	Greenland: [68]; USA: [49]	taxonomically unclear species (cf. [69,70])
	C. subanceps Petrov	E Russia	**20**	Russia (Chukotka): [71]	taxonomically unclear species
	C. heterophylla Pursh var. ***heterophylla***	Nova Scotia, USA, Mexico, Guatemala, Hispaniola	**20**	Canada: [72]; USA: (this variety?)	
			40	USA: [1]	the record 2n=10 for USA ([11], variety not specified) is doubtful
	C. heterophylla var. ***bolanderi*** (Hegelm.) Fassett	British Columbia to S California	**20**	Canada: [1,72]; USA: [1]	
	C. marginata Torr.	British Columbia to Gulf of California	**20**	Canada: [1]; USA: [1]	
	C. nuttallii Torr.	USA – Mexican Gulf area	**20**	USA: [1]	
	C. petriei R.Mason subsp. ***petriei***	New Zealand	**20**	New Zealand: [34]	
			30	New Zealand: [34]	2 records, in Dawson [73] reported as *C.* "aff. *petriei*"
	C. petriei subsp. ***chathamensis*** R. Mason	Chatham Islands	**20**	New Zealand (Chatham Islands): [34]	

Section	Taxon	Native distribution	2n	References	Notes
Callitriche	***C. brutia*** Petagna	W, NW and SW Europe, NW Africa, Middle East (?)	**28**	France: [12,19]; Germany: [29]; Morocco: [19]; Portugal: [15,19]; Spain: [40,74], [39] (Baleares); Wales: [26,41,75]	the number 2n=20 for Iceland [76] is doubtful
	C. hamulata* × *platycarpa?		**29**	Sweden: [59]	the single population; parental species of this hybrid were not reliably proved
	C. hamulata* × *cophocarpa?		**29**	Czech Republic: **this study**	
	C. hamulata Kütz. ex W.D.J. Koch	W, N & central Europe, Greenland	**38**	Czechoslovakia (Bohemia): [45,78]; Czech Republic: **this study**; Denmark: [4]; England: [5,77]; France: [12,27,47,79]; Germany: [12,27,29,47]; Great Britain: [6,26]; Iceland: [76]; Netherlands: [12,33]; Poland: [35]; Spain: [40]; Switzerland: [12,47]; Wales: [12,41,47]	the records of 2n=40 for Iceland [50,76] are doubtful; Wołek [80] states that he counted the material from S Poland, but without indicating the chromosome number
	C. trochlearis Fassett	Oregon, California	**40**	USA: [1]	
	C. antarctica Engelm. ex Hegelm.	Tasmania, Tierra del Fuego, Falklands & other subantarctic islands	**40**	Australia (Macquarie Island): [81]; Falkland Islands: [82]; New Zealand (Campbell Island): [34]	[81] – meiosis (n=20)
	C. aucklandica R. Mason	Auckland Islands	**40**	New Zealand (Auckland Islands): [34]	

The chromosome counts published by Villani et al. [83] from Italy were not included, because the plant determination was incorrect and based on inappropriate methodology. In light of these shortcomings, the doubtful count of *C. platycarpa* by Serra et al. [84] was also omitted, having the same authors as [83].

Callitriche truncata Guss. subsp. *truncata*, *C. truncata* subsp. *fimbriata* Schotsman and *C. transvolgensis* Tzvelev are the only European taxa with unknown chromosome numbers. Although Lansdown [85] provides chromosome numbers for these taxa (2n=6 for *C. truncata* subsp. *truncata*, 2n=10 for *C. truncata* subsp. *fimbriata* and *C. transvolgensis*), these numbers were reported in error (R.V. Lansdown, pers. comm. 2014). Martinsson [10] also states 2n=6 for *C. truncata* subsp. *truncata*, but does not list any sources. Actually, chromosome counts of these taxa probably have never been reliably published.

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TABLE S2: Locality details for 1076 *Callitriche* individuals from 494 localities, including reference number, number of analysed samples, genome size (three times-measured values are in bold, containing mean and standard deviation), geographic coordinates, altitude, type of habitat (with categories described in Materials and Methods), other *Callitriche* taxa recorded on the same locality, date of collection and collector name(s) (JanR = Jan Rydlo, JarR = Jaroslav Rydlo, JP = Jan Prančl, KK = Klára Kabátová, PT = Pavel Trávníček, ZK = Zdeněk Kaplan).

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C007-07	1	3.27	Czech Republic, distr. Tábor, Hamr, ditch of Vřesenský stream below overflow of Hluboký u Hamru fishpond	49°9'37"N, 14°46'15"E	425	ditch		28 June 2007 JP & ZK
C009-07a	2	3.18	Czech Republic, distr. Klatovy, Sušice, Roušarka stream on S edge of town	49°13'12.9"N, 13°30'33.5"E	480	stream (sand)	*C. hamulata*	29 June 2007 JP
C010-07	1	3.27	Czech Republic, distr. Nymburk, Libice nad Cidlinou, National Nature Reserve Libický luh, Bačovka stream	50°6'16"N, 15°10'9"E	185	stream (mud)		6 July 2007 JP
C015-07	1	3.20	Czech Republic, distr. Mělník, Kly, pool near right bank of Labe River	50°18'31.8"N, 14°29'47.1"E	160	exposed bottom		8 Aug. 2007 JP
C016-07	1	3.22	Czech Republic, distr. Mělník, Kly, Tuhaňská strouha canal in village	50°18'28.9"N, 14°30'8.1"E	160	stream (mud)		8 Aug. 2007 JP
C028-07	1	3.10	Czech Republic, distr. Nymburk, Loučeň, Knížecí fishpond	50°17'28.7"N, 15°0'28.7"E	245	exposed bottom		22 Aug. 2007 JP
C029-07	1	3.18	Czech Republic, distr. Nymburk, Loučeň, rivulet below small pond by spring Dobrá Voda	50°18'5"N, 15°1'48"E	250	stream (mud)		22 Aug. 2007 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
Co35-07	1	3.21	Czech Republic, distr. Jindřichův Hradec, Třeboň, Prostřední Stoka canal near railway station Třeboň lázně	49°0'24.0"N, 14°46'36.2"E	420	stream (mud)		28 June 2007 JP
Co01-08a	3	3.25	Czech Republic, Praha, city distr. Modřany, pools "Modřanské laguny" on right bank of Vltava River	49°59'48.3"N, 14°24'11.1"E	190	pool	*C. hamulata*	1 May 2008 JP
Co02-08	3	**3.25±0.03**	Czech Republic, Praha, city distr. Běchovice, Běchovický stream	50°5'1.0"N, 14°37'30.1"E	235	stream (mud)		8 May 2008 JP
Co03-08	6	**3.21±0.03**	Czech Republic, Praha, city distr. Běchovice, pool in littoral zone of Počernický fishpond	50°4'59.3"N, 14°35'46.9"E	230	pool		8 May 2008 JP
Co04-08	4	3.19	Czech Republic, Praha, city distr. Zbraslav, Nature Monument Krňák, Lipanský stream	49°58'23"N, 14°22'29"E	195	stream (mud)	*C. stagnalis*	15 May 2008 JP
Co06-08a	2	3.22	Czech Republic, distr. Chrudim, Rabštejnská Lhota, small pond on Podhůra stream	49°54'28.5"N, 15°46'31.6"E	315	fishpond	*C. hamulata*	23 May 2008 JP
Co09-08	1	3.18	Czech Republic, distr. Chrudim, Nasavrky, small pond in Nature Monument V Koutech	49°50'6.6"N, 15°47'40.5"E	530	pool		24 May 2008 JP
Co10-08	1	**3.18±0.01**	Czech Republic, distr. Chrudim, Trhová Kamenice, settlement Petrkov, forest fishpond	49°48'4.0"N, 15°50'35.4"E	585	fishpond		24 May 2008 JP
Co15-08	1	3.20	Czech Republic, distr. Chrudim, Jeníkov, ruts on forest path near solitude Lány	49°43'39.2"N, 15°56'42.1"E	610	puddle		25 May 2008 JP
Co16-08	2	**3.18±0.03**	Czech Republic, distr. Praha-východ, Káraný, Nature Reserve Lipovka – Grado, ditch	50°10'45.8"N, 14°46'2.0"E	175	ditch		30 May 2008 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. cophocarpa **Sendtn.**								
Co17-08b	2	3.24	Czech Republic, distr. Břeclav, ruts on forest path NE of chateau Pohansko	48°44'12"N, 16°54'20"E	155	puddle	*C. palustris*	11 June 2008 JP
Co18-18	1	3.28	Czech Republic, distr. Břeclav, ditch NE of chateau Pohansko	48°44'8"N, 16°54'11"E	150	ditch		11 June 2008 JP
Co19-08	1	3.26	Czech Republic, distr. Břeclav, pool Bornova jama near chateau Lány	48°42'56"N, 16°55'7"E	150	pool		11 June 2008 JP
Co21-08	1	3.25	Czech Republic, distr. Břeclav, ditch SSE of chateau Lány	48°40'57.3"N, 16°55'34.1"E	150	ditch		11 June 2008 JP
Co234-08	1	**3.22±0.03**	Czech Republic, distr. Břeclav, Kostice, Nature Reserve Stibůrkovská jezera, complex of pools	48°44'41"N, 16°59'54"E	145	pool		12 June 2008 JP
Co25-08	1	**3.22±0.01**	Czech Republic, distr. Klatovy, Horská Kvilda, tributary of Hamerský stream W of village	49°3'22.6"N, 13°32'23.6"E	1010	stream (sand)		2 July 2008 I. Prančlová
Co30-08	1	3.19	Czech Republic, distr. Jindřichův Hradec, Lutová, ditch along bank of Velký Holý fishpond	48°58'36.0"N, 14°52'42.4"E	435	ditch		10 July 2008 JP
Co37-08	4	**3.25±0.03**	Czech Republic, distr. Jindřichův Hradec, Lužnice, ditch under railway bridge	49°4'0.2"N, 14°44'9.6"E	425	ditch		12 July 2008 JP
Co47-08	1	3.26	Czech Republic, distr. Olomouc, Mladeč, pool in floodplain forest N of village	49°42'46"N, 17°1'22.E	240	pool		23 July 2008 JP
Co61-08	1	3.17	Czech Republic, distr. Cheb, Nová Ves, pool in kaolin quarry SSW of village	50°9'42"N, 12°24'42"E	440	pool	*C. hamulata*	3 Aug. 2008 JP
Co70-08	2	3.19	Czech Republic, distr. Prachatice, Volary, Volarský stream near bus station	48°54'34"N, 13°53'30"E	755	stream (mud)		6 Sept. 2008 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C071-08	6	3.19	Czech Republic, distr. Prachatice, Volary, Volarský stream under railway bridge	48°54'24.3"N, 13°53'42.1"E	750	stream (mud)		6 Sept. 2008 JP
C072-08	1	3.17	Czech Republic, distr. Prachatice, Volary, Planerův Dvůr settlement, small meadow reservoir	48°53'6"N, 13°54'9"E	760	pool		6 Sept. 2008 JP
C076-08	2	**3.16±0.03**	Czech Republic, distr. Prachatice, Volary, lower fishpond on Jedlový stream in Stögrova Huť settlement	48°54'52.4"N, 13°51'19.1"E	795	fishpond	*C. palustris*	6 Sept. 2008 JP
C080-08	1	3.18	Czech Republic, distr. Prachatice, Stožec, ditch along forest path SE of Na vrchu hill	48°50'56"N, 13°50'31"E	855	puddle		7 Sept. 2008 JP
C081-08	1	3.16	Czech Republic, distr. Prachatice, Stožec, former bed of Schwarzenberský canal near crossing with Jelení stream	48°49'33"N, 13°50'51"E	910	puddle		7 Sept. 2008 JP
C083-08	1	3.17	Czech Republic, distr. Prachatice, Jelení, puddle near bank of Hučina stream	48°49'42"N, 13°51'50"E	805	puddle		7 Sept. 2008 JP
C084-08	2	3.24	Czech Republic, distr. Prachatice, Jelení, Jelení jezírko reservoir	48°49'15.2"N, 13°53'32.2"E	945	reservoir		7 Sept. 2008 JP
C086-08	1	3.25	Czech Republic, distr. Žďár nad Sázavou, Radostín, stream flowing from peat-bog Radostínské rašeliniště	49°39'6"N, 15°53'14.2"E	620	stream (mud)		22 Sept. 2008 JP
C089-08	2	3.21	Czech Republic, distr. Žďár nad Sázavou, Karlov, ditch SW of village, near estuary to Velké Dářko fishpond	49°38'35"N, 15°54'7"E	615	ditch		22 Sept. 2008 JP
C092-08	1	3.24	Czech Republic, distr. Příbram, Voznice, Voznický stream under bridge of highway R4	49°49'0.6"N, 14°13'18.6"E	360	stream (mud)		6 Nov. 2008 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C093-08	2	3.18	Czech Republic, distr. Příbram, Voznice, Horní fishpond (Horní Tušiny), tall-segde vegetation in littoral zone	49°48'25"N, 14°12'46"E	400	exposed bottom		6 Nov. 2008 JP
C094-08	3	3.22	Czech Republic, distr. Příbram, Voznice, Charvátův fishpond	49°48'56.1"N, 14°11'34.3"E	415	exposed bottom		6 Nov. 2008 JP
C096-08	1	3.20	Czech Republic, distr. Příbram, Chouzavá, ruts on forest path W of hill Točná	49°50'12"N, 14°11'42"E	420	puddle	*C. stagnalis*	6 Nov. 2008 JP
C101-08	1	**3.21±0.04**	Czech Republic, distr. Prachatice, Stožec, puddle on forest path ESE of village	48°51'22"N, 13°50'2"E	850	puddle		7 Sept. 2008 JP
C102-08	1	3.16	Czech Republic, distr. Česká Lípa, Hradčany, Nature Reserve Hradčanské rybníky, Hradčanský stream below Černý fishpond	50°36'29.1"N, 14°45'38.6"E	279	stream (sand)		17 Sept. 2008 J. Sádlo & P. Petřík
C103-08	1	3.24	Czech Republic, distr. Hradec Králové, Běleč nad Orlicí, pool on left bank of Orlice River	50°11'54.0"N, 15°57'54.7"E	236	pool		25 Sept. 2008 ZK
C002-09	3	3.22	Czech Republic, distr. Litoměřice, Písty, pool on W edge of village	50°25'30"N, 14°8'45"E	160	pool		25 Apr. 2009 JP
C003-09	2	**3.22±0.03**	Czech Republic, distr. Klatovy, Rejštejn, former side bed of Losenice stream near Václavíkova sawmill	49°8'3"N, 13°32'14"E	605	pool		30 Apr. 2009 JP
C005-09	2	3.19	Czech Republic, distr. Mělník, Jelenice, forest marsh near National Nature Monument Polabská černava	50°20'26"N, 14°32'16"E	185	stream (mud)		8 May 2008 JP
C006-09	1	3.21	Czech Republic, distr. Mělník, Přívory, Košátecký stream	50°17'32"N, 14°34'57"E	175	stream (mud)		8 May 2008 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
Co07-09	4	**3.18±0.03**	Czech Republic, distr. Mělník, Kozly, ditch S of village	50°14'57.6"N, 14°33'43.6"E	165	ditch		8 May 2008 JP
Co08-09	2	3.18	Czech Republic, distr. Nymburk, Dvorce, in Mlynařice stream under road bridge E of village	50°12'28.9"N, 14°48'8.0"E	185	stream (mud)		9 May 2008 JP
Co10-09	1	3.19	Czech Republic, distr. Rakovník, Nové Strašecí, ditch on E edge of Nature Reserve Podhůrka	50°8'35"N, 13°53'24"E	405	ditch		22 May 2009 JP
Co13-09	1	3.18	Czech Republic, Praha, city distr. Dolní Chabry, trough close to fishpond in street Pod hrází	50°8'56.5"N, 14°26'25.0"E	205	stream (mud)		24 May 2009 JP
Co15-08	1	3.22	Czech Republic, distr. Nymburk, Semice, ditch on E edge of village	50°9'44.9"N, 14°52'57.3"E	175	ditch		29 May 2009 JP
Co19-09	1	**3.23±0.02**	Czech Republic, distr. Česká Lípa, Hradčany, Nature Reserve Hradčanské rybníky, marsh below overflow of Strážovský fishpond	50°36'39"N, 14°44'29"E	275	puddle	*C. hamulata*	8 June 2009 JP
Co22-09	1	3.20	Czech Republic, distr. Česká Lípa, Břehyně, National Nature Reserve Břehyně-Pecopala, ruts on path E of Břehyňský fishpond	50°34'43"N, 14°43'42"E	275	puddle	*C. platycarpa*	8 June 2009 JP
Co31-09	1	3.22	Czech Republic, distr. Litoměřice, Julčín, Nature Reserve Na Černčí, pit in forest	50°32'49.7"N 14°22'27.3"E	305	puddle		19 June 2009 JP
Co32-09	1	3.25	Czech Republic, distr. Litoměřice, Tetčiněves, Úštěcký stream in village	50°33'40."N, 14°20'49"E	200	stream (sand)		19 June 2009 JP
Co44-09	2	3.18	Czech Republic, distr. Karviná, Dolní Marklovice, small fishpond close to state border	49°53'7.3"N, 18°34'4.6"E	227	fishpond	*C. palustris*	9 Aug. 2009 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C045-09	1	**3.19±0.02**	Czech Republic, distr. Karviná, Dolní Marklovice, stream flowing out of small fishpond close to state border	49°53'5.8"N, 18°34'3.4"E	227	stream (mud)		9 Aug. 2009 JP
C047-09	1	3.21	Czech Republic, distr. Karviná, Dolní Marklovice, small fishpond near motocross track N of village	49°54'5.3"N, 18°34'10.5"E	258	fishpond		9 Aug. 2009 JP
C051-09	1	3.15	Czech Republic, distr. Karviná, Prstná, pool below outlet of fishpond on SW part of village	49°54'50.4"N, 18°33'35.6"E	236	pool		9 Aug. 2009 JP
C054-09	1	**3.11±0.03**	Czech Republic, distr. Nový Jičín, Hukovice, marsh beside main forest road in forest Roveň	49°37'19.3"N, 18°2'36.4"E	275	exposed bottom		11 Aug. 2009 JP
C075-09	2	3.19	Czech Republic, distr. Prostějov, Pohora, Podhora stream close to outlet of fishpond in village	49°33'36.6"N, 16°45'11.0"E	570	stream (mud)		25 Aug. 2009 JP
C078-09	1	3.20	Czech Republic, distr. Blansko, Benešov, puddle on intersection of forest paths WNW of hill Skalky	49°30'8.5"N, 16°47'2.3"E	710	puddle	*C. palustris*	25 Aug. 2009 JP
C083-09	1	8.83	Czech Republic, distr. Blansko, Žďárná, ruts on forest path SE of village	49°26'58.3"N, 16°47'10.2"E	575	puddle	*C. palustris,* *C. stagnalis*	26 Aug. 2009 JP
C091-09	1	3.22	Czech Republic, distr. Brno-venkov, Ochoz u Brna, pool on Říčka stream SW of Svobodův Mlýn solitude	49°14'3.8"N, 16°43'30.2"E	290	pool		30 Aug. 2009 JP
C092-09	1	3.14	Czech Republic, distr. České Budějovice, Staré Hutě, slough below dam of Mlýnský fishpond	48°42'35.0"N, 14°42'43.2"E	765	puddle		18 Sept. 2009 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C094-09b	1	**3.20±0.03**	Czech Republic, distr. České Budějovice, Staré Hutě, blind arm of Lužný stream WSW of settlement Mlýnský Vrch	48°42'27.7"N, 14°41'56.2"E	750	pool	*C. hamulata*	18 Sept. 2009 JP
C096-09	1	**3.20±0.02**	Czech Republic, distr. České Budějovice, Staré Hutě, small ditch on plot of house in village	48°43'11.9"N, 14°42'56.6"E	797	ditch		20 Sept. 2009 JP
C101-09	1	3.20	Czech Republic, distr. Pardubice, Dolní Jelení, ditch along forest path S of Oborecký fishpond	50°2'5.9"N, 16°7'15.5"E	285	puddle		8 Oct. 2009 JP
C102-09	3	**3.20±0.01**	Czech Republic, distr. Pardubice, Rousínov, side arm of Čermná stream close to Horní Pecák fishpond	50°2'57.1"N, 16°7'54.4"E	275	ditch	*C. stagnalis*	8 Oct. 2009 JP
C104-09	1	3.19	Czech Republic, distr. Pardubice, Korunka, Čermná stream in village	50°3'48.7"N, 16°8'27.2"E	265	stream (mud)		8 Oct. 2009 JP
C108-09b	1	3.22	Czech Republic, distr. Svitavy, Opatov, puddle on side of forest path S of railway station Semanín	49°50'17.2"N, 16°28'17.2"E	430	puddle	*C. hamulata*, *C. palustris*, *C. stagnalis*	19 Oct. 2009 JP
C111-09	1	3.24	Czech Republic, distr. Svitavy, Opatov, puddle on forest path N of pond Rebelant	49°50'1.3"N, 16°28'30.3"E	436	puddle		19 Oct. 2009 JP
C112-09	3	3.22	Czech Republic, distr. Svitavy, Opatov, small pond Rebelant	49°49'49.7"N, 16°28'28.0"E	440	reservoir		19 Oct. 2009 JP
C115-09	1	3.25	Czech Republic, distr. Svitavy, Opatov, ditch along forest road SW of pond Rebelant	49°49'32.5"N, 16°28'8.0"E	450	puddle		19 Oct. 2009 JP
C116-09	4	**3.17±0.00**	Czech Republic, distr. Svitavy, Opatovec, Terčový fishpond	49°48'58.4"N, 16°28'52.9"E	443	fishpond		19 Oct. 2009 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C003-10	2	3.14	Czech Republic, distr. Olomouc, Horka nad Moravou, Častava stream on edge of Nature Monument Častava	49°38'31.3"N, 17°12'52.4"E	215	stream (mud)		12 May 2010 JP
C004-10	1	**3.19±0.03**	Czech Republic, distr. Olomouc, Horka nad Moravou, Častava stream near Podbradský fishpond	49°38'39.5"N, 17°12'12.8"E	215	stream (mud)		12 May 2010 JP
C005-10	1	3.16	Czech Republic, distr. Olomouc, Hynkov, ditch SSE of village	49°39'44.9"N, 17°10'29.0"E	220	ditch		12 May 2010 JP
C006-10	1	3.16	Czech Republic, distr. Olomouc, Štěpánov, ditch WNW of railway station	49°40'33.2"N, 17°11'55.7"E	220	ditch		12 May 2010 JP
C016-10	3	**3.17±0.03**	Czech Republic, distr. Semily, Kadeřavec, small artificial pool WNW of Valdštejn castle	50°33'53.8"N, 15°9'23.0"E	375	pool		12 June 2010 JP
C019-10	1	**3.17±0.00**	Czech Republic, distr. Břeclav, Hlohovec, ditch between small fishponds W of Hlohovecký fishpond	48°46'46.6"N, 16°45'18.0"E	170	ditch		7 June 2010 PT
C022-10	3	**3.18±0.03**	Czech Republic, distr. Jičín, Ostroměř, millrace of Javorka stream	50°22'30.0"N, 15°32'52.3"E	265	stream (sand)	*C. hamulata*	26 June 2010 JP
C023-10	1	3.20	Czech Republic, distr. Jičín, Šárovcova Lhota, ditch near settlement Mezihoří	50°23'56.6"N, 15°32'34.1"E	275	ditch		26 June 2010 JP
C032-10	1	3.18	Czech Republic, distr. Zlín, Fryšták, stream in town distr. Dolní Ves	49°16'31.3"N, 17°40'29.9"E	246	stream (mud)		30 June 2010 JP
C034-10	2	**3.19±0.02**	Czech Republic, distr. Zlín, Lukov, Nature Monument Bezedník, Bezedník fishpond	49°17'59.1"N, 17°43'26.4"E	320	fishpond	*C. palustris*	1 July 2010 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C042-10	1	3.26	Czech Republic, distr. Zlín, Vlčková, puddle on forest path NE of hill Ondřejovsko	49°19'52.6"N, 17°44'6.2"E	570	puddle	*C. stagnalis*	1 July 2010 JP
C046-10	1	3.14	Czech Republic, distr. Domažlice, Mezholezy, small fishpond on Mezholezský stream W of village	49°37'21.2"N, 12°53'50.8"E	439	fishpond		5 July 2010 JP & ZK
C048-10	2	3.13	Czech Republic, distr. Domažlice, Mezholezy, small forest pond SE of rock formation Skalní mísy	49°37'28.7"N, 12°52'41.6"E	450	reservoir		5 July 2010 JP & ZK
C050-10	1	3.17	Czech Republic, distr. Tachov, Racov, Nature Monument Racovské rybníčky, overgrown northern pond	49°37'47.1"N, 12°51'25.0"E	485	pool		5 July 2010 JP & ZK
C064-10	2	3.18	Czech Republic, distr. Domažlice, Pila, Nature Monument Louka u Šnajberského rybníka, artificial pool N of Černý fishpond	49°24'42.7"N, 12°51'55.8"E	469	pool		9 July 2010 JP & M. Štech
C112-10	1	3.12	Czech Republic, distr. Nymburk, Kostomlaty nad Labem, Vlkava stream od SW edge of village	50°11'5.0"N, 14°56'46.6"E	180	stream (mud)		2 Aug. 2010 JP
C133-10	1	**3.20±0.02**	Czech Republic, distr. Rychnov nad Kněžnou, Čestice, meadow ditch W of railway station	50°7'50.0"N, 16°7'59.4"E	260	ditch		7 Aug. 2010 ZK
C135-10	1	3.23	Czech Republic, distr. Chrudim, Rohozná, rivulet in alder carr close to NW edge of Velký Rohozenský fishpond	49°48'37.8"N, 15°48'14.8"E	578	stream (mud)		27 Aug. 2010 JP & ZK
C137-10	2	3.22	Czech Republic, distr. Chrudim, Rohozná, Hubský fishpond	49°48'27.2"N, 15°49'22.0"E	575	exposed bottom	*C. hamulata*, *C. palustris*	27 Aug. 2010 JP & ZK

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C143-10	1	3.22	Czech Republic, distr. Havlíčkův Brod, Rovný, small forest fishpond NE of village	49°44'29.0"N, 15°48'16.2"E	570	fishpond		28 Aug. 2010 JP & ZK
C155-10	1	3.14	Czech Republic, distr. Klatovy, Prášily, on bank at confluence of Křemelná and Prášilský streams	49°8'5.2"N, 13°23'13.5"E	810	exposed bottom		25 Sept. 2010 JP
C156-10	2	3.18	Czech Republic, distr. Písek, Zátaví, Řežabinecký stream near estuary to Otava River	49°16'16.4"N, 14°5'35.8"E	367	stream (mud)		29 Sept. 2010 JP
C159-10	1	3.21	Czech Republic, distr. Tábor, Hamr, Smíchov II fishpond	49°9'36.7"N, 14°46'41.7"E	420	exposed bottom	*C. palustris*	20 Sept. 2010 S. Píšová
C001-11	2	3.18	Czech Republic, Praha, city distr. Klánovice, stream on W edge of village	50°05'53.5"N, 14°38'54.6"E	263	stream (mud)		11 May 2011 JP
C002-11	1	**3.13±0.02**	Czech Republic, Praha, city distr. Horní Počernice, pool between SE edge of village and nameless fishpond	50°05'56.0"N, 14°36'52.2"E	245	pool		11 May 2011 JP
C004-11	1	3.19	Czech Republic, distr. Ústí nad Orlicí, Horní Morava, on forest path NNE of quarry above village	50°9'49.7"N 16°49'27.5"E	795	puddle		14 May 2011 JP
C013-11	1	3.17	Czech Republic, distr. Frýdek-Místek, Horní Lomná, puddles of forest path S of hill Úplaz	49°31'0"N, 18°40'26"E	905	puddle		12 June 2011 J. Prach
C041-11	1	3.23	Poland, prov. Małopolskie, Jabłonka, marsh close to Czarna Orawa River WSW of village	49°28'07.8"N, 19°40'22.8"E	604	puddle		25 June 2011 JP & ZK

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C047-11	1	3.21	Slovakia, distr. Námestovo, Oravské Veselé, reservoir in the village	49°27'45.0"N, 19°23'0.5"E	711	reservoir		25 June 2011 JP & ZK
C062-11	1	3.13	Czech Republic, distr. Praha-východ, Cyrilov, Šestajovický stream close to estuary to garden pond on SE edge of village	50°6'3.7"N, 14°41'32.4"E	250	stream (mud)		13 Aug. 2011 JP
C002-12	2	3.19	Czech Republic, Praha, city distr. Horní Počernice, Xaverov, ditch NNW of hill Homole	50°5'36.8"N, 14°37'43.9"E	255	ditch		11 May 2012 JP
C004-12	1	3.24	Czech Republic, Praha, city distr. Běchovice, ditch between village parts Běchovice I and Běchovice II	50°4'53.1"N, 14°36'32.7"E	235	ditch		11 May 2012 JP
C005-12	1	3.18	Czech Republic, Mělník, Pšovka stream in town	50°21'22.6"N, 14°29'0.8"E	165	stream (sand)		13 May 2012 JP
C016-12	1	3.18	Czech Republic, distr. Rakovník, Třtice, littoral zone of small pond close to solitude Třtická Lísa	50°9'49.6"N, 13°50'20.4"E	450	exposed bottom	*C. hamulata*	12 June 2012 JP & A. Potůčková
C017-12	4	3.18	Czech Republic, distr. Rakovník, Třtice, Nature Reserve V Bahnách, artificial pool	50°10'27.2"N, 13°51'38.3"E	418	pool		12 June 2012 JP & A. Potůčková
C025-12	1	3.25	Slovakia, distr. Zlaté Moravce, Jelenec, on N bank of Jelenec fishpond	48°24'4.1"N, 18°12'13.6"E	234	exposed bottom	*C. palustris*	4 July 2012 JP
C033-12	5	**3.25±0.01**	Denmark, Jutland, reg. Syddanmark, Seem, estuary of field ditch to Ribeå River ESE of town	55°18'54.8"N, 08°50'04.0"E	1	stream (mud)		31 July 2012 JP & ZK

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C063-12	1	**3.16±0.04**	Czech Republic, distr. Ústí nad Orlicí, Brandýs nad Orlicí, Tichá Orlice River below bridge of Žerotínova street	50°0'4.9"N, 16°16'45.1"E	295	river (sand)	*C. hamulata, C. hamulata* × *C. cophocarpa?*	15 Aug. 2012 JP
C074-12	1	**3.12±0.02**	Poland, prov. Podlaskie/Mazowieckie, Tyszki-Gostery, Ruż stream near bridge of Droga Wojewódzka road NE of village	52°59'33.0"N, 21°54'58.4"E	111	stream (sand)		31 Aug. 2012 PT & B. Kubátová
C001-13	1	3.26	Czech Republic, distr. Olomouc, Jívová, pool in valley of Bystřice stream near settlement Panský Mlýn	49°42'21.3"N, 17°25'56.4"E	404	pool		29 Apr. 2013 JP & KK
C011-13	1	3.19	Czech Republic, distr. Hradec Králové, Pamětník, Mlýnská Cidlina stream in village	50°07'25.9"N, 15°26'51.2"E	210	stream (mud)		28 May 2013 JP
C013-13	1	3.20	Czech Republic, distr. Břeclav, Kostice, pools in forest Tvrdonické polesí SE of village	48°44'14.0"N, 17°00'21.9"E	150	pool	*C. hamulata*	4 June 2013 JP & M. Hrdinová
C015-13	2	3.20	Czech Republic, distr. Břeclav, Lanžhot, old separated arm of Morava River near border crossing of D2 highway	48°41'29.9"N, 16°59'34.6"E	150	pool		4 June 2013 JP & M. Hrdinová
C016-13	1	**3.24±0.03**	Slovakia, distr. Galanta, Pusté Úľany, Nový stream close to road bridge SW of village	48°13'35.2"N, 17°33'52.1"E	115	stream (mud)		5 June 2013 JP & M. Hrdinová
C017-13	3	3.24	Slovakia, distr. Senec, Hamuliakovo, canal in village near reservoir Hrušov on Dunaj River	48°2'1.9"N, 17°14'55.3"E	124	stream (sand)		6 June 2013 JP & M. Hrdinová

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
Co19-13	1	3.24	Czech Republic, distr. Příbram, Mokrovraty, puddle on side of forest path NE of hill Dolní Jezberná	49°48'45.5"N, 14°14'33.6"E	345	puddle		11 June 2013 JP & KK
Co36-13	2	3.13	Czech Republic, distr. Příbram, Mokrovraty, Dolní (Tušimský) fishpond	49°48'23.7"N, 14°13'50.6"E	380	fishpond	*C. hamulata*	11 June 2013 JP & KK
Co27-13	1	3.20	Czech Republic, distr. Svitavy, Třebařov, Rohlíček fishpond	49°50'40.5"N, 16°40'35.1"E	340	fishpond		20 June 2013 JP & ZK
Co29-13	2	3.25	Czech Republic, distr. Olomouc, Nové Zámky, old separated arm of Morava River in Nature Monument Pod Templem	49°43'14.8"N, 17°01'01.4"E	240	pool	*C. hamulata*	21 June 2013 JP & ZK
Co30-13	1	3.23	Czech Republic, distr. Přerov, Týn nad Bečvou, pool complex in N part of Nature Monument Týn nad Bečvou	49°31'37.2"N, 17°38'33.3"E	234	pool		21 June 2013 JP & ZK
Co31-13	1	3.25	Czech Republic, distr. Přerov, Tovačov, Splavská canal on SW edge of town	49°25'39.8"N, 17°17'03.3"E	199	stream (mud)		21 June 2013 JP & ZK
Co38-13	1	3.18	Czech Republic, distr. Jičín, Kbelnice, ditch in village	50°27'18"N, 15°20'48"E	280	ditch		4 July 2013 V. Sedláček & M. Chytrý
Co39-13	2	3.15	Czech Republic, distr. Trutnov, Borovnička, stream in alder carr SW of village	50°29'41"N, 15°39'5"E	470	stream (sand)		4 July 2013 P. Koutecký
Co46-13	1	3.16	Czech Republic, distr. Trutnov, Choustníkovo Hradiště, small fishpond on Hradišťský stream below caste in village	50°25'47"N, 15°52'46"E	300	fishpond		4 July 2013 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C063-13	1	**3.17±0.05**	Czech Republic, distr. Cheb, Nový Drahov, settlement Hájek, marsh on meadow in S part of National Nature Reserve Soos	50°8'39.8"N, 12°24'49.4"E	432	puddle		19 July 2013 KK & JarR
C066-13	2	**3.22±0.04**	Czech Republic, distr. Cheb, Dvorek, Stodolský stream by road bridge NE of village	50°8'19.5"N, 12°24'42.5"E	431	stream (sand)	*C. hamulata*, *C. stagnalis*	19 July 2013 KK & JarR
C071-13	1	**3.26**	Sweden, prov. Västernorrland, Bollstabruk, ditch along road no. 90 NNW of village	63°3'18.0"N, 17°36'49.2"E	98	ditch		3 Aug. 2013 JanR
C081-13	6	**3.22±0.02**	Czech Republic, distr. Jablonec nad Nisou, Bedřichov, Blatný rybník reservoir	50°48'36.9"N, 15°9'53.8"E	770	reservoir	*C. hamulata*	22 Aug. 2013 JP & KK
C088-13	1	3.27	Czech Republic, distr. Hradec Králové, Blešno, pool in alluvium of Orlice River S of village	50°12'33.1"N, 15°55'45.5"E	225	exposed bottom		1 Sept. 2013 JP & KK
C095-13	2	**3.19±0.03**	Czech Republic, distr. Ústí nad Orlicí, Choceň, town distr. Peliny, confluence of outlet of separated river arm to Tichá Orlice River	49°59'55.7"N, 16°14'17.4"E	295	river (mud)	*C. hamulata*	1 Sept. 2013 JP & KK
C119-13	1	**3.24±0.04**	Czech Republic, distr. Ústí nad Labem, Petrovice, artificial pool ENE of border crossing	50°48'55"N, 13°59'3"E	430	pool		27 Aug. 2013 JanR & JarR
C126-13	1	3.19	Czech Republic, distr. Nymburk, Libice nad Cidlinou, National Nature Reserve Libický luh, pool Malý Přerov near confluence of Labe and Cidlina Rivers	50°7'12.2"N, 15°9'43.7"E	185	pool		1 Sept. 2013 JarR
C128-13	5	3.15	Czech Republic, distr. Jihlava, Řásná, Velký Pařezitý fishpond	49°13'41.4"N, 15°22'44.5"E	675	fishpond	*C. hamulata*	8 Sept. 2013 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C131-13	1	3.21	Czech Republic, distr. Jihlava, Řídelov, ditch along forest path near SW bank of Pilný fishpond	49°14'08.7"N, 15°23'52.6"E	620	ditch	*C. stagnalis*	8 Sept. 2013 JP
C138-13	1	3.20	Czech Republic, distr. Frýdek-Místek, Horní Lomná, puddle on forest path NNE of Kalužný hill NNW of village	49°34'24.2"N, 18°37'37.4"E	975	puddle		16 Sept. 2013 JP & KK
C139-13	3	3.24	Czech Republic, distr. Frýdek-Místek, Horní Lomná, puddle on forest path near observation tower Kozubová	49°34'13.1"N, 18°40'24.7"E	975	puddle	*C. palustris*	16 Sept. 2013 JP & KK
C141-13	1	3.23	Slovakia (on borderline with Poland), distr. Námestovo, Vychylovka, puddle on ridge of forest road NE of Jaworzyna mountain	49°23'58.4"N, 19°02'34.2"E	1091	puddle		17 Sept. 2013 JP & KK
C142-13	1	3.24	Slovakia (on borderline with Poland), distr. Námestovo, Novoť, puddle on ridge of forest road N of Úšust (Ozsust) mountain	49°25'26.8"N, 19°11'30.2"E	958	puddle		18 Sept. 2013 JP & KK
C143-13	3	3.23	Slovakia (on borderline with Poland), distr. Námestovo, Mútňanská Píla, puddle on ridge of forest road WNW of village	49°28'49.9"N, 19°13'31.4"E	1074	puddle		17 Sept. 2013 JP & KK
C145-13	1	3.26	Czech Republic, Olomouc, field ditch SW of city distr. Holice	49°33'46"N, 17°17'19"E	205	ditch		23 Sept. 2013 JarR
C147-13	1	3.18	Czech Republic, distr. Nymburk, Veleliby, Liduška stream below bridge W of village	50°12'41"N, 15°1'1"E	195	stream (mud)		11 Oct. 2013. KK & JarR
C148-13	1	3.15	Czech Republic, distr. Nymburk, Veleliby, Liduška stream below Zdonín farm	50°12'13"N, 15°1'32"E	190	stream (mud)		11 Oct. 2013. KK & JarR

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. cophocarpa* Sendtn.**								
C149-13	1	3.16	Czech Republic, Nymburk, Liduška stream below railway bridge on NW edge of town	50°11'39"N, 15°1'52"E	185	stream (mud)		11 Oct. 2013. KK & JarR
***C. hamulata* Kütz. ex W.D.J. Koch**								
C009-07b	1	9.01	Czech Republic, distr. Klatovy, Sušice, Roušarka stream on S edge of town	49°13'12.9"N, 13°30'33.5"E	480	stream (sand)	*C. cophocarpa*	29 June 2007 JP
C017-07	1	8.83	Czech Republic, distr. Mělník, Hořín, pool on left bank of Vltava River	50°19'51.3"N, 14°28'35.1"E	160	exposed bottom		8 Aug. 2007 JP
C021-07	1	9.00	Czech Republic, distr. Rakovník, Řevničov, Nature Reserve Prameny Klíčavy, puddle on forest path	50°8'38.2"N, 13°49'37.2"E	420	puddle	*C. stagnalis*	16 Aug. 2007 JP
C023-07	1	8.88	Czech Republic, distr. Kladno, Lány, forest puddle near solitude Nový Dvůr	50°8'57"N, 13°55'37"E	425	puddle	*C. stagnalis*	16 Aug. 2007 JP
C036-07	1	9.05	Czech Republic, distr. České Budějovice, National Nature Reserve Brouskův mlýn, Stropnice stream	48°52'52"N, 14°40'58"E	450	stream (sand)		29 June 2007 ZK
C038-07	1	8.79	Czech Republic, distr. Tábor, Bechyňská Smoleč, puddle on forest path	49°18'35.6"N 14°33'33.7"E	490	puddle		15 July 2007 H. Chudáčková
C001-08b	1	8.99	Czech Republic, Praha, city distr. Modřany, pools "Modřanské laguny" on right bank of Vltava River	49°59'48.3"N, 14°24'11.1"E	190	pool	*C. cophocarpa*	1 May 2008 JP
C006-08b	1	8.90	Czech Republic, distr. Chrudim, Rabštejnská Lhota, small pond on Podhůra stream	49°54'28.5"N, 15°46'31.6"E	315	fishpond	*C. cophocarpa*	23 May 2008 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hamulata* Kütz. ex W.D.J. Koch**								
C011-08	2	8.87	Czech Republic, distr. Chrudim, Trhová Kamenice, settlement Petrkov, puddle on forest path	49°47'55.8"N, 15°50'29.7"E	575	puddle		24 May 2008 JP
C012-08	1	8.92	Czech Republic, distr. Chrudim, Hamry, Chrudimka River in village	49°44'22.6"N, 15°55'32.6"E	585	river (sand)		25 May 2008 JP
C013-08	1	**8.87±0.04**	Czech Republic, distr. Chrudim, Hamry, small fishpond SSE of village	49°43'52.5"N, 15°55'49.9"E	615	fishpond		25 May 2008 JP
C014-08	2	8.87	Czech Republic, distr. Chrudim, Hamry, Chrudimka River SSE of village	49°43'38.6"N, 15°56'8.5"E	605	river (sand)		25 May 2008 JP
C027-08	1	**8.85±0.06**	Czech Republic, distr. Děčín, Mezná, Kamenice stream in Divoká soutěska canyon	50°51'50.0"N, 14°19'10.5"E	170	stream (sand)		3 July 2008 JP
C029-08	1	**8.96±0.05**	Czech Republic, distr. Jindřichův Hradec, Majdalena, Lužnice River in National Nature Reserve Stará řeka	48°59'17"N, 14°50'54"E	435	river (sand)		10 July 2008 JP
C032-08	2	8.97	Czech Republic, distr. Jindřichův Hradec, Hamr, Koštěnický stream	48°57'8.3"N, 14°54'54.4"E	450	stream (sand)		10 July 2008 JP
C044-08	2	**8.88±0.03**	Czech Republic, distr. Praha-východ, Jevany, middle forest fishpond in Aldašín deer-park	49°58'6.6"N, 14°50'21.2"E	385	exposed bottom	*C. palustris*, *C. stagnalis*	19 July 2008 JP
C048-08	2	8.84	Czech Republic, distr. Cheb, Krapice, Podsedek fishpond	50°6'52"N, 12°19'26"E	440	exposed bottom		1 Aug. 2008 JP
C049-08	1	8.85	Czech Republic, distr. Cheb, Františkovy Lázně, settlement Lužná, puddle on forest path near Vražedný fishpond	50°6'19"N, 12°17'16"E	450	puddle	*C. stagnalis*	1 Aug. 2008 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. hamulata **Kütz. ex W.D.J. Koch**								
C052-08	1	8.86	Czech Republic, distr. Cheb, Ostroh, Slatinný stream	50°7'58"N, 12°16'54"E	490	stream (sand)		1 Aug. 2008 JP
C056-08	1	8.87	Czech Republic, distr. Cheb, Podílná, puddle on forest path NE of Čtvrtý fishpond	50°8'56"N, 12°13'27"E	575	puddle	*C. palustris*	2 Aug. 2008 JP
C059-08	1	8.97	Czech Republic, distr. Cheb, Skalná, Sázek stream near settlement Kateřina	50°9'34"N, 12°24'24"E	435	stream (sand)		3 Aug. 2008 JP
C060-08	1	8.81	Czech Republic, distr. Cheb, Nová Ves, pool in kaolin quarry SSW of village	50°9'42"N, 12°24'42"E	440	pool	*C. cophocarpa*	3 Aug. 2008 JP
C064-08	2	8.77	Czech Republic, distr. Sokolov, Kynšperk nad Ohří, Ohře River in camp	50°7'32.5"N, 12°31'46.3"E	415	river (sand)		3 Aug. 2008 JP
C069-08	2	8.84	Czech Republic, distr. Liberec, Černousy, Smědá stream S of railway station	51°0'6"N, 15°2'16"E	220	stream (mud)	*C. platycarpa*	19 Aug. 2008 JP
C077-08	2	8.72	Czech Republic, distr. Prachatice, Volary, settlement Soumarský Most, Vltava River in camp	48°54'28.0"N, 13°49'37.7"E	750	river (sand)		6 Sept. 2008 JP
C079-08	3	8.82	Czech Republic, distr. Prachatice, Stožec, millrace of Studená Vltava stream	48°51'31.8"N, 13°49'11.6"E	780	stream (sand)		6 Sept. 2008 JP
C085-08	1	9.02	Czech Republic, distr. Havlíčkův Brod, Hluboká, puddle on path NW of village	49°39'51"N, 15°50'50"E	560	puddle		22 Sept. 2008 JP
C088-08	1	8.97	Czech Republic, distr. Žďár nad Sázavou, Karlov, Stružný stream (Sázava) near estuary to Velké Dářko fishpond	49°38'48"N, 15°53'45"E	615	stream (sand)		22 Sept. 2008 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. hamulata **Kütz. ex W.D.J. Koch**								
C090-08	1	8.75	Czech Republic, distr. Žďár nad Sázavou, Tři Studně, on path near NE bank of Medlovský fishpond	49°36'52"N, 16°3'26"E	715	puddle		23 Sept. 2008 JP
C091-08	3	8.83	Czech Republic, distr. Žďár nad Sázavou, Milovy, Svratka stream, southernmost meander	49°40'11"N, 16°5'26"E	595	stream (sand)		23 Sept. 2008 JP
C105-08	1	8.86	Czech Republic, distr. Mladá Boleslav, Bělá pod Bezdězem, village distr. Vrchbělá, puddle on forest path	50°31'17"N, 14°46'38"E	315	puddle	*C. stagnalis*	28 Sept. 2008 J. Suda
C004-09	2	**8.83±0.08**	Czech Republic, distr. Klatovy, Rejštejn, Radešov settlement, Opolenecký stream near estuary to Otava River	49°9'11.1"N, 13°30'48.4"E	550	stream (sand)		30 Apr. 2009 JP
C016-09	1	9.01	Czech Republic, distr. Česká Lípa, Mimoň, Ploučnice River in town	50°39'32.8"N, 14°43'38.2"E	280	river (sand)		8 June 2009 JP
C018-09	2	**9.02±0.09**	Czech Republic, distr. Česká Lípa, Boreček, Ploučnice River S of settlement	50°37'51.6"N, 14°43'5.7"E	270	river (sand)		8 June 2009 JP
C020-09	1	9.16	Czech Republic, distr. Česká Lípa, Hradčany, Nature Reserve Hradčanské rybníky, marsh below overflow of Strážovský fishpond	50°36'39"N, 14°44'29"E	275	puddle	*C. cophocarpa*	8 June 2009 JP
C027-09	1	**9.04±0.07**	Czech Republic, Česká Lípa, Ploučnice River in town	50°40'58.4"N, 14°32'15.9"E	245	river (sand)		9 June 2009 JP
C046-09	1	**8.85±0.05**	Czech Republic, distr. Karviná, Dolní Marklovice, Petrůvka stream in village	49°53'31.8"N, 18°33'59.5"E	225	stream (sand)		9 Aug. 2009 JP
C058-09	3	8.84	Czech Republic, distr. Děčín, Mikulášovice, Mikulášovický stream in NW part of town	50°58'50.7"N, 14°20'36.7"E	385	stream (mud)	*C. platycarpa*	18 Aug. 2009 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hamulata* Kütz. ex W.D.J. Koch**								
C059-09	1	8.87	Czech Republic, distr. Děčín, Mikulášovice, former pond E of settlement Tomášov	50°57'24.8"N, 14°20'33.3"E	470	pool		18 Aug. 2009 JP
C060-09	1	8.89	Czech Republic, distr. Děčín, Mikulášovice, outdoor swimming pool, southern reservoir	50°57.5'N, 14°21.8'E	430	reservoir		18 Aug. 2009 JP
C061-09	1	8.89	Czech Republic, distr. Děčín, Mikulášovice, small fishpond S of railway	50°57'24.5"N, 14°22'33.4"E	455	exposed bottom		18 Aug. 2009 JP
C064-09	2	**8.88±0.08**	Czech Republic, distr. Děčín, Velký Šenov, Vilémovský stream in town	50°59'24.3"N, 14°22'36.4"E	350	stream (mud)	*C. platycarpa*	18 Aug. 2009 JP
C069-09	2	8.87	Czech Republic, distr. Děčín, Šluknov, fishpond on SE part of settlement Nové Hraběcí	51°0'40.3"N, 14°26'39.8"E	350	fishpond		19 Aug. 2009 JP
C071-09	5	8.97	Czech Republic, distr. Děčín, Šluknov, Stříbrný stream in town	51°0'19.9"N, 14°27'5.7"E	340	stream (sand)	*C. platycarpa*	19 Aug. 2009 JP
C072-09	2	8.95	Czech Republic, distr. Děčín, Šluknov, southern pond in chateau park	51°0'20.1"N, 14°27'17.6"E	340	fishpond		19 Aug. 2009 JP
C080-09	1	8.87	Czech Republic, distr. Blansko, Žďárná, on forest path NW of settlement Skelná Huť	49°28'42.4"N, 16°47'12.4"E	670	puddle		25 Aug. 2009 JP
C090-09	1	8.94	Czech Republic, distr. Blansko, Vilémovice, National Nature Reserve Vývěry Punkvy, reappearing of subterranean Punkva stream from Punkevní caves	49°22'14.0"N, 16°43'31.7"E	375	pool		28 Aug. 2009 JP
C093-09	1	**8.88±0.08**	Czech Republic, distr. České Budějovice, Staré Hutě, Lužný stream below outlet from Mlýnský fishpond	48°42'33.5"N, 14°42'45.2"E	767	exposed bottom		18 Sept. 2009 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. hamulata **Kütz. ex W.D.J. Koch**								
C094-09a	2	8.81	Czech Republic, distr. České Budějovice, Staré Hutě, blind arm of Lužný stream WSW of settlement Mlýnský Vrch	48°42'27.7"N, 14°41'56.2"E	750	pool	*C. cophocarpa*	18 Sept. 2009 JP
C099-09	3	**8.87±0.03**	Czech Republic, distr. Pardubice, Dolní Jelení, Oborecký fishpond	50°2'29.6"N, 16°7'14.4"E	280	exposed bottom	*C. palustris*	8 Oct. 2009 JP
C108-09a	2	9.01	Czech Republic, distr. Svitavy, Opatov, puddle on side of forest path S of railway station Semanín	49°50'17.2"N, 16°28'17.2"E	430	puddle	*C. cophocarpa, C. palustris, C. stagnalis*	19 Oct. 2009 JP
C109-09	1	**8.99±0.08**	Czech Republic, distr. Svitavy, Opatov, puddle on forest path NNW of pond Rebelant	49°50'2.7"N, 16°28'19.4"E	437	puddle	*C. stagnalis*	19 Oct. 2009 JP
C114-09	1	8.83	Czech Republic, distr. Svitavy, Opatov, ruts on forest path NW of pond Rebelant	49°49'53.5"N, 16°28'20.0"E	440	puddle	*C. stagnalis*	19 Oct. 2009 JP
C001-10	1	**8.83±0.08**	Czech Republic, distr. Příbram, Sychrov, ditch on edge of forest meadow NNE of village	49°46'8.2"N, 14°7'20.4"E	420	ditch		8 May 2010 JP
C007-10	1	**8.96±0.06**	Czech Republic, Praha, city distr. Modřany, pool connected to Vltava River	49°59'52.7"N, 14°24'13.0"E	185	pool		27 May 2010 JP
C012-10	1	**8.91±0.05**	Czech Republic, Most, Bílina River in city	50°30'57.0"N, 13°38'51.2"E	221	river (mud)		2 June 2010 JP
C014-10	3	**9.06±0.10**	Czech Republic, distr. Semily, Turnov, small ponds below castle Hrubý Rohozec	50°35'58.6"N, 15°9'35.6"E	246	reservoir	*C. palustris, C. platycarpa*	12 June 2010 JP
C021-10	3	**8.84±0.06**	Czech Republic, distr. Jičín, Ostroměř, millrace of Javorka stream	50°22'30.0"N, 15°32'52.3"E	265	stream (sand)	*C. cophocarpa*	26 June 2010 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. hamulata **Kütz. ex W.D.J. Koch**								
C026-10	1	8.75	Czech Republic, distr. Jičín, Lázně Bělohrad, Nature Monument Bělohradská bažantnice, pool Hraběnčino jezírko	50°25'30.4"N, 15°35'53.6"E	295	pool		26 June 2010 JP
C052-10	1	**9.02±0.02**	Czech Republic, distr. Domažlice, Mezholezy, forest pool near western bank of Mezholezský fishpond	49°37'45.6"N, 12°53'31.3"E	435	exposed bottom		5 July 2010 JP & ZK
C071-10	1	8.89	Czech Republic, Domažlice, Zubřina stream in town	49°26'18.8"N, 12°55'41.9"E	415	stream (sand)		5 July 2010 P. Koutecký
C079-10	1	8.85	Czech Republic, distr. Klatovy, Horská Kvilda, Hamerský stream WSW of village	49°3'18.7"N, 13°32'54.1"E	1024	stream (sand)		17 July 2010 JP
C082-10	1	8.93	Czech Republic, distr. Sokolov, Liboc, Libocký stream in village	50°7'18.5"N, 12°31'0.9"E	415	stream (sand)		19 July 2010 JP & ZK
C083-10	2	9.04	Czech Republic, distr. Sokolov, Chotíkov, Ohře River in village	50°6'58.0"N, 12°30'18.2"E	413	river (sand)		19 July 2010 JP & ZK
C084-10	1	8.96	Czech Republic, distr. Cheb, Mostov, Ohře River NW of village	50°7'1.2"N, 12°29'22.1"E	416	river (sand)		19 July 2010 JP & ZK
C085-10	1	8.98	Czech Republic, distr. Cheb, Mostov, Ohře River in village	50°6'54.3"N, 12°29'22.0"E	416	river (sand)		19 July 2010 JP & ZK
C086-10	1	9.02	Czech Republic, distr. Cheb, Mostov, blind arm of Ohře River	50°06'57.4"N, 12°29'16.2"E	416	pool		19 July 2010 JP & ZK
C091-10	1	8.87	Czech Republic, distr. Liberec, Hrádek nad Nisou, Lužická Nisa River in town	50°50'55.4"N, 14°50'50.3"E	245	river (sand)		28 July 2010 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. hamulata **Kütz. ex W.D.J. Koch**								
C093-10	2	8,89	Czech Republic, distr. Liberec, Jablonné v Podještědí, Panenský stream between Pivovarský and Markvartický fishponds	50°46'33.1"N, 14°46'54.1"E	310	stream (mud)	*C. platycarpa*	28 July 2010 JP
C094-10	1	8,81	Czech Republic, distr. Liberec, Jablonné v Podještědí, town distr. Zámecká, Panenský stream	50°45'44.6"N, 14°45'28.4"E	305	stream (sand)		28 July 2010 JP
C102-10	1	8,86	Czech Republic, distr. Česká Lípa, Kunratice u Cvikova, Svitávka stream in village	50°46'8.7"N, 14°40'43.4"E	320	stream (sand)		28 July 2010 JP
C104-10	3	8,97	Czech Republic, distr. Česká Lípa, Radvanec, Dobranovský stream close to estuary to Radvanecký fishpond	50°44'59.2"N, 14°35'43.7"E	294	stream (mud)	*C. platycarpa*	28 July 2010 JP
C106-10	4	8,72	Czech Republic, distr. Česká Lípa, Sloup v Čechách, Dobranovský stream in village	50°44'34.6"N, 14°35'32.2"E	290	stream (mud)	*C. platycarpa*	29 July 2010 JP
C118-10	2	8,78	Czech Republic, distr. Ústí nad Orlicí, Hnátnice, Tichá Orlice River close to railway station	50°0'7.6"N, 16°27'38.1"E	337	river (sand)		11 Aug. 2010 JP
C120-10	1	8,83	Czech Republic, Ústí nad Orlicí, Tichá Orlice River in town	49°58'30.5"N, 16°23'11.8"E	324	river (sand)		12 Aug. 2010 JP
C121-10	3	8,79	Czech Republic, distr. Ústí nad Orlicí, Sudislav nad Orlicí, Tichá Orlice River ESE of village	49°59'2.2"N, 16°20'6.6"E	315	river (sand)		12 Aug. 2010 JP
C136-10	2	8,91	Czech Republic, distr. Chrudim, Rohozná, Hubský fishpond	49°48'27.2"N, 15°49'22.0"E	575	exposed bottom	*C. cophocarpa*, *C. palustris*	27 Aug. 2010 JP & ZK
C139-10	1	**8.97±0.09**	Czech Republic, distr. Chrudim, Trhová Kamenice, Chrudimka River in village	49°47'9.2"N, 15°48'53.5"E	525	river (sand)		28 Aug. 2010 JP & ZK

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hamulata* Kütz. ex W.D.J. Koch**								
C142-10	1	8.95	Czech Republic, distr. Havlíčkův Brod, Kocourov, Návesní fishpond	49°45'9.5"N, 15°47'59.9"E	552	fishpond		28 Aug. 2010 JP & ZK
C145-10	3	**9.04±0.10**	Czech Republic, distr. Havlíčkův Brod, Stružinec, Januš fishpond	49°44'11.8"N, 15°50'43.5"E	548	exposed bottom	*C. palustris*	28 Aug. 2010 JP & ZK
C146-10	1	8.93	Czech Republic, distr. Chrudim, Vortová, Návesník fishpond	49°42'45.9"N, 15°55'45.3"E	617	fishpond		28 Aug. 2010 JP & ZK
C147-10	5	**8.89±0.03**	Czech Republic, distr. Žďár nad Sázavou, Račín, Nature Reserve Mlýnský potok Uhlířky, Losenický stream SW of village	49°36'33.7"N, 15°50'52.6"E	597	stream (sand)		29 Aug. 2010 JP & ZK
C151-10	1	9.02	Czech Republic, distr. Děčín, Šluknov, Spréva (Spree) stream on state border in former village Fukov	51°2'37"N, 14°30'15"E	300	stream (sand)	*C. platycarpa*	9 Sept. 2010 P. Bauer
C153-10	1	8.75	Czech Republic, distr. Prachatice, Borová Lada, Vltava River in village	48°59'26.5"N, 13°39'41.8"E	892	stream (sand)		25 Sept. 2010 JP
C014-11	2	8.72	Czech Republic, distr. Klatovy, Dobršín, Otava River S of village	49°15'18.5"N, 13°33'40.1"E	453	river (sand)		17 June 2011 JP & P. Koutecký
C015-11	1	8.84	Czech Republic, distr. Klatovy, Čepice, Otava River W of village	49°15'59.7"N, 13°35'17.1"E	450	river (sand)		17 June 2011 JP & P. Koutecký
C017-11	3	8.72	Czech Republic, distr. Klatovy, Čepice, blind arm on right bank of Otava River ESE of village	49°15'57.5"N, 13°36'3.7"E	445	pool	*C. ×vigens*	17 June 2011 JP & P. Koutecký

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hamulata* Kütz. ex W.D.J. Koch**								
C019-11	1	8.80	Czech Republic, distr. Klatovy, Čepice, blind arm on leftbank of Otava River ESE of village	49°15'58.2"N, 13°36'13.0"E	445	pool	*C. ×vigens*	17 June 2011 JP & P. Koutecký
C021-11	3	8.70	Czech Republic, distr. Klatovy, Velké Hydčice, blind arm of Otava River N of village	49°18'19.5"N, 13°40'9.8"E	426	pool	*C. ×vigens*	17 June 2011 JP & P. Koutecký
C026-11	1	8.83	Czech Republic, distr. Klatovy, Horažďovice, on alluvium of Otava River below weir on SE edge of town	49°18'52.9"N, 13°43'8.3"E	415	exposed bottom		18 June 2011 JP & P. Koutecký
C029-11	1	8.80	Czech Republic, distr. Strakonice, Střelské Hoštice, Otava River on S edge of village	49°17'38.8"N, 13°45'21.2"E	408	river (sand)		18 June 2011 JP & P. Koutecký
C030-11	1	**8.77±0.07**	Czech Republic, distr. Strakonice, Horní Poříčí, Otava River below weir	49°17'4.3"N, 13°46'47.7"E	405	river (sand)		18 June 2011 JP & P. Koutecký
C033-11	1	8.82	Czech Republic, distr. Strakonice, Katovice, on alluvium of Otava River below weir in village	49°16'20.7"N, 13°49'30.7"E	400	exposed bottom	*C. palustris*	18 June 2011 JP & P. Koutecký
C038-11	2	8.69	Czech Republic, Strakonice, town distr. Podskalí, blind arm on right bank of Otava River in camp	49°15'26.2"N, 13°53'19.8"E	390	pool	*C. ×vigens*	19 June 2011 JP & P. Koutecký

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. hamulata **Kütz. ex W.D.J. Koch**								
C040-11	3	8.83	Poland, prov. Małopolskie, Jabłonka, Czarna Orawa River WSW of village	49°28'07.8"N, 19°40'22.8"E	604	river (sand)		25 June 2011 JP & ZK
C045-11	8	**8.88±0.04**	Slovakia, distr. Námestovo, Rabča, Modré jazierko pool close to Polhoranka stream near S edge of village	49°27'32.7"N, 19°29'50.1"E	620	pool		24 June 2011 JP & ZK
C007-12	3	9.11	Czech Republic, distr. Mělník, Vrbno, pool on left side of Vltava River ENE of village	50°19'41.1"N, 14°28'34.5"E	160	pool		13 May 2012 JP
C014-12	1	8.89	Czech Republic, distr. Rakovník, Řevničov, puddle on forest path N of Horní Kracle fishpond	50°8'35.7"N, 13°50'30.4"E	410	puddle	*C. stagnalis*	12 June 2012 JP & A. Potůčková
C015-12	4	8.78	Czech Republic, distr. Rakovník, Třtice, littoral zone of small pond close to solitude Třtická Lísa	50°9'49.6"N, 13°50'20.4"E	450	exposed bottom	*C. cophocarpa*	12 June 2012 JP & A. Potůčková
C022-12	3	8.82	Czech Republic, distr. Mladá Boleslav, Dolení Kruhy, Jizera River NNW of village	50°33'45.6"N, 14°59'41.3"E	230	river (sand)	*C.* ×*vigens*	14 June 2012 JP & PT
C026-12	1	8.72	Czech Republic, distr. Český Krumlov, Vyšší Brod, Vltava River E of town	48°37'15.6"N, 14°19'38.1"E	550	river (sand)		19 July 2012 JP
C027-12	1	8.82	Czech Republic, distr. Český Krumlov, Hrudkov, Vltava River SE of village	48°37'36.9"N, 14°21'20.4"E	540	river (sand)		19 July 2012 JP
C029-12	1	8.90	Czech Republic, Český Krumlov, Vltava River on E edge of town	48°48'49.3"N, 14°19'47.0"E	495	river (sand)		21 July 2012 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hamulata* Kütz. ex W.D.J. Koch**								
C035-12	2	8.90	Denmark, Jutland, reg. Nordjylland, Trængstrup, Sonderupå stream near bridge of Suldrupvej road	56°51'08.5"N, 09°38'11.3"E	18	stream (sand)	*C. platycarpa*	1 Aug. 2012 JP & ZK
C039-12	1	9.27	Denmark, Jutland, reg. Midtjylland, Nees, Søndersund lake	56°23'57.0"N, 08°13'51.3"E	0	lake	*C. platycarpa*, *C. ×vigens*	1 Aug. 2012 JP & ZK
C043-12	3	8.98	Denmark, Jutland, reg. Midtjylland, Silkeborg, small pond between Lupinvej and Borgergade streets	56°10'57.8"N, 09°32'53.5"E	29	fishpond		1 Aug. 2012 JP & ZK
C045-12	1	**9.10±0.09**	Denmark, Jutland, reg. Midtjylland, Tarm, Sonderå River WNW of town	55°55'06.4"N, 08°28'58.0"E	1	river (mud)	*C. platycarpa*	3 Aug. 2012 JP & ZK
C047-12	1	9.22	Denmark, Jutland, reg. Syddanmark, Tange, Varde Å River NE of town	55°38'39.2"N, 08°32'37.7"E	4	river (mud)		3 Aug. 2012 JP & ZK
C050-12	1	9.20	Germany, Lower Saxony, Baven, Örtze River below the bridge of Zur Örtze road	52°50'41.9"N, 10°6'00.7"E	52	river (mud)	*C. platycarpa*	3 Aug. 2012 JP & ZK
C058-12	16	8.98	Czech Republic, Ústí nad Orlicí, Tichá Orlice River under railway bridge	49°58'17.0"N, 16°23'05.4"E	320	river (sand)		15 Aug. 2012 JP
C059-12	15	9.03	Czech Republic, Ústí nad Orlicí, town distr. Kerhartice, Tichá Orlice River above bridge of Sokolská street	49°58'19.6"N, 16°21'44.0"E	315	river (sand)		15 Aug. 2012 JP
C060-12	12	9.02	Czech Republic, Ústí nad Orlicí, town distr. Kerhartice, Tichá Orlice River W of bridge of Sokolská street	49°58'22.2"N, 16°21'24.6"E	315	river (sand)		15 Aug. 2012 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hamulata* Kütz. ex W.D.J. Koch**								
Co62-12	26	**8.85±0.09**	Czech Republic, distr. Ústí nad Orlicí, Brandýs nad Orlicí, Tichá Orlice River below bridge of Žerotínova street *(resampled for chromosome counting as Co94-13, 1 IX 2013 JP & KK)*	50°0'4.9"N, 16°16'45.1"E	295	river (sand)	*C. cophocarpa*, *C. hamulata* × *C. cophocarpa?*	15 Aug. 2012 JP
Co64-12	5	**8.78±0.08**	Czech Republic, distr. Ústí nad Orlicí, Choceň, Tichá Orlice River on SW edge of town	49°59'43.2"N, 16°12'36.1"E	270	river (sand)	*C. hamulata* × *C. cophocarpa?*	15 Aug. 2012 JP
Co67-12	10	**8.91±0.05**	Czech Republic, distr. Ústí nad Orlicí, Choceň, millrace of Tichá Orlice River on NW edge of town	50°0'4.5"N, 16°12'13.2"E	270	stream (sand)	*C. hamulata* × *C. cophocarpa?*	15 Aug. 2012 JP
Co69-12	1	8.94	Czech Republic, distr. Prachatice, Lenora, Teplá Vltava River below bridge in village	48°55'39.1"N, 13°47'41.1"E	765	stream (sand)		14 Aug. 2012 M. Hrdinová
Co71-12	1	8.65	Czech Republic, distr. Prachatice, Pěkná, Vltava River W of village	48°51'9.7"N, 13°55'11.4"E	730	river (sand)		15 Aug. 2012 M. Hrdinová
Co72-12	1	8.84	Czech Republic, distr. Prachatice, Ovesná, Vltava River ESE of railway station	48°48'18.5"N, 13°56'47.2"E	725	river (sand)		15 Aug. 2012 M. Hrdinová
Co73-12	4	8.83	Czech Republic, Liberec, Lužická Nisa River in city centre	50°46'01.1"N, 15°03'10.2"E	350	stream (sand)		26 Aug. 2012 JP
Co78-12	4	9.04	Czech Republic, distr. Liberec, Arnoltice, small blind arm of Bulovský stream close road bridge in village	50°57'47.4"N, 15°05'47.8"E	290	pool		11 Oct. 2012 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hamulata* Kütz. ex W.D.J. Koch**								
C079-12	3	8.85	Czech Republic, distr. Liberec, Horní Pertoltice, small pond SSE of village	50°58'30.7"N, 15°05'43.2"E	289	reservoir		11 Oct. 2012 JP
C080-12	1	8.94	Czech Republic, distr. Liberec, Horní Pertoltice, Šálkův fishpond	50°59'01.5"N, 15°06'12.5"E	319	fishpond	*C. palustris*	11 Oct. 2012 JP
C087-12	1	8.75	Czech Republic, distr. Svitavy, Jedlová, Pulec fishpond	49°39'37.5"N, 16°20'15.2"E	584	exposed bottom	*C. palustris*	16 Oct. 2012 JP
C091-12	1	8.86	Czech Republic, distr. Domažlice, Stráž, Zelenovský fishpond	49°24'36.8"N, 12°53'27.6"E	480	fishpond		29 Oct. 2013 JP
C014-13	3	**8.88±0.06**	Czech Republic, distr. Břeclav, Kostice, pools in forest Tvrdonické polesí SE of village	48°44'14.0"N, 17°00'21.9"E	150	pool	*C. cophocarpa*	4 June 2013 JP & M. Hrdinová
C022-13	1	**8.84±0.05**	Czech Republic, distr. Příbram, Mokrovraty, Dolní (Tušimský) fishpond	49°48'23.7"N, 14°13'50.6"E	380	fishpond	*C. cophocarpa*	11 June 2013 JP & KK
C025-13	1	8.86	Czech Republic, distr. Žďár nad Sázavou, Švařec, Svratka River in S part of village	49°31'03.0"N, 16°20'31.5"E	350	river (sand)		20 June 2013 JP & ZK
C028-13	2	**8.91±0.04**	Czech Republic, distr. Olomouc, Nové Zámky, old separated arm of Morava River in Nature Monument Pod Templem	49°43'14.8"N, 17°01'01.4"E	240	pool	*C. cophocarpa*	21 June 2013 JP & ZK
C033-13	1	8.74	Czech Republic, distr. Jindřichův Hradec, Lomy, fishpond in central part of village	49°6'39.6"N, 15°9'28.2"E	570	fishpond		25 June 2013 S. Píšová

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hamulata* Kütz. ex W.D.J. Koch**								
C044-13	3	8.79	Czech Republic, distr. Trutnov, Kohoutov, Rabiš fishpond	50°27'6.5"N, 15°53'14.2"E	470	fishpond	*C. palustris*	4 July 2013 JP
C047-13	5	8.88	Czech Republic, distr. Trutnov, Choustníkovo Hradiště, Hradišťský stream in village	50°25'34.6"N, 15°52'42.0"E	284	stream (sand)		4 July 2013 JP
C049-13	1	**8.86±0.01**	USA, Oregon, Multnomah County, Troutdale, Sandy River Delta Park, old separated arm of Delta River	45°33'20.3"N, 122°22'0.1"W	4	pool		25 July 2013 JP & T. Kávová
C050-13	1	**8.86±0.02**	USA, Oregon, Lane County, Eugene, canal by bridge of Seavey Loop Road in city	44°0'41.8"N, 123°0'19.1"W	139	stream (mud)		28 July 2013 JP & T. Kávová
C051-13	1	**8.85±0.07**	USA, Oregon, Lane County, Florence, Woahink Lake	43°55'41.7"N, 124°5'53.3"W	15	lake		28 July 2013 JP & T. Kávová
C052-13	2	8.97	USA, Oregon, Curry County, Langlois, Floras Lake	42°54'9.0"N, 124°30'9.3"W	4	lake		29 July 2013 JP & T. Kávová
C058-13	1	**8.73±0.05**	Czech Republic, distr. Cheb, Milhostov, Lužní stream on S edge of Nature Reserve Děvín	50°9'30.8"N, 12°25'41.5"E	440	stream (sand)		19 July 2013 KK & JarR
C059-13	1	8.76	Czech Republic, distr. Cheb, Milhostov, Lužní stream near S edge of Nature Reserve Děvín	50°9'25"N, 12°25'45"E	440	stream (sand)		19 July 2013 KK & JarR
C060-13	1	8.67	Czech Republic, distr. Cheb, Milhostov, Lužní stream WSW of village	50°9'12"N, 12°25'43"E	439	stream (sand)		19 July 2013 KK & JarR

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hamulata* Kütz. ex W.D.J. Koch**								
C061-13	1	8.67	Czech Republic, distr. Cheb, Povodí, Lužní stream on confluence with Sázek stream	50°8'57.4"N, 12°25'27.2"E	436	stream (sand)	*C. stagnalis*	19 July 2013 KK & JarR
C064-13	1	8.75	Czech Republic, distr. Cheb, Milhostov, small pond WSW of village	50°8'59.6"N, 12°25'33.1"E	438	reservoir		18 July 2013 KK & JarR
C065-13	7	**8.81±0.04**	Czech Republic, distr. Cheb, Dvorek, Stodolský stream by road bridge NE of village	50°8'19.5"N, 12°24'42.5"E	431	stream (sand)	*C. cophocarpa*, *C. stagnalis*	19 July 2013 KK & JarR
C069-13b	1	8.93	Czech Republic, distr. Domažlice, Nemanice, blind arm of Nemanický stream SW of settlement Nemaničky	49°25'48.6"N, 12°42'40.6"E	504	pool	*C. platycarpa*, *C. ×vigens*	10 Aug. 2013 M. Hrdinová
C080-13	2	**8.89±0.10**	Czech Republic, distr. Jablonec nad Nisou, Bedřichov, Blatný rybník reservoir	50°48'36.9"N, 15°9'53.8"E	770	reservoir	*C. cophocarpa*	22 Aug. 2013 JP & KK
C084-13	1	8.91	Czech Republic, distr. Klatovy, Horská Kvilda, Hamerský stream SW of village	49°3'15.6"N, 13°33'17.7"E	1030	stream (sand)		25 Aug. 2013 JP & KK
C086-13	1	8.95	Czech Republic, distr. Cheb, Prameny, Dlouhá stoka canal NE of village	50°03'43.2"N, 12°44'43.7"E	780	stream (sand)		21 Aug. 2013 J. Chrtek jr.
C090-13	19	**8.80±0.04**	Czech Republic, distr. Ústí nad Orlicí, Orlické Podhůří, Tichá Orlice River by railway bridge near settlement Luh	49°59'34.0"N, 16°20'9.5"E	315	river (sand)		1 Sept. 2013 JP & KK
C091-13	28	**8.82±0.04**	Czech Republic, distr. Ústí nad Orlicí, Orlické Podhůří, Tichá Orlice River under bridge in settlement Bezpráví	49°59'55.6"N, 16°20'1.2"E	315	river (sand)		1 Sept. 2013 JP & KK

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hamulata* Kütz. ex W.D.J. Koch**								
C096-13	1	8,85	Czech Republic, distr. Ústí nad Orlicí, Choceň, town distr. Peliny, confluence of outlet of separated river arm to Tichá Orlice River	49°59'55.7"N, 16°14'17.4"E	295	river (mud)	*C. cophocarpa*	1 Sept. 2013 JP & KK
C097-13	1	8,98	Czech Republic, distr. Cheb, Jindřichov, Ohře River near village	50°6'24"N, 12°23'47"E	435	river (sand)		24 Aug. 2013 JanR & JarR
C098-13	1	8,95	Czech Republic, distr. Cheb, Tršnice, Ohře River near village	50°6'50"N, 12°24'6"E	435	river (sand)		24 Aug. 2013 JanR & JarR
C099-13	1	8,90	Czech Republic, distr. Cheb, Vokov, Ohře River ESE of settlement Třídvoří	50°6'54"N, 12°26'39"E	425	river (sand)		24 Aug. 2013 JanR & JarR
C100-13	1	8,84	Czech Republic, distr. Cheb, Nebanice, Ohře River near village	50°6'41"N, 12°28'12"E	420	river (sand)		24 Aug. 2013 JanR & JarR
C101-13	1	8,68	Czech Republic, distr. Cheb, Dobroše, Ohře River near village	50°6'44"N, 12°31'3"E	415	river (sand)		24 Aug. 2013 JanR & JarR
C102-13	1	8,88	Czech Republic, distr. Sokolov, Liboc, Ohře River near village	50°7'13"N, 12°31'11"E	415	river (sand)		24 Aug. 2013 JanR & JarR
C103-13	1	8,71	Czech Republic, distr. Cheb, Dobroše, pool on right bank of Ohře River N of village	50°6'43"N, 12°30'55"E	415	pool		24 Aug. 2013 JanR & JarR

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. hamulata **Kütz. ex W.D.J. Koch**								
C104-13	1	8.89	Czech Republic, distr. Sokolov, Dasnice, Ohře River in village	50°8'46"N, 12°33'56.0"E	410	river (sand)		25 Aug. 2013 JanR & JarR
C105-13	1	8.79	Czech Republic, distr. Sokolov, Šabina, Ohře River in village	50°8'5"N, 12°34'45"E	410	river (sand)		25 Aug. 2013 JanR & JarR
C106-13	1	8.88	Czech Republic, Sokolov, Ohře River in town	50°11'0"N, 12°38'31"E	400	river (sand)		25 Aug. 2013 JanR & JarR
C107-13	1	8.75	Czech Republic, distr. Karlovy Vary, Dalovice, Ohře River near village	50°14'35"N, 12°53'36"E	370	river (sand)		29 Aug. 2013 JanR & JarR
C109-13	1	8.97	Czech Republic, distr. Karlovy Vary, Sedlečko, blind arm of Ohře River NNE of settlement Hubertus	50°14'27"N, 12°55'49"E	365	pool	*C. ×vigens*	29 Aug. 2013 JanR & JarR
C111-13	1	8.82	Czech Republic, distr. Ústí nad Labem, Ostrov, fishpond close to hotel Ostrov	50°48'15"N, 14°2'49"E	450	fishpond		28 Aug. 2013 JanR & JarR
C112-13	1	8.76	Czech Republic, distr. Ústí nad Labem, Ostrov, fishpond above hotel Ostrov	50°48'13"N, 14°2'51"E	450	fishpond		28 Aug. 2013 JanR & JarR
C113-13	1	8.92	Czech Republic, distr. Ústí nad Labem, Petrovice, fishpond on Olšový stream E of village	50°48'5"N, 13°59'32"E	475	fishpond		27 Aug. 2013 JanR & JarR
C117-13	1	**8.92±0.01**	Czech Republic, distr. Ústí nad Labem, Ostrov, Ostrovský fishpond	50°48'20"N, 14°2'47"E	445	fishpond		28 Aug. 2013 JanR & JarR

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hamulata* Kütz. ex W.D.J. Koch**								
C118-13	1	8,82	Czech Republic, distr. Děčín, Maxičky, Rybníček fishpond	50°48'29"N, 14°10'55"E	435	fishpond		26 Aug. 2013 JanR & JarR
C120-13	1	8,78	Czech Republic, distr. Ústí nad Labem, Libouchec, upper fishpond in Nature Reserve Libouchecké rybníčky	50°45'59"N, 14°3'34"E	330	fishpond		27 Aug. 2013 JanR & JarR
C129-13	5	8,88	Czech Republic, distr. Jihlava, Řásná, Velký Pařezitý fishpond	49°13'41.4"N, 15°22'44.5"E	675	fishpond	*C. cophocarpa*	8 Sept. 2013 JP
C132-13b	1	8,81	Germany, Bayern, Hohenfels, Follerenbach stream between Hohenfels and Kalmünz villages	49°11'7.2"N, 11°54'45.7"E	364	stream (sand)	*C. ×vigens*	7 Sept. 2013 KK
C133-13	2	8,92	Czech Republic, distr. Praha-východ, Kostelec nad Černými Lesy, puddle on forest path SSW of town	49°58'13.6"N, 14°50'47.7"E	390	puddle	*C. stagnalis*	9 Sept. 2013 JP
C144-13	1	8,81	Czech Republic, distr. Olomouc, Domašov nad Bystřicí, Bystřice stream in village	49°44'30"N, 17°26'47.0"E	495	stream (sand)		25 Sept. 2013 JarR
C146-13	1	8,90	Czech Republic, distr. Trutnov, Arnultovice, on forest path ENE of village	50°33'24"N, 15°44'23"E	465	puddle		25 Sept. 2013 JanR
***C. hermaphroditica* L.**								
C097-09	1	**1.92±0.02**	Czech Republic, Institute of Botany ASCR in Třeboň, cultivated (probably originates from Eastern Bohemia)					23 Sept. 2009 JP
C088-12	2	**1.94±0.01**	Czech Republic, distr. Svitavy, Jedlová, Ráček I pond	49°40'7.0"N, 16°19'59.5"E	595	reservoir		11 Oct. 2012 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. hermaphroditica* L.**								
C089-12	3	**1.94±0.02**	Czech Republic, distr. Svitavy, Jedlová, Ráček III pond	49°40'7.3"N, 16°19'53.7"E	595	reservoir		11 Oct. 2012 JP
C090-12	1	**1.93±0.01**	Czech Republic, distr. Svitavy, Jedlová, Ráček II pond	49°40'14.3"N, 16°19'52.5"E	595	reservoir		11 Oct. 2012 JP
C127-13	1	**2.00±0.02**	Sweden, prov. Östergötland, Tranås, Lake Sommen, part Torpasjön, Stora Bianäset	57°58'49.4"N, 15°6'55.0"E	153	lake		25 Aug. 2013 A. Svenson
***C. lenisulca* Clavaud**								
C004-13	1	**3.59±0.02**	Italy, prov. Siena, Chiusi, ditch near road Strada Provinciale 300, N of town	43°2'51.0"N, 11°56'8.4"E	250	ditch		23 May 2013 PT & B. Kubátová
C005-13	1	**3.58±0.04**	Italy, prov. Ravenna, Ravenna, Nature Reserve Bardello, pool E of Strade Statale Romea road	44°32'26.2"N, 12°14'19.3"E	-1	pool		24 May 2013 PT & B. Kubátová
C006-13	1	**3.61±0.01**	Italy, prov. Ravenna, Ravenna, ditch in fields near road Via Bevano, S of city	44°16'37.2"N, 12°14'29.5"E	1	ditch		23 May 2013 PT & B. Kubátová
***C. obtusangula* Le Gall**								
C034-12	5	**3.87±0.02**	Denmark, Jutland, reg. Syddanmark, Varming, drainage ditch near Ribeå River E of town	55°19'17.5"N, 08°50'09.9"E	1	ditch		31 July 2012 JP & ZK
C048-12	1	**3.88±0.02**	Denmark, Jutland, reg. Syddanmark, Højer, Højer Havn, system of drainage ditches SSW of village	54°56'16.2"N, 08°41'12.1"E	0	ditch		3 Aug. 2012 JP & ZK

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. obtusangula* Le Gall**								
C049-12	20	**3.85±0.02**	Germany, Lower Saxony, Garstedt, Luhe River below bridge of Vierhöfener Straße	53°16'47.1"N, 10°10'59.7"E	16	river (sand)		3 Aug. 2012 JP & ZK
C052-12	1	**3.85±0.04**	Netherlands, prov. Noord-Brabant, Breda, Turfvaart canal on SW edge of town	51°33'50.1"N, 4°44'3.3"E	30	stream (mud)		1 Aug. 2012 PT & B. Kubátová
C053-12	1	**3.88±0.02**	Belgium, Flemish Region, Retie, canal below bridge of Postelsebaan street	51°16'38.4"N, 5°9'2.7"E	30	stream (mud)		31 July 2012 PT & B. Kubátová
C007-13	1	**3.76±0.00**	Italy, prov. Rieti, Colli sul Velino, race of Fiume Velino River near Hotel Relais Villa D'Assio	42°29'30.0"N, 12°48'7.1"E	379	stream (sand)		23 May 2013 PT & B. Kubátová
C008-13	1	**3.71±0.01**	Italy, prov. Salerno, Sala Consilina, ditch near Strada Provinciale 11i Road	40°22'5.2"N, 15°35'58.6"E	453	ditch		21 May 2013 PT & B. Kubátová
C009-13	1	**3.73±0.02**	Italy, prov. Salerno, Sala Consilina, stream near Strada Provinciale 11i road	40°22'1.9"N, 15°35'53.3"E	453	stream (sand)		21 May 2013 PT & B. Kubátová
***C. palustris* L.**								
C006-07	1	3.87	Czech Republic, distr. Jindřichův Hradec, Lomnice nad Lužnicí, Velký Lomnický fishpond	49°5'33"N, 14°42'35"E	415	exposed bottom		27 June 2007 JP & ZK
C018-13	1	3.91	Czech Republic, distr. Rakovník, Lužná, fishpond near solitude Krásná dolina	50°7'26.7"N, 13°47'48.3"E	370	fishpond		15 Aug. 2007 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. palustris* L.**								
C037-07	1	3.91	Czech Republic, distr. Klatovy, Hartmanice, ruts on path S of solitude Dobrá Voda	49°8'43.7"N, 13°26'8.1"E	940	puddle		1 July 2007 JP
C008-08	1	**3.86±0.05**	Czech Republic, distr. Chrudim, Nasavrky, Horní fishpond	49°50'23.5"N, 15°47'57.6"E	520	fishpond		23 May 2008 JP
C017-08a	1	3.93	Czech Republic, distr. Břeclav, ruts on forest path NE of chateau Pohansko	48°44'12"N, 16°54'20"E	155	puddle	*C. cophocarpa*	11 June 2008 JP
C022-08	2	**3.88±0.03**	Czech Republic, distr. Břeclav, Lanžhot, puddles on forest path NE of town	48°43'56"N, 16°59'30"E	145	puddle		12 June 2008 JP
C028-08	1	3.91	Czech Republic, distr. Jindřichův Hradec, Třeboň, alder carr by Kaprový fishpond	48°59'28"N, 14°46'59"E	435	puddle		10 July 2008 JP
C035-08	1	3.93	Czech Republic, distr. Jindřichův Hradec, Žíteč, Černá Cepáků fishpond	49°0'39"N, 14°53'30"E	445	fishpond		11 July 2008 JP
C036-08	2	**3.90±0.03**	Czech Republic, distr. Jindřichův Hradec, Lužnice, Namšal fishpond	49°3'26.5"N, 14°45'20.8"E	425	exposed bottom		12 July 2008 JP
C038-08	3	3.91	Czech Republic, distr. Jindřichův Hradec, Lomnice nad Lužnicí, Malý Lomnický fishpond	49°5'35"N, 14°43'1"E	425	fishpond		12 July 2008 JP
C042-08	1	3.93	Czech Republic, distr. Praha-východ, Jevany, ruts on side of path E of village	49°58'19"N, 14°51'5"E	370	puddle	*C. stagnalis*	19 July 2008 JP
C045-08	1	3.88	Czech Republic, distr. Praha-východ, Jevany, middle forest fishpond in Aldašín deer-park	49°58'6.6"N, 14°50'21.2"E	385	exposed bottom	*C. hamulata*, *C. stagnalis*	19 July 2008 JP
C054-08	1	3.86	Czech Republic, distr. Cheb, Podílná, puddle on forest path WNW of Čtvrtý fishpond	50°8'52"N, 12°13'7"E	550	puddle		2 Aug. 2008 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. palustris* L.**								
C055-08	1	3.85	Czech Republic, distr. Cheb, Podílná, puddle on forest path NE of Čtvrtý fishpond	50°8'56"N, 12°13'27"E	575	puddle	*C. hamulata*	2 Aug. 2008 JP
C073-08	1	3.83	Czech Republic, distr. Prachatice, Volary, forest path SE of town	48°53'43"N, 13°52'47"E	755	puddle		6 Sept. 2008 JP
C075-08	1	3.92	Czech Republic, distr. Prachatice, Volary, lower fishpond on Jedlový stream in Stögrova Huť settlement	48°54'52.4"N, 13°51'19.1"E	795	fishpond	*C. cophocarpa*	6 Sept. 2008 JP
C082-08	1	3.89	Czech Republic, distr. Prachatice, Stožec, puddle on forest path near rock formation Gabrielstein	48°49'49"N, 13°51'3"E	895	puddle		7 Sept. 2008 JP
C098-08	1	3.95	Czech Republic, distr. Praha-východ, Kytín, pudle on crossroads of forest paths WSW of village	49°50'48.0"N, 14°11'51.0"E	470	puddle	*C. stagnalis*	6 Nov. 2008 JP
C009-09	1	3.87	Czech Republic, distr. České Budějovice, Koloděje nad Lužnicí, fishpond by road on S edge of village	49°14'50"N, 14°25'13"E	375	exposed bottom		16 May 2009 JP
C014-09	1	**3.93±0.03**	Czech Republic, distr. Praha-východ, Podolanka, biggest fishpond on SE edge of village	50°9'18.1"N, 14°36'3.7"E	220	exposed bottom		24 May 2009 JP
C021-09	1	3.83	Czech Republic, distr. Česká Lípa, Hradčany, Nature Reserve Hradčanské rybníky, puddle on forest path near Strážovský fishpond	50°36'31"N, 14°44'30"E	275	puddle		8 June 2009 JP
C025-09	1	3.93	Czech Republic, distr. Česká Lípa, Rybnov, alluvium of Bobří stream between Jílovka and Velká Komora fishponds	50°37'21"N, 14°30'40"E	260	exposed bottom		9 June 2009 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. palustris* L.**								
C029-09	1	**3.93±0.02**	Czech Republic, distr. Litoměřice, Děkovka, puddle on forest path NE of hill Hrádek	50°29'29.1"N, 13°55'10.6"E	495	puddle		17 June 2009 JP
C037-09	1	3.90	Czech Republic, distr. Karlovy Vary, Přestání, on intersection of forest paths W of village	50°4'25.1"N, 13°4'18.7"E	610	puddle	*C. stagnalis*	6 Aug. 2009 JP
C042-09	1	3.92	Czech Republic, distr. Karlovy Vary, Toužim, Velká Studna fishpond	50°3'28.1"N, 12°59'58.0"E	615	fishpond		6 Aug. 2009 JP
C043-09	2	3.93	Czech Republic, distr. Karviná, Dolní Marklovice, small fishpond close to state border	49°53'7.3"N, 18°34'4.6"E	227	fishpond	*C. cophocarpa*	9 Aug. 2009 JP
C048-09	1	**3.91±0.03**	Czech Republic, distr. Karviná, Prstná, on intersection of paths N of settlement Kempy	49°54'38.5"N, 18°34'9.4"E	270	puddle		9 Aug. 2009 JP
C074-09	1	3.96	Czech Republic, distr. Prostějov, Šubířov, puddle on forest path E of village	49°36'1.0"N, 16°49'53.1"E	601	puddle		24 Aug. 2009 JP
C079-09	1	3.92	Czech Republic, distr. Blansko, Benešov, puddle on intersection of forest paths WNW of hill Skalky	49°30'8.5"N, 16°47'2.3"E	710	puddle	*C. cophocarpa*	25 Aug. 2009 JP
C082-09	1	3.92	Czech Republic, distr. Blansko, Žďárná, ruts on forest path SE of village	49°26'58.3"N, 16°47'10.2"E	575	puddle	*C. cophocarpa*, *C. stagnalis*	26 Aug. 2009 JP
C100-09	2	**3.87±0.04**	Czech Republic, distr. Pardubice, Dolní Jelení, Oborecký fishpond	50°2'29.6"N, 16°7'14.4"E	280	exposed bottom	*C. hamulata*	8 Oct. 2009 JP
C105-09	1	**3.89±0.04**	Czech Republic, distr. Svitavy, Opatov, marsh below dam of Nový fishpond	49°50'28.7"N, 16°29'24.2"E	423	exposed bottom		19 Oct. 2009 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. palustris* L.**								
C106-09	1	3.92	Czech Republic, distr. Svitavy, Opatov, puddle on side of forest path S of railway station Semanín	49°50'17.2"N, 16°28'17.2"E	430	puddle	*C. cophocarpa, C. hamulata, C. stagnalis*	19 Oct. 2009 JP
C002-10	1	**3.87±0.04**	Czech Republic, distr. Příbram, Obořiště, Přívaží fishpond	49°45'14.2"N, 14°8'48.5"E	398	exposed bottom		8 May 2010 JP
C009-10	1	**3.87±0.03**	Czech Republic, distr. Teplice, Duchcov, fishpond Hájská I	50°36'51.4"N, 13°43'32.2"E	230	exposed bottom		1 June 2010 JP
C015-10	1	**3.90±0.02**	Czech Republic, distr. Semily, Turnov, small ponds below castle Hrubý Rohozec	50°35'58.6"N, 15°9'35.6"E	246	reservoir	*C. hamulata, C. platycarpa*	12 June 2010 JP
C025-10	1	3.86	Czech Republic, distr. Jičín, Lukavec u Hořic, puddle on forest path E of Zákopský fishpond	50°24'40.6"N, 15°37'8.8"E	336	puddle	*C. stagnalis*	26 June 2010 JP
C027-10	1	3.88	Czech Republic, distr. Zlín, Zboženska, puddle on forest path NNW of village	49°14'33.4"N, 17°36'48.4"E	265	puddle		30 June 2010 JP
C029-10	1	3.92	Czech Republic, Zlín, city distr. Mladcová, puddle on forest path S of Zadní vrch hill	49°15'30.2"N, 17°38'9.7"E	360	puddle		30 June 2010 JP
C030-10	1	**3.85±0.05**	Czech Republic, Zlín, city distr. Zlínské Paseky, puddle on forest path NW of settlement Mezihoří	49°15'17.6"N, 17°39'29.5"E	343	puddle		30 June 2010 JP
C031-10	1	3.94	Czech Republic, Zlín, city distr. Zlínské Paseky, puddle on side of forest road N of settlement Kocanda	49°15'9.5"N, 17°40'22.4"E	330	puddle		30 June 2010 JP
C033-10	1	3.91	Czech Republic, distr. Zlín, Lukov, Nature Monument Bezedník, Bezedník fishpond	49°17'59.1"N, 17°43'26.4"E	320	fishpond	*C. cophocarpa*	1 July 2010 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. palustris* L.**								
C038-10	1	3.96	Czech Republic, distr. Zlín, Vlčková, puddle on forest path ENE of hill Bzová	49°19'16.5"N, 17°44'27.1"E	563	puddle	*C. stagnalis*	1 July 2010 JP
C041-10	1	**3.96±0.02**	Czech Republic, distr. Zlín, Vlčková, puddle on intersection of forest paths NNE of hill Ondřejovsko	49°19'39.3"N, 17°43'43.7"E	560	puddle	*C. stagnalis*	1 July 2010 JP
C058-10	1	3.96	Czech Republic, distr. Domažlice, Novosedly, ruts on forest path SSW of castle ruins Starý Herštejn	49°27'29.5"N, 12°42'28.7"E	560	puddle	*C. stagnalis*	6 July 2010 JP & P. Koutecký
C065-10	1	3.91	Czech Republic, distr. Domažlice, Pila, Hadrovec fishpond	49°24'48.4"N, 12°52'5.5"E	465	fishpond		9 July 2010 JP & M. Štech
C098-10	1	3.91	Czech Republic, distr. Česká Lípa, Velký Valtinov, puddle on path ESE of Zaječí fishpond	50°45'7.6"N, 14°43'30.6"E	325	puddle	*C. platycarpa*, *C. stagnalis*	28 July 2010 JP
C101-10	1	3.89	Czech Republic, distr. Česká Lípa, Kunratice u Cvikova, ruts on forest path SW of Kamenáč hill	50°45'27.0"N, 14°42'27.3"E	333	puddle	*C. platycarpa*, *C. stagnalis*	28 July 2010 JP
C131-10	1	3.90	Czech Republic, distr. Znojmo, Olbramkostel, puddles along forest path SE of castle ruins Šimperk	48°55'33.2"N, 15°55'17.2"E	387	puddle	*C. stagnalis*	16 Aug. 2010 JP
C138-10	1	3.88	Czech Republic, distr. Chrudim, Rohozná, Hubský fishpond	49°48'27.2"N, 15°49'22.0"E	575	exposed bottom	*C. cophocarpa*, *C. hamulata*	27 Aug. 2010 JP & ZK
C140-10	1	3.93	Czech Republic, distr. Havlíčkův Brod, Kocourov, puddles on dam of Návesní fishpond	49°45'13.7"N, 15°48'1.4"E	553	puddle	*C. stagnalis*	28 Aug. 2010 JP & ZK

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. palustris* L.**								
C144-10	2	3.88	Czech Republic, distr. Havlíčkův Brod, Stružinec, Januš fishpond	49°44'11.8"N, 15°50'43.5"E	548	exposed bottom	*C. hamulata*	28 Aug. 2010 JP & ZK
C158-10	1	**3.95±0.04**	Czech Republic, distr. Tábor, Hamr, Smíchov II fishpond	49°9'36.7"N, 14°46'41.7"E	420	exposed bottom	*C. cophocarpa*	20 Sept. 2010 S. Píšová
C006-11	2	3.91	Czech Republic, distr. Nymburk, Loučeň, puddle on forest path E of spring Dobrá Voda	50°18'02.7"N, 15°02'07.3"E	264	puddle	*C. stagnalis*	30 May 2011 JP
C024-11	1	**3.87±0.03**	Czech Republic, distr. Klatovy, Horažďovice, on alluvium in blind arm of Otava River on SW edge of town	49°19'16.1"N, 13°41'29.0"E	420	exposed bottom		17 June 2011 JP & P. Koutecký
C034-11	1	3.85	Czech Republic, distr. Strakonice, Katovice, on alluvium of Otava River below weir in village	49°16'20.7"N, 13°49'30.7"E	400	exposed bottom	*C. hamulata*	18 June 2011 JP & P. Koutecký
C042-11	2	**3.90±0.03**	Slovakia, distr. Námestovo, Zubrohlava, exposed bottom on bank of Orava reservoir	49°25'06.4"N, 19°30'50.9"E	595	exposed bottom		24 June 2011 JP & ZK
C044-11	2	3.90	Slovakia, distr. Námestovo, Bobrov, exposed bottom on bank of Orava reservoir	49°24'48.2"N, 19°31'47.4"E	595	exposed bottom		24 June 2011 JP & ZK
C046-11	1	3.88	Slovakia, distr. Námestovo, Mútňanská Píla, meadow ditch near NW edge of village	49°28'06.8"N, 19°17'28.3"E	785	exposed bottom		25 June 2011 JP & ZK
C060-11	1	3.88	Czech Republic, distr. Třebíč, Číměř, on bank of water reservoir Dalešice ENE of village	49°12'03.5"N, 16°00'27.0"E	385	exposed bottom		7 July 2011 P. Koutecký

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. palustris* L.**								
C019-12	1	3.91	Czech Republic, distr. Mladá Boleslav, Březina, Oběšenec fishpond	50°32'20.2"N, 15°3'36.4"E	238	fishpond		14 June 2012 JP & PT
C023-12	1	3.85	Slovakia, distr. Zlaté Moravce, Mankovce, Mankovce reservoir	48°25'11.0"N, 18°19'55.0"E	230	reservoir		4 July 2012 JP
C024-12	2	3.93	Slovakia, distr. Zlaté Moravce, Jelenec, on N bank of Jelenec fishpond	48°24'4.1"N, 18°12'13.6"E	234	exposed bottom	*C. cophocarpa*	4 July 2012 JP
C081-12	1	**3.85±0.01**	Czech Republic, distr. Liberec, Horní Pertoltice, Šálkův fishpond	50°59'01.5"N, 15°06'12.5"E	319	fishpond	*C. hamulata*	11 Oct. 2012 JP
C082-12	1	3.85	Czech Republic, distr. Liberec, Dolní Pertoltice, puddle of path on forest edge NNW of village	50°59'07.4"N, 15°03'49.5"E	290	puddle		11 Oct. 2012 JP
C086-12	1	3.93	Czech Republic, distr. Svitavy, Jedlová, Pulec fishpond	49°39'37.5"N, 16°20'15.2"E	584	exposed bottom	*C. hamulata*	16 Oct. 2012 JP
C098-12	1	3.91	Czech Republic, distr. Svitavy, Jedlová, small pool in Zlatý stream in village near Dubovec fishpond	49°39'21.4"N, 16°19'49.6"E	580	pool		16 Oct. 2012 JP
C021-13	1	3.89	Czech Republic, distr. Příbram, Mokrovraty, puddle on meadow path NE of hill Dolní Jezberná	49°48'43.8"N, 14°14'44.7"E	335	puddle	*C. stagnalis*	11 June 2013 JP & KK
C032-13	1	3.85	Czech Republic, distr. Jindřichův Hradec, Lomy, fishpond in W part of village	49°06'39.1"N, 15°09'24.5"E	570	exposed bottom		25 June 2013 S. Píšová
C034-13	1	3.81	Czech Republic, distr. Jindřichův Hradec, Nová Olešná, puddles on meadow near NE bank of Zadní Obecní fishpond	49°09'49.7"N, 15°09'37.6"E	554	puddle		27 June 2013 ZK

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. palustris* L.**								
C043-13	1	3.80	Czech Republic, distr. Trutnov, Kocbeře, puddle on forest path between village and Rabiš fishpond	50°27'09.2"N, 15°52'42.4"E	470	puddle	*C. stagnalis*	4 July 2013 JP
C045-13	2	3.85	Czech Republic, distr. Trutnov, Kohoutov, Rabiš fishpond	50°27'6.5"N, 15°53'14.2"E	470	fishpond	*C. hamulata*	4 July 2013 JP
C048-13	1	**3.96±0.00**	USA, Washington, Snohomish County, Cathcart, lake S of Shadow Lake near Shohomish River	47°51'33.8"N, 122°5'30.3"W	6	lake		15 July 2013 JP & T. Kávová
C073-13	1	**3.94±0.04**	Norway, prov. Sør-Trøndelag, Hjerkinn/Kongsvoll/Drivdalen Protection Area, path near Gåvålivatnet lake	62°16'26.3"N, 9°37'29.1"E	941	puddle		4 Aug. 2013 KK
C140-13	2	3.82	Czech Republic, distr. Frýdek-Místek, Horní Lomná, puddle on forest path near observation tower Kozubová	49°34'13.1"N, 18°40'24.7"E	975	puddle	*C. cophocarpa*	16 Sept. 2013 JP & KK
C150-13	1	3.93	Sweden, prov. Lappland, Karesuando, Munionjoki River on N edge of village	68°26'33"N, 22°28'54"E	320	river (mud)		6 Aug. 2013 ZK
***C. platycarpa* Kütz.**								
C065-08	5	**6.18±0.02**	Czech Republic, distr. Liberec, Kunratice, small retention reservoir E of village	50°55'31.4"N, 15°0'44.8"E	300	reservoir		19 Aug. 2008 JP
C067-08	3	**6.16±0.04**	Czech Republic, distr. Liberec, Višňová, Višňovský stream in village	50°57'59.3"N, 15°1'32.3"E	235	stream (mud)		19 Aug. 2008 JP
C068-08	1	**6.15±0.05**	Czech Republic, distr. Liberec, Černousy, Smědá stream S of railway station	51°0'6"N, 15°2'16"E	220	stream (mud)	*C. hamulata*	19 Aug. 2008 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. platycarpa* Kütz.**								
C001-09	1	**6.17±0.05**	Czech Republic, distr. Louny, Stradonice, side arm of Debeřský stream	50°22'21"N, 13°57'48"E	200	stream (mud)		25 Apr. 2009 JP
C023-09	2	**6.21±0.06**	Czech Republic, distr. Česká Lípa, Břehyně, National Nature Reserve Břehyně-Pecopala, ruts on path E of Břehyňský fishpond	50°34'43"N, 14°43'42"E	275	puddle	*C. cophocarpa*	8 June 2009 JP
C057-09	2	**6.19±0.02**	Czech Republic, distr. Děčín, Mikulášovice, Mikulášovický stream in NW part of town	50°58'50.7"N, 14°20'36.7"E	385	stream (mud)	*C. hamulata*	18 Aug. 2009 JP
C062-09	3	**6.26±0.06**	Czech Republic, distr. Děčín, Mikulášovice, ditch on E part of town	50°57'36.7"N, 14°23'28.0"E	455	ditch		18 Aug. 2009 JP
C063-09	2	**6.18±0.02**	Czech Republic, distr. Děčín, Velký Šenov, settlement Leopoldka, ruts on forest patk SSE of Černý fishpond	50°58'43.7"N, 14°23'33.4"E	395	puddle		18 Aug. 2009 JP
C065-09	2	**6.22±0.07**	Czech Republic, distr. Děčín, Velký Šenov, Vilémovský stream in town	50°59'24.3"N, 14°22'36.4"E	350	stream (mud)	*C. hamulata*	18 Aug. 2009 JP
C066-09	1	**6.22±0.05**	Czech Republic, distr. Děčín, Velký Šenov, slough beside path NNE of Partyzánský vrch hill	51°0'29.3"N, 14°24'50.2"E	385	puddle		19 Aug. 2009 JP
C070-09	5	**6.18±0.02**	Czech Republic, distr. Děčín, Šluknov, Stříbrný stream in town	51°0'19.9"N, 14°27'5.7"E	340	stream (sand)	*C. hamulata*	19 Aug. 2009 JP
C008-10	2	6.11	Czech Republic, distr. Teplice, Duchcov, outfall from storage ponds to fishpond Hranáč	50°36'43.0"N, 13°43'57.0"E	226	ditch		1 June 2010 JP
C010-10	2	**6.15±0.05**	Czech Republic, distr. Teplice, Háj u Duchcova, Hájský stream near Salát fishpond	50°37'5.2"N, 13°43'14.2"E	234	stream (mud)		1 June 2010 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. platycarpa* Kütz.**								
C013-10	4	**6.16±0.06**	Czech Republic, distr. Semily, Turnov, small ponds below castle Hrubý Rohozec	50°35'58.6"N, 15°9'35.6"E	246	reservoir	*C. hamulata*, *C. palustris*	12 June 2010 JP
C018-10	3	6.18	Czech Republic, distr. Semily, Mašov, fishpond in village	50°34'19.6"N, 15°8'43.0"E	257	fishpond		12 June 2010 JP
C057-10	2	6.09	Czech Republic, distr. Domažlice, Novosedly, rivulet in alder carr NNE of village	49°27'25.1"N, 12°41'56.2"E	533	stream (mud)		6 July 2010 JP & P. Koutecký
C062-10	3	6.12	Czech Republic, distr. Domažlice, Babylon, millrace of Teplá Bystřice stream close to railway station	49°23'54.4"N, 12°52'9.2"E	483	stream (mud)		9 July 2010 JP & M. Štech
C063-10	12	**6.20±0.03**	Czech Republic, distr. Domažlice, Babylon, Babylon fishpond	49°23'58.4"N, 12°51'53.2"E	465	fishpond		9 July 2010 JP & M. Štech
C074-10	2	6.14	Czech Republic, distr. Domažlice, Novosedly, Nemanický stream NW of village	49°26'46.7"N, 12°42'10.1"E	510	stream (sand)		7 July 2010 ZK
C087-10	2	6.23	Czech Republic, distr. Cheb, Odrava, ditch near Ohře River NW of village	50°6'27.1"N, 12°28'29.6"E	420	ditch		19 July 2010 JP & ZK
C092-10	4	6.15	Czech Republic, distr. Liberec, Jablonné v Podještědí, Panenský stream between Pivovarský and Markvartický fishponds	50°46'33.1"N, 14°46'54.1"E	310	stream (mud)	*C. hamulata*	28 July 2010 JP
C097-10	1	**6.27±0.02**	Czech Republic, distr. Česká Lípa, Velký Valtinov, puddle on path ESE of Zaječí fishpond	50°45'7.6"N, 14°43'30.6"E	325	puddle	*C. palustris*, *C. stagnalis*	28 July 2010 JP
C100-10	1	6.18	Czech Republic, distr. Česká Lípa, Kunratice u Cvikova, ruts on forest path SW of Kamenáč hill	50°45'27.0"N, 14°42'27.3"E	333	puddle	*C. palustris*, *C. stagnalis*	28 July 2010 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. platycarpa* Kütz.**								
C103-10	3	6.17	Czech Republic, distr. Česká Lípa, Radvanec, Dobranovský stream close to estuary to Radvanecký fishpond	50°44'59.2"N, 14°35'43.7"E	294	stream (mud)	*C. hamulata*	28 July 2010 JP
C105-10	3	6.19	Czech Republic, distr. Česká Lípa, Sloup v Čechách, Dobranovský stream in village	50°44'34.6"N, 14°35'32.2"E	290	stream (mud)	*C. hamulata*	29 July 2010 JP
C108-10	2	6.14	Czech Republic, distr. Česká Lípa, Horní Kamenice, small reservoir on little stream N of village	50°48'31.3"N, 14°26'43.3"E	355	pool		29 July 2010 JP
C109-08	1	6.24	Czech Republic, distr. Česká Lípa, Kamenická Nová Víska, bank of Kamenice stream NNE of village	50°48'5.2"N, 14°22'48.1"E	250	exposed bottom		29 July 2010 JP
C111-10	2	**6.19±0.02**	Czech Republic, distr. Česká Lípa, Janská, flooded depression under rock overhang in gorge of Olešnička stream S of village	50°48'0.8"N, 14°21'43.1"E	245	pool		29 July 2010 JP
C150-10	2	6.19	Czech Republic, distr. Děčín, Šluknov, Spréva (Spree) stream on state border in former village Fukov	51°2'37"N, 14°30'15"E	300	stream (sand)	*C. hamulata*	9 Sept. 2010 P. Bauer
C010-12	1	6.23	Czech Republic, distr. Most, Louka u Litvínova, on S bank of Plutovský fishpond	50°35'24.2"N, 13°37'57.3"E	295	fishpond		27 May 2012 JP
C011-12	3	6.22	Czech Republic, distr. Most, Mariánské Radčice, Radčický stream E of village	50°34'18.9"N, 13°40'45.8"E	250	stream (mud)		27 May 2012 JP
C032-12	3	6.30	Denmark, Jutland, Hajstrup, reg. Syddanmark, Grønå stream above bridge of Slogsherredsvej road	54°56'00.6"N, 09°06'53.7"E	12	stream (mud)		31 July 2012 JP & ZK

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. platycarpa* Kütz.**								
C036-12	2	6.24	Denmark, Jutland, reg. Nordjylland, Trængstrup, Sonderupå stream near bridge of Suldrupvej road	56°51'08.5"N, 09°38'11.3"E	18	stream (sand)	*C. hamulata*	1 Aug. 2012 JP & ZK
C037-12	1	6.19	Denmark, Jutland, reg. Nordjylland, Bislev, Binderupå stream near the bridge of Jordemodervej road	56°56'14.8"N, 09°38'15.7"E	22	stream (sand)		1 Aug. 2012 JP & ZK
C040-12	3	6.25	Denmark, Jutland, reg. Midtjylland, Nees, Søndersund lake	56°23'57.0"N, 08°13'51.3"E	0	lake	*C. hamulata*, *C.* ×*vigens*	1 Aug. 2012 JP & ZK
C042-12	2	6.11	Denmark, Jutland, reg. Midtjylland, Nees, Indfjorden lake	56°24'38.3"N, 08°13'50.0"E	0	lake		1 Aug. 2012 JP & ZK
C044-12	1	**6.24±0.04**	Denmark, Jutland, reg. Midtjylland, Tarm, Sonderå River WNW of town	55°55'06.4"N, 08°28'58.0"E	1	river (mud)	*C. hamulata*	3 Aug. 2012 JP & ZK
C046-12	2	**6.29±0.03**	Denmark, Jutland, reg. Syddanmark, Tange, ditch between Varde Å River and Lundvej Road NE of town	55°38'41.1"N, 08°32'35.7"E	6	ditch		3 Aug. 2012 JP & ZK
C051-12	3	6.21	Germany, Lower Saxony, Baven, Örtze River below the bridge of Zur Örtze road	52°50'41.9"N, 10°6'00.7"E	52	river (mud)	*C. hamulata*	3 Aug. 2012 JP & ZK
C054-12	1	6.22	Germany, Niedersachsen, Nordstemmen, Leine River below bridge of Reichsstraße street	52°8'50.1"N, 9°46'3.9"E	76	stream (mud)		3 Aug. 2012 PT & B. Kubátová
C077-12	1	6.11	Germany, Bayern, Potterstein, Weihersbach stream near Gasthof Schüttersmühle	49°45'5.2"N, 11°25'37.9"E	411	stream (mud)		10 Sept. 2012 A. Knotek

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. platycarpa* Kütz.**								
C096-12	2	6.20	Czech Republic, distr. Děčín, Šluknov, puddle on forest path in forest complex between town distr. Království and former settlement Fukov	51°1'19.8"N, 14°29'41.9"E	380	puddle		31 Dec. 2012 JP
C097-12	1	6.19	Czech Republic, distr. Děčín, Šluknov, puddle on crossroads of paths near cemetery of former settlement Fukov	51°2'32.9"N, 14°30'6.9"E	310	puddle		31 Dec. 2012 JP
C010-13	1	**6.06±0.01**	Italy, prov. Cosenza, Acri, ditch by waterlogged meadow near Strada Statale 660 road, N of Lago di Cecita	39°26'18.5"N, 16°31'29.2"E	1207	ditch		20 May 2013 PT & B. Kubátová
C069-13a	2	6.27	Czech Republic, distr. Domažlice, Nemanice, blind arm of Nemanický stream SW of settlement Nemaničky	49°25'48.6"N, 12°42'40.6"E	504	pool	*C. hamulata*, *C. ×vigens*	10 Aug. 2013 M. Hrdinová
C070-13	1	6.24	Czech Republic, distr. Domažlice, Novosedly, Nemanický stream SW of village	49°26'21.4"N, 12°42'27.4"E	510	stream (sand)		11 Aug. 2013 M. Hrdinová
C079-13	3	**6.11±0.01**	Czech Republic, distr. Liberec, Černousy, stream along second uppermost fishpond in pond cascade E of settlement V Poli	50°59'35.3"N, 15°03'26.4"E	242	stream (mud)	*C. stagnalis*	22 Aug. 2013 JP & KK
C122-13	1	**6.16±0.00**	Czech Republic, distr. Děčín, Zadní Ves, pool opposite restaurant Hraniční Bouda in village	50°47'51"N, 14°5'0,5"E	560	pool		28 Aug. 2013 JanR & JarR
C124-13	3	**6.10±0.02**	Czech Republic, distr. Děčín, Zadní Ves, Oborský fishpond	50°48'21"N, 14°4'20"E	525	fishpond		28 Aug. 2013 JanR & JarR

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. stagnalis* Scop.**								
C008-07	1	3.01	Czech Republic, distr. Jindřichův Hradec, Horusice, National Nature Reserve Ruda, puddle on path near Horusický fishpond	49°9'8"N, 14°41'22"E	415	puddle		28 June 2007 JP & ZK
C014-07	1	2.99	Czech Republic, distr. Příbram, Čenkov, Běštínský stream below spring	49°47'23"N, 14°1'41"E	550	stream (mud)		29 July 2007 JP
C019-07	1	2.93	Czech Republic, distr. Rakovník, Lužná, puddle on forest path near solitude Krásná dolina	50°7'42"N, 13°48'3"E	385	puddle		15 Aug. 2007 JP
C020-07	1	3.01	Czech Republic, distr. Rakovník, Řevničov, Nature Reserve Prameny Klíčavy, puddle on forest path	50°8'38.2"N, 13°49'37.2"E	420	puddle	*C. hamulata*	16 Aug. 2007 JP
C024-07	1	2.99	Czech Republic, distr. Kladno, Lány, forest puddle near solitude Nový Dvůr	50°8'57"N, 13°55'37"E	425	puddle	*C. hamulata*	16 Aug. 2007 JP
C027-07	1	2.95	Czech Republic, distr. Kladno, Běleč, forest terrain depression near solitude Fialka	50°3'47.5"N, 13°57'55.9"E	395	puddle		16 Aug. 2007 JP
C030-07	1	3.03	Czech Republic, distr. Mladá Boleslav, Jabkenice, puddle on forest path near Erichův fishpond	50°18'58"N, 15°2'46"E	250	puddle		22 Aug. 2007 JP
C005-08	1	**3.02±0.01**	Czech Republic, Praha, city distr. Zbraslav, Nature Monument Krňák, Lipanský stream	49°58'23"N, 14°22'29"E	195	stream (mud)	*C. cophocarpa*	15 May 2008 JP
C007-08	1	**3.02±0.02**	Czech Republic, distr. Chrudim, Kochánovice, puddle on forest path near Hluboký fishpond	49°54'2"N, 15°47'17.8"E	330	puddle		23 May 2008 JP
C026-08	1	3.01	Czech Republic, distr. Děčín, Mezná, on forest path E of village	50°52'0"N, 14°19'17"E	265	puddle		3 July 2008 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. stagnalis* Scop.**								
C033-08	1	3.04	Czech Republic, distr. Jindřichův Hradec, Lutová, ruts on path near Safl fishpond	49°0'11"N, 14°53'9"E	440	puddle		11 July 2008 JP
C034-08	1	3.00	Czech Republic, distr. Jindřichův Hradec, Lutová, puddle on forest path near Humlenský fishpond	49°0'2"N, 14°52'29"E	440	puddle		11 July 2008 JP
C039-08	1	3.02	Czech Republic, distr. Praha-východ, Krupá, ruts on forest path WSW of village	50°0'56"N, 14°51'46"E	275	puddle		19 July 2008 JP
C040-08	1	3.02	Czech Republic, distr. Praha-východ, Svatbín, puddle on forest path in forest Brník	49°59'27"N, 14°53'35"E	370	puddle		19 July 2008 JP
C041-08	2	**2.98±0.03**	Czech Republic, distr. Praha-východ, Svatbín, ditch along forest path in forest Brník	49°59'19"N, 14°53'35"E	370	puddle		19 July 2008 JP
C043-08	1	3.03	Czech Republic, distr. Praha-východ, Jevany, ruts on side of path E of village	49°58'19"N, 14°51'5"E	370	puddle	*C. palustris*	19 July 2008 JP
C046-08	1	**3.03±0.00**	Czech Republic, distr. Praha-východ, Jevany, middle forest fishpond in Aldašín deer-park	49°58'6.6"N, 14°50'21.2"E	385	exposed bottom	*C. hamulata*, *C. palustris*	19 July 2008 JP
C050-08	1	2.95	Czech Republic, distr. Cheb, Františkovy Lázně, settlement Lužná, puddle on forest path near Vražedný fishpond	50°6'19"N, 12°17'16"E	450	puddle	*C. hamulata*	1 Aug. 2008 JP
C053-08	2	**3.00±0.01**	Czech Republic, distr. Cheb, Libá, puddle on forest path NW of Kamenný fishpond	50°8'13"N, 12°13'7"E	540	puddle		2 Aug. 2008 JP
C057-08	1	2.99	Czech Republic, distr. Cheb, Mýtinka, puddle on forest path E of village	50°8'47"N, 12°19'4"E	505	puddle		2 Aug. 2008 JP
C066-08	1	3.00	Czech Republic, distr. Liberec, Víska, ditch along road in village	50°56'51"N, 15°1'45.0"E	240	ditch		19 Aug. 2008 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. stagnalis **Scop.**								
C074-08	1	2.93	Czech Republic, distr. Prachatice, Volary, ruts on forest path in railway triangle SE of town	48°53'27"N, 13°52'11"E	750	puddle		6 Sept. 2008 JP
C087-08	1	2.99	Czech Republic, distr. Žďár nad Sázavou, Karlov, puddle on forest path near Velké Dářko fishpond	49°38'47.9"N, 15°53'45.4"E	615	puddle		22 Sept. 2008 JP
C095-08	1	3.03	Czech Republic, distr. Příbram, Voznice, puddle on path close to N edge of Charvátův fishpond	49°48'58"N, 14°11'37"E	415	puddle		6 Nov. 2008 JP
C097-08	1	3.01	Czech Republic, distr. Příbram, Chouzavá, ruts on forest path W of hill Točná	49°50'12"N, 14°11'42"E	420	puddle	*C. cophocarpa*	6 Nov. 2008 JP
C099-08	1	2.99	Czech Republic, distr. Praha-východ, Kytín, pudle on crossroads of forest paths WSW of village	49°50'48.0"N, 14°11'51.0"E	470	puddle	*C. palustris*	6 Nov. 2008 JP
C104-08	1	2.99	Czech Republic, distr. Mladá Boleslav, Bělá pod Bezdězem, village distr. Vrchbělá, puddle on forest path	50°31'17"N, 14°46'38"E	315	puddle	*C. hamulata*	28 Sept. 2008 J. Suda
C106-08	1	3.01	Czech Republic, distr. Louny, Domoušice, on path NNE of railway station Mutějovice	50°13'20.1"N, 13°42'23.9"E	520	puddle		29 Sept. 2008 H. Chudáčková
C107-08	1	3.01	Czech Republic, distr. Brno-venkov, Moravské Knínice, puddle on forest path SW of village	49°16'43.1"N, 16°29'19.7"E	327	puddle		2 June 2008 H. Chudáčková
C024-09	1	**3.02±0.03**	Czech Republic, distr. Česká Lípa, Doksy, Okenský stream in town	50°34'1"N, 14°39'20"E	270	stream (mud)		9 June 2009 JP
C034-09	1	2.99	Czech Republic, distr. Karlovy Vary, Údrč, on forest path near Malý Pásmovský fishpond	50°7'37.9"N, 13°6'34.7"E	625	puddle		5 Aug. 2009 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. stagnalis **Scop.**								
C035-09	1	2.97	Czech Republic, distr. Karlovy Vary, Veselov, puddle on side of path NE of hill Havraní vrch	50°7'23.5"N, 13°6'49.4"E	645	puddle		5 Aug. 2009 JP
C036-09	1	2.98	Czech Republic, distr. Karlovy Vary, Přestání, on intersection of forest paths W of village	50°4'25.1"N, 13°4'18.7"E	610	puddle	*C. palustris*	6 Aug. 2009 JP
C038-09	1	**2.98±0.01**	Czech Republic, distr. Karlovy Vary, Přestání, puddle on forest path W of village	50°4'21.8"N, 13°4'22.7"E	600	puddle		6 Aug. 2009 JP
C039-09	1	2.99	Czech Republic, distr. Karlovy Vary, Radyně, puddle on forest path NNE of village	50°4'22.7"N, 13°3'20.3"E	610	puddle		6 Aug. 2009 JP
C041-09	1	2.95	Czech Republic, distr. Karlovy Vary, Radyně, puddle on path NNW of village	50°4'17.2"N, 13°1'57.5"E	615	puddle		6 Aug. 2009 JP
C049-09	1	2.98	Czech Republic, distr. Karviná, Prstná, ruts on forest path SE of village	49°54'41.1"N, 18°34'4.1"E	270	puddle		9 Aug. 2009 JP
C053-09	1	**2.99±0.03**	Czech Republic, distr. Nový Jičín, Hukovice, ruts on intersection of forest paths in forest Roveň	49°37'35.7"N, 18°2'39.0"E	275	puddle		11 Aug. 2009 JP
C055-09	1	2.97	Czech Republic, distr. Nový Jičín, Libhošť, puddle on intersection of forest path in forest Roveň	49°36'59.0"N, 18°2'43.0"E	285	puddle		11 Aug. 2009 JP
C056-09	1	2.97	Czech Republic, distr. Nový Jičín, Libhošť, puddle beside main forest road in forest Roveň	49°36'41.5"N, 18°2'49.9"E	295	puddle		11 Aug. 2009 JP
C067-09	1	**2.99±0.01**	Czech Republic, distr. Děčín, Velký Šenov, ruts on forest path SSE of Špičák hill	51°0'39.8"N, 14°24'35.6"E	385	puddle		19 Aug. 2009 JP
C068-09	1	3.01	Czech Republic, distr. Děčín, Šluknov, puddle beside forest path WSW of settlement Nové Hraběcí	51°0'51.1"N, 14°25'0.0"E	410	puddle		19 Aug. 2009 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. stagnalis **Scop.**								
C077-09	1	2.96	Czech Republic, distr. Blansko, Benešov, puddle on forest path SE of village	49°30'15.4"N, 16°46'50.5"E	700	puddle		25 Aug. 2009 JP
C081-09	1	3.00	Czech Republic, distr. Blansko, Žďárná, ruts on forest path SE of village	49°26'58.3"N, 16°47'10.2"E	575	puddle	*C. cophocarpa, C. palustris*	26 Aug. 2009 JP
C098-09	2	2.99	Czech Republic, distr. Pardubice, Horní Jelení, ditch of Čermná stream on E edge of town	50°2'55.6"N, 16°5'41.5"E	285	ditch		8 Oct. 2009 JP
C103-09	2	**3.01±0.01**	Czech Republic, distr. Pardubice, Rousínov, side arm of Čermná stream close to Horní Pecák fishpond	50°2'57.1"N, 16°7'54.4"E	275	ditch	*C. cophocarpa*	8 Oct. 2009 JP
C107-09	1	3.03	Czech Republic, distr. Svitavy, Opatov, puddle on side of forest path S of railway station Semanín	49°50'17.2"N, 16°28'17.2"E	430	puddle	*C. cophocarpa, C. hamulata, C. palustris*	19 Oct. 2009 JP
C110-09	1	2.99	Czech Republic, distr. Svitavy, Opatov, puddle on forest path NNW of pond Rebelant	49°50'2.7"N, 16°28'19.4"E	437	puddle	*C. hamulata*	19 Oct. 2009 JP
C113-09	1	3.04	Czech Republic, distr. Svitavy, Opatov, ruts on forest path NW of pond Rebelant	49°49'53.5"N, 16°28'20.0"E	440	puddle	*C. hamulata*	19 Oct. 2009 JP
C017-10	2	**3.00±0.01**	Czech Republic, distr. Semily, Kacanovy, puddle on forest path SW of Valdštejn castle	50°33'31.1"N, 15°9'46.5"E	345	puddle		12 June 2010 JP
C020-10	1	2.99	Czech Republic, distr. Děčín, Mikulášovice, meadow spring SE of town	50°57'1"N, 14°22'41"E	450	puddle		8 June 2010 J. Hadinec & H. Härtel

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. stagnalis* Scop.**								
C024-10	1	3.02	Czech Republic, distr. Jičín, Lukavec u Hořic, puddle on forest path E of Zákopský fishpond	50°24'40.6"N, 15°37'8.8"E	336	puddle	*C. palustris*	26 June 2010 JP
C035-10	1	**3.02±0.02**	Czech Republic, distr. Zlín, Lukov, ditch along forest path N of Bezedník fishpond	49°18'23.2"N, 17°43'32.9"E	360	puddle		1 July 2010 JP
C037-10	1	**2.95±0.01**	Czech Republic, distr. Zlín, Lukov, puddle on forest path NNE of hill Velá	49°18'57.8"N, 17°43'32.0"E	455	puddle		1 July 2010 JP
C039-10	1	2.97	Czech Republic, distr. Zlín, Vlčková, puddle on forest path ENE of hill Bzová	49°19'16.5"N, 17°44'27.1"E	563	puddle	*C. palustris*	1 July 2010 JP
C040-10	1	2.99	Czech Republic, distr. Zlín, Vlčková, puddle on intersection of forest paths NNE of hill Ondřejovsko	49°19'39.3"N, 17°43'43.7"E	560	puddle	*C. palustris*	1 July 2010 JP
C043-10	1	2.96	Czech Republic, distr. Zlín, Vlčková, puddle on forest path NE of hill Ondřejovsko	49°19'52.6"N, 17°44'6.2"E	570	puddle	*C. cophocarpa*	1 July 2010 JP
C049-10	1	2.96	Czech Republic, distr. Tachov, Racov, Nature Monument Racovské rybníčky, place digged up by forest animals	49°37'37.1"N, 12°51'9.7"E	495	puddle		5 July 2010 JP & ZK
C059-10	1	3.03	Czech Republic, distr. Domažlice, Novosedly, ruts on forest path SSW of castle ruins Starý Herštejn	49°27'29.5"N, 12°42'28.7"E	560	puddle	*C. palustris*	6 July 2010 JP & P. Koutecký
C061-10	1	3.00	Czech Republic, distr. Plzeň-jih, Zemětice, artificial pool E of Dražský fishpond	49°34'54.0"N, 13°12'8.5"E	385	pool		8 July 2010 JP & J. Chrtek
C088-10	1	3.01	Czech Republic, distr. Plzeň-sever, Hůrky, on forest path N of village	49°53'37.7"N, 13°11'4.6"E	542	puddle		19 July 2010 JP & ZK

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. stagnalis* Scop.**								
C096-10	1	2.97	Czech Republic, distr. Česká Lípa, Velký Valtinov, puddle on path ESE of Zaječí fishpond	50°45'7.6"N, 14°43'30.6"E	325	puddle	*C. palustris*, *C. platycarpa*	28 July 2010 JP
C099-10	1	2.96	Czech Republic, distr. Česká Lípa, Kunratice u Cvikova, ruts on forest path SW of Kamenáč hill	50°45'27.0"N, 14°42'27.3"E	333	puddle	*C. palustris*, *C. platycarpa*	28 July 2010 JP
C107-10	1	**2.99±0.03**	Czech Republic, distr. Česká Lípa, Horní Kamenice, puddle on forest path N of village	50°48'24.0"N, 14°26'47.5"E	355	puddle		29 July 2010 JP
C124-10	1	2.96	Czech Republic, distr. Znojmo, Lesná, puddle on forest path SSW of villa Lusthaus	48°53'37.6"N, 15°51'13.5"E	475	puddle		15 Aug. 2010 JP
C125-10	3	2.98	Czech Republic, distr. Znojmo, Čížov, right bank of Dyje River S of village	48°51'38.0"N, 15°52'27.8"E	280	river (mud)		16 Aug. 2010 JP
C126-10	1	2.96	Czech Republic, distr. Znojmo, Čížov, puddle on forest path E of village	48°52'46.0"N, 15°52'55.1"E	425	puddle		16 Aug. 2010 JP
C128-10	1	**2.99±0.02**	Czech Republic, distr. Znojmo, Šumná, puddle on forest path SSW of castle ruins Šimperk	48°55'29.0"N, 15°54'28.1"E	395	puddle		16 Aug. 2010 JP
C132-10	1	2.99	Czech Republic, distr. Znojmo, Olbramkostel, puddles along forest path SE of castle ruins Šimperk	48°55'33.2"N, 15°55'17.2"E	387	puddle	*C. palustris*	16 Aug. 2010 JP
C141-10	1	2.97	Czech Republic, distr. Havlíčkův Brod, Kocourov, puddles on dam of Návesní fishpond	49°45'13.7"N, 15°48'1.4"E	553	puddle	*C. palustris*	28 Aug. 2010 JP & ZK
C152-10	3	**2.97±0.03**	Czech Republic, distr. Prachatice, Lipka, small fishpond in settlement Stará Lipka	49°1'29.1"N, 13°43'57.1"E	875	fishpond		25 Sept. 2010 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. stagnalis* Scop.**								
C005-11	2	2.99	Czech Republic, distr. Nymburk, Loučeň, puddle on forest path E of spring Dobrá Voda	50°18'02.7"N, 15°02'07.3"E	264	puddle	*C. palustris*	30 May 2011 JP
C007-11	1	**3.00±0.03**	Czech Republic, distr. Nymburk, Chudíř, on forest path near SW bank of Nový fishpond	50°17'58.0"N, 15°00'45.6"E	230	puddle		30 May 2011 JP
C061-11	1	2.99	Czech Republic, distr. Třebíč, Jackov, puddle on forest path NW of Hrachovec fishpond	49°2'56.4"N, 15°44'5.7"E	470	puddle		8 July 2011 JP
C013-12	1	2.97	Czech Republic, distr. Rakovník, Řevničov, puddle on forest path N of Horní Kracle fishpond	50°8'35.7"N, 13°50'30.4"E	410	puddle	*C. hamulata*	12 June 2012 JP & A. Potůčková
C030-12	1	2.97	Czech Republic, distr. Sokolov, Rotava, puddle on forest path near rock formation Rotavské varhany	50°18'12"N, 12°34'30"E	600	puddle		29 July 2012 J. Hadinec
C038-12	1	3.05	Denmark, Jutland, reg. Midtjylland, Nees, pasture near between Søndersund lake and Ulfsundvej road	56°23'57.2"N, 08°13'56.8"E	0	puddle		1 Aug. 2012 JP & ZK
C075-12	1	**2.95±0.03**	Czech Republic, distr. Ústí nad Labem, Petrovice, puddle on path near former settlement Hladov	50°48'45"N, 13°59'45"E	480	puddle		13 Sept. 2012 J. Hadinec & P. Bauer
C076-12	1	**2.97±0.02**	Czech Republic, distr. Děčín, Brtníky, puddle on forest path S of village	50°56'32"N, 14°26'14.9"E	455	puddle		13 Sept. 2012 J. Hadinec & P. Bauer
C092-12	1	3.01	Czech Republic, distr. Domažlice, Babylon, ruts on crossroads of forest paths near settlement Na Pohodnici	49°24'18.4"N, 12°52'38.4"E	520	puddle		29 Oct. 2013 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. stagnalis* Scop.**								
C002-13	2	3.01	Czech Republic, distr. Mělník, Želízy, Želízská svodnice ditch in village	50°25'21.1"N, 14°27'51.2"E	176	ditch		3 May 2013 JP
C003-13	3	**3.03±0.03**	Czech Republic, distr. Mělník, Tupadly, Nature Reserve Mokřady Dolní Liběchovky, pool in valley of Liběchovka stream	50°26'12"N, 14°28'13"E	180	pool		3 May 2013 JarR
C012-13	1	3.01	Czech Republic, distr. Nymburk, Kněžičky, puddle on forest path in deer-park Kněžičky S of village	50°09'35.6"N, 15°20'39.3"E	245	puddle		29 May 2013 JP
C018-13	1	3.03	Czech Republic, distr. Nymburk, Loučeň, pool on bottom of long-term drained fishpond Lutovník	50°17'34.6"N, 15°0'9.0"E	234	pool		7 June 2013 JP
C020-13	1	3.01	Czech Republic, distr. Příbram, Mokrovraty, puddle on meadow path NE of hill Dolní Jezberná	49°48'43.8"N, 14°14'44.7"E	335	puddle	*C. palustris*	11 June 2013 JP & KK
C024-13	1	3.07	Czech Republic, distr. Tábor, Hrnčíře, puddle on forest path NW of village	49°35'04.8"N, 14°51'11.2"E	453	puddle		18 June 2013 KK
C035-13	1	2.97	Czech Republic, distr. Jindřichův Hradec, Nová Olešná, puddles on forest path below dam of Vydloubal fishpond	49°09'50.0"N, 15°09'41.0"E	554	puddle		27 June 2013 ZK
C040-13	1	2.97	Czech Republic, distr. Jičín, Mlázovice, puddle on forest path in Kamenický forest WNW of village	50°25'11.7"N, 15°30'9.0"E	315	puddle		4 July 2013 M. Štefánek
C041-13	1	2.97	Czech Republic, distr. Trutnov, Kocbeře, puddle on path on forest glade E of village	50°27'05.7"N, 15°52'13.4"E	448	puddle		4 July 2013 JP

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. stagnalis* Scop.**								
C042-13	1	2.95	Czech Republic, distr. Trutnov, Kocbeře, puddle on forest path between village and Rabiš fishpond	50°27'09.2"N, 15°52'42.4"E	470	puddle	*C. palustris*	4 July 2013 JP
C053-13	1	**2.97±0.01**	USA, Oregon, Coos County, Coos Bay, Mingus Park, garden pond in park	43°22'18.5"N, 124°13'26.4"W	3	reservoir		29 July 2013 JP & T. Kávová
C054-13	3	**2.99±0.03**	USA, Oregon, Coos County, Bandon, Lost Lake	43°1'23.9"N, 124°25'51.8"W	9	lake		30 July 2013 JP & T. Kávová
C062-13	1	**2.94±0.05**	Czech Republic, distr. Cheb, Povodí, Lužní stream on confluence with Sázek stream	50°8'57.4"N, 12°25'27.2"E	436	stream (sand)	*C. hamulata*	19 July 2013 KK & JarR
C067-13	2	**2.98±0.03**	Czech Republic, distr. Cheb, Dvorek, Stodolský stream by road bridge NE of village	50°8'19.5"N, 12°24'42.5"E	431	stream (sand)	*C. hamulata*, *C. cophocarpa*	19 July 2013 KK & JarR
C072-13	1	**2.99±0.04**	Norway, prov. Møre og Romsdal, Runde Island, wet places on path on NW part of island	62°24'29.0"N, 5°35'47.4"E	246	puddle		7 Aug. 2013 KK
C078-13	2	2.98	Czech Republic, distr. Liberec, Černousy, stream along second uppermost fishpond in pond cascade E of settlement V Poli	50°59'35.3"N, 15°03'26.4"E	242	stream (mud)	*C. platycarpa*	22 Aug. 2013 JP & KK
C087-13	1	3.05	Czech Republic, distr. Cheb, Prameny, puddle on forest path NE–ENE of village	50°03'41.3"N, 12°45'36.3"E	760	puddle		21 Aug. 2013 J. Chrtek jr.

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. stagnalis* Scop.**								
C089-13	1	**3.04±0.03**	Czech Republic, distr. Rychnov nad Kněžnou, Petrovice, puddle on forest path SE of railway station	50°10'17.4"N, 16°3'4.8"E	250	puddle		1 Sept. 2013 JP & KK
C110-13	1	2.99	Czech Republic, distr. Ústí nad Labem, Ostrov, small fishpond below dam of Ostrovský fishpond	50°48'24"N, 14°2'47"E	445	fishpond		28 Aug. 2013 JanR & JarR
C114-13	1	3.01	Czech Republic, distr. Děčín, Maxičky, Vlčí jezero fishpond	50°48'35"N, 14°8'0"E	480	fishpond		28 Aug. 2013 JanR & JarR
C121-13	1	2.97	Czech Republic, distr. Ústí nad Labem, Libouchec, artificial pool in Nature Reserve Libouchecké rybníčky	50°46'1"N, 14°3'32"E	330	pool		27 Aug. 2013 JanR & JarR
C123-13	1	2.99	Czech Republic, distr. Děčín, Zadní Ves, puddle on forest path near state border N of village	50°48'35"N, 14°4'53"E	507	puddle		28 Aug. 2013 JanR & JarR
C130-13	2	2.99	Czech Republic, distr. Jihlava, Řídelov, ditch along forest path near SW bank of Pilný fishpond	49°14'08.7"N, 15°23'52.6"E	620	ditch	*C. cophocarpa*	8 Sept. 2013 JP
C134-13	1	3.04	Czech Republic, distr. Praha-východ, Kostelec nad Černými Lesy, puddle on forest path SSW of town	49°58'13.6"N, 14°50'47.7"E	390	puddle	*C. hamulata*	9 Sept. 2013 JP
***C. stagnalis* autotriploid**								
C125-13	1	**4.55±0.03**	Czech Republic, distr. Děčín, Zadní Ves, on forest path N of village	50°48'26"N, 14°4'45"E	525	puddle		28 Aug. 2013 JanR & JarR

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
C. ×vigens **K. Martinsson [*C. cophocarpa* × *C. platycarpa*]**								
C058-08	3	**4.69±0.01**	Czech Republic, distr. Cheb, Nový Drahov, settlement Hájek, National Nature Reserve Soos, pool near spring Věra	50°8'59.7"N, 12°23'59.3"E	435	pool		3 Aug. 2008 JP
C017-09	3	**4.72±0.04**	Czech Republic, distr. Česká Lípa, Mimoň, ditch in park	50°39'26"N, 14°43'44"E	280	ditch		8 June 2009 JP
C026-09	2	**4.72±0.02**	Czech Republic, distr. Česká Lípa, Zahrádky, National Nature Monument Peklo, side arm of Robečský stream	50°38'37"N, 14°30'35"E	250	pool		9 June 2009 JP
C030-09	3	**4.72±0.05**	Czech Republic, distr. Litoměřice, Kostelec nad Ohří, Nature Reserve Myslivna, ditch in floodplain forest	50°23'42.6"N 14°05'0.6"E	165	ditch		18 June 2009 JP
C011-10	3	**4.65±0.04**	Czech Republic, Most, city distr. Kopisty, Bílina River	50°32'31.3"N, 13°37'2.8"E	227	river (mud)		2 June 2010 JP
C157-10	2	**4.64±0.05**	Czech Republic, distr. Písek, Zátaví, right bank of Otava River near settlement Zátavský Mlýn	49°16'20.9"N, 14°5'50.6"E	365	river (mud)		29 Sept. 2010 JP
C016-11	5	**4.62±0.03**	Czech Republic, distr. Klatovy, Čepice, blind arm on right bank of Otava River ESE of village	49°15'57.5"N, 13°36'3.7"E	445	pool	*C. hamulata*	17 June 2011 JP & P. Koutecký
C018-11	2	4.60	Czech Republic, distr. Klatovy, Čepice, blind arm on leftbank of Otava River ESE of village	49°15'58.2"N, 13°36'13.0"E	445	pool	*C. hamulata*	17 June 2011 JP & P. Koutecký
C020-11	4	4.63	Czech Republic, distr. Klatovy, Velké Hydčice, blind arm of Otava River N of village	49°18'19.5"N, 13°40'9.8"E	426	pool	*C. hamulata*	17 June 2011 JP & P. Koutecký

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
***C. ×vigens* K. Martinsson [*C. cophocarpa* × *C. platycarpa*]**								
C022-11	1	4.55	Czech Republic, distr. Klatovy, Týnec, on alluvium of Otava River below weir	49°18'48.3"N, 13°40'34.6"E	425	exposed bottom		17 June 2011 JP & P. Koutecký
C023-11	1	4.65	Czech Republic, distr. Klatovy, Týnec, blind arm of Otava River E of village	49°18'53.0"N 13°40'44.0"E	425	pool		17 June 2011 JP & P. Koutecký
C035-11	1	4.66	Czech Republic, distr. Strakonice, Pracejovice, blind arm of Otava River NE of village	49°15'38.2"N, 13°51'34.4"E	395	pool		18 June 2011 JP & P. Koutecký
C036-11	1	4.61	Czech Republic, distr. Strakonice, Virt, Nature Reserve Bažantnice u Pracejovic, blind arm of Otava River S of village	49°15'41.8"N, 13°52'0.1"E	390	pool		18 June 2011 JP & P. Koutecký
C037-11	3	4.69	Czech Republic, Strakonice, town distr. Podskalí, blind arm on right bank of Otava River in camp	49°15'26.2"N, 13°53'19.8"E	390	pool	*C. hamulata*	19 June 2011 JP & P. Koutecký
C039-11	2	**4.63±0.04**	Czech Republic, Strakonice, separated arm of Otava River on E edge of town	49°15'30.0"N, 13°55'48.7"E	385	pool		19 June 2011 JP & P. Koutecký
C021-12	5	**4.64±0.02**	Czech Republic, distr. Mladá Boleslav, Dolení Kruhy, Jizera River NNW of village	50°33'45.6"N, 14°59'41.3"E	230	river (sand)	*C. hamulata*	14 June 2012 JP & PT
C041-12	2	**4.62±0.02**	Denmark, Jutland, reg. Midtjylland, Nees, Søndersund lake	56°23'57.0"N, 08°13'51.3"E	0	lake	*C. hamulata*, *C. platycarpa*	1 Aug. 2012 JP & ZK

Reference no.	No. of indivi-duals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitu-de (m)	Type of habitat	Other taxa	Date + Collec-tor(s)
***C. ×vigens* K. Martinsson [*C. cophocarpa* × *C. platycarpa*]**								
C055-13	1	**4.62±0.07**	Czech Republic, distr. Cheb, Skalná, National Nature Reserve Soos, lake E of settlement Kateřina	50°9'21.2"N, 12°24'37.4"E	447	lake		18 July 2013 KK & JarR
C056-13	1	**4.64±0.06**	Czech Republic, distr. Cheb, Nový Drahov, settlement Hájek, National Nature Reserve Soos, pool NE of railway station	50°8'53.5"N, 12°24'0.5"E	439	pool		18 July 2013 KK & JarR
C068-13	3	**4.64±0.04**	Czech Republic, distr. Domažlice, Nemanice, blind arm of Nemanický stream SW of settlement Nemaničky	49°25'48.6"N, 12°42'40.6"E	504	pool	*C. hamulata*, *C. platycarpa*	10 Aug. 2013 M. Hrdinová
C082-13	7	**4.63±0.03**	Czech Republic, distr. Jablonec nad Nisou, Bedřichov, water reservoir on N edge of village	50°47'43.1"N, 15°8'34.8"E	733	reservoir		22 Aug. 2013 JP & KK
C108-13	2	**4.72±0.02**	Czech Republic, distr. Karlovy Vary, Sedlečko, blind arm of Ohře River NNE of settlement Hubertus	50°14'27"N, 12°55'49"E	365	pool	*C. hamulata*	29 Aug. 2013 JanR & JarR
C115-13	1	**4.65±0.06**	Czech Republic, distr. Děčín, Maxičky, lowermost fishpond S of village	50°48'19"N, 14°10'58"E	405	fishpond		28 Aug. 2013 JanR & JarR
C116-13	1	**4.67±0.05**	Czech Republic, distr. Děčín, city distr. Přípeř, fishpond close to Drážďanská street	50°47'13"N, 14°12'10"E	180	fishpond		26 Aug. 2013 JanR & JarR
C132-13a	1	4,74	Germany, Bayern, Hohenfels, Follerenbach stream between Hohenfels and Kalmünz villages	49°11'7.2"N, 11°54'45.7"E	364	stream (sand)	*C. hamulata*	7 Sept. KK

Reference no.	No. of individuals	2C-value (pg) + s.d.	Locality	Coordinates (WGS 84)	Altitude (m)	Type of habitat	Other taxa	Date + Collector(s)
hybrid from Tichá Orlice River [probably *C. hamulata* × *C. cophocarpa*]								
Co61-12	5	**7.60±0.02**	Czech Republic, distr. Ústí nad Orlicí, Brandýs nad Orlicí, Tichá Orlice River below bridge of Žerotínova street	50°0'4.9"N, 16°16'45.1"E	295	river (sand)	*C. cophocarpa, C. hamulata*	15 Aug. 2012 JP
Co65-12	1	**7.61±0.08**	Czech Republic, distr. Ústí nad Orlicí, Choceň, Tichá Orlice River on SW edge of town	49°59'43.2"N, 16°12'36.1"E	270	river (sand)	*C. hamulata*	15 Aug. 2012 JP
Co66-12	6	**7.64±0.08**	Czech Republic, distr. Ústí nad Orlicí, Choceň, millrace of Tichá Orlice River on NW edge of town	50°0'4.5"N, 16°12'13.2"E	270	stream (sand)	*C. hamulata*	15 Aug. 2012 JP

PAPER II

Prančl J., Koutecký P., Trávníček P., Jarolímová V., Lučanová M., Koutecká E. & Kaplan Z. (2018): Cytotype variation, cryptic diversity and hybridization in *Ranunculus* sect. *Batrachium* revealed by flow cytometry and chromosome numbers. – Preslia 90: 195–223.

A rare hybrid *Ranunculus aquatilis* × *R. peltatus*. Photo P. Koutecký, "Choryňský mokřad" protected area, Czech Republic.

Cytotype variation, cryptic diversity and hybridization in *Ranunculus* sect. *Batrachium* revealed by flow cytometry and chromosome numbers

Cytotypová variabilita, kryptická diverzita a hybridizace u lakušníků (*Ranunculus* sect. *Batrachium*) odhalená pomocí cytometrických a karyologických analýz

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ABSTRACT

Water-crowfoots (*Ranunculus* sect. *Batrachium*) are one of the taxonomically most intricate groups of aquatic plants. Investigation of its species is complicated by morphological reduction and phenotypic plasticity, as well as by the frequent occurrence of polyploidy and hybridization, giving rise to numerous intergrading or morphologically undetectable taxa. We used, for the first time in this group, flow cytometry to gain an insight into evolutionary processes and genome size differentiation in *Ranunculus* sect. *Batrachium*. Flow cytometry complemented by confirmation of chromosome counts was applied to an extensive dataset of 3354 individuals from 612 localities in 13 mainly central-European countries, of which the genome size was estimated for 1032 individuals. In total, 34 *Batrachium* cytotypes of five ploidy levels ranging from diploids to hexaploids were detected. The results indicate that the genome size is a reliable marker for distinguishing most of the traditionally recognized species, including those with identical chromosome numbers. Although variation in chromosome numbers is reported for six of eight central-European species, we detected only two taxa variable at the ploidy level (*R. fluitans* 2x + 3x, *R. penicillatus* 4x + 6x). However, the situation is much more challenging due to the presence of numerous hybrids and cryptic lineages. Cryptic variation was revealed within *R. trichophyllus*, in which three distinct tetraploid cytotypes were detected, which differ in their ecology and distribution. The allopolyploid complex of running-water taxa referred to as *R. penicillatus* is even more complex, including six different cytotypes. We also revealed 16 cytotypes of hybrid origin, which accounted for ca 15% of the individuals studied. Seven of the hybrid cytotypes were identified as F_1 hybrids and the rest are of unknown or uncertain origin. In the *R. penicillatus* group, the occurrence of a large hybrid swarm was documented. Hybrids with variable genome sizes frequently arise also from hybridization of the tetraploids *R. peltatus* and *R. trichophyllus* in which the gene flow tends to be largely unidirectional towards *R. peltatus*.

KEYWORDS: aquatic plants, chromosome number, cryptic variation, determination, flow cytometry, genome size, hybridization, karyology, ploidy level, polyploidy, *Ranunculus* sect. *Batrachium*

INTRODUCTION

Beyond doubt, aquatic plants are one of the taxonomically most intricate angiosperms. Although phylogenetically unrelated, many aquatics share similar environmental adaptations, which altogether predetermine the formation of hardly detectable diversity (Sculthorpe 1967, Barrett et al. 1993, Kaplan 2002). Their simplified morphology combined with high phenotypic plasticity results in species, which although well separated reproductively, geographically or ecologically, may be indistinguishable morphologically, i.e. they are cryptic (Crawford et al. 2001, Thum et al. 2011, Kaplan & Marhold 2012). The same phenomena may also result in the parallel evolution of similar traits in unrelated taxa (Jobson et al. 2003, Kaplan & Štěpánek 2003, Kaplan & Fehrer 2011). On that account, traditional morphologically-based classification of numerous aquatic plant groups is rather misleading and unable to reflect the actual evolutionary relationships. Furthermore, additional microevolutionary processes can substantially shape the diversification of aquatic plants. In 80% of the aquatic genera, for which chromosome numbers are available, there is intrageneric variation in chromosome number. In the majority of them (68% of the total) this can be attributed to polyploidy (Les & Philbrick 1993; comparable with 61% estimated for all vascular plant genera; Wood et al. 2009). The great importance of hybridization became obvious with the advent of molecular techniques (e.g. Les & Philbrick 1993, Kaplan et al. 2009, Zuellig & Thum 2012). In certain genera, the number of hybrids may even exceed the number of species, as documented in *Potamogeton* (Kaplan et al. 2013). Newly established polyploids and hybrids often persist due to prolific clonal growth and fast vegetative spread (Grace 1993).

Water-crowfoots (*Ranunculus* sect. *Batrachium* according to Hörandl & Emadzade 2012, *Ranunculaceae*; hereafter referred to as *Batrachium*) is evolutionarily one of the most complex groups of aquatic plants. The section consists of about 30 species, occurring predominantly in the Northern

Hemisphere (Wiegleb et al. 2017). The world diversity centre is believed to be in Europe, where 14–18 species are reported (Cook 1966, Wiegleb et al. 2017), of which eight occur in central Europe (Tables 1, 2). *Batrachium* taxa occupy a wide range of wetland habitats from eutrophic lowland lakes and fishponds to high alpine oligotrophic lakes and from standing to fast running water, which is reflected in their extensive phenotypic plasticity. The group is well known for the occurrence of heterophylly, i.e. presence of two completely different types of leaves: laminar leaves with a flat, more or less lobate and dentate lamina, which usually float on water and capillary leaves with lamina consisting of branched capillary segments, which are mostly submerged. Presence or absence of heterophylly closely correspond to different life strategies of particular taxa (e.g. Cook 1966, 1969, Hong 1991). Flower size differs among species of *Batrachium* and is associated with the mode of reproduction: smallflowered taxa are considered largely autogamous or even cleistogamous whereas bigflowered species exhibit several adaptations for outbreeding and particular taxa may even be self-incompatible (Cook 1966, Turała-Szybowska 1978, Hong 1991).

Polyploidy is frequent within *Batrachium*, covering five ploidy levels ranging from diploids (2n = 16) to hexaploids (2n = 48) (Table 1). Variation in ploidy level is reported for about a half of the European species (e.g. Cook 1966, Wiegleb et al. 2017). Aneuploid chromosome numbers are also reported but are rare (Cook 1966, Dahlgren 1991, Diosdado et al. 1993). Interspecific hybridization occurs frequently and probably all taxa are potentially capable of crossing. Many hybrids are at least partially fertile (Cook 1966, 1970, Dahlgren 1991). Clonal growth and autogamy are considered to be important factors in the persistence of hybrids and establishment of new polyploids (Cook 1966, Wiegleb & Herr 1983). The combination of multiple polyploidization and hybridization events may even result in the formation of extremely complex allopolyploid conglomerates. An example of such a group is *Ranunculus penicillatus*. This obligatory runningwater "species" actually consists of various crosses and allopolyploids arising from hybridization of *R. fluitans* with several other species (namely *R. peltatus*, *R. aquatilis*, *R. trichophyllus* and perhaps *R. baudotii*), apparently including backcrossing and introgressive hybridization among particular taxa (Wiegleb & Herr 1983, Webster 1988, Dahlgren 1993, Zalewska-Gałosz et al. 2014). Ploidy level is probably one of the crucial factors influencing the direction of gene flow and consequently even the identity of taxa. This is particularly evident in the case

TABLE 1. List of central-European species of *Ranunculus* sect. *Batrachium* and the chromosome numbers previously published for each taxon. Note that *R. pseudofluitans* (Syme) Newbould ex Baker et Foggitt and *R. kauffmannii* Clerc are included in *R. penicillatus* while *R. confervoides* (Fr.) Fr. is classified as *R. trichophyllus* subsp. *eradicatus*, following Cook (1966).

Taxon	**Distribution**	**Ecology**	**2n**	**Selected references**
R. aquatilis L.	most of Europe, Asia (rarely), N Africa, W of North and South America	predominantly standing water	32	Dahlgren 1993 (Denmark)
			48	Cook 1966 (Denmark, England, Germany, Japan), Hong 1991 (Sweden), Májovský 1978 (Czechoslovakia),Turała 1969 (Poland)
R. baudotii Godr.	coastal parts of Europe, rarely inland	brackish and mineral-rich water	16	Diosdado et al. 1993 (Spain)
			32	Cook 1966 (Austria, Denmark, England, Finland), Dahlgren 1991 (Aegean Islands), Dahlgren 1993 (Denmark), Dvořák & Dadáková 1984 (Czechoslovakia), Hong 1991 (Sweden), Turała 1969 (Poland)
R. circinatus Sibth.	temperate Europe, temperate and boreal (?) Asia	predominantly standing water	16	Cook 1966 (England, Germany, Poland), Dahlgren 1993 (Denmark), Hong 1991 (Sweden), Měsíček & Jarolímová 1992 (Czechoslovakia), Turała 1969 (Poland)
R. fluitans Lam.	NW and Central Europe	rivers, bigger streams	16	Cook 1966 (England, Germany, Sweden), Hong 1991 (Sweden), Měsíček & Jarolímová 1992 (Czechoslovakia), Turała 1969 (Poland), Turała-Szybowska 1977 (Germany)
			24	Cook 1962 (Germany), Měsíček & Jarolímová 1992 (Czechoslovakia), Turała-Szybowska 1977 (Germany)
			32	Cook 1962 (England), Turała-Szybowska 1977 (Czechoslovakia, Germany, Lithuania)
R. peltatus Schrank	most of Europe, N Africa	both standing and running water	16	Cook 1966 (Portugal, Spain), Dahlgren 1991 (Aegean Islands), Ferarella et al. 1981 (Italy), Fernandez Bernaldo de Quirós 1987 (Spain)

Taxon	Distribution	Ecology	2n	Selected references
			32	Cook 1966 (Denmark, Germany), Dahlgren 1991 (Aegean Islands), Diosdado et al. 1993 (Spain), Fernandez Bernaldo de Quirós 1987 (Spain), Hong 1991 (Sweden), Murín et Májovsky 1978 (Czechoslovakia), Turała 1969 (Poland)
			48	England (Cook 1966)
R. penicillatus (Dum.) Bab.	most of Europe	rivers, big streams	16	Diosdado et al. 1993 (Spain), Dahlgren 1991 (Aegean Islands – var. *pseudofluitans*)
			24	Dahlgren 1993 (Denmark)
			32	Cook 1966 (Germany - both var. *penicillatus* and *pseudofluitans*), Dahlgren 1993 (Denmark, both var. *penicillatus* and *pseudofluitans*), Diosdado et al. 1993 (Spain), Turała 1969 (Poland)
			48	Cook 1966 (England, var. *pseudofluitans*, *vertumnus*; Germany - var. *pseudofluitans*; Ireland - var. *penicillatus*)
R. rionii Lagger	central and E Europe, Middle East, Tibet	mineral-rich standing water	16	Cook 1962 (Austria, Iraq), Dahlgren 1991 (Aegean Islands), Gadnidze et al. 1998 (Georgia), Podlech & Dieterle 1969 (Afghanistan)
R. trichophyllus subsp. *eradicatus* (Laest) C. D. K. Cook	Arctic and alpine regions of Europe, Asia and North America	oligotrophic lakes	32	Cook 1966 (Germany, Greenland, Iceland), Löve & Kjellqvist 1974 (Spain)
R. trichophyllus Chaix subsp. *trichophyllus*	Europe, Asia (except of S parts and Japan), N and S Africa, N and S America, SE Australia, New Zealand	both standing and running water	16	Diosdado et al. 1993 (Spain), Löve & Solbrig 1964 (Canada), Roberts 1976 (USA)
			32	Cook 1966 (Algeria, Canada, England, Germany), Dahlgren 1991 (Aegean Islands, Turkey), Favarger et al. 1979 (Morocco), Larsen & Laegaard 1971 (Sicily), Murín & Záborský 1976 (Czechoslovakia),Turała 1969 (Poland)

TABLE 2. Diagnostic characters of central-European species of *Ranunculus* sect. *Batrachium*, based on a combination of the most relevant taxonomic studies (e.g. Cook 1966, Englmaier 2016,Wiegleb et al. 2017) and our observations. The most useful diagnostic characters are in bold. The length of capillary leaves refers to leaves in the middle part of generative shoots. To determine the hairiness of fruits it is necessary to examine young achenes and the occurrence of laminar leaves has to be studied in adult (flowering or fruiting) plants.

Taxon	Capillary leaves	Laminar leaves	Peduncles	Corolla length	Nectar pits	Receptacles	Achenes	Other characters
R. aquatilis	mostly flaccid, densely branched, 2–6 cm long, usually shorter than adjacent internode	sometimes present; with 3–5 primary lobes, dissected up to 2/3 of the lamina or deeper (or even divided into leaflets), **secondary lobes 17–26,** margin dentate, **basal sinus of the lamina 0–90°**	1–6 cm, straight to recurved	4–10 mm	circular	hairy	ca 20–50, hairy	
R. baudotii	somewhat rigid, densely branched, 1–10 cm long, shorter or as long as adjacent internode	sometimes present; **mostly with 3 primary lobes**, dissected up to 2/3 of the lamina or deeper (or even divided into leaflets), secondary lobes 8–14, margin acutely crenate to dentate, basal sinus of the lamina 90–180°	3–18 cm, **strongly recurved in fruit**	4–10 mm	variable, lunate to pyriform, often cup-shaped	sometimes elongating in fruit, hairy	ca 25–60, glabrous to sparsely hairy, **often narrowly winged when dry**	plants pale green with whitish, often fleshy stems
R. circinatus	**rigid,** markedly shorter than the adjacent internode, circular to semicircular, 1–3 cm long, **segments lying in one plane**	absent	2–10 cm, straight to slightly recurved	6–10 mm	lunate	hairy	ca 20–50, hairy	
R. fluitans (both 2x and 3x)	**rigid, sparsely branched (only 2–4 divisions), 8–40 cm long, usually longer than the adjacent internode**	absent	4–10 cm, straight to slightly curved	7–15 mm	pyriform	**glabrous to sparsely hairy**	**mostly undeveloped**	**stems very long (up to 6 m); petals often more than 5 (up to 13)**

Taxon	Capillary leaves	Laminar leaves	Peduncles	Corolla length	Nectar pits	Receptacles	Achenes	Other characters
R. peltatus	mostly flaccid, densely branched, 3–8 cm long, usually shorter than adjacent internode	usually present; with 3–5 primary lobes, dissected up to 1/3 to 2/3 of the lamina, secondary lobes 7–15, **margin most often obtusely crenate**, basal sinus of the lamina 45–180°	3.5–10 cm, straight to slightly curved	8–15 mm	pyriform	hairy	ca 20–50, hairy	
R. penicillatus (cytotypes A–F)	mostly flaccid, densely branched (4–8 divisions), **5–20 cm long, usually longer than the adjacent internode**	only present in some populations of the cytotype A; very variable, with 3–5 primary lobes, dissected up to 1/2 of the lamina or deeper, secondary lobes 5–11, margin almost entire to dentate, basal sinus of the lamina 90–180°	5–20 cm, straight to slightly curved	8–15 mm	variable, most often pyriform	hairy	ca 20–50, hairy	**stems very long (up to 5 m)**
R. rionii	mostly flaccid, densely branched, 1–3 cm long, shorter than adjacent internode	absent	1–3 cm, recurved	2–5 mm	lunate, rarely pyriform	hairy	**ca 30–80, glabrous**	
R. trichophyllus A	flaccid, sparsely to densely branched, 1–3 cm long, shorter than adjacent internode, segments fine	absent	1–3 cm, recurved	2–4 mm, rarely cleistogamous	lunate	hairy	ca 10–30, hairy	often flowering underwater
R. trichophyllus B	mostly flaccid, sparsely to densely branched, 1–5 cm long, shorter than adjacent internode	absent	1.5–5 cm, recurved	3.5–7 mm, rarely cleistogamous	lunate	hairy	ca 15–40, hairy	often flowering underwater
R. trichophyllus subsp. *eradicatus*	flaccid, sparsely branched, 1–3 cm long, shorter than adjacent internode, segments extremely fine	absent	1–3 cm, recurved	2–4, but **flowers mostly cleistogamous**	lunate	hairy	ca 5–15, hairy	delicate plants with **creeping shoots rooting at nodes**, mostly flowering underwater

of *R. trichophyllus* (Table 1). In northern Europe, *R. trichophyllus* is reported to be represented by an hexaploid cytotype that freely hybridizes with hexaploid *R. aquatilis* and the subsequent introgressive hybridization blurs the boundaries between these species (Hong 1991). In contrast, central-European *R. trichophyllus* populations are predominantly tetraploid, which raises questions about their potential to hybridize with *R. aquatilis*.

Despite this group showing notable morphological and ecological diversity as a result of various evolutionary processes, only a few biosystematic studies have included *Batrachium* since Cook's comprehensive monograph (Cook 1966). Recent molecular studies of European water-crowfoots are mostly confined to small regions and only three studies employed molecular markers to analyse genetic variation of more than two species (British plants by Telford et al. 2011, Polish plants by Zalewska-Gałosz et al. 2014 and north-European plants by Bobrov et al. 2015, all of them using a combination of cpDNA and the ITS region). Despite their limited scope, all three studies reveal substantial genetic variation in the morphologically strongly reduced species *R. trichophyllus*. Phylogenetic studies that focus on the entire genus *Ranunculus* include only a few samples of *Batrachium* (e.g. Hörandl et al. 2005, Emadzade et al. 2010, Hörandl & Emadzade 2012). The reticulate evolution of *Batrachium*, however, makes research on its phylogeny very difficult (Hörandl & Emadzade 2012, Bobrov et al. 2015).

Because of a high degree of phenotypic plasticity and probably underestimated occurrence of hybrids, various taxonomic concepts are adopted across Europe (e.g. Cook 1966, Cook et al. 1986, Husák et al. 1988, Pizzaro 1995, Dahlgren & Jonsell 2001, Englmaier 2016, Wiegleb et al. 2017). In an attempt to change this disappointing state of knowledge, we decided to analyse the variation in ploidy level and genome size in an extensive set of *Batrachium* populations using flow cytometry (FCM), which (i) is an efficient tool for analysing a large number of individuals rapidly, allowing to reveal the structure of populations and to detect rare cytotypes and hybrids, (ii) is frequently used in studies of evolutionarily and taxonomically intricate groups of plants, including polyploid and hybridogenous complexes (e.g. Suda et al. 2010, Trávníček et al. 2011a, b, 2012, Chumová et al. 2015, Flatscher et al. 2015, Lepší et al. 2015, Kobrlová et al. 2016, Vít et al. 2016, Bressler et al. 2017, Feulner et al. 2017) and homoploid plants (Loureiro et al. 2010, Prančl et al. 2014), (iii) has been successfully used as a basic method for taxonomic determination and delimitation of other complex aquatic taxa, such as

Callitriche (Prančl et al. 2014) and *Nymphaea* (Volkova et al. 2010, Kabátová et al. 2014), and (iv) has never been used to investigate *Batrachium*, with the exception of a single *R. trichophyllus* population (Hidalgo et al. 2015).

We used flow cytometry combined with chromosome counting to improve our understanding and identification of water-crowfoots in central Europe. Specifically, we addressed the following questions: (i) Is the genome size a suitable marker for reliable determination of *Batrachium* taxa? (ii) What are the patterns in cytotype and ploidy level variation in the area studied? Does the variation in genome size correlate with current taxonomic concepts? (iii) What is the frequency of interspecific hybridization? (iv) Is there any evidence of cryptic variation within morphologically recognized species?

MATERIALS AND METHODS

Field sampling

Plant material was collected in Austria (38 localities), Czech Republic (495 localities), Denmark (18 localities), Germany (16 localities), Hungary (13 localities) and Slovakia (17 localities), additional individual samples were obtained from Lithuania, Moldova, Poland, Romania, Slovenia, Switzerland and United Kingdom (in total 15 localities). The sampling was carried out to include all eight central-European species from the widest possible range of aquatic habitats and covering the whole range of morphological variation. If possible, multiple individuals were collected from each population. The number of individuals per population depended on population size and the extent of observed phenotypic variation. In total, 3354 individuals from 612 localities were obtained (for locality details, see Electronic Appendix 1). Voucher specimens are preserved in the herbaria of Charles University in Prague (acronym PRC), Faculty of Science of the University of South Bohemia in České Budějovice (CBFS) and National Museum in Prague (PR).

Morphological identification

We included only well-developed plants that were identified based on a combination of the most relevant taxonomic studies and our field experience (see Table 2 for diagnostic characters). The accepted taxonomic concept mostly follows Cook (1966). In the case of *Ranunculus penicillatus*, we did not adopt any intraspecific units because the observed variation is too complex and did not fully correspond to any of the currently used names (see below). Our tentative determinations were then compared with the genome sizes obtained from the FCM analysis. Plants that could not be identified unambiguously were cultivated in garden tanks in order to obtain the optimal morphological stage of development for their reliable determination.

Flow cytometry

Genome size was estimated using flow cytometry. If multiple individuals were collected from a population, they were first screened for genome size homogeneity using bulked samples of up to five individuals in a single run. Subsequently, selected individuals (usually three individuals per each morphological species and cytotype within a population, and all individuals that had divergent genome sizes; in total 1707 individuals) were analysed individually. The number of individuals measured from each population is summarized in Electronic Appendix 1.

The sample preparation followed the simplified two-step procedure described by Doležel et al. (2007). About 0.25 cm^2 of leaf tissue (one individual or a mixture from several individuals) was chopped together with an appropriate volume of the internal standard using a sharp razor blade in a Petri dish containing 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20). *Bellis perennis* L. was selected as a primary reference standard, as it has a similar but non-overlapping genome size with all the samples studied. The crude suspension was filtered through a 42-μm nylon mesh and incubated for about 5 min at room temperature. After incubation, isolated nuclei were stained with 1 ml of Otto II buffer (0.4 M $Na_2HPO_4.12H_2O$) supplemented with 2-mercaptoethanol (2 μg/ml) and either 4,6-diamidino-2-phenylindole (DAPI) (4 μg/ml) or propidium iodide (PI) and RNase IIA (both 50 μg/ml). Samples were run on the flow cytometer after about one minute of staining. The DAPI-stained samples were analysed using a Partec PA II flow cytometer (Partec GmbH, Münster, Germany, now Sysmex) equipped with a mercury arc lamp as the UV light excitation source. The fluorescence intensity of 3000–5000 particles was recorded. The PI-stained samples were analysed using a Partec CyFlow instrument equipped with a green diode-pumped solid-state laser (Cobolt Samba, 532 nm, 150 mW output power) and the fluorescence intensity of 5000 particles was recorded. Selected samples for precise genome size measurement were analysed with propidium iodide and the analyses repeated 2–3 times on different days to account for random measurement error; if the range of variation of the repeated measurements exceeded the 2% threshold, the outlying value was discarded and the sample reanalysed. Histograms were evaluated using FloMax software, ver. 2.4d (Partec GmbH) or FlowJo 10 (TreeStar Inc.). In total, exact genome size was estimated for 1032 individuals, for which repeated analyses of appropriate quality were available.

The genome size was expressed as the ratio of the mean fluorescence of the sample and the internal standard. For PI-stained samples, the genome size in absolute units was calculated based on the genome size of *Bellis perennis* (2C = 3.38 pg, Schönswetter et al. 2007). Basic statistics (mean, standard error, standard deviation and variation range) were calculated. The extent of the total variation in intraspecific genome size was calculated as a percentage of the difference between the highest and lowest genome size value and expressed as % of the minimum. Subsequently, 1C values, and for taxa with known chromosome counts, also 1Cx-values were derived from the mean 2C values. For the taxa having overlapping genome sizes, these were compared using analysis of variance; genome sizes were log10-transformed and TukeyHSD test was used for multiple comparisons if more than 2 groups were present. All these statistical analyses were performed in R, version 3.4.3 (R Development Core Team 2017).

Chromosome counts

Attempts were made to count the number of chromosomes for at least one sample of each cytotype detected using flow cytometry. Selected plants were cultivated in a garden tank until they formed adventive roots on their stems, which were used for counting chromosomes. Alternatively, when suitable adventive roots were not available, chromosomes were counted using roots of seedlings. First, the ripe achenes were obtained from plants analysed using flow cytometry. The achenes were then placed on moistened filter paper in Petri dishes at room temperature to induce germination. If they germinated, the youngest primary roots about 2 mm long were collected (usually 6 days after germination).

The roots were pre-treated in a saturated water solution of p-dichlorobenzene at room temperature for approximately three hours, then fixed in a freshly prepared 3:1 mixture of 96% ethanol and acetic acid and stored at –20°C until further processing. Before chromosome preparation, the material was macerated in a 1:1 mixture of ethanol and hydrochloric acid for

10 s, then transferred onto a microscope slide, non-meristematic tissues were removed, and the meristem stained in a drop of lacto-propionic orcein, covered with a coverslip and squashed. The preparations were examined under a Carl Zeiss Jena NU microscope equipped with an Olympus E – 510 Digital SRL Camera or an Olympus BX 51 microscope equipped with DP-71 Olympus digital camera with the DP Controller imaging software 3.1 (Olympus Corp.). Only the slides, on which at least 5 mitoses were found, were considered. Altogether, chromosome numbers were determined for 37 individuals of 21 cytotypes. For the remaining cytotypes recognized using flow cytometry, the chromosome counting was unsuccessful or we did not have usable material (living plants or seeds).

Niche differentiation

Environmental parameters based on climatic data from the WorldClim database (Hijmans et al. 2005; http://www.worldclim.org) were assessed to elucidate the degree of ecological divergence among morphologically similar but ecologically different couples *R. aquatilis* – *R. peltatus* and *R. trichophyllus* A – *R. trichophyllus* B. Spatial stratification, conducted via spThin R script (Aiello-Lammens et al. 2014), was used prior to the analysis to eliminate unequal sampling across the area. Minimum spatial threshold for sample elimination was set to 20 km and consequently 67 georeferenced locations of *R. peltatus*, 20 of *R. aquatilis*, 32 of *R. trichophyllus* A and 29 of *R. trichophyllus* B were included in the analysis. For these locations we extracted elevation (see Electronic Appendix 1) and data from a database of 19 bioclimatic variables representing annual trends, seasonality, and extreme and limiting environmental factors (Electronic Appendix 2). Data from raster layers for each of the 19 bioclimatic variables were extracted using the *extract* function in the raster package in R (Hijmans et al. 2016).

First, principal trends in the variation in bioclimatic variables were detected by principal component analysis. Next, all variables were used for linear discriminant analysis. The analyses were performed separately for each of above-mentioned pairs of taxa. All analyses were done using the MorphoTools R scripts for multivariate data handling (Koutecký 2015).

RESULTS

Cytotype diversity and chromosome counts of traditionally recognized species

Most of the traditionally recognized central-European species are well-defined in terms of mean genome size and ploidy level (Tables 3, 4, Figs 1, 2). About 85% of the individuals can be attributed to dominant cytotypes of the morphological species and the remaining ~15% to hybrids and minority cytotypes (note that we use the term "cytotype" for each taxonomic entity defined by a specific range of genome sizes, which reflect differences in chromosome number, structure or size, as well as for each entity defined unequivocally by the combination of specific genome size and morphology). The detected 2C-values varied 3.59-fold from 3.85 pg in diploid *R. fluitans* up to 13.84 pg in hexaploid *R. penicillatus* F (Fig 1A). Monoploid genome sizes (1Cx-values) were also highly variable, ranging from 1.63 pg in a hybrid cytotype from the upper Vltava river (see below) to 2.83 pg in *R. circinatus* (Fig. 1B).

No substantial variation in genome size was revealed in *R. aquatilis* (hexaploid), *R. baudotii* (tetraploid), *R. circinatus* and *R. rionii* (both diploid). In *R. fluitans*, diploid and triploid populations were detected, with diploids being more frequent in our dataset (52 and 15, respectively). Both cytotypes share almost identical monoploid genome size. In all other taxa, we revealed several cytotypes of different ploidy levels or different genome sizes within a ploidy level, or both.

In *R. peltatus*, all populations studied consist of one dominant cytotype, which is tetraploid. However, aberrant plants with the appearance of *R. peltatus* were rarely detected among ordinary tetraploids, which had a genome size corresponding to the putative pentaploids (three individuals in populations K10-06 and K15-19) or hexaploids (one individual in population B15-053). Unfortunately, we were not able to detect the true ploidy level by direct chromosome counting in these extremely rare individuals.

Two distinct cytotypes, both tetraploid, were recognized within *R. trichophyllus*. Their mean genome sizes differ by 11.0% (Fig. 3A) and henceforth we tentatively designate them as *R. trichophyllus* A and *R. trichophyllus* B (with smaller and bigger genome sizes, respectively). The cytotype *R. trichophyllus* B is somewhat more robust in overall morphology and appears to be ± intermediate between *R. trichophyllus* A and hexaploid *R. aquatilis* (Table 2). However, *R. trichophyllus* B is intermediate neither in ploidy nor in genome size. Both cytotypes of *R. trichophyllus* are fully fertile, with most of the achenes developing normally. The variation within *R. trichophyllus* may be even more extensive. Genome size of plants from oligotrophic alpine lakes, which correspond to the traditionally recognized *R. trichophyllus* subsp. *eradicatus* (Table 2), overlaps the lower half of the genome size range of *R. trichophyllus* A. Due to distinct ecologies and minute but constant morphological differences we treat these plants as a third group within *R. trichophyllus*. Furthermore, the *R. trichophyllus* B sample with the largest genome size (B14-119, Hallstättersee, Austria) is distinct from other samples of this cytotype (bifurcated peaks in simultaneous analyses; Fig. 3B).

Extraordinary variation was revealed within *R. penicillatus*, in which the genome size spans from 7.27 to 13.84 pg and six distinct cytotypes were detected (referred to as *R. penicillatus* A–F). Three of these cytotypes, including the most frequently occurring, were found to be tetraploids, whereas the single population with the largest genome size among all *Batrachium* taxa was hexaploid. Unfortunately, the chromosome counting of the remaining cytotypes failed as the preparations contained only mitotically inactive cells or the observations were inconclusive.

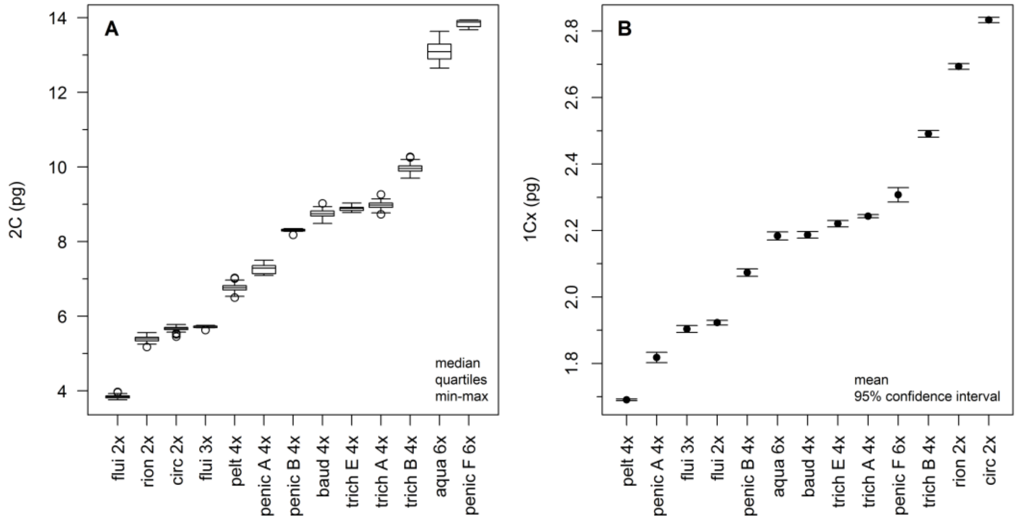

FIG. 1. Box-and-whisker plots showing the genome size variation in 13 cytotypes of sect. *Batrachium*, for which the chromosome numbers were determined: **(A)** holoploid genome sizes (2C-values); **(B)** monoploid genome sizes (1Cx-values). Species abbreviations: aqua – *R. aquatilis*; baud – *R. baudotii*; circ – *R. circinatus*; flui – *R. fluitans*; pelt – *R. peltatus*; penic – *R. penicillatus* (cytotypes A, B and F); rion – *R. rionii*; trich – *R. trichophyllus* (cytotypes A and B; E = subsp. *eradicatus*). After cytotype abbreviations, ploidy levels are indicated.

TABLE 3. Summary of flow cytometric genome size estimations (propidium iodide staining). ***N*** – number of individuals; note that for precision only individuals measured repeatedly on different days are included. ***Ratio*** – mean ratio with the internal standard *Bellis perennis*. ***2C*** – mean genome size (2C value) in pg of DNA. SE – standard error of mean (pg of DNA). ***Min, Max, % var*** – minimum and maximum 2C values and the difference between them expressed as % of the minimum. ***Ploidy*** – ploidy level; in some hybrids the ploidy level is not supported by chromosome counts and is only inferred from ploidy levels of the parental species (marked by *). ***1Cx*** – monoploid genome size in pg of DNA calculated from the mean 2C value and the ploidy level.

Taxon / Cytotype	N	Ratio	2C	SE	Min	Max	% var	Ploidy	1Cx
R. aquatilis	57	3.88	13.1	0.03	12.65	13.63	7.8	6x	2.18
R. baudotii	35	2.59	8.75	0.02	8.49	9.02	6.3	4x	2.19
R. circinatus	61	1.68	5.67	0.01	5.46	5.78	6	2x	2.83
R. fluitans (2x)	56	1.14	3.85	0.01	3.76	3.97	5.6	2x	1.92
(3x)	8	1.69	5.71	0.01	5.63	5.76	2.3	3x	1.9
R. peltatus	307	2.01	6.76	0.01	6.5	7.03	8.2	4x	1.69
(putative 5x)	3	2.51	8.47	0.08	8.31	8.54	2.8	?	
(putative 6x)	1	3	10.1					?	
(aneuploid)	2	1.92	6.48	0.11	6.38	6.59	3.3	?	
R. penicillatus A	18	2.15	7.27	0.03	7.09	7.5	5.8	4x	1.82
B	7	2.45	8.29	0.02	8.18	8.35	2	4x	2.07
C	1	2.68	9.06					?	
D	1	2.85	9.63					4x	2.41
E	1	3.21	10.9					?	
F	4	4.1	13.8	0.06	13.68	13.94	1.9	6x	2.31
R. rionii	86	1.6	5.39	0.01	5.18	5.56	7.4	2x	2.69
R. trichophyllus A	115	2.66	8.97	0.01	8.73	9.26	6.1	4x	2.24
B	47	2.95	9.96	0.02	9.7	10.28	5.9	4x	2.49
subsp. *eradicatus*	18	2.63	8.88	0.02	8.78	9.03	2.9	4x	2.22
Hybrids									
R. aquatilis × *R. peltatus* (F_1)	10	2.96	9.98	0.04	9.85	10.3	4.5	5x	2.01
R. aquatilis × *R. peltatus* (hybrid 1)	1	2.15	7.27					?	
(hybrid 2)	1	3.21	10.8					?	
R. aquatilis × *R. trichophyllus* B (F_1)	2	3.39	11.5	0.04	11.43	11.5	0.6	5x	2.29
R. circinatus × *R. rionii* (F_1)	1	1.63	5.52					2x*	2.76
R. circinatus × *R. trichophyllus* A (F_1)	8	2.17	7.34	0.04	7.26	7.57	4.3	3x	2.45
R. peltatus hybr.	54	2.01	6.79	0.01	6.62	7.18	8.4	?	
R. peltatus hybrid (Váh river)	4	2.68	9.07	0.02	9.02	9.1	0.9	?	
R. peltatus hybrid (Vltava river)	8	2.89	9.78	0.05	9.6	9.99	4.1	6x	1.63
R. peltatus-penicillatus A	56	2.05	6.93	0.02	6.66	7.16	7.5	4x	1.73
R. peltatus × *R. trichophyllus* A (F_1)	31	2.33	7.88	0.01	7.71	8.05	4.5	4x	1.97
R. peltatus × *R. trichophyllus* A (other hybrids)	23	2.25	7.6	0.1	7.02	8.52	21.4	4x*	1.9
R. penicillatus F × *R. trichophyllus* B (F_1)	1	3.54	12					5x*	2.4
R. penicillatus F (F_1 hybrid?)	1	2.06	6.97					3x*	2.32
R. rionii × *R. trichophyllus* A (F_1)	1	2.14	7.23					3x*	2.41
R. trichophyllus hybr.?	2	2.9	9.79	0.25	9.54	10.04	5.2	?	

TABLE 4. Chromosome numbers of 21 water-crowfoot cytotypes recorded in this study, sorted according toploidy level. For locality details, see Electronic Appendix 1.

Taxon	Ref. no.	Locality	Ploidy	2n
R. circinatus	B14-043	Czech Republic, Tvrdonice: Hnátkovská jezera pools	2x	16
R. fluitans	B14-036	Czech Republic, Lindava: Svitavka stream	2x	16
R. rionii	B11-004	Czech Republic, Chudíř: Nový fishpond	2x	16
R. rionii	B12-008	Czech Republic, Mariánské Radčice: pool in mining area	2x	16
R. circinatus × *R. trichophyllus* A (F_1)	B14-118	Austria, Hallstatt: Hallstättersee lake	3x	24
R. fluitans	B12-006	Czech Republic, Praha: Vltava river	3x	24
R. baudotii	B14-038	Austria, Sankt Andrä am Zicksee: Zicksee lake	4x	32
R. baudotii	B14-073	Czech Republic, Buškovice: fish storage ponds	4x	32
R. baudotii	B14-049	Czech Republic, Hořátev: Hliňovka fishpond	4x	32
R. baudotii	B13-010	Czech Republic: Pečky, Výrovka stream	4x	32
R. baudotii	B12-048	Denmark, Ormslev: Aarhuså stream	4x	32
R. baudotii	B12-057	Denmark, Rønge: Gudenå river	4x	32
R. peltatus	K15-19	Czech Republic, Horní Poříčí: unnamed fishpond	4x	32
R. peltatus	B12-009	Czech Republic, Řevničov: Horní Kracle fishpond	4x	32
R. peltatus	B12-045	Denmark, Ribe: artificial pool in town	4x	32
R. peltatus	K11-15	Czech Republic, Majdalena: Cep sand pit	4x	32
R. peltatus hybrid	B12-060	Denmark, Tarm: Sonderå river	4x	32
R. peltatus × *R. trichophyllus* A (F_1)	B13-037	Czech Republic, Švařec: Svratka river	4x	32
R. peltatus × *R. trichophyllus* A (F_1)	K13-23d	Czech Republic, Vlkov: Vlkovská pískovna sand pit	4x	32
R. peltatus-penicillatus A	B15-039	Czech Republic, Okounov: Ohře river	4x	32
R. penicillatus A	B14-031	Czech Republic, Mimoň: Panenský stream	4x	32
R. penicillatus B	B15-043	Czech Republic, Vysoké Mýto: Loučná river	4x	32
R. penicillatus D	B12-050	Denmark, Skivum: Sønderup Å stream	4x	32
R. trichophyllus A	B12-010	Czech Republic, Březina: Oběšenec fishpond	4x	32
R. trichophyllus A	B14-035	Czech Republic, Krásná Lípa: unnamed fishpond	4x	32
R. trichophyllus B	B14-122	Austria, Grünau im Almtal: Almsee lake	4x	32
R. trichophyllus B	K15-15	Austria, Mattighofen: Kühbach brook	4x	32
R. trichophyllus B	B12-005	Czech Republic, Litoměřice: unnamed pond	4x	32
R. trichophyllus B	K15-021	Czech Republic, Makarov: unnamed fishpond	4x	32
R. trichophyllus subsp. *eradicatus*	B14-121	Austria, Grünau im Almtal: Almsee lake	4x	32
R. aquatilis × *R. peltatus* (F_1)	B14-021	Czech Republic, Lanžhot: Bornova jama pool	5x	40
R. aquatilis × *R. trichophyllus* B (F_1)	B14-013	Czech Republic, Holasice: Ludmila pool	5x	40
R. aquatilis	B11-005	Czech Republic, Loučeň: Lutovník fishpond	6x	48
R. aquatilis	B13-034	Czech Republic, Hradec Králové: Na Plachtě pools	6x	48
R. aquatilis	B13-021	Slovakia, Malacky: Marhecké rybníky fishponds	6x	48
R. peltatus hybrid (Vltava river)	K15-17	Czech Republic, Bližní Lhota: Hamerský stream	6x	ca 48
R. penicillatus F	B15-020	Austria, Bogenhofen: unnamed stream	6x	ca 48

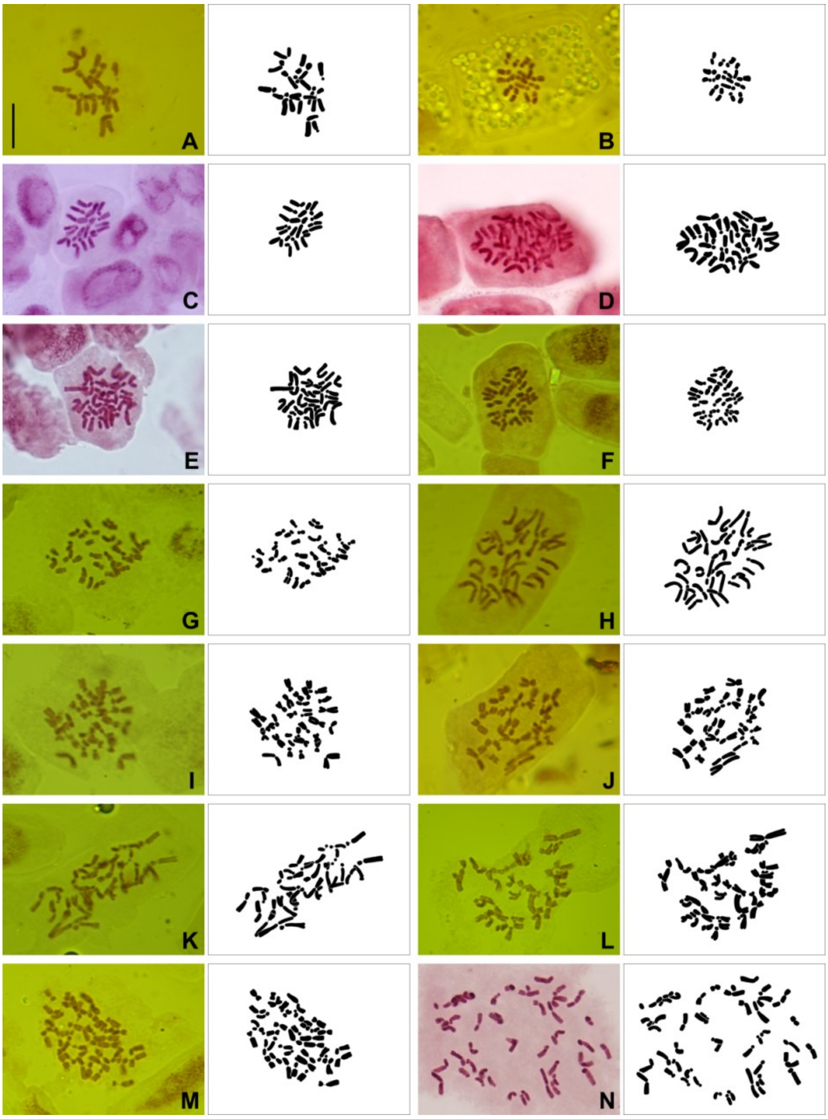

FIG. 2. Chromosomes (photograph of cytological preparation on the left with its interpretation on the right) of selected cytotypes of *Ranunculus* sect. *Batrachium* at mitotic metaphase in somatic cells, arranged according to chromosome number: **A** – *Ranunculus circinatus*, sample B14-043, 2n = 16; **B** – *R. fluitans*, sample B14-036, 2n = 16; **C** – *R. rionii*, sample B12-008, 2n = 16; **D** – *R. baudotii*, sample B12-048, 2n = 32; **E** – *R. baudotii*, sample B14-073, 2n = 32; **F** – *R. peltatus*, sample B12-045, 2n = 32; **G** – *R. peltatus*, sample B12-009, 2n = 32; **H** – *R. penicillatus* B, sample B15-043, 2n = 32; **I** – *R. trichophyllus* B, sample B14-122, 2n = 32; **J** – *R. trichophyllus* A, sample B14-035, 2n = 32; **K** – *R. trichophyllus* A, sample B12-010, 2n = 32; **L** – *R. peltatus* × *R. trichophyllus* A, sample K13-23d, 2n = 32; **M** – *R. aquatilis* × *R. trichophyllus* B, sample B14-013, 2n = 40; **N** – *R. aquatilis*, sample B13-021, 2n = 48. Scale bar identical for all figures = 10 µm.

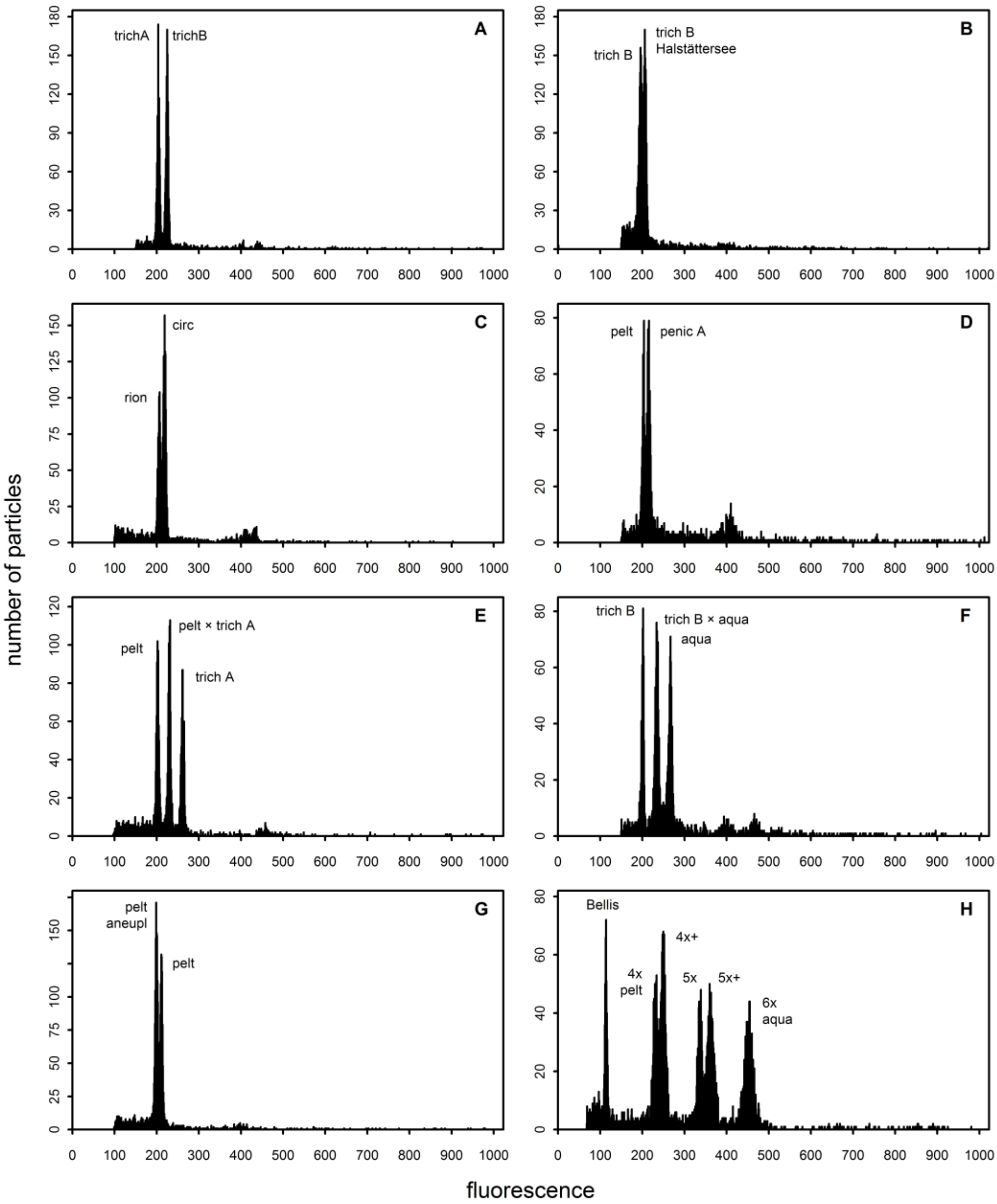

FIG. 3. Flow cytometric histograms showing simultaneous analyses of selected sect. *Batrachium* taxa: **A** – simultaneous analysis of *R. trichophyllus* A (sample B14-035) and *R. trichophyllus* B (sample B12-005) documenting 11.0% divergence between these tetraploid taxa; **B** – simultaneous analysis of two *R. trichophyllus* B accessions (samples B14-122, Almsee, AT, and B14-119, Hallstattersee, AT) resulting in a bifurcated peak; **C** – simultaneous analysis of two diploid species with similar genome sizes, *R. rionii* and *R. circinatus* (samples B14-082 and B14-087); **D** –

bifurcated peaks documenting the slight difference between the genome sizes of tetraploid taxa *R. peltatus* (sample B12-009) and *R. penicillatus* A (sample B14-031); **E** – simultaneous analysis of the tetraploid taxa *R. peltatus* (sample B14-075), *R. trichophyllus* A (sample B14-076) and their hybrid (sample B14-077) from the locality "Bražecke hliňaky", CZ; **F** – simultaneous analysis of tetraploid *R. trichophyllus* B (sample B14-011), hexaploid *R. aquatilis* (sample B14-010) and their pentaploid hybrid (sample
B14-013) from the pool "Ludmila", CZ; **G** – slightly different genome sizes of two individuals of *R. peltatus* (Javorensky fishpond, CZ; samples B14-080 and B14-079), i.e. the putative aneuploid with the smaller genome size and "typical" *R. peltatus*; **H** – simultaneous analysis of several individuals from "Choryňsky mokřad", CZ, resulting in five peaks, represented by *R. peltatus* (sample K13-18), putative backcross towards *R. peltatus* (sample K13-18-11), two individuals of the hybrid *R. peltatus* × *aquatilis* with slightly different genome sizes (sample K13-20) and *R. aquatilis* (sample K13-19), respectively. All analyses were performed using DAPI fluorescence dye, except for histogram H for which PI staining was used.

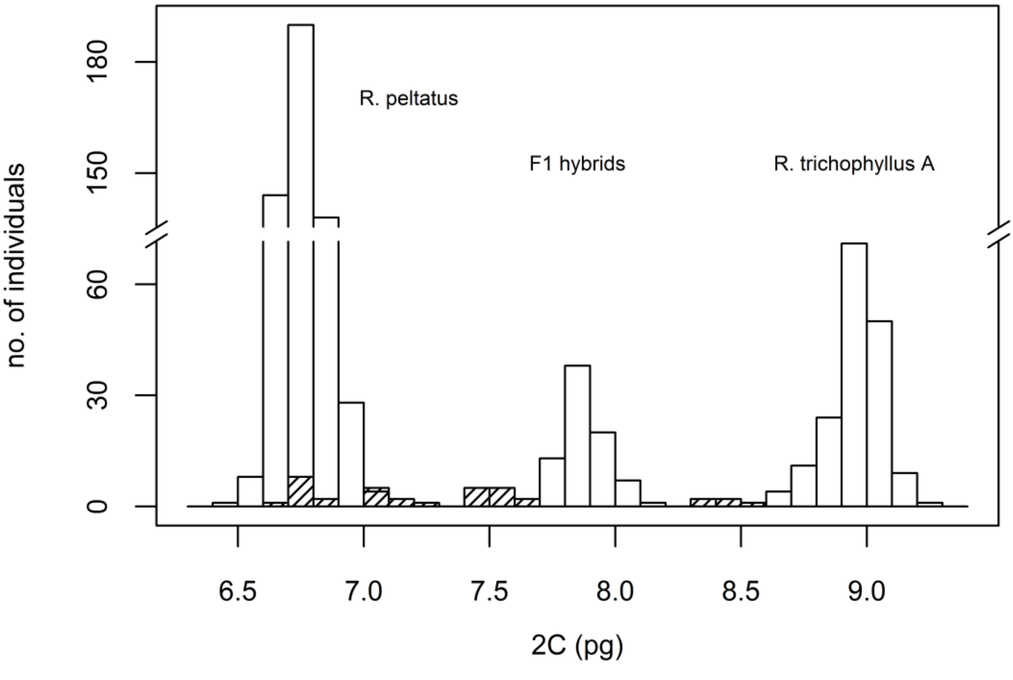

FIG 4. Histogram of the distribution of the genome sizes (2C value) of *Ranunculus peltatus*, *R. trichophyllus* A and all products of hybridization between these taxa: empty columns – individuals classified as *R. peltatus*, F1 hybrid *R. peltatus* × *trichophyllus* Aand *R. trichophyllus* A; hatched columns – various hybrids other than F1 determined as such based on morphology.

The majority of *Batrachium* species (including the lineages A and B of *R. trichophyllus* as separate groups and taking only the most widespread cytotype A of *R. penicillatus* into account) have distinct DNA contents (2C-values) with almost non-overlapping ranges (Figs 1A, 3C, D, Table 3). The only two exceptions showing a considerable overlap are (i) *R. circinatus* and triploid *R. fluitans* and (ii) *R. baudotii* and *R. trichophyllus* (the cytotype A and the alpine populations marked as subsp. *eradicatus*). However, even within these groups the differences are statistically significant ($P < 0.05$).

Hybridization

Besides the taxonomically pure species (some including several cytotypes), numerous putative hybrids were found based on a combination of morphology and genome size. These hybrids accounted for ~15% of the individuals analysed and were recorded in 14% of the populations analysed, making the overall pattern in the cytotype variation very complicated. At least 16 different hybrid cytotypes were revealed (Table 3). Five of these cytotypes were identified as F_1 hybrids, being morphologically ± intermediate, with genome sizes intermediate between the putative parents (Figs 3E, F) and usually being only partially fertile (only a small number of achenes develop normally, the rest of the carpels on receptacles is shrivelled). Among them, only *R. peltatus* × *R. trichophyllus* A (tetraploid, like its parental taxa) occurred relatively frequently, being found at 17 localities (at seven localities co-occurring with both parental species, four localities where *R. trichophyllus* A was absent and six localities where the hybrid occurred without any of the parental species). The remaining hybrid combinations, represented by triploid and pentaploid hybrids that resulted from heteroploid crosses, were rarely detected. We found two additional putative F_1 hybrids but do not have firm evidence for their unequivocal identification. A single plant (B16-036) with slightly reduced fertility, found within a mixed population of *R. circinatus* and *R. rionii*, was morphologically intermediate between these species. However, due to small difference in the parental 2C-values, it is not possible to distinguish the putative hybrid from individuals at the extremes of the variation of either parent. The second case was a single plant growing in a stand of *R. trichophyllus* A, which had a genome size intermediate between *R. trichophyllus* A and *R. rionii*, but the latter species was not detected at the locality. This young individual was in the initial stages of flowering and bore no fruits at that time, which prevented reliable morphological identification. In both cases, further analyses using molecular methods are needed.

Apart from F_1 hybrids, we recorded several plants of *R. peltatus* × *R. trichophyllus* A appearance that had various 2C-values ranging between that of *R. peltatus* and the F_1 hybrid *R. peltatus* × *R. trichophyllus* A (Fig. 4; referred to as "other hybrids" in Table 3 and Electronic Appendix 1). These plants often co-occurred with *R. peltatus* (whereas *R. trichophyllus* A was sometimes absent). Rarely morphologically hybrid populations were found with unexpected patterns of cytotype variation, such as individuals with a genome size larger than that of *R. trichophyllus* A (referred to as "*R. trichophyllus* hybr.?") and several putative aneuploid or hybrid plants of *R. peltatus* morphology, but with a genome size slightly smaller than that of *R. peltatus* (Fig. 3G; referred to as "*R. peltatus* aneuploid").

A more complex case of hybridization was detected also in one of the two explored populations of the pentaploid hybrid *R. aquatilis* × *R. peltatus* (K13-20, Choryňský mokřad marsh, Czech Republic). Genome size data indicate the occurrence of the parental species, F_1 hybrids and two other hybrid individuals with non-intermediate genome sizes (referred to as "hybrid 1" and "hybrid 2" in Table 3), as also revealed by the simultaneous flow cytometric analysis of selected plants that resulted in five separate peaks (Fig. 3H).

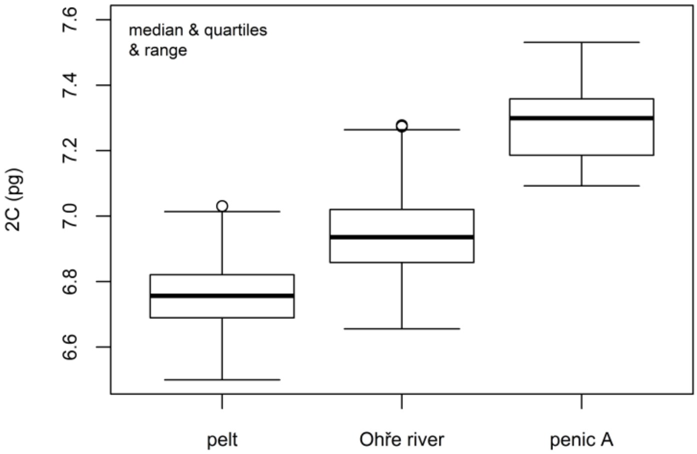

FIG. 5. Box-and-whisker plots showing the genome size variation of *Ranunculus peltatus*, *R. penicillatus* A and a putative hybrid swarm in the Ohře river and its tributary Odrava (referred as to "*R. peltatus-penicillatus* A"). Line, box and whiskers refer to median, quartiles and non-outlier range, respectively.

In the Ohře river and its tributaries (north-western Czech Republic), numerous abundant populations were found that showed a continuous variation in morphology between *R. peltatus* and *R. penicillatus* A (hereafter referred to as *R. peltatus-penicillatus* A). Compared to "pure" *R. peltatus* and *R. penicillatus* A sampled at other sites, genome sizes of the Ohře populations are transitional, with the entire range of values between these species (Fig. 5).

Finally, populations of morphologically strange infertile or only partly fertile plants were found in several rivers. These populations were not clearly attributable to any parental species, based on either morphology or genome size, although *R. peltatus* parentage may be expected in all cases. These includes plants from several rivers in Moravia, southern Bohemia (Czech Republic) and Upper Austria (e.g. Moravice, Lužnice, Stropnice, Malše / Maltsch, Große Mühl) whose genome size is virtually indistinguishable from that of *R. peltatus,* but which have a set of characters typical of hybrids and very unusual for *R. peltatus* (partial absence of laminar leaves when in flower, frequent formation of intermediate types of leaves or presence of deeply dissected laminar leaves with irregularly and sharply dentate margins, unusually long peduncles, small number of developed achenes); we further refer to them as "*R. peltatus* hybr.". A unique cytotype occurs in the Váh river (Slovakia) and another one in the upper course of the Vltava river (Czech Republic). Remarkably, the plants from the Vltava were found to be hexaploid and have the smallest monoploid genome size of all the taxa analysed.

Distribution and ecology

By far the most abundant taxon in our dataset was *R. peltatus*, which was recorded at 209 of the 612 localities, followed by *R. circinatus* (77 localities) and *R. trichophyllus* A (73 localities). Only two species (*R. fluitans* and *R. penicillatus*) are confined to running water, whereas *R. peltatus* and several hybrids regularly occur both in running and still water. The remaining species were detected rather rarely in running-water habitats or are absent from them.

Regarding species with several cytotypes, the distributions of particular cytotypes do not overlap much. In *R. fluitans*, the occurrence of both (2x, 3x) cytotypes was detected in just three rivers (Berounka, Sázava and Želivka rivers, Czech Republic) and in only a single population was there a mixture of cytotypes (K14-75; however, plants at this site included some unrooted individuals caught on the bank of the river, probably originating from upstream). Among a total of 115 *R. trichophyllus* sites, there were only two at which both cytotypes A and B co-occurred. In the Czech Republic, where the largest part of our samples was collected, both cytotypes show a distinct distribution pattern: whereas cytotypeBoccurs predominantly in warm areas atlow elevations, cytotypeA was recorded mostly at middle and rather high elevations and tended to absent in lowlands. The same pattern was observed also for the morphologically similar pair of species *R. aquatilis* and *R. peltatus* (the former at lower and the latter at higher elevations), which were found growing together at only four localities. The discriminant analyses of Bioclime data performed on these taxa indicate clear (although by far not complete) separation of both taxa couples on the basis of their fundamental bioclimatic characteristics (Fig. 6; Electronic Appendix 2).

However, several taxa often co-occurred in mixed populations. This is particularly true for the pair *R. peltatus* – *R. trichophyllus* A, which were found growing together at 26 localities (i.e., 35.6% of the localities of the latter).

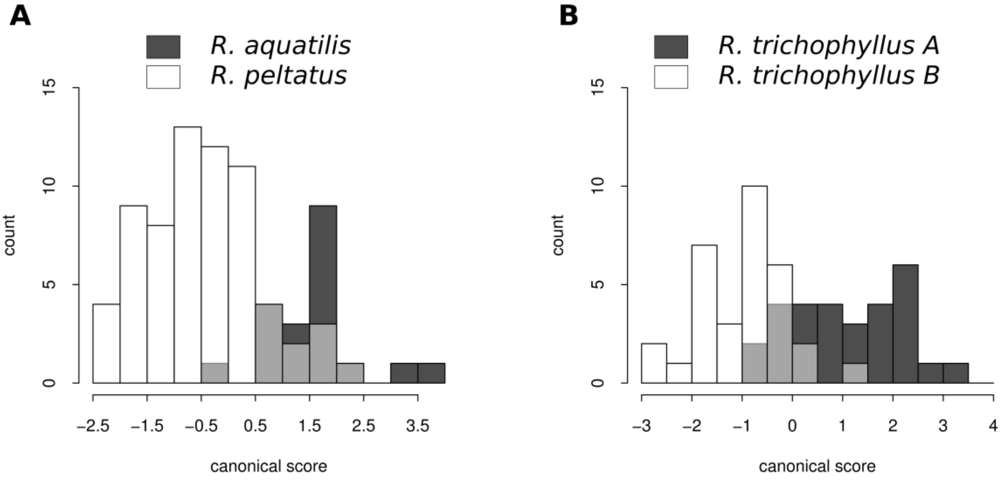

FIG. 6. Linear discriminant analysis of range-wide climatic niche, calculated for the taxa couples *Ranunculus aquatilis* – *R. peltatus* (**A**; 87 populations) and *R. trichophyllus* A – *R. trichophyllus* B (**B**; 61 populations). In classes where both groups are present the less frequent one is depicted in grey (“semitransparent”). The model is based on 19 environmental variables (for details, see Electronic Appendix 2).

DISCUSSION

Genome size as a tool for identification of traditionally recognized species of Batrachium

We proved that flow cytometry combined with morphology is a reliable, fast, inexpensive and easy tool for identifying central-European taxa of *Batrachium* (Table 3, Figs 1, 3). Even homoploid species are recognizable on the basis of their genome size. Only a few cases of genome size overlap were detected. However, the identification of *R. circinatus* and the triploid cytotype of *R. fluitans* is easy even using vegetative characters as they are probably the most distinctive species of *Batrachium* in Europe. Last but not least, these two species differ fundamentally ecologically and were never found co-occurring. The second group of taxa with overlapping genome sizes consists of *R. baudotii*, *R. trichophyllus* A and *R. trichophyllus* subsp. *eradicatus*. These taxa also differ ecologically and we have never found them growing together. While *R. baudotii* is a species of mineral-rich water in lowlands and is also characteristic of brackish water in coastal regions of Europe (Cook 1966), *R. trichophyllus* A seems to prefer more acidic habitats. As for *R. trichophyllus* subsp. *eradicatus*, it differs only slightly morphologically from *R. trichophyllus* A (Table 2) but is, however, distinguished also by its unusual habitat (oligotrophic alpine lakes).

Despite the large number of different chromosome numbers reported in the literature (Table 1), most of the central-European species of *Batrachium* are well-defined and uniform in ploidy level (Tables 3, 4). It seems likely that at least a part of the reported variation is due to misidentification of the counted material. Chromosome counting combined with genome size determination could eliminate most of this sort of confusion in the future.

Monoploid genome sizes and effect of polyploidy on the overall variation

Polyploidization is the main driver of sympatric speciation in plants (Otto & Whitton 2000). Differences in ploidy level often have significant effects on phenotypic and reproductive traits (Husband et al. 2013). Our study confirmed *Batrachium* as an extraordinarily remarkable group in terms of variation in ploidy levels and revealed a total of five ploidy levels within central-European representatives (2x, 3x, 4x, 5x, 6x; see Table 4, Fig. 2). Among them, pentaploid ($2n = 40$) counts were rarely recorded in hybrids, whereas other ploidy levels were recorded for both pure species and hybrids.

The biggest part of the variation in genome size was detected at the tetraploid level, both in terms of the number of taxa and variation attributable to hybridization.

In addition to the variation in ploidy level, we also detected notable variation within individual ploidy levels, which reflects the 1.69-fold variation in monoploid genome sizes (1Cx-values) (Table 3, Fig. 1B). This prevents genome size (2C-value) being straightforwardly used for estimating ploidy level. For example, diploid *R. circinatus* (2C = 5.67 pg of DNA) has nearly the same genome size as triploid *R. fluitans* (2C = 5.71 pg), and a hexaploid hybrid from the Vltava river (2C = 9.78 pg) has a smaller genome size than the tetraploid *R. trichophyllus* B (2C = 9.96 pg) and pentaploid hybrid *R. aquatilis* × *R. peltatus* (2C = 10.06 pg). An unambiguous ploidy determination, therefore, relies on chromosome counting. Such a situation is not rare and several other examples of ecologically different congeners with genome sizes being incongruent with ploidy levels are currently known (e.g. *Chenopodium*, Mandák et al. 2012; *Callitriche*, Prančl et al. 2014; *Anthoxanthum*, Chumová et al. 2015).

The monoploid genome size reflects evolutionary relationships in many groups of plants (e.g. Hohmann et al. 2014, Mandák et al. 2016, Krahulcová et al. 2017). Among species of water-crowfoots, the largest 1Cx-values are for the pair of diploid species, *R. circinatus* and *R. rionii*, which corresponds to their isolated phylogenetic position in the sect. *Batrachium* (Bobrov et al. 2015). Monoploid genome sizes may also reflect the origin of polyploids, i.e. monoploid genome sizes of the parents and the number of copies of their chromosome complements (e.g. Kúr et al. 2012, Mandák et al. 2012, Chumová et al. 2015). We assume an autopolyploid origin of the triploid *R. fluitans*, whose monoploid genome size is almost identical to that of the diploid cytotype of this species (1Cx = 1.92 pg and 1Cx = 1.90 pg, respectively). We also did not notice any obvious morphological differences between these cytotypes, which would be expected if the triploids were allopolyploid. The triploid cytotype may have originated by syngamy of reduced and unreduced gametes of a diploid or could be a descendant of hybridization between a diploid and hypothetical autotetraploid. Tetraploid *R. fluitans* is repeatedly reported from various regions in Europe including our area of interest (see Table 1). Nevertheless, we did not find any tetraploids during our fieldwork and the single report of tetraploid *R. fluitans* from the Czech Republic (Teplá river near the confluence with the Ohře river; Turała-Szybowska 1977) is questionable and may be an identification error because we have found only *R. peltatus* in the former river and *R. peltatus-R. penicillatus* A in the latter river.

Spontaneous formation of autopolyploids within populations of a lower ploidy level is not a rare phenomenon (e.g. Kolář et al. 2017) and is also recorded in aquatic plants (e.g. *Potamogeton*, Kaplan et al. 2013; *Callitriche*, Prančl et al. 2014). In this study, we recorded a single plant of *R. peltatus* (B15-053), growing within a population of tetraploids, whose genome size corresponded to that of a hexaploid (Table 3). This individual could have arisen by syngamy of reduced (2x) and unreduced (4x) gametes of *R. peltatus*. We recorded, however, several plants with the appearance of *R. peltatus*, whose genome size corresponded to putative pentaploids (three individuals in two populations: K10-06, K15-19; Electronic Appendix 1, Table 3) and the occasional occurrence of putative aneuploids. In these cases, there is no simple explanation of how these individuals might have arisen from tetraploid parental species. A further study employing molecular methods is in progress.

The most well-known case of allopolyploid species in *Batrachium* is the *R. penicillatus* complex, for which four ploidy levels are reported (Table 1). In this study, most populations morphologically assignable to *R.penicillatus* were identified as tetraploids (Tables 3, 4) and only a single population was hexaploid. The genome size of the most widespread cytotypeA is consistentwith its known allopolyploid origin from*R. fluitans*and *R. peltatus* (two chromosome sets from each; calculated 2C = 7.23 pg which is nearly identical to the recorded value of 7.27 pg). In the hexaploid *R. penicillatus* F (population K15-12, Bogenhofen, Austria), the genome size is consistent with the sum of the genome sizes of *R. fluitans* (two copies) and *R. trichophyllus* B (four copies): the calculated value is 13.81 pg, while the recorded value is 13.84 pg. The hexaploid *R. penicillatus* thus might be an allopolyploid that arose from these two species either via the union of two unreduced gametes or via a triploid F_1 hybrid that polyploidized. Indeed, *R. trichophyllus* B still occurs at the site and one individual there had a genome size intermediate between the putative parents (i.e., half of the genome size of *R. penicillatus* F), which might be a F_1 hybrid. However, all these hypotheses have to be confirmed by the ongoing molecular study.

Cryptic variation

Cryptic variation in organisms is genetic variation that is not reflected in morphology but may be an fundamental source of physiological and evolutionary potential (Bickford et al. 2007, Paaby & Rockman 2014). Water-crowfoots have a unique potential for investigating patterns of cryptic diversity, which share a number of evolutionary phenomena that can blur the morphological borders among biological taxa. Indeed, we found cryptic variation in two evolutionarily distinct lineages: *R. trichophyllus* and *R. penicillatus*.

The morphologically strongly reduced species *R. trichophyllus* in our data set includes three tetraploid lineages: ecologically highly specialized alpine plants (subsp. *eradicatus*, sometimes recognized at the species level as *R. confervoides*) and two non-alpine cytotypes A and B, which differ significantly in their genome size. These two cytotypes also differ ecologically (see above) and we have not revealed any signs of hybridization between them. However, the cryptic variation in the *R. trichophyllus* group may be even more complex. Recent molecular studies revealed four haplotypes and five ribotypes within this species, some of them seemingly unrelated (Zalewska-Gałosz et al. 2014, Bobrov et al. 2015). In addition, a sample of *R. trichophyllus* B from low elevations in the Alps (B14-119) had a markedly larger genome size than other samples assigned to this cytotype, which raises doubts about their taxonomic homogeneity. The variation in *R. trichophyllus* in peri-alpine rivers and lakes, which were only marginally covered by our sampling, and elsewhere outside central Europe, thus, needs further study.

On the other hand, we have not confirmed the reported presence (see Table 1) of the hexaploid *R. trichophyllus* in central Europe. During the fieldwork, however, we recorded extensive morphological variation in several hexaploid populations (e.g. B13085, K14-15) that contained both typical *R. aquatilis* plants with relatively big flowers and laminar leaves, small-flowered homophyllous *R. trichophyllus*-like individuals and various intermediates. We consider these plants as extremes of the variation in *R. aquatilis* instead of two putative taxa and hybrids, because (1) we never recorded *R. trichophyllus*like hexaploids forming populations on their own, and (2) the genome size of these plants are consistent with a hexaploid derived from *R. trichophyllus* A and is much smaller than that of a hexaploid based on *R. trichophyllus* B but the habitat conditions at these sites (lowlands, mineral-rich water) match only that of *R. trichophyllus* B while

R. trichophyllus A has never been found in such a habitat. Indeed, frequent occurrence of only homophyllous plants of *R. aquatilis* is known (e.g. Cook 1966, Englmaier 2016, Wiegleb et al. 2017). Therefore, it is possible that reports of hexaploid *R. trichophyllus* in central Europe are based on misidentification of atypical *R. aquatilis*.

Another group, in which we recorded cryptic variation, is the *R. penicillatus* complex. In most of Europe, two taxa of *R. penicillatus* s. lat. are traditionally recognized and classified either as the distinct species *R. penicillatus* s. str. and *R. pseudofluitans* (e.g. Englmaier 2016, Wiegleb et al. 2017) or at the subspecies level (e.g. Webster 1988, Dahlgren 1993). The former is defined as a heterophyllous taxon, believed to have originated by hybridization between *R. fluitans* and *R. peltatus*, while the latter is characterized by the absence of laminar leaves and *R. fluitans* along with *R. aquatilis* or *R. trichophyllus* are considered to be its putative parental species (Cook 1966). In this study we found that the actual situation is considerably more complicated. We revealed a total of six cytotypes, including at least two ploidy levels (Tables 3, 4). In central Europe, we detected two tetraploid and one hexaploid cytotype but only tetraploid cytotype A is widespread while other tetraploids are confined to two rivers (cytotype B: Loučná river, Czech Republic; Pisz river, Poland) and the hexaploid (cytotype F) is found only at a single locality (Bogenhofen, Austria). Within the widespread *R. penicillatus* A, populations with heterophyllous individuals prevailed. However, both heterophyllous and homophyllous plants of this cytotype were often found growing together and in some rivers only homophyllous plants occurred (e.g., Opava river, Czech Republic; Freiberger Mulde and Zschopau rivers, Germany). Thus, presence/absence of laminar leaves seems to be a feature of lower taxonomic value than was previously assumed and the current taxonomic concept of *R. penicillatus* s. lat. does not correlate with the actual diversity, which remains largely unrecognized.

As previously reported, it is often difficult to distinguish stabilized hybridogenous taxa of *R. penicillatus* s. lat. from recent hybrids of *R. fluitans* with other species (Cook 1966, Zalewska-Gałosz et al. 2014). During our study, we did not record any populations with the appearance of *R. penicillatus* with markedly reduced fertility. Therefore, we include all these populations under *R. penicillatus*.

Interspecific hybridization

In aquatic plants, newly formed interspecific hybrids often persist by vegetative reproduction for a long time (even after one or both parental species have disappeared from a site) and may also spread to other sites and become an important part of the overall biodiversity (e.g. Moody & Les 2007, Kaplan et al. 2009, Prančl et al. 2014). Morphological reduction of many aquatic plants combined with extensive phenotypic plasticity result in (i) aquatic plant hybrids often being neglected and (ii) extreme morphotypes of "pure" species erroneously being considered to be hybrids. For *Batrachium*, an extraordinary number of hybrids is reported in the literature (e.g. Cook 1966, 1970, Dahlgren 1991, 1995, Hong 1991, Wiegleb et al. 2017). Our study confirmed the large effect of hybridization in this group. We detected hybrids in almost 14% of the populations studied and revealed at least 16 cytotypes of putative hybrid origin, which makes the overall variation much more complicated (Table 3, Figs 3E, F, H, Figs 4, 5). Seven of them we consider to be F_1 hybrids based on both their intermediate morphology and genome sizes.

Intensity of hybridization in the *Batrachium* group seems to be species-specific. The diploid species appear to rarely form hybrids. For the polyploid taxa, the frequency of hybridization is uneven. The ecologically specialized species *R. baudotii* grows alone without the presence of other species at most of the localities where it occurs and no hybrids of *R. baudotii* were recorded. The same holds true for the alpine *R. trichophyllus* subsp. *eradicatus*. The ecologically similar taxa *R. peltatus* and *R. trichophyllus* A, however, often grow in mixed populations and the F_1 hybrids of them are relatively frequent, being found at 17 localities, sometimes occurring even without one or both parents (four and six localities, respectively). This quite frequent and morphologically conspicuous hybrid is reported only sporadically (cf. Wiegleb et al. 2017). This may be due to frequent confusion with the morphologically similar but not closely related *R. aquatilis*. In *R. penicillatus* A (the only widespread cytotype of this species) the pattern of hybridization is rather unexpected. Although it co-occurs with *R. peltatus* and sometimes also with *R. fluitans* in many of the rivers sampled we found *R. penicillatus* hybrids only in the Ohře river and its tributary Odrava in the Czech Republic. In this river many plants show transitional appearance between *R. peltatus* and *R. penicillatus*, occurring in a 150 km stretch of the river. These plants most likely represent a fertile hybrid swarm, which may include F_1 hybrids, subsequent filial generations and backcrosses. Other species of *Batrachium* hybridize only rarely because of their rare co-occurrence. However, the breeding barriers might be week, as exemplified by the occurrence hybrids at two out of the four sites sampled where *R. aquatilis* and *R. peltatus* co-occur, and at two out of the five sites where *R. aquatilis* and *R. trichophyllus* B co-occurred.

Most of F_1 hybrids were found to be at least partially fertile. We revealed marked genome size variation in *R. peltatus* × *R. penicillatus* A (Fig. 5, see above), *R. peltatus* × *R. trichophyllus* A (Fig. 4) and *R. aquatilis* × *R. peltatus* (Fig. 3H). Hybrid individuals with genome sizes not halfway between the parents may be either backcrosses or F_2 or later-generation hybrids, which may be variable due to the segregation of homeologous chromosomes of different sizes even in homoploid crosses (e.g. Hutchinson et al. 1979; analogous is also the within-species variation in *Festuca pallens* as documented by Šmarda et al. 2008). In progeny of triploid and pentaploid hybrids (if not sterile), additional variation may be introduced by the unbalanced number of chromosomes in gametes. In the most frequent hybrid combination at the tetraploid level, *R. peltatus* × *R. trichophyllus*, the genome size of hybrids is biased towards *R. peltatus* (Fig. 4). We hypothesize that this is more likely the result of backcrossing than variation in F_2 and later generation hybrids. The rather unidirectional gene flow might be driven by a large difference in flower size: the big-flowered *R. peltatus* is more likely to be a pollen donor than the small-flowered *R. trichophyllus*. We have even repeatedly encountered populations of plants of definite hybrid appearance (see above; named as "*R. peltatus* hybr." here), which have, however, a genome size within the rangeof "pure" *R. peltatus*. We hypothesize that these plants might be multiple backcrosses towards *R. peltatus*. It is possible that such multiple backcrosses are quite frequent and over time become indistinguishable from *R. peltatus* in terms of morphology, karyology and genome size. In any case, a molecular analyses or in situ hybridization techniques could bring more clarity to this issue in the future.

Rivers and streams may be an "evolutionary incubator" for newly arising hybrids and polyploids, because even completely sterile individuals can spread effectively downstream via clonal propagation. Due to the heterosis effect, hybrids can survive for a very long time in streams even after the disappearances there of their parents, as frequently documented for aquatic plants (e.g. Preston et al. 1998, Kaplan & Fehrer 2011, Prančl et al. 2014, Kaplan et al. 2018). Except for putative multiple backcrosses of *R. peltatus* we recorded two unique cytotypes of unknown origin (upper Vltava river, Czech Republic; Váh river, Slovakia; Table 3), which are both heterophyllous and apparently derived from *R. peltatus*, forming plentiful populations without the presence of any other *Batrachium* taxa. The upper Vltava hybrid is especially remarkable, because this hexaploid cytotype has the lowest monoploid genome size so far recorded in *Batrachium* and it is lower than in any of its potential parental species (Table 3). This completely sterile hybrid occupies an approximately 25 km long section of this submontane river above the Lipno I dam; however, downstream we surprisingly recorded only *R. peltatus*.

Ecological differences among Batrachium taxa

Our study revealed clear ecological difference between the morphologically similar and often confused species *R. aquatilis* and *R. peltatus* (Fig. 6A). This finding is in accordance with the observations of the monographer C. D. K. Cook, who states that he has never seen these two species growing together at the same site (Cook 1966). Comparison of bioclimatic characteristic showed that the temperature-related variables are the most informative characters, i.e. *R. aquatilis* prefers warmer areas than *R. peltatus*. The same pattern was observed also for the pair of cryptic *R. trichophyllus* cytotypes A and B, with the latter being more thermophilous (Fig. 6B). However, other factors than temperature may be involved or even the determining ones. In general, the distribution of species of aquatic plants is less dependent on temperature than that of terrestrial plants (Sculthorpe 1967). In the area where most samples were collected, temperature is clearly correlated with elevation and the bedrock: the warm lowlands are composed mainly of mineral-rich sediments while in the colder highlands more acidic (both sedimentary and igneous) bedrock dominates. Nevertheless, there are also several cases of central-European species of aquatic plants with climatically limited distributions regardless of the substrate pH, such as *Hottonia palustris* in lowlands or *Eleocharis mamillata* subsp. *austriaca* in mountainous areas (Kaplan et al. 2015, 2016).

We also noticed a difference in the distribution of the cytotypes of *R. fluitans*. While we found triploids predominantly in large rivers, diploids occur in all types of habitats including small and mid-sized water-courses. If both cytotypes occur in the same river (a total of three cases, see Electronic Appendix 1), diploids were found in the upper and triploids in the lower sections of the river. A detailed study focusing on rivers with common occurrence of both cytotypes would be useful to confirm this pattern.

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SOUHRN

Lakušníky (*Ranunculus* sect. *Batrachium*) jsou často považovány za taxonomicky nejsložitější skupinu vodních rostlin v Evropě. Jejich výzkum je komplikován zejména celkovou redukcí tělní stavby, vysokou měrou fenotypové plasticity, výskytem vnitrodruhové ploidní variability a častou hybridizací. Pomocí průtokové cytometrie doplněné o počítání chromozomů jsme hodnotili rozsáhlý soubor 3354 jedinců z 612 lokalit, shromážděných na území 13 převážně středoevropských zemí, absolutní velikost genomu byla určena u 1032 jedinců. Celkem jsme zaznamenali 34 cytotypů o pěti různých ploidiích, od diploidů po hexaploidy. Velikost jaderného genomu se ukázala být spolehlivým znakem pro rozlišení většiny tradičně uznávaných druhů, včetně taxonů o stejné ploidní úrovni. Ačkoli byla variabilita v ploidním stupni udávána pro většinu evropských i středoevropských druhů, naše studie odhalila větší počet ploidních úrovní pouze u dvou druhů, konkrétně u *R. fluitans* (2x, 3x) a *R. penicillatus* (4x, 6x). Situace ve střední Evropě je nicméně značně komplikovaná kvůli četnému výskytu hybridních a kryptických taxonů. Kryptická variabilita byla objevena u druhu *R. trichophyllus*, u kterého byly zaznamenány tři signifikantně odlišné tetraploidní cytotypy, lišící se I ekologicky. Ještě složitější je situace u allopolyploidního komplexu *R. penicillatus*. U tohoto říčního taxonu bylo zaznamenáno celkem šest cytotypů. Dále jsme objevili 16 různých cytotypů hybridního původu, ke kterým patří asi 15 % analyzovaných jedinců. Sedm z těchto cytotypů bylo identifikováno jako F_1 kříženci díky intermediární velikosti genomu, zbylé cytotypy jsou neznámého nebo nejistého původu. V řece Ohři se *R. penicillatus* podílí na vzniku rozsáhlého hybridního roje. Četní kříženci s variabilní velikostí genomu byli odhaleni také ve společných populacích druhů *R. peltatus* a *R. trichophyllus*, přičemž křížení pravděpodobně probíhá převážně jednosměrně směrem k *R. peltatus*. Průtoková cytometrie je ideální metodou pro základní výzkum diverzity lakušníků, a rovněž je schopna odhalit mnohé evoluční procesy, které v této skupině probíhají.

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ELECTRONIC APPENDIX 1. Locality details for 3354 *Batrachium* individuals from 612 localities. ***Ref. no.*****:** reference number unique for each combination of Taxon and Locality (i.e., if there are more taxa at one site, they have different reference numbers). ***Locality no.*****:** identification number of the locality (the same for all taxa at the site). ***N*****:** number of all plants studied by flow cytometry, including bulked samples and using either PI or DAPI fluorochromes. ***N (GS)*****:** Number of plants measured individually and with PI fluorochrome only; populations means and standard errors are calculated from these data. ***N repeats*****:** Number of individuals measured individually with PI fluorochrome repeatedly on different days; taxon statistics (Table 3 in the main text) are based only on this subset of the most precise data. *Ratio to* Bellis: Ratio of the mean fluorescences of the sample and the internal standard (mean ± SE). ***2C-value (pg)*****:** 2C value calculated from the sample / standard ratio, based on *Bellis perennis*, 2C = 3.38 pg (mean ± SE). ***Other taxa*****:** Other taxa present at the locality (reported under different Reference numbers). ***Collected*****:** Date of collection and collector name(s); JP = Jan Prančl, ML = Magdalena Lučanová, PK = Petr Koutecký, PT = Pavel Trávníček, ZK = Zdeněk Kaplan.

Ref. no.	**Locality no.**	**Taxon / Cytotype**	**N**	**N (GS)**	**N repeats**	**Ratio to *Bellis***	**2C-value (pg)**	**Locality**	**Coordinates (WGS 84)**	**Elevation (m)**	**Other taxa**	**Collected**	**Notes**
K17-15	1	*R. aquatilis*	3	3	3	3.925 ± 0.012	13.268 ± 0.040	Austria; Niederösterreich (Lower Austria); Marchegg: Mühlbach brook (the ancient lower reach of the Weidenbach), west of Marchegg center, at the bridge of the federal road B49	48°16'39"N, 16°53'44"E	143	*R. trichophyllus* B	2017-06-05 PK & Englmaier P.	
K15-16	2	*R. aquatilis*	5	3	3	3.908 ± 0.013	13.209 ± 0.044	Austria; Oberösterreich, Innviertel; Utzenaich: a small pond in the centre of the village	48°16'30"N, 13°27'43"E	400		2015-06-13 PK, JP & Hohla M.	

Ref. no.	Loca-lity no.	Taxon / Cytotype	N	N (GS)	N rep-eats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Other taxa	Collected	Notes
B14-010	3	*R. aquatilis*	10	10	2	3.848 ± 0.016	13.005 ± 0.052	Czech Republic; distr. Brno-venkov; Holasice: "Ludmila" oxbow	49°4'1.6"N, 16°36'23.9"E	185	*R. rionii*, *R. trichophyllus* B, *R. aquatilis* × *trichophyllus* B	2014-05-19 JP	
B14-019	4	*R. aquatilis*	11	11	3	3.812 ± 0.015	12.885 ± 0.051	Czech Republic; distr. Břeclav; Břeclav: pool Bornova jama near chateau Lány, ca 4.6 km SSE of railway station	48°42'54.5"N, 16°55'2.5"E	152	*R. peltatus*, *R. aquatilis* × *peltatus* (F1)	2014-05-21 JP	
B10-006	5	*R. aquatilis*	15	4	1	3.899 ± 0.015	13.179 ± 0.051	Czech Republic; distr. Břeclav; Hlohovec, Aloch III pond ESE of village	48°46'7.4"N, 16°47'47.4"E	175	*R. trichophyllus* B, *R. aquatilis* × *trichophyllus* B (F1)	2010-06-07 PT	
K17-48	6	*R. aquatilis*	2	2	0	4.024 ± 0.008	13.601 ± 0.027	Czech Republic; distr. Břeclav; Lanžhot: ca 7.4 SSW of the church, abandoned sand pit "U Ruských domků"	48°39'36"N, 16°56'31"E	150		2017-06-17 ZK & Lučanová M.	
B11-015	7	*R. aquatilis*	1	1	0	3.897	13.172	Czech Republic; distr. Břeclav; Lanžhot: flooded field NE of town, exposed bottom	48°43'47.2"N, 16°59'17.2"E	155		2011-06-27 JP	
B14-041	8	*R. aquatilis*	1	1	1	3.760	12.709	Czech Republic; distr. Břeclav; Lanžhot: Košárské louky meadow complex S of town, southernmost pool	48°37'40.2"N, 16°55'58.6"E	147		2014-06-07 ZK	
B14-042	9	*R. aquatilis*	1	1	1	3.801	12.847	Czech Republic; distr. Břeclav; Lanžhot: Lánské louky meadow complex ca 1.5 km SSE of chateau Lány, shallow pool	48°42'2.1"N, 16°55'40.6"E	153		2014-06-07 ZK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B13-015	10	*R. aquatilis*	10	2	0	3.969 ± 0.014	13.415 ± 0.047	Czech Republic; distr. Břeclav; Lanžhot: oxbow of Morava river SSE of town	48°47'37.1"N, 17°02'37.1"E	152		2013-05-20 Urfus T.; 2013-06-04 JP & Hrdinová M.	
B14-022	11	*R. aquatilis*	5	1	0	3.850	13.013	Czech Republic; distr. Břeclav; Lanžhot: pool near forest road N of chateau Lány	48°43'23.0"N, 16°55'17.4"E	155		2014-05-21 JP	
K14-15	12	*R. aquatilis*	9	9	1	3.903 ± 0.025	13.193 ± 0.085	Czech Republic; distr. Břeclav; Lanžhot: Soutok area, ca 10.1 km S of the church, abandoned sand pit "Dědova louka"	48°37'59"N, 16°57'34"E	150	*R. rionii*	2014-06-07 PK & ZK	markedly variable population, from small-flowered homophyllous plant (*R. trichophyllus*-like) to typical *R. aquatilis* with floating leaves and various transitions
K14-14	13	*R. aquatilis*	3	3	0	3.924 ± 0.032	13.264 ± 0.109	Czech Republic; distr. Břeclav; Lanžhot: Soutok area, ca 3.4 km SW of the church; ca 0.9 km SE of "Lány" castle, a small pond partly drying during the summer	48°42'27"N, 16°55'41"E	150		2014-06-07 PK & ZK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K14-13	14	*R. aquatilis*	3	3	0	3.850 ± 0.036	13.014 ± 0.122	Czech Republic; distr. Břeclav; Lanžhot: Soutok area, ca 3.7 km SW of the church; ca 0.6 km SE of "Lány" castle, a small pond Dolečky partly drying during the summer	48°42'30"N, 16°55'23"E	150	*R. peltatus*	2014-06-07 PK & ZK	
B11-014	15	*R. aquatilis*	1	1	0	3.913	13.226	Czech Republic; distr. Břeclav; Lanžhot: wet field depression near highway D2	48°43'52.1"N, 16°58'53.7"E	154		2011-06-27 JP	
B14-018	16	*R. aquatilis*	20	1	1	3.837	12.969	Czech Republic; distr. Břeclav; Týnec: old oxbow of Morava river SE of village	48°45'41.3"N, 17°2'5.2"E	156		2014-05-20 JP	
B14-015	17	*R. aquatilis*	7	1	1	3.799	12.841	Czech Republic; distr. Hodonín; Vracov: periodically flooded ditch W of town	48°58'17.7"N, 17°10'23.3"E	195		2014-05-20 JP	
B13-034	18	*R. aquatilis*	60	5	4	3.807 ± 0.013	12.869 ± 0.045	Czech Republic; distr. Hradec Králové; Hradec Králové: "Na Plachtě" area on SE edge of town, several adjacent pools	50°11'21.6"N, 15°51'28.9"E	239	*R. peltatus* hybr. (rarely, probably affected by introgression)	2013-06-17 JP & Křístková M.	markedly variable homophyllous population, from small-flowered plants (*R. trichophyllus*-like) to big-flowered (somewhat *R. peltatus*-like)
B15-047	19	*R. aquatilis*	20	3	3	3.776 ± 0.018	12.762 ± 0.060	Czech Republic; distr. Hradec Králové; Hrádek: forest pond NE of castle, exposed bottom	50°13'38.2"N, 15°40'26.7"E	287		2015-09-03 JP	

Ref. no.	**Locality no.**	**Taxon / Cytotype**	**N**	**N (GS)**	**N repeats**	**Ratio to *Bellis***	**2C-value (pg)**	**Locality**	**Coordinates (WGS 84)**	**Elevation (m)**	**Other taxa**	**Collected**	**Notes**
B13-055	20	*R. aquatilis*	16	1	1	3.870	13.081	Czech Republic; distr. Jičín; Železnice: Hluboký fishpond	50°28'58.4"N, 15°22'19.6"E	320		2013-07-02 Nunvářová Kabátová K.	
K17-71	21	*R. aquatilis*	3	1	0	3.894	13.162	Czech Republic; distr. Kroměříž; Chvalčov: lake in an abandoned stone quarry ca 1.8 km SE of the chapel in the village	49°22'39"N, 17°43'31"E	440		2017-07-16 Lučanová M.	
B16-038	22	*R. aquatilis*	1	1	1	3.917	13.239	Czech Republic; distr. Mladá Boleslav; Mohelnice nad Jizerou: Mohelka stream in village	50°33'38"N, 14°58'41"E	226		2016-07-05 Velebil J.	
K16-09	23	*R. aquatilis*	2	2	0	3.907 ± 0.002	13.206 ± 0.007	Czech Republic; distr. Mladá Boleslav; Žehrov: SW of part of a fishpond in "V Dubech" natural monument, ca 0.57 km WSW of the centre of the village	50°31'45"N, 15°05'33"E	245		2016-07-05 Štech M.	
PT12-01	24	*R. aquatilis*	7	7	0	3.956 ± 0.003	13.373 ± 0.011	Czech Republic; distr. Nymburk; Hradištko: "Kerské rybníčky", small forest pond ca 4.1 km S–SSW of chapel in village	50°7'50.3"N, 14°55'30.8"E	192		2012-04-26 PT	
B13-014	25	*R. aquatilis*	20	1	1	3.812	12.885	Czech Republic; distr. Nymburk; Kněžičky: Čihadelské rybníky fishponds in deer-park Kněžičky, SSE of village, middle pond	50°9'19.1"N, 15°20'57.1"E	245		2013-05-20 JP	
K11-03	26	*R. aquatilis*	3	3	3	3.977 ± 0.014	13.368 ± 0.046	Czech Republic; distr. Nymburk; Kněžičky: Žehuňská obora area, Kopičácký fishpond, 1.7 km SSW of the village	50°09'45"N, 15°19'57"E	235	*R. circinatus*	2011-05-27 Kaštovský J.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B13-117	27	*R. aquatilis*	1	1	0	3.937	13.307	Czech Republic; distr. Nymburk; Libice nad Cidlinou: Malý Přerov pool in Libický luh alluvial forest, near confluence of Labe and Cidlina rivers	50°7'10.4"N, 15°9'46.3"E	187		2013-09-01 Rydlo Jar.	
B11-005	28	*R. aquatilis*	14	6	4	3.867 ± 0.027	13.072 ± 0.092	Czech Republic; distr. Nymburk; Loučeň: shallow pool on the bottom of former fishpond Lutovník, ca 1.7 km NW of the castle in the village	50°17'34.6"N, 15°0'9.0"E	234	*R. circinatus*	2011-05-30 JP; 2013-06-07 JP; 2016-07-07 PK	
B13-101	29	*R. aquatilis*	7	2	2	3.868 ± 0.027	13.074 ± 0.091	Czech Republic; distr. Rychnov nad Kněžnou; Čestice: ditch between Alba canal and railway, ca 1.0 km W of railway station	50°07'49.8"N, 16°08'01.9"E	260		2010-08-07 ZK; 2013-07-10 ZK	
B13-039	30	*R. aquatilis*	17	2	1	3.904 ± 0.031	13.196 ± 0.105	Czech Republic; distr. Svitavy; Moravská Třebová: town distr. Boršov, small fishpond near road Boršov – Hřebeč	49°44'47.5"N, 16°35'41.1"E	429		2012-05-26 Lustyk P.; 2013-06-20 JP & ZK	
B13-044	31	*R. aquatilis*	15	1	1	3.815	12.895	Czech Republic; distr. Šumperk; Moravičany: pool complex along railway in Kačení louka wetland	49°45'5.2"N, 16°59'52.0"E	243		2013-06-20 JP & ZK	
PT10-05	32	*R. aquatilis*	1	1	1	3.916	13.236	Czech Republic; distr. Ústí nad Orlicí; Nové Hrady: small pond on NE edge of village	49°51'12"N, 16°8'55"E	384		2010-05-29 PT	
PT10-06	33	*R. aquatilis*	6	6	6	4.002 ± 0.013	13.528 ± 0.045	Czech Republic; distr. Ústí nad Orlicí; Nové Hrady: small spring pond Kapalice ca 1.8 km WSW of castle in village	49°50'45"N, 16°07'21"E	357		2010-06 PT; 2017-06-18 Lepší M.	

Ref. no.	Loca-lity no.	Taxon / Cytotype	N	N (GS)	N rep-eats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Other taxa	Collected	Notes
K13-19	34	*R. aquatilis*	4	4	4	3.930 ± 0.012	13.284 ± 0.042	Czech Republic; distr. Vsetín; Choryně: "Choryňský mokřad" area, ca 2.5 km N of the village, small artificial / restored pond	49°31'03"N, 17°54'25"E	275	*R. peltatus*, *R. aquatilis × peltatus* (F1 and other hybrids)	2013-06-13 PK	without floating leaves
B16-012	35	*R. aquatilis*	1	1	0	3.715	12.557	Hungary; Csongrád county; Dóc: wet temporary pool in puszta near NE edge of village	46°26'32"N, 20°8'30"E	85		2016-05-21 Kolář F. et al.	
B14-025	36	*R. aquatilis*	1	1	1	3.826	12.932	Hungary; Heves County; Felsőtárkány: pond on the NE edge of village	47°58'54"N, 20°26'3"E	239		2014-05-13 Mesterházy A.	
B12-072	37	*R. aquatilis*	2	2	2	3.934 ± 0.011	13.297 ± 0.037	Poland; Podlaskie Voivodeship; Kolno County; Wincenta, Wincenta stream near mouth to Pisz river	53°27'40.9"N, 21°52'17.8"E	110		2012-08-31 PT & Kubátová B.	
B13-021	38	*R. aquatilis*	4	4	4	3.871 ± 0.018	13.083 ± 0.062	Slovakia; distr. Malacky; Malacky: Marhecké rybníky fishponds, 3rd westernmost fishpond	48°24'34.9"N, 17°1'22.1"E	168		2013-06-06 JP & Hrdinová M.	
B13-022	39	*R. aquatilis*	5	1	1	3.926	13.270	Slovakia; distr. Malacky; Malacky: Marhecké rybníky fishponds, 6th westernmost fishpond	48°24'40.7"N, 17°1'50.7"E	170		2013-06-06 JP & Hrdinová M.	
B14-021	4	*R. aquatilis × R. peltatus* (F1)	9	9	4	2.932 ± 0.016	9.911 ± 0.055	Czech Republic; distr. Břeclav; Břeclav: pool Bornova jama near chateau Lány, ca 4.6 km SSE of railway station	48°42'54.5"N, 16°55'2.5"E	152	*R. peltatus*, *R. aquatilis*	2014-05-21 JP	heterophyllous, partially fertile

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K13-20	34	*R. aquatilis* × *R. peltatus* (F1)	7	7	6	2.946 ± 0.005	9.963 ± 0.016	Czech Republic; distr. Vsetín; Choryně: "Choryňský mokřad" area, ca 2.5 km N of the village, small artificial / restored pond	49°31'03"N, 17°54'25"E	275	*R. aquatilis*, *R. peltatus*, *R. aquatilis* × *R. peltatus* (other hybrids)	2010-06-04 PK; 2013-06-13 PK	morphologically variable, both with *R. peltatus*-like floating leaves and without them
K13-18-06	34	*R. aquatilis* × *R. peltatus* (hybrid 1)	1	1	1	3,205	10,833	Czech Republic; distr. Vsetín; Choryně: "Choryňský mokřad" wetland area, ca 2.5 km N of the village, small artificial pond	49°31'03"N, 17°54'25"E	275	*R. aquatilis*, *R. peltatus*, *R. aquatilis* × *peltatus* (F1 and other hybrids)	2013-06-13 PK	
K13-18-11	34	*R. aquatilis* × *R. peltatus* (hybrid 2)	1	1	1	2.151	7.270	Czech Republic; distr. Vsetín; Choryně: "Choryňský mokřad" wetland area, ca 2.5 km N of the village, small artificial pond	49°31'03"N, 17°54'25"E	275	*R. aquatilis*, *R. peltatus*, *R. aquatilis* × *peltatus* (F1 and other hybrids)	2013-06-13 PK	
B14-013	3	*R. aquatilis* × *R. trichophyllus* B (F1)	1	1	1	3.403	11.502	Czech Republic; distr. Brno-venkov; Holasice: "Ludmila" oxbow	49°4'1.6"N, 16°36'23.9"E	185	*R. aquatilis*, *R. rionii*, *R. trichophyllus B*	2014-05-19 JP	partially fertile
PT10-09	5	*R. aquatilis* × *R. trichophyllus* B (F1)	1	1	0	3.433	11.604	Czech Republic; distr. Břeclav; Hlohovec: Aloch III pond ESE of village	48°46'7.4"N, 16°47'47.4"E	175	*R. aquatilis*, *R. trichophyllus* B	2010-06-07 PT	
B14-029	35	*R. aquatilis* × *R. trichophyllus* B (F1)	1	1	1	3.382	11.431	Czech Republic; distr. Kolín; Zbraň: pool in reed bed SE of Žehuňský fishpond	50°08'31.6"N, 15°21'03.1"E	204		2014-05-26 ZK	

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B14-038	36	*R. baudotii*	1	1	1	2.511	8.487	Austria; Burgenland; Sankt Andrä am Zicksee: Seewinkelhauptkanal canal near mouth to Zicksee lake	47°47'49"N, 16°54'6"E	119	*R. rionii*	2014-05-23 Mesterházy A.	
K17-94	37	*R. baudotii*	4	1	0	2.580	8.720	Austria; Burgenland; Zurndorf: pond in a former gravel pit c. 1 km NE of Friedrichshof near Zurndorf	47°57'20"N, 16°57'08"E	165		2017-09-15 Shau H.	
K17-11	38	*R. baudotii*	5	3	3	2.581 ± 0.007	8.725 ± 0.024	Austria; Niederösterreich (Lower Austria); Angern an der March: at the SSE outskirts of the village, large oxbow of Morava river artificially deepened as a bathing pond	48°22'23"N, 16°50'18"E	150		2017-06-05 PK & Englmaier P.	
B14-106	39	*R. baudotii*	1	1	1	2.602	8.795	Czech Republic; distr. Brno-venkov; Pasohlávky: flooded sand-pit Velká laguna	48°53'49.2"N, 16°34'2.6"E	171		2014-07-20 JP	
B14-104	40	*R. baudotii*	5	2	1	2.614 ± 0.003	8.835 ± 0.010	Czech Republic; distr. Břeclav; Rakvice: flooded sand-pit on SE edge of village	48°51'5.4"N, 16°49'29.4"E	159		2011-06-28 JP; 2014-07-20 JP	
B14-048	41	*R. baudotii*	1	1	1	2.614	8.835	Czech Republic; distr. Hradec Králové; Obědovice: flooded sand-pit near N edge of village	50°09'59.2"N, 15°34'54.6"E	225		2014-06-15 ZK	
B13-011	42	*R. baudotii*	5	3	2	2.610 ± 0.021	8.821 ± 0.069	Czech Republic; distr. Hradec Králové; Stará Voda: flooded sand-pit NE of village	50°9'35.0"N, 15°32'32.4"E	223		2010-08-12 ZK; 2011-06-10 ZK; 2013-05-28 JP	
B14-096	43	*R. baudotii*	1	1	1	2.548	8.612	Czech Republic; distr. Chomutov; Dobřenec: fishpond ca 1.0 km E of village	50°14'43.0"N, 13°16'08.2"E	425	*R. rionii*	2014-07-03 ZK	
B14-083	44	*R. baudotii*	11	2	1	2.579 ± 0.007	8.717 ± 0.024	Czech Republic; distr. Chomutov; Sedlec u Radonic: Sedlec reservoir	50°16'12.7"N, 13°14'41.4"E	419		2014-07-03 JP & ZK	

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B13-010	45	*R. baudotii*	17	8	1	2.600 ± 0.015	8.787 ± 0.050	Czech Republic; distr. Kolín; Pečky: Výrovka stream NE of town	50°6'10.3"N, 15°2'44.0"E	188		2012-04-26 PT; 2013-05-27 JP	
B14-070	46	*R. baudotii*	1	1	1	2.569	8.683	Czech Republic; distr. Louny; Buškovice: Dolánecký stream in village	50°13'32"N, 13°22'21"E	337		2014-06-30 Rydlo Jan	
B14-073	47	*R. baudotii*	14	1	1	2.596	8.774	Czech Republic; distr. Louny; Buškovice: fish storage ponds on Dolánecký stream near Rybnický mill	50°13'41.0"N, 13°20'43.5"E	365		2014-07-01 ZK & Nunvářová Kabátová K.	
B14-091	48	*R. baudotii*	10	1	1	2.600	8.788	Czech Republic; distr. Louny; Buškovice: flooded kaolin quarry 1 km SSW of village	50°13'6.8"N, 13°22'8.6"E	350		2014-07-04 JP	
B14-094	49	*R. baudotii*	12	2	1	2.575 ± 0.002	8.704 ± 0.007	Czech Republic; distr. Louny; Buškovice: flooded kaolin quarry Velký Olpram on SE edge of village	50°13'17.6"N, 13°22'44.4"E	335		2014-07-03 ZK	
B14-092	50	*R. baudotii*	10	1	1	2.563	8.663	Czech Republic; distr. Louny; Buškovice: small sludge lagoon between kaolin quarries SSW of village	50°13'8.8"N, 13°22'9.4"E	353		2014-07-04 JP	
B14-098	51	*R. baudotii*	2	2	2	2.562 ± 0.011	8.660 ± 0.037	Czech Republic; distr. Louny; Dětaň: flooded kaolin quarry SW of village	50°11'18.5"N, 13°18'11.0"E	463		2014-07-03 ZK	
B14-071	52	*R. baudotii*	1	1	1	2.617	8.845	Czech Republic; distr. Louny; Nepomyšl: Nepomyšlský fishpond	50°12'41"N, 13°18'48"E	436	*R. circinatus*	2014-06-30 Rydlo Jan	
B14-089	53	*R. baudotii*	1	1	1	2.561	8.656	Czech Republic; distr. Louny; Podbořany: flooded kaolin quarry Malý Olpram SW of town	50°13'7.1"N, 13°23'20.7"E	331		2014-07-04 JP	

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B14-049	54	*R. baudotii*	8	8	2	2.656 ± 0.014	8.976 ± 0.046	Czech Republic; distr. Nymburk; Hořátev: Hliňovka fishpond	50°8'53.7"N, 15°3'3.2"E	185		2012-04-26 PT; 2014-05-22 Z. Hroudová; 2014-06-15 ZK	
B13-071	55	*R. baudotii*	14	2	1	2.598 ± 0.018	8.781 ± 0.061	Czech Republic; distr. Uherské Hradiště; Ostrožská Nová Ves: NE tip of big sand-pit W of village	49°0'53.3"N, 17°25'25.2"E	172	*R. rionii*	2013-09-07 A. Knotek; 2015-05-17 JP	
B15-004	56	*R. baudotii*	10	1	1	2.566	8.673	Czech Republic; distr. Uherské Hradiště; Ostrožská Nová Ves: S tip of sand-pit WNW of village	49°0'59.4"N, 17°25'31.7"E	172	*R. rionii*	2015-05-17 JP	
K14-24	57	*R. baudotii*	16	5	5	2.609 ± 0.007	8.819 ± 0.023	Czech Republic; Jevišovice: restored pond in the village, ca 0.45 km SSW of the church	48°59'02"N, 15°59'17"E	366		2014-06-13 PK	
B12-056	58	*R. baudotii*	2	1	0	2.657	8.981	Denmark; reg. Midtjylland; Bjerringbro: Gudenå river near E edge of town	56°22'33.2"N, 09°41'10.0"E	6		2012-08-02 JP & ZK	
B12-048	59	*R. baudotii*	3	1	1	2.534	8.565	Denmark; reg. Midtjylland; Ormslev: Aarhuså stream near Årslev Engsø lake	56°08'05.8"N, 10°02'25.9"E	4		2012-08-01 JP & ZK	
B12-057	60	*R. baudotii*	2	1	0	2.665	9.008	Denmark; reg. Midtjylland; Rønge: Gudenå river SW of village	56°23'00.7"N, 09°44'32.3"E	5		2012-08-02 JP & ZK	
B12-052	61	*R. baudotii*	1	1	1	2.670	9.025	Denmark; reg. Midtjylland; Virksund: Ørslevkloster sø lake	56°36'20.0"N, 09°15'54.9"E	0		2012-08-01 JP & ZK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B12-058	62	*R. baudotii*	5	1	1	2.619	8.852	Denmark; reg. Midtjylland; Voervadsbro: Gudenå river in the village	56°00'50.6"N, 09°41'49.3"E	33		2012-08-03 JP & ZK	
B12-093	63	*R. baudotii*	1	1	1	2.601	8.791	Germany; Niedersachsen (Lower Saxony); Salzgitter: sand-pit Steterburgergraben	52°11'55.4"N, 10°26'33.9"E	82		2012-07-23 PT & Kubátová B.	
B14-100	64	*R. baudotii*	1	1	0	2.481	8.386	Hungary; Bács-Kiskun County; Szabadszállás: small swamp SSW of village	46°50'13"N, 19°12'21"E	92	*R. trichophyllus* B	2014-06-19 Mesterházy A.	
B14-026	65	*R. baudotii*	1	1	1	2.587	8.744	Hungary; Vas County; Mesterháza: gravel-pits NW of village	47°22'40"N, 16°51'2"E	159		2014-05-15 Mesterházy A.	
K17-19	66	*R. circinatus*	5	3	3	1.683 ± 0.003	5.688 ± 0.010	Austria; Niederösterreich (Lower Austria); Himberg: Neubach brook at the SW edge of the village	48°04'36"N, 16°25'59"E	170		2017-06-06 PK	
B14-024	67	*R. circinatus*	1	1	0	1.716	5.800	Austria; Niederösterreich; Tulln an der Donau: pond in botanical garden	48°19'46.2"N, 16°1'56.9"E	178		2014-08-20 Kubátová B.	
K15-38	68	*R. circinatus*	5	3	3	1.684 ± 0.003	5.691 ± 0.011	Austria; Oberösterreich, Innviertel; Humertsham: Engelbach brook at the S edge of the village	48°06'41"N, 13°06'06"E	430		2015-06-13 PK & JP & Hohla M.	
B14-144	69	*R. circinatus*	1	1	1	1.649	5.574	Austria; Oberösterreich, Innviertel; Kirchdorf am Inn: reservoir W of village	48°17'36.8"N, 13°15'58.2"E	320		2014-08-09 Hrdinová M.	
K15-37	70	*R. circinatus*	2	2	0	1.676 ± 0.002	5.663 ± 0.005	Austria; Oberösterreich, Innviertel; Mamling: fishpond by Obersunzig settlement, N of the village	48°17'23"N, 13°11'12"E	330		2015-06-13 PK & JP & Hohla M.	
B14-143	71	*R. circinatus*	1	1	1	1.672	5.651	Austria; Oberösterreich, Innviertel; Mining: canal near Inn river N of village	48°16'54.0"N, 13°9'42.8"E	330		2014-08-09 Hrdinová M.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K15-36	72	*R. circinatus*	1	1	1	1.684	5.692	Austria; Oberösterreich, Innviertel; Mining: drainage channel along the S bank of the Inn river, NW of the town	48°16'45"N, 13°08'52"E	330		2015-06-13 PK & JP & Hohla M.	
B14-120	73	*R. circinatus*	1	1	1	1.668	5.638	Austria; Oberösterreich; Hallstatt: Hallstättersee lake	47°36'40.8"N, 13°37'58.0"E	508	*R. trichophyllus* B, *R. circinatus* × *trichophyllus* A (F1)	2014-07-27 ZK	
B16-002	74	*R. circinatus*	2	2	2	1.682 ± 0.004	5.683 ± 0.012	Czech Republic; distr Jičín; Jičíněves: fishpond near football field in village	50°22'15"N, 15°20'23"E	246	*R. rionii*	2016-05-26 JP & Hanzlíčková J.	
B13-016	75	*R. circinatus*	1	1	1	1.662	5.618	Czech Republic; distr. Břeclav; Moravská Nová Ves, gravel-pit Štěrkovna SE of village	48°47'41.2"N 17°2'18.4"E	156		2014-06-09 Píšová S.	
B14-044	76	*R. circinatus*	3	3	2	1.652 ± 0.008	5.584 ± 0.027	Czech Republic; distr. Břeclav; Moravská Nová Ves, smaller gravel-pit near gravel-pit Štěrkovna SE of village	48°47'37.1"N, 17°02'37.1"E	156		2013-06-04 JP & Hrdinová M.	
B14-043	77	*R. circinatus*	1	1	0	1.678	5.672	Czech Republic; distr. Břeclav; Tvrdonice, Hnátkovská jezera pools SE of village	48°44'56.7"N 17°0'11.2"E	154		2014-09-06 Píšová S.	
B14-033	78	*R. circinatus*	1	1	1	1.682	5.685	Czech Republic; distr. Česká Lípa; Bukovany, Kachní fishpond	50°42'40.0"N, 14°35'4.4"E	266	*R. trichophyllus* A	2014-05-31 JP	
B13-118	79	*R. circinatus*	1	1	1	1.687	5.702	Czech Republic; distr. Česká Lípa; Hradčany, flooded mining area near airfield	50°36'58.0"N, 14°43'54.7"E	273		2013-06-13 ZK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B13-091	80	*R. circinatus*	1	1	0	1.690	5.712	Czech Republic; distr. Česká Lípa; Máchovo jezero fishpond near protected area Swamp	50°34'35"N, 14°40'7"E	265		2013-06-20 Nunvářová Kabátová K.	
B16-044	81	*R. circinatus*	3	3	3	1.687 ± 0.001	5.701 ± 0.003	Czech Republic; distr. Havlíčkův Brod; Krucemburk: Řeka fishpond, SW tip	49°40'13"N, 15°50'51"E	550	*R. trichophyllus* A	2016-09-08 Šumberová K.	
B10-027	82	*R. circinatus*	2	2	1	1.689 ± 0.005	5.709 ± 0.017	Czech Republic; distr. Chrudim; Podlažice, Horecký fishpond	49°53'28.0"N, 15°56'38.8"E	276	*R. trichophyllus* A	2010-08-27 JP & ZK; 2011-06-08 ZK	
K11-24	83	*R. circinatus*	10	5	5	1.676 ± 0.005	5.634 ± 0.017	Czech Republic; distr. Chrudim; Třemošnice: Čeperka fishpond at the S edge of the village	49°51'42"N, 15°34'53"E	295		2011-06-27 PK	
B10-008	84	*R. circinatus*	1	1	1	1.710	5.780	Czech Republic; distr. Jičín; Lukavec u Hořic, Zákopský fishpond	50°24'41.5"N, 15°36'47.2"E	321		2010-06-25 JP	
B14-087	85	*R. circinatus*	1	1	1	1.650	5.577	Czech Republic; distr. Karlovy Vary; military training area Hradiště, small pond near settlement Hájovna	50°12'5.0"N, 13°12'5.9"E	674	*R. peltatus*, *R. trichophyllus* A	2014-07-03 Kolář F.	
B12-031	86	*R. circinatus*	1	1	0	1.688	5.705	Czech Republic; distr. Karviná; Karviná: city distr. Darkov, canal connecting Karvinské moře reservoir and Olše river	49°50'21.9"N, 18°32'44.1"E	227		2012-07-13 JP	
B12-032	87	*R. circinatus*	1	1	0	1.695	5.729	Czech Republic; distr. Karviná; Karviná: city distr. Lázně Darkov, lagoon near flooded mining area Karvinské moře	49°50'13.1"N, 18°32'57.0"E	228		2012-07-13 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K13-17	88	*R. circinatus*	3	3	0	1.712 ± 0.006	5.787 ± 0.021	Czech Republic; distr. Karviná; Karviná: flooded depression (after coal mining) by the Loucká Mlýnka brook, by the bridge on the road to Stonava, S of the town	49°49'44"N, 18°33'41"E	235		2013-06-12 PK	
B15-018	89	*R. circinatus*	1	1	0	1.684	5.692	Czech Republic; distr. Karviná; Karviná: Pod Farou fishpond SW of city	49°50'4.3"N, 18°29'26.3"E	229		2015-05-27 Rydlo Jar.	
K13-16	90	*R. circinatus*	3	3	0	1.659 ± 0.003	5.609 ± 0.011	Czech Republic; distr. Karviná; Karviná: town part Fryšták, artificial lake in the park in the centre of the town	49°50'55"N, 18°32'11"E	225		2013-06-12 PK	
B13-096	91	*R. circinatus*	1	1	0	1.674	5.658	Czech Republic; distr. Kladno; Lány: Pánovka fishpond S of village	50°6'28.7"N, 13°56'43.9"E	413		2013-06-25 Hrdinová M.	
K11-17	92	*R. circinatus*	2	2	2	1.681 ± 0.004	5.650 ± 0.013	Czech Republic; distr. Klatovy; Týnec (by Horažďovice): ca 1.3 km E of the village, oxbow of the Otava river	49°18'53"N, 13°40'44"E	425		2011-06-17 PK & JP	
K11-16	93	*R. circinatus*	2	2	2	1.680 ± 0.002	5.647 ± 0.007	Czech Republic; distr. Klatovy; Velké Hydčice: oxbow by the camping ca 0.8 km NNE of the railway station	49°18'20"N, 13°40'10"E	425		2011-06-17 PK & JP	
B14-168	52	*R. circinatus*	1	1	1	1.666	5.631	Czech Republic; distr. Louny; Nepomyšl, Nepomyšlský fishpond	50°12'41"N, 13°18'48"E	436	*R. baudotii*	2014-06-30 Rydlo Jan	
B12-001	94	*R. circinatus*	2	2	0	1.697 ± 0.024	5.736 ± 0.081	Czech Republic; distr. Mělník; Hořín: pools on left bank of Vltava river	50°19'38.8"N, 14°28'34.6"E	157		2012-05-13 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B13-090	95	*R. circinatus*	1	1	0	1.685	5.695	Czech Republic; distr. Mělník; Střemy: Lhotka fishpond in Kokořínský důl valley	50°23'31"N, 14°32'48"E	207		2013-06-20 Nunvářová Kabátová K.	
B12-013	96	*R. circinatus*	1	1	0	1.681	5.682	Czech Republic; distr. Mladá Boleslav; Žehrov: Hájenský fishpond	50°31'59.5"N, 15°5'3.9"E	240		2014-06-14 JP & PT	
B12-011	97	*R. circinatus*	1	1	0	1.691	5.716	Czech Republic; distr. Mladá Boleslav; Žehrov: Lápek fishpond	50°31'57.4"N, 15°5'16.4"E	240		2014-06-14 JP & PT	
B12-014	98	*R. circinatus*	3	3	0	1.633 ± 0.004	5.518 ± 0.015	Czech Republic; distr. Mladá Boleslav; Žehrov: small pond near settlement Arnoštice	50°31'43.3"N, 15°5'10.6"E	249		2014-06-14 JP & PT	
K13-15	99	*R. circinatus*	5	3	0	1.714 ± 0.000	5.792 ± 0.001	Czech Republic; distr. Nový Jičín; Studénka: oxbow lake by the NW dyke of Kotvice fishpond, ca 0.9 km ESE of the railway station	49°42'17"N, 18°04'43"E	240		2013-06-11 PK	
K11-04	26	*R. circinatus*	6	4	3	1.696 ± 0.007	5.710 ± 0.033	Czech Republic; distr. Nymburk; Kněžičky: Kopičácký fishpond in deer-park Kněžičky	50°09'45.7"N, 15°19'58.6"E	232	*R. aquatilis*	2011-05-27 Kaštovský J.; 2011-06-15 ZK	
K16-11	28	*R. circinatus*	3	1	0	1.682	5.685	Czech Republic; distr. Nymburk; Loučeň: shallow pool on the bottom of former fishpond Lutovník, ca 1.7 km NW of the castle in the village	50°17'35"N, 15°00'10"E	235	*R. aquatilis*	2016-07-07 PK	
B10-002	100	*R. circinatus*	1	1	1	1.681	5.682	Czech Republic; distr. Olomouc; Horka nad Moravou: flooded sand-pit Poděbrady SE of village	49°37'34.4"N, 17°13'38.5"E	215		2010-05-12 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B15-027	101	*R. circinatus*	1	1	0	1.668	5.638	Czech Republic; distr. Olomouc; Olomouc: city distr. Chomoutov, flooded sand-pit Chomoutovské jezero	49°39'11.3"N, 17°13'52.2"E	216		2015-07-13 JP	
B12-033	102	*R. circinatus*	1	1	0	1.672	5.651	Czech Republic; distr. Opava; Děhylov: Štěpán fishpond	49°51'36.3"N, 18°11'40.5"E	212	*R. trichophyllus* A	2012-07-14 JP & Pachmanová L.	
B14-117	103	*R. circinatus*	1	1	1	1.674	5.658	Czech Republic; distr. Pardubice; Dolany: flooded sand-pit Oplatil	50°6'29.8"N, 15°43'50.2"E	219		2014-07-22 Hrdinová M.	
B16-007	104	*R. circinatus*	2	2	2	1.693 ± 0.013	5.721 ± 0.042	Czech Republic; distr. Pardubice; Lázně Bohdaneč: Bohdanečský fishpond, shallow lagoones near E bank	50°5'42"N, 15°41'13"E	220	*R. trichophyllus* A, *R. peltatus*	2016-05-26 JP & Hanzlíčková J.	
B16-005	105	*R. circinatus*	4	4	3	1.697 ± 0.001	5.735 ± 0.003	Czech Republic; distr. Pardubice; Stéblová: flooded sand-pit Jezero NNW of village	50°6'40"N, 15°44'49"E	220		2016-05-26 JP & Hanzlíčková J.	
B16-021	106	*R. circinatus*	1	1	1	1.690	5.712	Czech Republic; distr. Plzeň-jih; Oselce: Pihovatý fishpond	49°25'56"N, 13°41'12"E	559	*R. trichophyllus* A	2016-06-11 JP & ZK	
B15-016	107	*R. circinatus*	1	1	0	1.690	5.712	Czech Republic; distr. Praha; Praha: city distr. Háje, Milíčovský fishpond	50°1'33.6"N, 14°32'27.4"E	274		2015-05-20 Nunvářová Kabátová K.	
B14-051	108	*R. circinatus*	1	1	1	1.681	5.682	Czech Republic; distr. Praha; Praha: city distr. Šeberov, Kovařský fishpond, exposed bottom	50°0'50.1"N, 14°30'49.9"E	291		2014-06-16 JP	
K13-08	109	*R. circinatus*	10	4	0	1.690 ± 0.007	5.712 ± 0.024	Czech Republic; distr. Přerov; abandoned gravel pits "Malé laguny" on the right bank of Bečva river, at the N edge of the town	49°27'34"N, 17°27'58"E	215		2013-06-08 PK; 2015-05-18 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K15-11	110	*R. circinatus*	1	1	0	1.699	5.743	Czech Republic; distr. Přerov; Týn nad Bečvou: oxbow lake ca 2.5 km NE of the church	49°31'34"N, 17°38'35"E	230	*R. peltatus* (2013)	2015-06-10 PK	
B12-020	111	*R. circinatus*	1	1	0	1.711	5.783	Czech Republic; distr. Rakovník; Řevničov: Horní Kracle fishpond SE of railway station	50°8'29.4"N, 13°50'33.1"E	409	*R. peltatus*	2012-06-12 JP & A. Potůčková	
B15-046	112	*R. circinatus*	10	3	3	1.673 ± 0.003	5.655 ± 0.009	Czech Republic; distr. Rychnov nad Kněžnou; Čermná nad Orlicí: Tichá Orlice river below weir on N edge of village	50°4'40.4"N, 16°8'14.0"E	259		2015-09-03 JP	
B15-045	113	*R. circinatus*	1	1	1	1.697	5.736	Czech Republic; distr. Rychnov nad Kněžnou; Korunka, Tichá Orlice river below weir E of village	50°3'44.9"N, 16°9'27.2"E	262		2015-09-03 JP	
B16-013	114	*R. circinatus*	1	1	1	1.683	5.689	Czech Republic; distr. Strakonice; Březí: Kozor fishpond	49°29'39"N, 13°49'0"E	514	*R. trichophyllus* A	2016-06-10 JP & ZK	
K12-28	115	*R. circinatus*	1	2	0	1.669 ± 0.005	5.610 ± 0.017	Czech Republic; distr. Strakonice; Lnářský Málkov: Podkadovský fishpond, ca 0.5 km N of the village	49°23'43"N, 13°46'38"E	490		2012-06-28 PK	
K11-21	116	*R. circinatus*	1	1	1	1.685	5.664	Czech Republic; distr. Strakonice; Strakonice: oxbow ca 0.95 km ENE of the railway station	49°15'30"N, 13°55'49"E	385		2011-06-18 PK & JP	
K14-37	117	*R. circinatus*	3	3	0	1.710 ± 0.004	5.779 ± 0.015	Czech Republic; distr. Strakonice; Únice: a small fishpond S of Barborka fishpond, ca 1 km WSW of the village	49°18'38"N, 13°51'16"E	450		2014-07-10 PK	
B13-042	118	*R. circinatus*	1	1	1	1.631	5.513	Czech Republic; distr. Svitavy; Třebařov: Rohlíček fishpond	49°50'40.5"N, 16°40'35.1"E	339	*R. peltatus*, *R. rionii*	2013-06-20 JP & ZK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K15-07	119	*R. circinatus*	1	1	0	1.710	5.780	Czech Republic; distr. Třebíč; Kralice nad Oslavou: Pivovárek fishpond at the N edge of the town	49°12'11"N, 16°12'15"E	410		2015-06-08 PK	
B12-066	120	*R. circinatus*	1	1	0	1.681	5.682	Czech Republic; distr. Ústí nad Orlicí; Brandýs nad Orlicí: Tichá Orlice river below bridge of Žerotínova street	50°0'4.9"N, 16°16'45.1"E	297	*R. peltatus* (2013)	2012-08-15 JP	
B12-067	121	*R. circinatus*	1	1	0	1.706	5.766	Czech Republic; distr. Ústí nad Orlicí; Choceň: millrace of Tichá Orlice river on W edge of town	50°0'4.5"N, 16°12'13.2"E	283		2012-08-15 JP	
B12-065	122	*R. circinatus*	1	1	0	1.676	5.665	Czech Republic; distr. Ústí nad Orlicí; Kerhartice: Tichá Orlice river below weir on E edge of village	49°58'19.6"N, 16°21'44.0"E	317	*R. peltatus*	2012-08-15 JP	
B13-067	123	*R. circinatus*	1	1	0	1.668	5.638	Czech Republic; distr. Ústí nad Orlicí; Orlické Podhůří: Tichá Orlice river by railway bridge near settlement Luh	49°59'34.0"N, 16°20'9.5"E	310	*R. peltatus*	2013-09-01 JP & Nunvářová Kabátová K.	
PT10-04	124	*R. circinatus*	1	1	1	1.693	5.722	Czech Republic; distr. Ústí nad Orlicí; Prochody: Prochodský fishpond	50°2'1.1"N, 16°9'3.9"E	295		2010-05-27 PT	
B10-024	125	*R. circinatus*	1	1	1	1.680	5.678	Czech Republic; distr. Ústí nad Orlicí; Sudislav nad Orlicí: Tichá Orlice river ESE of village	49°59'2.2"N, 16°20'6.6"E	312		2010-08-12 JP	
B15-007	126	*R. circinatus*	5	1	1	1.681	5.682	Czech Republic; distr. Zlín; Spytihněv: big flooded sand-pit E of village	49°8'06.8"N, 17°31'5.3"E	178	*R. rionii*	2015-05-17 JP	
B15-041	127	*R. circinatus*	1	1	0	1.661	5.614	Denmark; reg. Hovedstaden; Lynge: Buresø lake	55°49'40.0"N, 12°13'0.8"E	35		2015-07-22 Kubátová B. & Kávová T.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B12-054	128	*R. circinatus*	1	1	1	1.669	5.641	Denmark; reg. Midtjylland; Bøvlingbjerg: Indfjorden lake	56°24'38.3"N, 8°13'50.0"E	4		2012-08-01 JP & ZK	
B15-040	129	*R. circinatus*	1	1	0	1.634	5.523	Denmark; reg. Sjaelland; Haraldsted: Haraldsted Sø lake	55°28'52.1"N, 11°45'16.7"E	23		2015-07-16 Kubátová B. & Kávová T.	
B12-021	130	*R. circinatus*	1	1	1	1.699	5.743	Denmark; reg. Syddanmark; Højer: system of drainage ditches ca 2.8 km SSW of church in village	54°56'16.2"N, 8°41'12.1"E	0		2012-08-03 JP & ZK	
B13-070	131	*R. circinatus*	1	1	0	1.685	5.695	Germany; Bayern (Bavaria); Hohenfels: Follerenbach stream between Hohenfels and Kalmünz villages	49°11'7.2"N, 11°54'45.7"E	364		2013-07-09 Nunvářová Kabátová K.	
B14-123	132	*R. circinatus*	1	1	1	1.672	5.651	Germany; Bayern (Bavaria); Pocking: reservoir on SW edge of village	48°23'31.4"N, 13°18'16.5"E	321		2014-07-24 Hrdinová M.	
B12-098	133	*R. circinatus*	3	1	1	1.665	5.628	Lithuania; Alytus County; Veisiejai: Ančia lake	54°6'1.6"N, 23°40'55.3"E	115		2012-08-29 PT & Kubátová B.	
B12-099	134	*R. circinatus*	1	1	0	1.655	5.594	Poland; Podlaskie Voivodeship; Sejny County; Ogrodniki: Jezioro Hołny	54°7'55.5"N, 23°27'22.7"E	130		2012-08-28 PT & Kubátová B.	
B12-100	135	*R. circinatus*	1	1	0	1.651	5.580	Poland; Podlaskie Voivodeship; Suvałki County; Burdeniszki: Dowcień lake	54°4'35.5"N, 23°7'0.6"E	136		2012-08-28 PT & Kubátová B.	
B14-047	136	*R. circinatus*	1	1	0	1.654	5.591	Slovakia; distr. Komárno; Dedina Mládeže: Kolárovský canal WNW of village	47°57'26.0"N, 17°57'52.0"E			2014-05-21 Rydlo Jar.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B13-020	137	*R. circinatus*	1	1	1	1.614	5.455	Slovakia; distr. Komárno; Kameničná: canal NW of settlement Balvany	47°50'32.7"N, 18°00'22.8"E	111		2013-06-06 JP & Hrdinová M.	
B11-019	138	*R. circinatus*	1	1	0	1.663	5.621	Slovakia; distr. Senica; Sekule: flooded gravel-pit Mláky	48°36'48.7"N, 17°00'02.0"E	155		2011-06-26 JP & ZK	
B13-017	139	*R. circinatus*	1	1	1	1.700	5.746	Slovakia; distr. Šaľa; Žihárec: fishpond near NW edge of village	48°4'44.7"N, 17°52'20.7"E	114		2013-06-05 JP & Hrdinová M.	
B16-036	140	*R. circinatus* × *R. rionii* (F1)	1	1	1	1.634	5.523	Czech Republic; distr. Kroměříž; Hulín: flooded sand-pit Hulínská pískovna, S bank	49°17'47"N, 17°26'56"E	185	*R. rionii*	2016-06-18 Trávníček B.	reduced fertility, intermediate appearance; also pure *R. circinatus* proved to occur on the locality (photo and several herbarium specimens B. Trávníček, OL)
B14-118	73	*R. circinatus* × *R. trichophyllus* A (F1)	8	8	8	2.173 ± 0.012	7.343 ± 0.042	Austria; Oberösterreich; Hallstatt: Hallstättersee lake	47°36'40.8"N, 13°37'58.0"E		*R. circinatus, R. trichophyllus* B	2014-07-27 ZK	infertile, intermediate appearance

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K17-29	141	*R. fluitans* (2x)	5	3	2	1.119 ± 0.004	3.782 ± 0.013	Austria; Niederösterreich (Lower Austria); Sittendorf: Kamp river, ca 1.5 km WSW of the centre of the village	48°26'05"N, 15°42'59"E	190		2017-06-06 PK	
PT10-16	142	*R. fluitans* (2x)	1	1	1	1.124	3.799	Czech Republic; distr. Benešov; Radonice: Blanice river near bridge WNW of village	49°47'19.0"N, 14°55'58.4"E	309		2010-07-15 PT	
B12-106	143	*R. fluitans* (2x)	1	1	0	1.129	3.816	Czech Republic; distr. Brno-venkov: Jamolice, Oslava river near bridge ca 2.3 km NNW of church in village	49°5'34.8"N, 16°15'0.3"E	232		2012-06-10 Lučanová M.	
K16-12	144	*R. fluitans* (2x)	5	2	0	1.156 ± 0.002	3.906 ± 0.008	Czech Republic; distr. Brno-venkov; Hrubšice: Jihlava river N of the village (quickly flowing water)	49°05'44"N, 16°18'22"E	220		2016-07-12 Šumberová K.	1 individual had developed achenes
K13-27	145	*R. fluitans* (2x)	3	0	0			Czech Republic; distr. Brno-venkov; Senorady: Jihlava river, ca 3 km S of the village	49°05'49"N, 16°14'46"E	240		2014-03-08 PK	
B12-074	146	*R. fluitans* (2x)	10	1	1	1.136	3.840	Czech Republic; distr. Brno-venkov; Skryje: Bobrůvka stream W of village	49°23'34.5"N, 16°17'44.3"E	341		2012-09-16 JP	
B14-006	147	*R. fluitans* (2x)	12	3	3	1.138 ± 0.009	3.833 ± 0.025	Czech Republic; distr. Brno-venkov; Tišnov: Svratka river near bridge of Cáhlovská street at the SW part of the town	49°20'34.2"N, 16°25'15.4"E	246		2010-10-17 PK; 2014-05-19 JP	
B14-036	148	*R. fluitans* (2x)	10	1	1	1.124	3.799	Czech Republic; distr. Česká Lípa; Lindava: Svitavka stream on SW edge of village	50°44'8.4"N, 14°38'17.8"E	288		2014-06-01 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K10-26	149	*R. fluitans* (2x)	2	2	0	1.137 ± 0.004	3.822 ± 0.013	Czech Republic; distr. České Budějovice; České Budějovice: Mlýnská stoka stream W of the historical centre of the towm, ca 150 m E of the confluence with Vltava river	48°58'38"N, 14°28'09"E	385	*R. peltatus*	2010-07 PK	
K12-24	150	*R. fluitans* (2x)	5	3	3	1.138 ± 0.002	3.826 ± 0.006	Czech Republic; distr. České Budějovice; Heřmaň: Blanice river, by the bridge S of the village	49°14'19"N, 14°08'14"E	395		2012-08-09 PK	
K15-33	151	*R. fluitans* (2x)	6	6	6	1.140 ± 0.001	3.848 ± 0.006	Czech Republic; distr. České Budějovice; Roudné: Malše river, by the bridge at the E edge of the village	48°56'05"N, 14°29'23"E	390		2012-10-14 Konečná M.; 2015-08-04 PK	1 individual had developed achenes
B14-129	152	*R. fluitans* (2x)	1	1	1	1.144	3.867	Czech Republic; distr. Děčín; Brtníky: Křinice stream near state border ca 4.0 km SW of railway station	50°55'23.4"N, 14°24'11.5"E	290		2014-07-24 Rydlo Jan & Rydlo Jar.	
B14-151	153	*R. fluitans* (2x)	1	1	0	1.153	3.897	Czech Republic; distr. Děčín; Hřensko: Kamenice stream in village	50°52'28.7"N, 14°14'37.6"E	122		2014-08-19 Rydlo Jan & Rydlo Jar.	
K14-44	154	*R. fluitans* (2x)	3	3	0	1.161 ± 0.002	3.923 ± 0.006	Czech Republic; distr. Děčín; Jetřichovice: Kamenice river, by the former mill "Dolský", ca 3.25 km WSW of the church	50°50'57"N, 14°20'51"E	195		2014-10-25 PK	
B14-130	155	*R. fluitans* (2x)	1	1	1	1.139	3.850	Czech Republic; distr. Děčín; Jetřichovice: Křinice (Kirnitszch) stream on state border near former village Zadní Jetřichovice, ca 5.6 km NNW of church in village	50°53'55.4"N, 14°21'26.4"E	242		2014-07-24 Rydlo Jan & Rydlo Jar.	

Ref. no.	Loca-lity no.	Taxon / Cytotype	N	N (GS)	N rep-eats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Other taxa	Collected	Notes
B14-128	156	*R. fluitans* (2x)	1	1	1	1.142	3.860	Czech Republic; distr. Děčín; Kyjov: Křinice stream ca 1 km under Turistický most bridge	50°55'17.1"N, 14°24'54.0"E	300		2014-07-24 Rydlo Jan & Rydlo Jar.	
B14-127	157	*R. fluitans* (2x)	1	1	1	1.142	3.860	Czech Republic; distr. Děčín; Kyjov: Křinice stream near W edge of village	50°54'48.2"N, 14°27'25.3"E	351		2014-07-24 Rydlo Jan & Rydlo Jar.	
B14-150	158	*R. fluitans* (2x)	1	1	0	1.160	3.921	Czech Republic; distr. Děčín; Vysoká Lípa: Jetřichovická Bělá stream near confluence with Kamenice stream	50°50'55.9"N, 14°21'1.8"E	200		2014-08-19 Rydlo Jan & Rydlo Jar.	
B16-040	159	*R. fluitans* (2x)	2	2	2	1.151 ± 0.009	3.890 ± 0.030	Czech Republic; distr. Havlíčkův Brod; Havlíčkův Brod: Sázava river ca 1,1 km SE of railway station Pohledští Dvořáci	49°36'22"N, 15°37'41"E	418		2016-07-29 Šumberová K.	
PT10-15	160	*R. fluitans* (2x)	1	1	1	1.171	3.958	Czech Republic; distr. Havlíčkův Brod; Světlá nad Sázavou: Sázava river near railway station Stvořidla, ca 6.0 km W of náměstí Trčků z Lípy square in town	49°40'14.1"N, 15°19'6.7"E	355		2010-06-20 PT	
PT10-18	161	*R. fluitans* (2x)	1	1	1	1.159	3.917	Czech Republic; distr. Havlíčkův Brod; Víska: Doubrava stream between Víska and Maleč villages	49°46'16.4"N, 15°39'37.0"E	376		2010-08-27 PT	
B10-026	162	*R. fluitans* (2x)	3	1	1	1.127	3.809	Czech Republic; distr. Chrudim; Nasavrky: Chrudimka river near settlement V Pekle N of town	49°51'38.8"N, 15°48'22.1"E	346		2010-08-27 JP & ZK	
K10-24	163	*R. fluitans* (2x)	2	2	2	1.151 ± 0.014	3.869 ± 0.047	Czech Republic; distr. Chrudim; Spačice: Doubrava river, by the bridge in the village	49°49'02"N, 15°35'43"E	340		2010-06-29 PK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B15-012	164	*R. fluitans* (2x)	1	1	1	1.151	3.890	Czech Republic; distr. Chrudim; Svídnice: Chrudimka river below bridge ENE of village	49°53'31.1"N, 15°49'16.8"E	286		2015-05-17 Nunvářová Kabátová K.	
PT10-08	165	*R. fluitans* (2x)	3	1	1	1.122	3.792	Czech Republic; distr. Chrudim; Svídnice: Chrudimka river below Křižanovice II dam	49°52'53.3"N, 15°48'37.9"E	300		2010-06-03 PT	
B12-073	166	*R. fluitans* (2x)	5	1	1	1.129	3.816	Czech Republic; distr. Jablonec nad Nisou; Železný Brod: Jizera river in town	50°38'32.3"N, 15°15'29.6"E	274		2012-09-09 JP	
B10-022	167	*R. fluitans* (2x)	3	1	1	1.126	3.806	Czech Republic; distr. Liberec; Hrádek nad Nisou: Lužická Nisa river in town	50°50'55.4"N, 14°50'50.3"E	245		2010-08-27 JP	
B12-017	168	*R. fluitans* (2x)	5	5	0	1.120 ± 0.008	3.786 ± 0.025	Czech Republic; distr. Mladá Boleslav; Dolení Kruhy: Jizera river NNW of village	50°33'45.6"N, 14°59'41.3"E	224		2012-06-14 JP & PT	
B16-037	169	*R. fluitans* (2x)	3	3	2	1.152 ± 0.012	3.895 ± 0.039	Czech Republic; distr. Mladá Boleslav; Skorkov: Jizera river near Podbrahy village	50°13'39"N, 14°44'57"E	174		2016-07-08 JP	
ZK3270	170	*R. fluitans* (2x)	3	1	1	1.129	3.816	Czech Republic; distr. Náchod; Mstětín: Úpa river ca 1.1 km NW of village centre	50°27'51.6"N, 16°03'05.2"E	315		2015-06-06 Samková V.	
PT11-01	171	*R. fluitans* (2x)	1	1	0	1.131	3.823	Czech Republic; distr. Náchod; Petrovičky: Metuje river near bridge E of village	50°30'35.1"N, 16°12'40.6"E	395		2011-07-15 PT	
K11-05	172	*R. fluitans* (2x)	3	3	3	1.131 ± 0.008	3.802 ± 0.025	Czech Republic; distr. Náchod; Žernov: Úpa river, ca 0.85 km SW of the centre of the village	50°25'32"N, 16°02'53"E	290		2011-06 Ekrt L.	
B15-025	173	*R. fluitans* (2x)	2	1	1	1.132	3.826	Czech Republic; distr. Pardubice; Semín: Opatovický canal in village	50°03'12.1"N, 15°31'12.4"E	210		2015-06-20 ZK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
PT12-04	174	*R. fluitans* (2x)	3	3	3	1.158 ± 0.003	3.913 ± 0.010	Czech Republic; distr. Pelhřimov; Křelovice: Trnava stream near bridge SSE of village	49°31'46.7"N, 15°10'18.0"E	422		2012-08-13 PT	
K14-04	175	*R. fluitans* (2x)	1	1	1	1.143	3.863	Czech Republic; distr. Pelhřimov; Senožaty: Želivka river, by the bridge E of the village	49°34'12"N, 15°14'26"E	380	*R. fluitans* (3x)	2014-05-31 PK	one individual caught on the river bank, probably coming from upstream
B10-033	176	*R. fluitans* (2x)	1	1	1	1.117	3.775	Czech Republic; distr. Písek; Lhota u Kestřan: Otava river near bridge on N edge of village	49°15'47.4"N, 14°4'28.3"E	368	*R. peltatus*	2010-09-26 JP	
B14-134	177	*R. fluitans* (2x)	2	2	1	1.133 ± 0.029	3.830 ± 0.098	Czech Republic; distr. Plzeň-město; Plzeň: city district Bílá Hora, Berounka river below U Papírny weir	49°46'16"N, 13°25'3"E	298		2013-10-04 Nunvářová Kabátová K. & Rydlo Jar.; 2014-08-08 Nunvářová Kabátová K. & Rydlo Jar.	
B13-115	178	*R. fluitans* (2x)	1	1	0	1.121	3.789	Czech Republic; distr. Plzeň-sever; Dolany: Berounka river near road bridge	49°47'55.5"N, 13°28'6.9"E	292		2013-10-04 Nunvářová Kabátová K. & Rydlo Jar.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected
K10-29	179	*R. fluitans* (2x)	1	1	0	1.128	3.792	Czech Republic; distr. Praha-východ: Káraný: Jizera river, by the bridge at the N edge of the village	50°11'02"N, 14°43'52"E	175		2010-07-02 PK
ZK2254	180	*R. fluitans* (2x)	1	1	1	1.156	3.907	Czech Republic; distr. Rychnov nad Kněžnou; Štěnkov: Orlice river S–SSW of village	50°10'34.2"N, 15°59'48.1"E	240		2010-07-11 ZK
ZK3271	181	*R. fluitans* (2x)	1	1	1	1.142	3.860	Czech Republic; distr. Trutnov; Havlovice: Úpa river near wood bridge on N edge of village	50°29'42.4"N, 16°01'34.0"E	327		2015-06-07 ZK
ZK3269	182	*R. fluitans* (2x)	1	1	1	1.138	3.846	Czech Republic; distr. Trutnov; Suchovršice: Úpa river ca 1.3 km W–WNW of railway station	50°32'06.1"N, 15°59'14.1"E	355		2015-06-06 ZK
ZK3268	183	*R. fluitans* (2x)	1	1	1	1.117	3.775	Czech Republic; distr. Trutnov; Suchovršice: Úpa river on N edge of village	50°32'07.1"N, 15°59'50.0"E	355		2015-06-06 ZK
ZK2239	184	*R. fluitans* (2x)	1	1	1	1.141	3.857	Czech Republic; distr. Ústí nad Orlicí; Litice nad Orlicí: Divoká Orlice river near railway station	50°05'11.9"N, 16°21'24.0"E	365		2010-05-11 ZK
K10-30	185	*R. fluitans* (2x)	1	1	1	1.132	3.805	Czech Republic; distr. Žďár nad Sázavou; Strážek: Bobrůvka river, by the bridge ca 0.55 km NW of the town	49°26'31"N, 16°11'23"E	430		2010-08 Kolář F.
B12-091	186	*R. fluitans* (2x)	1	1	0	1.142	3.860	Germany; Niedersachsen; distr. Hildesheim; Nordstemmen: Leine river near bridge of Reichsstraße street	52°8'50.1"N, 9°46'3.9"E	75		2012-08-04 PT & Kubátová B.

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B14-152	187	*R. fluitans* (2x)	1	1	0	1.130	3.819	Germany; Sachsen (Saxony); distr. Sächsische Schweiz-Osterzgebirge; Sebnitz: Kirnitszch stream between Mitteldorfen and Ostrauer mills, SE of Alterdorf village	50°55'48"N, 14°11'49"E	170		2014-08 Rydlo Jan & Rydlo Jar.	
B14-153	188	*R. fluitans* (2x)	1	1	1	1.123	3.796	Germany; Sachsen (Saxony); distr. Sächsische Schweiz-Osterzgebirge; Sebnitz: Kirnitszch stream S of Lichtenhein village	50°55'36"N, 14°14'12"E	198		2014-08 Rydlo Jan & Rydlo Jar.	
B14-154	189	*R. fluitans* (2x)	1	1	1	1.130	3.819	Germany; Sachsen (Saxony); distr. Sächsische Schweiz-Osterzgebirge; Sebnitz: Kirnitszch stream SSE of Ottendorf village	50°55'35"N, 14°18'2"E	242		2014-08 Rydlo Jan & Rydlo Jar.	
B14-155	190	*R. fluitans* (2x)	1	1	1	1.129	3.816	Germany; Sachsen (Saxony); distr. Sächsische Schweiz-Osterzgebirge; Sebnitz: Kirnitszch stream SSW of Saupsdorf village	50°54'59"N, 14°18'41"E	242		2014-08 Rydlo Jan & Rydlo Jar.	
B10-036	191	*R. fluitans* (2x)	1	1	1	1.146	3.873	Germany; Sachsen-Anhalt (Saxony-Anhalt); distr. Harz; Wegeleben: Bode river near bridge	51°53'17.6"N 11°11'00.9"E	96		2010-06 Kolář F.	
B17-003	192	*R. fluitans* (2x)	1	1	1	1.119	3.782	Slovakia; distr. Stará Ľubovňa; Orlov: Poprad river above bridge in village	49°16'56.1"N, 20°51'55.5"E	478		2017-06-30 P. Baláži	
B10-035	193	*R. fluitans* (3x)	2	2	1	1.686 ± 0.021	5.697 ± 0.069	Czech Republic; distr. Benešov; Sázava: Sázava river below weir in town	49°52'50.7"N, 14°54'36.7"E	285		2010-09-12 JP	
B10-023	194	*R. fluitans* (3x)	3	1	1	1.703	5.756	Czech Republic; distr. Česká Lípa; Česká Lípa: Ploučnice river in town	50°40'59.0"N, 14°32'4.4"E	244		2010-07-29 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B14-126	195	*R. fluitans* (3x)	1	1	0	1.704	5.760	Czech Republic; distr. Litoměřice; Píšťany: fish-ladder around weir of Labe river near village	50°31'4.8"N, 14°4'26.8"E	143		2014-07-20 Rydlo Jar.	
K14-05	175	*R. fluitans* (3x)	3	1	0	1.765	5.966	Czech Republic; distr. Pelhřimov; Senožaty: Želivka river, by the bridge E of the village	49°34'12"N, 15°14'26"E	380	*R. fluitans* (2x)	2014-05-31 PK	the co-occuring R. fluitans (2x) was only one individual caught on the river bank, probably coming from upstream
B14-137	196	*R. fluitans* (3x)	1	1	0	1.712	5.787	Czech Republic; distr. Plzeň-sever; Bohy: Berounka river S of village	49°55'35.6"N, 13°34'15.3"E	267		2014-08-09 Nunvářová Kabátová K. & Rydlo Jar.	
B14-135	197	*R. fluitans* (3x)	1	1	1	1.695	5.729	Czech Republic; distr. Plzeň-sever; Nadryby: Berounka river below weir Valentovský mlýn, SSE of village	49°48'59"N, 13°31'42"E	285		2014-08 Nunvářová Kabátová K. & Rydlo Jar.	
B12-006	198	*R. fluitans* (3x)	10	1	0	1.715	5.797	Czech Republic; distr. Praha; Praha: city distr. Troja, Vltava river near Císařský ostrov island	50°6'52.5"N, 14°25'5.6"E	176		2012-05-26 JP	
B13-072	199	*R. fluitans* (3x)	2	1	1	1.684	5.692	Czech Republic; distr. Praha-východ: Stříbrná Skalice: Sázava river below weir near railway station	49°52'55.8"N, 14°51'27.8"E	279		2013-05-17 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B14-139	200	*R. fluitans* (3x)	1	1	1	1.694	5.726	Czech Republic; distr. Rakovník; Hřebečníky: Berounka river near castle ruin Týřov, between Skryje and Týřovice villages	49°58'27"N, 13°47'13"E	246		2014-08-10 Nunvářová Kabátová K. & Rydlo Jar.	
B14-138	201	*R. fluitans* (3x)	1	1	1	1.695	5.729	Czech Republic; distr. Rokycany; Liblín: Berounka river below weir near castle ruin Libštejn, S–SSE of village	49°54'8"N, 13°32'46"E	271		2014-08-09 Nunvářová Kabátová K. & Rydlo Jar.	
B14-136	202	*R. fluitans* (3x)	1	1	0	1.730	5.847	Czech Republic; distr. Rokycany; Liblín: Berounka river below weir near Liblínský Mlýn mill NE of village	49°55'21.1"N, 13°33'14.2"E	269		2014-08-09 Nunvářová Kabátová K. & Rydlo Jar.	
B10-025	203	*R. fluitans* (3x)	3	1	1	1.685	5.695	Czech Republic; distr. Znojmo; Čížov: Dyje river below weir near bridge to Hardegg (AT)	48°51'8.8"N, 15°51'51.5"E	284		2010-08-15 JP	
K10-17	204	*R. fluitans* (3x)	4	4	0	1.706 ± 0.006	5.765 ± 0.020	Czech Republic; distr. Znojmo; Havraníky: Dyje river ca 2,6 km NW of the church	48°49'25"N, 15°58'43"E	240		2014-06-10 PK	
PT10-07	205	*R. fluitans* (3x)	1	1	1	1.698	5.739	Czech Republic; distr. Znojmo; Strachotice: millrace of Dyje river in village	48°47'50.4"N, 16°10'35.2"E	194		2010-06-01 PT	
K14-25	206	*R. fluitans* (3x)	5	3	0	1.655 ± 0.008	5.594 ± 0.028	Czech Republic; distr. Znojmo; Tasovice: Dyje river by the bridge at the S edge of the village	48°49'53"N, 16°09'14"E	200		2014-06-13 PK	
K17-83	207	*R. peltatus*	7	7	0	1.974 ± 0.002	6.674 ± 0.008	Austria; Niederösterreich (Lower Austria); Etzen (distr. Zwettl): Forstteich near Josefsdorf, between Merzenstein and Etzen, western lakeshore	48°33'57"N, 15°03'51"E	665		2017-08-27 Pachschwöll C. & Pochynok T.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K17-30	208	*R. peltatus*	8	4	4	1.992 ± 0.007	6.734 ± 0.025	Austria; Niederösterreich (Lower Austria); Rudmanns (distr. Zwettl): exposed bottom of Rudmannser Teich fishpond, SSE of the village	48°35'28"N, 15°21'52"E	590	*R. trichophyllus A*, *R. peltatus* × *R. trichophyllus A* (F1)	2017-06-06 PK; 2017-08-12 Pachschwöll C.	
K15-23	209	*R. peltatus*	4	3	3	2.022 ± 0.005	6.833 ± 0.017	Czech Republic; distr Prachatice; Borová Lada, Knížecí Pláně: a small fishpond ca 0.65 km NW of the ruin of the church in the former village	48°57'21"N, 13°36'35"E	1015	*R. peltauts* (aneuploid)	2015-06-27 PK	
B14-167	210	*R. peltatus*	2	2	0	1.969 ± 0.003	6.654 ± 0.012	Czech Republic; distr. Benešov; Dolní Kralovice: water reservoir Švihov on Sázava river near Střítež village	49°40'9"N, 15°9'25"E	376		2014-07-26 Lučanová M.	
B13-036	211	*R. peltatus*	1	1	0	2.080	7.030	Czech Republic; distr. Benešov; Sedlice: water reservoir Švihov on Želivka river near bridge of D1 highway	49°41'20.3"N, 15°6'7.7"E	376		2013-06-16 Nunvářová Kabátová K.	
K12-17	212	*R. peltatus*	5	3	3	2.010 ± 0.013	6.757 ± 0.043	Czech Republic; distr. Bruntál; Liptaň: the lower fishpond on Liptaňský brook, ca 0.8 km S of the church	50°12'53"N, 17°36'09"E	345		2012-07-10 PK	
B14-020	4	*R. peltatus*	16	11	3	2.014 ± 0.008	6.808 ± 0.029	Czech Republic; distr. Břeclav; Břeclav: pool Bornova jama near chateau Lány, ca 4.6 km SSE of railway station	48°42'54.5"N, 16°55'2.5"E	153	*R. aquatilis*, *R. aquatilis* × *R. peltatus* (F1)	2014-05-21 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K14-17	14	*R. peltatus*	2	2	0	1.978 ± 0.012	6.686 ± 0.041	Czech Republic; distr. Břeclav; Lanžhot: Soutok area, ca 3.7 km SW of the church; ca 0.6 km SE of "Lány" castle, a small pond Dolečky partly drying during the summer	48°42'30"N, 16°55'23"E	150	*R. aquatilis*	2014-06-07 PK& ZK	
B14-030	213	*R. peltatus*	8	1	1	2.018	6.821	Czech Republic; distr. Česká Lípa; Okna, Robečský stream on SW edge of village	50°31'30.8"N, 14°40'15.2"E	279		2014-05-31 JP	
K12-01	214	*R. peltatus*	1	2	0	1.981 ± 0.009	6.657 ± 0.029	Czech Republic; distr. České Budějovice: Nový Vrbenský fishpond, W of the town part České Vrbné	49°0'31"N, 14°26'42"E	385		2012-05 Štech M.	
B12-097	215	*R. peltatus*	1	1	0	1.997	6.750	Czech Republic; distr. České Budějovice; Boršov nad Vltavou: Vltava river between road and railway bridges	48°55'29.1''N, 14°26'14.5''E	399		2012-08-15 Hrdinová M.	
K11-12	216	*R. peltatus*	16	6	6	2.012 ± 0.008	6.763 ± 0.025	Czech Republic; distr. České Budějovice; Branišov: Návesný fishpond, 1 km N of the village	48°59'16"N, 14°23'48"E	390	*R. peltatus* (aneuploid), *R. trichophyllus* A, *R. peltatus* × *trichophyllus* A (various hybrids)	2011-06-07 PK	some individuals with relatively small flowers
K16-02	217	*R. peltatus*	1	1	1	1.995	6.743	Czech Republic; distr. České Budějovice; České Budějovice: artificial pond in the campus of the Faculty of Science, Univ. of South Bohemia, in the NW part of the town	48°58'41"N, 14°26'41"E	390		2016-05-16 PK	the species appeared spotaneously one year after construction of the pond

Ref. no.	**Locality no.**	**Taxon / Cytotype**	**N**	**N (GS)**	**N repeats**	**Ratio to *Bellis***	**2C-value (pg)**	**Locality**	**Coordinates (WGS 84)**	**Elevation (m)**	**Other taxa**	**Collected**	**Notes**
K10-28	218	*R. peltatus*	1	1	0	1.982	6.662	Czech Republic; distr. České Budějovice; České Budějovice: Mlýnská stoka stream N of the historical centre of the towm	48°58'40"N, 14°28'29"E	385		2010-07 PK	
K10-27	149	*R. peltatus*	2	2	0	1.985 ± 0.003	6.671 ± 0.012	Czech Republic; distr. České Budějovice; České Budějovice: Mlýnská stoka stream W of the historical centre of the town, ca 150 m E of the confluence with Vltava river	48°58'38"N, 14°28'09"E	385	*R. fluitans* (2x)	2010-07 PK	
K15-26	219	*R. peltatus*	2	2	2	2.027 ± 0.008	6.851 ± 0.027	Czech Republic; distr. České Budějovice; České Budějovice: Velký Vávrovský fishpond, at the NW edge of the town	48°59'35"N, 14°26'16"E	385	*R. trichophyllus* A	2015-07-01 PK	
K17-41	220	*R. peltatus*	1	1	1	2.019	6.824	Czech Republic; distr. České Budějovice; Doubravice: small garden pond ca 0.5 km NW of the centre of the village	48°56'17"N, 14°30'16"E	435		2017-06-15 PK	
K11-10	221	*R. peltatus*	2	2	2	1.988 ± 0.002	6.681 ± 0.008	Czech Republic; distr. České Budějovice; Dubné: fishpond at the NE edge of the town, by the road to Čakov	48°58'40"N, 14°21'07"E	425		2011-06-04 PK	
K12-11	222	*R. peltatus*	10	5	5	1.994 ± 0.009	6.703 ± 0.029	Czech Republic; distr. České Budějovice; Hluboká u Borovan: Žemlička fishpond, 1 km SE of the centre of the town	48°53'30"N, 14°41'23"E	470		2012-06-02 PK	
K17-93	223	*R. peltatus*	4	1	0	1.967	6.648	Czech Republic; distr. České Budějovice; Horní Stropnice: Bedřich fishpond, ca 1.3 km WNW of the church	48°45'55"N, 14°43'13"E	560		2017-09-20 Šumberová K.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K15-06	224	*R. peltatus*	5	5	5	2.016 ± 0.005	6.814 ± 0.018	Czech Republic; distr. České Budějovice; Koloděje nad Lužnicí: fishpond by the road to Týn nad Vltavou, at the S edge of the village	49°14'50"N, 14°25'12"E	360		2015-05-31 PK	
K17-90	225	*R. peltatus*	3	1	0	1.955	6.608	Czech Republic; distr. České Budějovice; Lišov: Koníř fishpond, ca 1.9 km S of the church	48°59'55"N, 14°36'18"E	515		2017-09-18 Šumberová K.	
K17-89	226	*R. peltatus*	3	1	0	1.994	6.740	Czech Republic; distr. České Budějovice; Lišov: Vortínovec fishpond, ca 2.5 km S of the church	48°59'32"N, 14°36'07"E	505		2017-09-18 Šumberová K.	
K11-02	227	*R. peltatus*	6	4	4	1.991 ± 0.004	6.691 ± 0.015	Czech Republic; distr. České Budějovice; Nové Hrady: Terčino údolí, JV od města obnažené dno rybníka	48°46'49"N, 14°45'46"E	525		2011-05 Kubátová B.	
K17-92	228	*R. peltatus*	1	1	0	2.007	6.784	Czech Republic; distr. České Budějovice; Nové Hrady-Vyšné: Modráček fishpond, ca 0.3 km W of the village	48°47'23"N, 14°51'37"E	490		2017-09-20 Šumberová K.	
K16-17	229	*R. peltatus*	3	3	3	1.945 ± 0.011	6.574 ± 0.037	Czech Republic; distr. České Budějovice; Strýčice: fishpond ca 0.9 km SW of the church	49°02'27"N, 14°15'21"E	430	*R. trichophyllus* A	2016-08-15 Šumberová K.	
K17-33	230	*R. peltatus*	3	1	0	1.968	6.652	Czech Republic; distr. České Budějovice; Záblatí: Dolní Kozlovna fishpond, ca 1.1 km NNE of the centre of the village	49°08'34"N, 14°17'27"E	405		2017-06-09 Šumberová K.	
B16-048	231	*R. peltatus*	3	3	3	2.007 ± 0.013	6.783 ± 0.044	Czech Republic; distr. České Budějovice; Žabovřesky: Dehtář fishpond, artificial pool on isthmus of peninsula in NW part of fishpond	49°0'33"N, 14°16'54"E	405		2016-09-14 Šumberová K.	

Ref. no.	**Loca-lity no.**	**Taxon / Cytotype**	**N**	**N (GS)**	**N rep-eats**	**Ratio to *Bellis***	**2C-value (pg)**	**Locality**	**Coordinates (WGS 84)**	**Eleva-tion (m)**	**Other taxa**	**Collected**	**Notes**
K17-34	232	*R. peltatus*	1	1	0	2.007	6.784	Czech Republic; distr. Český Krumlov; Bukovsko: Čirý fishpond, ca 1.4 km W of the centre of the village	48°40'23"N, 14°31'19"E	655	*R. peltatus* × *R. trichophyllus* A (F1)	2017-06-08 Šumberová K.	
B12-104	233	*R. peltatus*	1	1	0	2.003	6.770	Czech Republic; distr. Český Krumlov; Černá v Pošumaví: Pláničský fishpond, ca 3.6 km ESE of the town	48°43'22"N, 14°9'22"E	750		2012-08-12 Kolář F.	
B12-039	234	*R. peltatus*	1	1	0	1.979	6.689	Czech Republic; distr. Český Krumlov; Čeřín: Vltava river near mouth of Rožmitálský stream S of village	48°42'05.1"N, 14°21'14.6"E	509		2012-07-20 JP	
K10-09	235	*R. peltatus*	1	1	1	2.014	6.770	Czech Republic; distr. Český Krumlov; Český Krumlov: Vltava river by the camping by the town part Nové Spolí, S of the town	48°47'55"N, 14°18'36"E	485		2010-06-01 PK	
B12-041	236	*R. peltatus*	5	1	0	2.004	6.774	Czech Republic; distr. Český Krumlov; Český Krumlov: Vltava river near E edge of town	48°48'49.3"N, 14°19'47.0"E	472		2012-07-21 JP	
B12-040	237	*R. peltatus*	1	1	0	1.967	6.648	Czech Republic; distr. Český Krumlov; Hašlovice: Vltava river in camp NE of village	48°44'58.3"N, 14°19'30.7"E	496		2012-07-20 JP	
B11-032	238	*R. peltatus*	4	4	0	1.981 ± 0.010	6.696 ± 0.032	Czech Republic; distr. Český Krumlov; Holubov: Vltava river below castle ruin Dívčí Kámen	48°53'26.1"N, 14°21'25.4"E	422		2011-07-20 Krejčíková J.; 2012-08-15 Hrdinová M.	
B12-037	239	*R. peltatus*	5	1	0	1.994	6.740	Czech Republic; distr. Český Krumlov; Hrudkov: Vltava river SE of village	48°37'36.9"N, 14°21'20.4"E	537		2012-07-19 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K17-36	240	*R. peltatus*	2	2	0	1.968 ± 0.006	6.650 ± 0.022	Czech Republic; distr. Český Krumlov; Jaroměř: Malý Hodonický fishpond, ca 1.95 km NE of the centre of the village	48°42'17"N, 14°33'21"E	630		2017-06-08 Šumberová K.	
B12-042	241	*R. peltatus*	5	1	1	1.987	6.716	Czech Republic; distr. Český Krumlov; Rájov: Vltava river ESE of bridge in village	48°50'31.3"N, 14°22'24.0"E	456		2012-07-21 JP	
B12-038	242	*R. peltatus*	5	1	1	1.981	6.696	Czech Republic; distr. Český Krumlov; Rožmberk nad Vltavou: Vltava river near footbridge N of castle	48°39'27.9"N, 14°21'51.9"E	525		2012-07-20 JP	
K10-11	243	*R. peltatus*	1	1	1	1.986	6.676	Czech Republic; distr. Český Krumlov; Rožmberk nad Vltavou: Vltava river, ca 0.9 km NNW of the castle	48°39'48"N, 14°21'38"E	530		2010-06-01 PK	
K10-12	244	*R. peltatus*	2	2	2	2.008 ± 0.006	6.748 ± 0.018	Czech Republic; distr. Český Krumlov; Studánky: Studánecký fishpond, NE of the village	48°35'39"N, 14°19'58"E	625		2010-06-01 PK	
K10-33	245	*R. peltatus*	2	2	2	2.003 ± 0.005	6.731 ± 0.018	Czech Republic; distr. Český Krumlov; Svatý Kámen (by Rychnov nad Malší: shallow fishpond by the Obecní brook, SE of the church	48°38'47"N, 14°30'20"E	625		2010-08-26 PK	
K13-26	246	*R. peltatus*	5	5	5	1.980 ± 0.007	6.694 ± 0.024	Czech Republic; distr. Český Krumlov; Třísov: Vltava river, ca 1 km ENE of the village	48°53'09"N, 14°21'34"E	435		2013-08-31 PK	
B12-036	247	*R. peltatus*	5	1	0	2.047	6.919	Czech Republic; distr. Český Krumlov; Vyšší Brod: Vltava river NNE of railway station Těchoraz	48°37'15.6"N, 14°19'38.1"E	546		2012-07-19 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K10-10	248	*R. peltatus*	1	1	0	1.979	6.652	Czech Republic; distr. Český Krumlov; Zátoňské Dvory: Vltava river by the bridge N of the town	48°44'12"N, 14°19'38"E	500		2010-06-01 PK	
K15-32	249	*R. peltatus*	5	5	5	2.024 ± 0.007	6.840 ± 0.024	Czech Republic; distr. Český Krumlov; Zlatá Koruna: small fishponds in the Kokotínského brook valey, ca 1.15 km W of the monastery	48°51'24"N, 14°21'18"E	475		2015-08-01 PK	
K10-16	250	*R. peltatus*	2	2	2	1.979 ± 0.001	6.652 ± 0.003	Czech Republic; distr. Český Krumlov; Zlatá Koruna: Vltava river N of the town	48°51'28"N, 14°22'11"E	450		2010-06-09 Karásek J.	
B12-089	251	*R. peltatus*	2	2	1	2.054 ± 0.008	6.943 ± 0.027	Czech Republic; distr. Děčín; Šluknov: Spréva (Spree) stream on state border in former village Fukov	51°2'37"N, 14°30'15"E	299	*R. penicillatus* A (2016)	2010-09-09 Bauer P.; 2012-05-25 Hadinec J.	
B10-011	252	*R. peltatus*	3	1	1	2.004	6.774	Czech Republic; distr. Domažlice; Mezholezy: small fishpond on Mezholezský stream W of village	49°37'21.2"N, 12°53'50.8"E	439		2010-07-05 JP & ZK	
K10-14	253	*R. peltatus*	2	2	2	1.975 ± 0.008	6.639 ± 0.027	Czech Republic; distr. Frýdek-Místek; Skalice: small fishponds by Záhoří settlement, ca 1.4 km SE of the church	49°38'38"N, 18°25'47"E	355		2010-06-04 PK	
B10-030	254	*R. peltatus*	5	4	3	2.019 ± 0.015	6.806 ± 0.061	Czech Republic; distr. Havlíčkův Brod; Ždírec nad Doubravou: Januš fishpond ca 1.0 km NE of railway station Stružinec, exposed bottom	49°44'11.8"N, 15°50'43.5"E	548		2010-08-28 JP & ZK; 2011-06 Kúr P.	
B13-086	255	*R. peltatus*	1	1	1	1.970	6.659	Czech Republic; distr. Hradec Králové; Hradec Králové: Češík fishpond near SSE edge of city	50°11'14.6"N, 15°51'57.3"E	239		2013-05-27 Rydlo Jan	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K12-08	256	*R. peltatus*	10	3	3	1.977 ± 0.002	6.646 ± 0.006	Czech Republic; distr. Cheb; Bříza: Studna u Lužné fishpond, N of the village	50°06'16"N, 12°17'05"E	450		2012-06 Kaštovský J.	
K12-15	257	*R. peltatus*	8	6	6	2.027 ± 0.005	6.815 ± 0.016	Czech Republic; distr. Cheb; Kladská: small stream in a wetland W of Kladský fishpond, E of the village	50°01'31"N, 12°40'25"E	815		2012-06-23 PK	
B14-062	258	*R. peltatus*	12	1	1	1.960	6.625	Czech Republic; distr. Cheb; Krásná: upper fishpond in settlement Černý Luh	50°14'39.0"N, 12°9'38.8"E	651		2014-06-19 JP	
B14-063	259	*R. peltatus*	5	1	0	2.002	6.767	Czech Republic; distr. Cheb; Lipoltov: Lipoltovský stream below bridge in village	50°5'14.8"N, 12°29'57.3"E	429		2014-06-19 JP	
B14-109	260	*R. peltatus*	1	1	0	2.024	6.841	Czech Republic; distr. Cheb; Prameny: Lesní stream near Giselin and Rudolfův spring SW of village	50°2'41.4"N, 12°43'25.9"E	744		2014-07-19 Čertner M.	
B15-050	261	*R. peltatus*	10	3	0	2.027 ± 0.012	6.851 ± 0.041	Czech Republic; distr. Chrudim; Ctětín: fishpond SW of village, exposed bottom	49°49'34.8"N, 15°50'1.7"E	527		2015-10-08 JP	
B10-028	262	*R. peltatus*	6	2	0	2.055 ± 0.021	6.944 ± 0.069	Czech Republic; distr. Chrudim; Rohozná: Hubský fishpond, exposed bottom	49°48'27.2"N, 15°49'22.0"E	579		2010-08-27 JP & ZK; 2011-06-08 ZK	
B14-068	263	*R. peltatus*	1	1	1	1.989	6.723	Czech Republic; distr. Chrudim; Rváčov: Chrudimka river	49°45'24.2"N, 15°51'21.3"E	539		2014-06-22 ZK	
B10-029	264	*R. peltatus*	3	1	1	2.049	6.926	Czech Republic; distr. Chrudim; Trhová Kamenice: Chrudimka river near bridge of Havlíčkova street in village	49°47'9.2"N, 15°48'53.5"E	523		2010-08-28 JP & ZK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K13-03	265	*R. peltatus*	5	3	0	2.031 ± 0.010	6.866 ± 0.034	Czech Republic; distr. Chrudim; Trpišov: Boušovka fishpond, ca 1,6 km SSW of the village	49°52'36"N, 15°47'28"E	375		2013-05-25 Skácelová O. & Lepš J.	
B15-013	266	*R. peltatus*	2	2	2	2.020 ± 0.000	6.826 ± 0.002	Czech Republic; distr. Chrudim; Vranov: middle fishpond S of village	49°48'4.2"N, 15°50'33.2"E	580		2014-06-20 ZK; 2015-05-17 Nunvářová Kabátová K.	
B17-015	267	*R. peltatus*	1	1	1	2.021	6.831	Czech Republic; distr. Jeseník; Zlaté Hory: Černé jezero protected area, lower reservoir ca 2.3 km SSW of railway station	50°14'19.6"N, 17°22'36.5"E	544		2017-10-15 JP	
B17-013	268	*R. peltatus*	8	8	8	2.011 ± 0.003	6.797 ± 0.011	Czech Republic; distr. Jeseník; Zlaté Hory: Zlaté jezero reservoir ca 2.0 km NNE of railway station	50°16'35.0"N, 17°23'41.0"E	381	*R. peltatus* × *R. trichophyllus* A (F1)	2017-10-15 JP	
B14-103	269	*R. peltatus*	1	1	1	1.980	6.692	Czech Republic; distr. Jihlava; Borovná: fishpond cascade SE of village, 2nd uppermost fishpond	49°9'41.9"N, 15°24'8.8"E	531		2014-07-20 JP	
K10-03	270	*R. peltatus*	2	2	0	2.024 ± 0.004	6.802 ± 0.012	Czech Republic; distr. Jihlava; Dolní Cerekev: Jihlava river, by the bridge near the railway station	49°20'37"N, 15°27'44"E	530		2010-05-29 PK	
K15-05	271	*R. peltatus*	5	3	0	2.017 ± 0.012	6.816 ± 0.039	Czech Republic; distr. Jihlava; Milíčov: Sviták fishpond, ca 1.6 km ENE of the village	49°23'55"N, 15°24'50"E	620		2015-05-30 Ekrt L.	
B14-023	272	*R. peltatus*	5	1	1	1.976	6.679	Czech Republic; distr. Jihlava; Plandry: Jihlava river near Brandlův Mlýn mill	49°24'58.0"N, 15°32'01.1"E	480		2014-05-21 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K13-04	273	*R. peltatus*	29	9	7	1.967 ± 0.004	6.632 ± 0.018	Czech Republic; distr. Jihlava; Popice: Horní Okrouhlík fishpond, ca 1.4 km NE of the village	49°21'28"N, 15°33'12"E	565	*R. peltatus* (5x?)	2010-05-30 PK; 2013-05-26 PK	
K14-02	274	*R. peltatus*	1	1	0	2.008	6.787	Czech Republic; distr. Jihlava; Popice: Popický fishpond, ca 1.2 km NE of the village	49°21'32"N, 15°33'02"E	570		2014-05-25 Ekrt L.	
K10-05	275	*R. peltatus*	2	2	2	1.987 ± 0.011	6.677 ± 0.035	Czech Republic; distr. Jihlava; Vysoká: Lužný fishpond, ca 0.9 km ENE of the village	49°22'31"N, 15°32'56"E	570		2010-05-30 PK	
K14-27	276	*R. peltatus*	4	1	0	1.984	6.706	Czech Republic; distr. Jihlava; Zbilidy: Maršovský brook by the settlement "U Šeredů", SE of the village	49°25'46"N, 15°26'48"E	580		2014-06-14 Ekrt L.	
B13-126	277	*R. peltatus*	1	1	0	2.019	6.824	Czech Republic; distr. Jindřichův Hradec; Červená Lhota: Dub fishpond	49°14'52.2"N, 14°52'18.9"E	483	*R. trichophyllus* A, *R. trichophyllus* hybr.	2013-06-02 Lučanová M.	
K12-05	278	*R. peltatus*	3	2	2	2.005 ± 0.004	6.738 ± 0.015	Czech Republic; distr. Jindřichův Hradec; Červená Lhota: Třešňovec fishpond, ca 1.2 km NNW of the village	49°15'24"N, 14°52'26"E	495		2012-05-26 Skácelová O.	
B17-004	279	*R. peltatus*	1	1	1	2.000	6.760	Czech Republic; distr. Jindřichův Hradec; Český Rudolec: Baruch fishpond near W edge of Matějovec village	49°3'43.7"N, 15°15'43.1"E	644		2017-07-03 L. Lippl	
K16-05	280	*R. peltatus*	1	1	1	1.986	6.713	Czech Republic; distr. Jindřichův Hradec; Český Rudolec: Bochník fishpond, ca 7 km WSW of the village, ca 0.7 km SSW from the small village of Rožnov	49°03'19"N, 15°13'49"E	670		2016-06-18 Lepšová O.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B17-007	281	*R. peltatus*	1	1	1	2.023	6.838	Czech Republic; distr. Jindřichův Hradec; Člunek: Námětek fispond ca 1.65 km ESE–SE of church in village, exposed bottom	49°6'18.5"N, 15°8'49.8"E	563		2017-07-06 JP	
K15-04	282	*R. peltatus*	3	3	0	2.016 ± 0.006	6.815 ± 0.021	Czech Republic; distr. Jindřichův Hradec; Člunek: Pleso fishpond, ca 2.5 km NE of the village	49°07'27"N, 15°09'30"E	560		2015-05-30 Ekrt L.	
K14-01	283	*R. peltatus*	10	5	0	1.974 ± 0.005	6.672 ± 0.017	Czech Republic; distr. Jindřichův Hradec; Dvory nad Lužnicí: small sand pit ca 400 m SE of the railway station	48°51'12"N, 14°54'07"E	455		2014-05-24 PK	
B17-009	284	*R. peltatus*	4	2	1	2.036 ± 0.008	6.882 ± 0.027	Czech Republic; distr. Jindřichův Hradec; Hamr: Vizír fishpond ca 2.1 km NE of railway station Majdalena	48°57'49.8"N, 14°53'22.0"E	443		2015-10-24 PK; 2017-07-03 J. Hanzlíčková	
B17-005	285	*R. peltatus*	2	2	2	2.030 ± 0.004	6.860 ± 0.012	Czech Republic; distr. Jindřichův Hradec; Hatín: Zedník fishpond ca 1.25 km NW of chapel in village	49°7'0.9"N, 14°53'55.1"E	459	*R. peltatus* × *R. trichophyllus* A (other hybrids)	2017-07-04 JP, M. Lučanová & J. Hanzlíčková	
B13-092	286	*R. peltatus*	1	1	0	1.956	6.611	Czech Republic; distr. Jindřichův Hradec; Hospříz: Horní Rokle fishpond	49°07'08.6"N, 15°05'32.4"E	557		2013-06-26 ZK	
B17-021	287	*R. peltatus*	1	1	1	2.014	6.807	Czech Republic; distr. Jindřichův Hradec; Jarošov nad Nežárkou: Rybníky u Lovětína protected area, eastern fishpond ca 550 m SSE of chapel in Lovětín village	49°12'21.5"N, 15°3'19.2"E	499		2017-07-05 Hanzlíčková J.	

Ref. no.	**Locality no.**	**Taxon / Cytotype**	**N**	**N (GS)**	**N repeats**	**Ratio to *Bellis***	**2C-value (pg)**	**Locality**	**Coordinates (WGS 84)**	**Elevation (m)**	**Other taxa**	**Collected**	**Notes**
B17-019	288	*R. peltatus*	1	1	1	2.000	6.760	Czech Republic; distr. Jindřichův Hradec; Jindřichův Hradec: Vysečený fishpond ca 860 m NW of railway station Dolní Radouň	49°12'0.2"N, 15°1'35.0"E	503		2017-07-07 J. Hanzlíčková	
K10-19	289	*R. peltatus*	2	2	2	1.999 ± 0.009	6.718 ± 0.029	Czech Republic; distr. Jindřichův Hradec; Klikov: Dračice river, by the bridge at the W edge of the village	48°54'18"N, 14°54'27"E	450		2010-06-13 PK	
K11-15	290	*R. peltatus*	10	5	5	1.996 ± 0.002	6.711 ± 0.008	Czech Republic; distr. Jindřichův Hradec; Majdalena: northern part of the sand pit "Cep"	48°56'51"N, 14°52'32"E	445		2011-06-11 PK	
B17-006	291	*R. peltatus*	1	1	1	2.014	6.807	Czech Republic; distr. Jindřichův Hradec; Nová Bystřice: small fishpond ca 540 m NE of chapel in village Hradiště	49°2'19.0"N, 15°5'8.0"E	555		2017-07-04 J. Prach	
B13-093	292	*R. peltatus*	1	1	1	1.957	6.615	Czech Republic; distr. Jindřichův Hradec; Nová Olešná: Rosolů fishpond	49°09'56.5"N, 15°09'44.1"E	557		2013-07-26 ZK	
B12-085	293	*R. peltatus*	1	1	0	2.012	6.801	Czech Republic; distr. Jindřichův Hradec; Plasná: Králek fishpond	49°10'34.2"N, 14°53'49.5"E	477		2011-07-28 Píšová S.	
B17-011	294	*R. peltatus*	1	1	1	2.018	6.821	Czech Republic; distr. Jindřichův Hradec; Plavsko: sand pit ca 1.6 km SW of chapel in village	49°4'31"N, 14°53'12"E	438		2017-07-03 Rydlo Jan	
K13-02	295	*R. peltatus*	5	3	0	1.988 ± 0.009	6.719 ± 0.029	Czech Republic; distr. Jindřichův Hradec; Pleše: exposed bottom of a small fishpond W of Chlumský fishpond, ca 2 km W of the village	49°11'38"N, 14°47'39"E	450		2013-05-18 PK	

Ref. no.	Loca-lity no.	Taxon / Cytotype	N	N (GS)	N rep-eats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Other taxa	Collected	Notes
K11-08	296	*R. peltatus*	2	2	2	1.993 ± 0.005	6.697 ± 0.015	Czech Republic; distr. Jindřichův Hradec; Polště: exposed bottom of Polom fishpond, E of the village	49°06'40"N, 14°56'33"E	495		2011-06 Kúr P.	
B12-088	297	*R. peltatus*	1	1	0	1.959	6.621	Czech Republic; distr. Jindřichův Hradec; Přeseka: Velký Tisý Fishpond, Na Lůsech peninsula	49°03'12.7''N, 14°43'40.1''E	424	*R. trichophyllus* A	2011-07-26 Píšová S.	
K10-20	298	*R. peltatus*	2	2	2	1.987 ± 0.005	6.677 ± 0.015	Czech Republic; distr. Jindřichův Hradec; Suchdol nad Lužnicí: abandoned sand pit "Malá Tušť", E of the village	48°53'46"N, 14°54'27"E	450		2010-06-13 PK	
B12-107	299	*R. peltatus*	1	1	0	2.028	6.855	Czech Republic; distr. Jindřichův Hradec; Suchdol nad Lužnicí: N edge of flooded sand-pit Tušť	48°54'9.7"N, 14°53'33.4"E	444		2012-06-15 Lučanová M.	
B14-074	300	*R. peltatus*	12	1	0	2.009	6.790	Czech Republic; distr. Karlovy Vary; Bražec: "Bražecké hliňáky" ponds WSW of village, 2nd northernmost pond	50°10'24.9"N, 13°2'15.8"E	695		2014-07-02 JP & Nunvářová Kabátová K.	
B14-075	301	*R. peltatus*	1	1	1	1.995	6.743	Czech Republic; distr. Karlovy Vary; Bražec: "Bražecké hliňáky" ponds WSW of village, easternmost pond	50°10'19.0"N, 13°2'22.0"E	690	*R. trichophyllus A*, *R. peltatus* × *R. trichophyllus A* (F1)	2014-07-02 JP & Melichar V.	
B14-079	302	*R. peltatus*	10	4	4	1.996 ± 0.001	6.747 ± 0.004	Czech Republic; distr. Karlovy Vary; Bražec: Javorenský fishpond, exposed bottom	50°10'37.4"N, 13°3'50.9"E	733	*R. peltatus* aneuploid, *R. trichophyllus* A	2014-07-02 JP	
B14-060	303	*R. peltatus*	11	1	1	2.022	6.834	Czech Republic; distr. Karlovy Vary; Březová: Teplá stream near footbridge in village	50°11'53.5"N, 12°52'16.1"E	401		2014-06-19 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B14-061	304	*R. peltatus*	15	1	1	2.030	6.861	Czech Republic; distr. Karlovy Vary; Karlovy Vary: town distr. Stará Role, E fishpond near NW edge of town	50°15'18.5"N, 12°48'49.5"E	398		2014-06-19 JP	
B14-057	305	*R. peltatus*	16	1	1	2.037	6.885	Czech Republic; distr. Karlovy Vary; Luhov: Malý Luhovský fishpond	50°1'31.1"N, 13°2'22.2"E	647		2014-06-18 JP	
B14-088	306	*R. peltatus*	3	3	3	2.017 ± 0.026	6.816 ± 0.089	Czech Republic; distr. Karlovy Vary; military training area Hradiště: pond above fishpond Tišina	50°12'54"N, 13°6'18"E	807		2014-07-03 Rydlo Jan	
B14-085	85	*R. peltatus*	1	1	1	1.968	6.652	Czech Republic; distr. Karlovy Vary; military training area Hradiště: small pond near settlement Hájovna	50°12'5.0"N, 13°12'5.9"E	674	*R. circinatus*, *R. trichophyllus* A	2014-07-03 Kolář F.	
B15-021	307	*R. peltatus*	9	3	1	2.022 ± 0.022	6.835 ± 0.075	Czech Republic; distr. Karlovy Vary; Ostrov: 2nd westernmost pond between fishponds Horní štít and Ottův	50°17'53.4"N, 12°55'5.3"E	416		2015-06-17 JP	
B15-022	308	*R. peltatus*	3	3	3	1.994 ± 0.005	6.739 ± 0.016	Czech Republic; distr. Karlovy Vary; Ostrov: westernmost pond between fishponds Horní štít and Ottův	50°17'52.3"N, 12°54'58.8"E	417		2015-06-17 JP	
B10-018	309	*R. peltatus*	1	1	1	2.017	6.817	Czech Republic; distr. Karlovy Vary; Teleč: Dolní Telečský fishpond	50°7'45.0"N, 13°3'57.4"E	626		2010-07-19 JP & ZK	
JH17-002	310	*R. peltatus*	8	4	4	2.038 ± 0.009	6.888 ± 0.031	Czech Republic; distr. Karlovy Vary; Toužim: Felixův fishpond ca 1.25 km WSW of railway station	50°2'47.8"N, 12°58'27.3"E	627		2017-05-06 Hanzlíčková J.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
JH17-001	311	*R. peltatus*	5	3	3	2.018 ± 0.008	6.820 ± 0.026	Czech Republic; distr. Karlovy Vary; Toužim: Lesní fishpond near settlement Luhov	50°1'26.2"N, 13°2'36.2"E	652		2017-05-06 Hanzlíčková J.	
B10-017	312	*R. peltatus*	1	1	1	1.999	6.757	Czech Republic; distr. Karlovy Vary; Údrč: Kopinský fishpond	50°7'57.2"N, 13°4'19.0"E	631		2010-07-19 JP & ZK	
B10-016	313	*R. peltatus*	1	1	1	2.080	7.030	Czech Republic; distr. Karlovy Vary; Údrč: Malý Kopinský fishpond	50°7'50.4"N, 13°4'17.9"E	628		2010-07-19 JP & ZK	
B11-007	314	*R. peltatus*	5	1	0	1.988	6.719	Czech Republic; distr. Klatovy; Čepice: Otava river ca 1.0 kmSW of W bridge in village	49°15'38.6"N, 13°34'53.9"E	449		2011-06-17 JP & PK	
K11-18	315	*R. peltatus*	6	6	5	2.000 ± 0.008	6.727 ± 0.024	Czech Republic; distr. Klatovy; Dobršín: Otava river, S of the village, by the Dobršínského Mlýn settlement	49°15'19"N, 13°33'40"E	435		2011-06-17 PK & JP	
B14-056	316	*R. peltatus*	1	1	1	2.001	6.763	Czech Republic; distr. Klatovy; Dolní Lhota: pond in protected area Luňáky NE of village	49°22'33.3"N, 13°14'40.6"E	395		2014-06-18 JP	
B15-048	317	*R. peltatus*	3	3	3	2.020 ± 0.009	6.826 ± 0.030	Czech Republic; distr. Klatovy; Hamry: water reservoir Nýrsko, exposed bottom on S tip of reservoir	49°14'36.8"N, 13°09'24.2"E	521		2015-09-12 JP	
K11-20	318	*R. peltatus*	6	6	5	2.012 ± 0.011	6.769 ± 0.032	Czech Republic; distr. Klatovy; Horažďovice: Otava river, ca 1.9 km SE of the railway station Horažďovice	49°18'32"N, 13°43'44"E	410		2011-06-17 PK & JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B13-066	319	*R. peltatus*	4	2	0	2.022 ± 0.011	6.834 ± 0.037	Czech Republic; distr. Klatovy; Horská Kvilda: Hamerský stream WSW of village	49°3'15.6"N, 13°33'17.7"E	1030		2010-07-17 JP; 2013-08-25 JP & Nunvářová Kabátová K.	
B16-033	320	*R. peltatus*	1	1	0	1.996	6.746	Czech Republic; distr. Klatovy; Chanovice: Hladoměř fishpond SW of Újezd u Chanovic village	49°23'49"N, 13°41'19"E	504	*R. trichophyllus* A	2016-06-11 JP, ZK, Hanzlíčková J. & Rybka V.	
B14-053	321	*R. peltatus*	10	1	1	2.020	6.828	Czech Republic; distr. Klatovy; Chanovice: small fishpond NE of Starý fishpond	49°24'49.2"N, 13°43'43.9"E	520	*R. trichophyllus A*, *R. peltatus* × *R. trichophyllus A* (F1)	2014-06-18 JP	
B16-023	322	*R. peltatus*	3	3	3	2.012 ± 0.006	6.802 ± 0.022	Czech Republic; distr. Klatovy; Kvášňovice: Jámský fishpond	49°24'27"N, 13°39'4"E	523	*R. trichophyllus* A	2016-06-11 JP, ZK & Hanzlíčková J.	
B16-025	323	*R. peltatus*	1	1	1	1.999	6.757	Czech Republic; distr. Klatovy; Pačejov: Buxín fishpond near railway station Pačejov, NW tip	49°23'41"N, 13°38'18"E	522	*R. trichophyllus* A	2016-06-11 JP, ZK & Hanzlíčková J.	
B16-027	324	*R. peltatus*	1	1	1	1.994	6.740	Czech Republic; distr. Klatovy; Pačejov: Nový Pačejov fishpond between Pačejov and Pačejov-nádraží villages, NE tip	49°22'58"N, 13°37'46"E	509		2016-06-11 JP, ZK & Hanzlíčková J.	
B17-012	325	*R. peltatus*	3	3	3	2.035 ± 0.007	6.877 ± 0.023	Czech Republic; distr. Klatovy; Předslav: Dolní Hladoměř fishpond near SE edge of Měcholupy village	49°26'17.6"N, 13°22'40.5"E	418		2017-08-16 K. Šumberová	

Ref. no.	Loca-lity no.	Taxon / Cytotype	N	N (GS)	N rep-eats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Other taxa	Collected	Notes
K11-19	326	*R. peltatus*	5	5	5	2.004 ± 0.006	6.736 ± 0.020	Czech Republic; distr. Klatovy; Žichovice: Otava river, ca 1.1 km NE of the chapel in the village	49°16'31"N, 13°38'24"E	435		2011-06-17 PK & JP	
B15-026	327	*R. peltatus*	1	1	1	2.013	6.804	Czech Republic; distr. Kutná Hora; Zdeslavice: Pastvický fishpond	49°51'17.1"N, 15°11'46.6"E	437		2015-06-21 ZK	
B12-078	328	*R. peltatus*	1	1	0	2.039	6.892	Czech Republic; distr. Liberec; Černousy: pond cascade near settlement V Poli, 2nd uppermost pond	50°59'35.0"N, 15°3'24.0"E	242		2012-10-11 JP	
B13-065	329	*R. peltatus*	1	1	0	1.982	6.699	Czech Republic; distr. Liberec; Pertoltice: Šálkův fishpond	50°59'01.5"N, 15°06'12.5"E	319	*R. trichophyllus* A (2012)	2013-08-24 JP & Nunvářová Kabátová K.	
B10-004	330	*R. peltatus*	3	1	1	2.029	6.858	Czech Republic; distr. Most; Litvínov: pool on former slag heap SSW of railway station Litvínov	50°35'10.6"N, 13°36'30.5"E	276		2010-06-01 JP	
K13-13	331	*R. peltatus*	5	5	5	2.015 ± 0.006	6.811 ± 0.021	Czech Republic; distr. Nový Jičín; Jistebník: pond in the pastures S of Bezruč fishpond, ca 1.5 km S of the railway station	49°44'06"N, 18°09'08"E	225		2013-06-11 PK	
B15-029	332	*R. peltatus*	1	1	0	2.004	6.774	Czech Republic; distr. Olomouc; Moravský Beroun: Důlní stream N of town	49°48'37.5"N, 17°26'56.7"E	551		2015-07-14 Dančák M. & Tkáčiková J.	
B15-028	333	*R. peltatus*	5	1	1	1.974	6.672	Czech Republic; distr. Olomouc; Olomouc: Oskava stream NE of city district Chomoutov	49°38'50.2"N, 17°14'41.8"E	213		2015-07-13 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K13-12	334	*R. peltatus*	10	10	5	2.020 ± 0.007	6.828 ± 0.023	Czech Republic; distr. Opava; Jilešovice: the western from the two ponds ca 0.65 km WNW of the railway station	49°54'00"N, 18°08'00"E	225		2013-06-10 PK	
K15-08	335	*R. peltatus*	5	3	3	1.994 ± 0.007	6.740 ± 0.023	Czech Republic; distr. Opava; Vítkov: "Bělidla" fishponds, the 4th fishpond from the upstream, ca 1.4 km E of the centre of the town	49°46'34"N, 17°46'18"E	470		2015-06-09 PK	
K13-14	336	*R. peltatus*	4	4	4	2.021 ± 0.006	6.830 ± 0.022	Czech Republic; distr. Ostrava-město; Polanka nad Odrou: Kačírek fishpond, ca 1.3 km SSW of the railway station	49°46'02"N, 18°11'09"E	220	*R. peltatus* × *R. trichophyllus* A (other hybrids)	2013-06-11 PK	
B16-006	104	*R. peltatus*	4	4	2	2.005 ± 0.011	6.776 ± 0.036	Czech Republic; distr. Pardubice; Lázně Bohdaneč: Bohdanečský fishpond, shallow lagoones near E bank	50°5'42"N, 15°41'13"E	220	*R. circinatus*, *R. trichophyllus* A	2016-05-26 JP & Hanzlíčková J.	
K17-08	337	*R. peltatus*	1	1	0	1.972	6.665	Czech Republic; distr. Pelhřimov; Bedřichov: Kostroun fishpond, ca 0.7 km SW of the village	49°28'17"N, 14°57'11"E	455		2017-05-18 Šumberová K.	
K14-06	338	*R. peltatus*	5	5	0	2.002 ± 0.008	6.767 ± 0.026	Czech Republic; distr. Pelhřimov; Pravíkov: fishpond ca 1 km SSW of the village	49°19'35"N, 15°05'46"E	580		2014-06-01 PK	
B10-032	176	*R. peltatus*	1	1	1	1.989	6.723	Czech Republic; distr. Písek; Lhota u Kestřan: Otava river near bridge on N edge of village	49°15'47.4"N, 14°4'28.3"E	368	*R. fluitans* (2x)	2010-09-26 JP	
K17-09	339	*R. peltatus*	1	1	0	1.971	6.662	Czech Republic; distr. Plzeň-jih; Březí: fishpond ca 150 m WNW of the centre of the village	49°30'08"N, 13°27'48"E	555		2017-05-20 Hauer T.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B16-019	340	*R. peltatus*	1	1	1	1.990	6.726	Czech Republic; distr. Plzeň-jih; Čížkov: Oborský fishpond near Chynín village	49°33'28"N, 13°42'14"E	648		2016-06-10 JP & ZK	
K13-06	341	*R. peltatus*	10	3	0	2.013 ± 0.010	6.805 ± 0.034	Czech Republic; distr. Plzeň-jih; Klášter: a small fishpond by the main road Nepomuk-Plzeň, ca 1 km WSW of the village	49°29'49"N, 13°33'53"E	440		2013-06-02 PK	
B16-031	342	*R. peltatus*	5	3	3	1.993 ± 0.013	6.737 ± 0.043	Czech Republic; distr. Plzeň-jih; Nepomuk: Nový fishpond ca 3.4 km W of náměstí Augustina Němejce square in town	49°29'8"N, 13°32'5"E	470		2016-06-12 JP & Hanzlíčková J.	
B10-038	343	*R. peltatus*	1	1	1	2.021	6.831	Czech Republic; distr. Prachatice; Kvilda: small pond near Kvildský stream on N edge of village	49°1'19.1"N, 13°34'51.3"E	1047		2010-07-30 Kubátová B.	
K13-07	344	*R. peltatus*	10	3	0	2.012 ± 0.010	6.802 ± 0.032	Czech Republic; distr. Prachatice; Lhenice: Koubovský fishpond, ca 2 km SE of the village	48°58'51"N, 14°10'12"E	535		2013-06-05 PK	
K15-28	345	*R. peltatus*	3	3	0	2.038 ± 0.012	6.890 ± 0.040	Czech Republic; distr. Prachatice; Nová Pec: north-western (upstrem) end of Lipno dam, the former channel of Vltavy river, ca 1.3 km N of the village	48°48'04"N, 13°56'50"E	730		2015-07-02 PK	
K12-21	346	*R. peltatus*	20	5	5	2.001 ± 0.004	6.725 ± 0.014	Czech Republic; distr. Prachatice; Prachatice: Fefrovské fishponds, small fishponds on the Fefrovský brook, ca 2.2 km SSW of the town centre	48°59'38"N, 13°59'22"E	665		2012-07-17 PK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K12-20	347	*R. peltatus*	3	3	3	2.023 ± 0.004	6.800 ± 0.013	Czech Republic; distr. Prachatice; Strunkovice nad Blanicí: Blanice river, S of the village	49°04'42"N, 14°03'09"E	450		2012-07-17 PK	
K12-19	348	*R. peltatus*	5	3	3	2.002 ± 0.003	6.730 ± 0.010	Czech Republic; distr. Prachatice; Vlachovo Březí: Libotyňský brook in the eastern part of the town	49°04'39"N, 13°56'38"E	560		2012-07-10 Štech M.	
B14-110	349	*R. peltatus*	1	1	0	1.975	6.676	Czech Republic; distr. Prachatice; Volary: lower pond in settlement Stögrova Huť	48°54'52"N, 13°51'19"E	791		2014-07-17 Rydlo Jar.	
B14-111	350	*R. peltatus*	1	1	0	1.994	6.740	Czech Republic; distr. Prachatice; Volary: middle pond in settlement Stögrova Huť	48°54'56"N, 13°51'20"E	798		2014-07-17 Rydlo Jar.	
K12-22	351	*R. peltatus*	20	5	5	1.983 ± 0.006	6.666 ± 0.020	Czech Republic; distr. Prachatice; Volary: Volarský brook, 0-500 m W of the bridge by Planerův Dvůr settlement, S of the town	48°53'24"N, 13°53'35"E	740	*R. trichophyllus* A	2012-07-17 PK	
B13-047	110	*R. peltatus*	2	2	1	2.001 ± 0.026	6.763 ± 0.088	Czech Republic; distr. Přerov; Týn nad Bečvou: pool complex near Bečva river NE of village	49°31'37.2"N, 17°38'33.3"E	234	*R. circinatus* (2015)	2013-06-21 JP & ZK	
B16-018	352	*R. peltatus*	1	1	1	1.997	6.750	Czech Republic; distr. Příbram; Hvoždany: fishpond Háj, SW tip, exposed bottom	49°31'26"N, 13°47'52"E	533		2016-06-10 JP & ZK	
B16-015	353	*R. peltatus*	4	4	4	2.033 ± 0.012	6.872 ± 0.042	Czech Republic; distr. Příbram; Hvoždany: Raputovský fishpond	49°31'55"N, 13°50'33"E	580	*R. trichophyllus*, *R. peltatus* × *R. trichophyllus* A (various hybrids)	2016-06-10 JP & ZK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B14-169	354	*R. peltatus*	9	2	1	2.012 ± 0.004	6.801 ± 0.014	Czech Republic; distr. Příbram; Malá Hraštice: small fishpond NW of railway station	49°48'45.1"N, 14°15'43.1"E	354		2014-07-11 Nunvářová Kabátová K.	
B13-050	355	*R. peltatus*	12	12	2	1.977 ± 0.005	6.681 ± 0.016	Czech Republic; distr. Příbram; Mokrovraty: Dolní fishpond NW of village	49°48'25.8"N, 14°14'3.7"E	377	*R. peltatus* × *trichophylus* A (F1 and other hybrids)	2013-06-29 JP & Nunvářová Kabátová K.	
B13-027	356	*R. peltatus*	20	1	1	2.021	6.831	Czech Republic; distr. Příbram; Mokrovraty: Střední fishpond WNW of village	49°48'26.6"N, 14°13'16.9"E	385		2013-06-11 JP & Nunvářová Kabátová K.	
B13-026	357	*R. peltatus*	1	1	1	1.977	6.682	Czech Republic; distr. Příbram; Voznice: Velký fishpond	49°49'7.5"N, 14°13'4.6"E	364		2013-06-11 JP & Nunvářová Kabátová K.	
B12-009	111	*R. peltatus*	10	1	1	2.028	6.855	Czech Republic; distr. Rakovník; Řevničov: Horní Kracle fishpond SE of railway station	50°8'29.4"N, 13°50'33.1"E	409	*R. circinatus*	2012-06-12 JP & Potůčková A.	
ZK2263	358	*R. peltatus*	1	1	1	1.977	6.682	Czech Republic; distr. Rychnov nad Kněžnou; Lípa nad Orlicí: drainage canal NNE of village	50°09'03.8"N, 16°06'23.4"E	263		2010-08-07 ZK	
B14-059	359	*R. peltatus*	13	1	1	1.988	6.719	Czech Republic; distr. Sokolov; Nová Ves: Velký Novoveský fishpond	50°4'46.2"N, 12°46'37.2"E	732		2014-06-19 JP	
B15-001	360	*R. peltatus*	1	1	1	1.991	6.730	Czech Republic; distr. Strakonice; Drahenický Málkov: ditch below Paštický fishpond	49°27'49.6"N, 13°54'19.6"E	476		2015-05-10 Nunvářová Kabátová K.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K15-18	361	*R. peltatus*	4	3	3	2.014 ± 0.011	6.808 ± 0.036	Czech Republic; distr. Strakonice; Horní Poříčí: small fishpond W of Pohodnice settlement, SE of the village	49°16'48"N, 13°48'00"E	410	*R. peltatus* (5x?)	2015-06-23 PK	
K15-02	362	*R. peltatus*	3	3	0	1.988 ± 0.009	6.719 ± 0.031	Czech Republic; distr. Strakonice; Kadov: artificial pool W of the Smyslov fishpond, ca 2.8 km NW of the village	49°25'22"N, 13°48'03"E	470		2015-05-25 Hauer T.	
K14-35	363	*R. peltatus*	6	6	0	1.971 ± 0.005	6.648 ± 0.015	Czech Republic; distr. Strakonice; Leskovice: Malduchy fishpond SW of the village, exposed sandy souther bank of the eastern part of the fishpond	49°19'12"N, 13°54'51"E	470	*R. trichophyllus A, R. peltatus* × *R. trichophyllus A* (F1)	2012-06 Kúr P.; 2014-07-10 PK	
K11-23	364	*R. peltatus*	10	5	5	2.023 ± 0.008	6.801 ± 0.025	Czech Republic; distr. Strakonice; Modlešovice: a small pond ca 500 m WNW of the railway station	49°15'28"N, 13°58'08"E	385		2011-06-22 PK	
B11-030	365	*R. peltatus*	3	3	0	1.973 ± 0.007	6.670 ± 0.024	Czech Republic; distr. Strakonice; Slaník: Otava river ca 0.3 km SW of chappel in village	49°15'57.7"N, 13°56'59.2"E	385		2011-07-14 Chudáčková H.	
B11-031	366	*R. peltatus*	2	2	0	2.021 ± 0.000	6.829 ± 0.002	Czech Republic; distr. Strakonice; Strakonice: Otava river near E edge of town	49°15'36.2"N, 13°55'42.9"E	388		2011-07-14 Chudáčková H.	
K15-01	367	*R. peltatus*	1	1	0	2.058	6.956	Czech Republic; distr. Strakonice; Tchořovice: Hadí fishpond, asi 3 km SE of the village	49°24'53"N, 13°49'37"E	440		2015-05-25 Hauer T.	
B15-054	368	*R. peltatus*	1	1	0	2.033	6.872	Czech Republic; distr. Svitavy; Jedlová: Na Drenáži fishpond	49°39'44.1"N, 16°19'35.4"E	589		2015-10-08 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B17-017	369	*R. peltatus*	5	5	5	2.015 ± 0.006	6.809 ± 0.021	Czech Republic; distr. Svitavy; Jedlová: newly established fishpond ca 830 m W of church in village	49°39'42.0"N, 16°17'35.7"E	626		2017-10-15 JP	
B13-038	370	*R. peltatus*	25	2	2	2.027 ± 0.029	6.850 ± 0.096	Czech Republic; distr. Svitavy; Jedlová: Ráček I pond	49°40'7.0"N, 16°19'59.5"E	595	*R. trichophyllus* A (2012)	2013-06-20 JP & ZK	some individuals with medium-sized flowers and without floating leaves – possibly introgresants
B15-052	371	*R. peltatus*	14	8	2	2.001 ± 0.006	6.764 ± 0.020	Czech Republic; distr. Svitavy; Modřec: Modřecký fishpond, exposed bottom	49°41'33.0"N, 16°17'46.5"E	595	*R. peltatus* (6x?)	2015-10-08 JP	
B15-015	372	*R. peltatus*	2	2	2	1.980 ± 0.011	6.691 ± 0.035	Czech Republic; distr. Svitavy; Opatov: stream flowing into W cove of Nový fishpond	49°50'16.9"N, 16°28'56.3"E	427		2015-05-17 Chrtek J.	
B13-041	118	*R. peltatus*	16	1	1	2.028	6.855	Czech Republic; distr. Svitavy; Třebařov: Rohlíček fishpond	49°50'40.5"N, 16°40'35.1"E	339	*R. circinatus*, *R. rionii*	2013-06-20 JP & ZK	
K14-08	373	*R. peltatus*	5	5	0	2.018 ± 0.011	6.822 ± 0.037	Czech Republic; distr. Tábor; Drahov: exposed bottom of Smíchov II fishpond, ca 2.6 km SE of the church	49°09'37"N, 14°46'41"E	425	*R. trichophyllus* A, *R. peltatus* × *trichophyllus* A (various hybrids; 2010)	2014-06-02 PK	

Ref. no.	**Locality no.**	**Taxon / Cytotype**	**N**	**N (GS)**	**N repeats**	**Ratio to *Bellis***	**2C-value (pg)**	**Locality**	**Coordinates (WGS 84)**	**Elevation (m)**	**Other taxa**	**Collected**	**Notes**
K12-02	374	*R. peltatus*	22	5	5	2.005 ± 0.007	6.739 ± 0.023	Czech Republic; distr. Tábor; Hamr: Hluboký u Hamru fishpond, SE part, ca 1,05 km ENE of the town	49°09'34"N, 14°46'21"E	415	*R. trichophyllus* A	2012-05-26 PK	
B16-009	375	*R. peltatus*	1	1	0	2.009	6.790	Czech Republic; distr. Tábor; Tábor: city distr. Zahrádka, unnamed fishpond W of Pochytil fishpond	49°25'11"N, 14°37'17"E	466	*R. trichophyllus* A	2016-06-02 JP	
B14-065	376	*R. peltatus*	1	1	1	2.024	6.841	Czech Republic; distr. Tachov; Diana: Kateřinský stream above small reservoir SW of village	49°37'7.8"N, 12°33'55.1"E	496		2014-06-20 JP	
B14-157	377	*R. peltatus*	5	1	1	1.974	6.672	Czech Republic; distr. Tachov; Chodová Planá: small fishpond near road Chodová Planá - Výškov	49°53'31.5"N, 12°45'26.3"E	565		2014-06-30 Čertner M.	
B14-107	378	*R. peltatus*	1	1	1	2.036	6.882	Czech Republic; distr. Tachov; Kyjov: fishpond near road Kyjov – Planá SE of village	49°52'51.7"N, 12°41'45.2"E	504	*R. trichophyllus* A	2014-07-19 Čertner M.	
K12-16	379	*R. peltatus*	10	5	5	2.000 ± 0.003	6.722 ± 0.010	Czech Republic; distr. Tachov; Planá: fishpond at the S edge of the town, ca 0.7 km SE of the church	49°51'42"N, 12°44'33"E	490		2012-06-24 PK	
B14-064	380	*R. peltatus*	1	1	1	2.033	6.872	Czech Republic; distr. Tachov; Tachov: Mže stream near bridge of Vodní street in town	49°47'36.8"N, 12°38'1.6"E	476		2014-06-20 JP	
B15-023	381	*R. peltatus*	10	3	2	1.973 ± 0.006	6.669 ± 0.019	Czech Republic; distr. Teplice; Košťany: flooded quarry Otakar	50°39'1.7"N, 13°44'33.5"E	259		2015-06-18 JP	
K17-84	382	*R. peltatus*	2	2	0	2.004 ± 0.002	6.774 ± 0.007	Czech Republic; distr. Třebíč; Domamil: Partyzánský fishpond, ca 1.8 km NW of the church in the village	49°05'25"N, 15°40'26"E	520		2017-09-02 Ekrt L.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K17-39	383	*R. peltatus*	5	1	0	1.957	6.615	Czech Republic; distr. Třebíč; Studenec: exposed bottom of Štičí fishpond, along its N coast, ca 1.95 km NW of the railway station Studenec	49°13'48"N, 16°02'42"E	450	*R. trichophyllus* A	2017-05-25 Šumberová K.	
K11-01	384	*R. peltatus*	5	0	0			Czech Republic; distr. Třebíč; Studenec: Hlad fishpond, ca 2.7 km N of the village	49°13'26"N, 16°03'21"E	435	*R. trichophyllus* A (2017)	2011-07-04 Štěpánek J.	
B13-069	120	*R. peltatus*	1	1	0	2.003	6.770	Czech Republic; distr. Ústí nad Orlicí; Brandýs nad Orlicí: Tichá Orlice river below bridge of Žerotínova street	50°0'4.9"N, 16°16'45.1"E	297	*R. circinatus* (2012)	2013-09-01 JP & Nunvářová Kabátová K.	
B15-044	385	*R. peltatus*	3	1	1	2.016	6.814	Czech Republic; distr. Ústí nad Orlicí; Choceň: Tichá Orlice river near wastewater treatment plant NW of town	50°0'36.8"N, 16°11'57.8"E	279		2015-09-03 JP	
B12-064	122	*R. peltatus*	2	2	0	2.037 ± 0.022	6.885 ± 0.074	Czech Republic; distr. Ústí nad Orlicí; Kerhartice: Tichá Orlice river below weir on E edge of village	49°58'19.6"N, 16°21'44.0"E	317	*R. circinatus*	2012-08-15 JP	
B13-068	123	*R. peltatus*	4	2	0	2.013 ± 0.009	6.804 ± 0.030	Czech Republic; distr. Ústí nad Orlicí; Orlické Podhůří: Tichá Orlice river by railway bridge near settlement Luh	49°59'34.0"N, 16°20'9.5"E	310	*R. circinatus*	2013-09-01 JP & Nunvářová Kabátová K.	
PT10-17	386	*R. peltatus*	1	1	1	2.014	6.807	Czech Republic; distr. Ústí nad Orlicí; Zářecká Lhota: small fishpond on W edge of village	49°59'27.9"N, 16°14'15.7"E	331		2010-08-13 PT	
K13-18	34	*R. peltatus*	22	11	11	1.994 ± 0.005	6.735 ± 0.017	Czech Republic; distr. Vsetín; Choryně: "Choryňský mokřad" wetland area, ca 2.5 km N of the village, small artificial pond	49°31'03"N, 17°54'25"E	275	*R. aquatilis*, *R. aquatilis* × *peltatus* (F1 and other hybrids)	2010-06-04 PK; 2013-06-13 PK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K13-01	387	*R. peltatus*	3	3	0	1.945 ± 0.003	6.573 ± 0.011	Czech Republic; distr. Źďár nad Sázavou; Znětínek: Staroborský fishpond, ca 1.2 km SW of the village	49°27'51"N, 15°54'47"E	560		2013-05 Ekrt L.	
K10-01	388	*R. peltatus*	1	1	1	2.018	6.783	Czech Republic; distr. Znojmo; Čížov: artificial pond in the wetlend west of Čížovský fishpond, ca 1.3 km N of the village	48°53'26"N, 15°52'34"E	400		2010-06 Ekrt L.	
K12-12	389	*R. peltatus*	2	2	2	2.009 ± 0.032	6.751 ± 0.106	Czech Republic; distr. Znojmo; Tavíkonice: Kacíř fishpond, SE of the village	49°01'47"N, 16°06'32"E	360		2012-06-03 Štech M.	
B16-046	390	*R. peltatus*	2	2	2	2.006 ± 0.000	6.779 ± 0.002	Czech Republic; distr. Žďár nad Sázavou; Radostín: Doubravník fishpond, near dam	49°39'2"N, 15°50'48"E	600		2016-09-08 Šumberová K.	
K16-13	391	*R. peltatus*	5	3	2	1.975 ± 0.004	6.676 ± 0.013	Czech Republic; distr. Žďár nad Sázavou; Žďár nad Sázavou: Převorský fishpond, at N edge of the town, right bank of Sázava river, downstream from the Pilská dam	49°35'02"N, 15°55'45"E	575		2016-07-14 Šumberová K.	
K16-14	392	*R. peltatus*	1	1	1	1.975	6.676	Czech Republic; distr. Žďár nad Sázavou; Žďár nad Sázavou: small fish storage ponds at N edge of the town, right bank of Sázava river, downstream from the Pilská dam	49°35'07"N, 15°55'47"E	575		2016-07-14 Šumberová K.	
B11-024	393	*R. peltatus*	2	2	0	2.010 ± 0.002	6.792 ± 0.008	Denmark; Jutland; reg. Syddanmark; Randbøldal: arm of the Vejle Å stream ENE of village	55°41'45.0 "N, 9°15'31.1"E	47		2011-08-19 Kolář F.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B12-045	394	*R. peltatus*	5	1	0	2.056	6.949	Denmark; Jutland; reg. Syddanmark; Ribe: artificial pool on right bank of Ribeå river at SE edge of town	55°19'26"N, 08°47'03"E	1		2012-07-31 JP & ZK	
B12-044	395	*R. peltatus*	1	1	1	2.019	6.824	Denmark; Jutland; reg. Syddanmark; Seem: estuary of field ditch to Ribeå river ESE of town	55°18'54.8"N, 08°50'04.0"E	1		2012-07-31 JP & ZK	
B12-061	396	*R. peltatus*	1	1	1	2.074	7.010	Denmark; Jutland; reg. Syddanmark; Tange: Varde Å river NE of town	55°38'39.2"N, 08°32'37.7"E	4		2012-08-03 JP & ZK	
B12-063	397	*R. peltatus*	5	1	1	2.044	6.909	Germany; Niedersachsen; Baven: Örtze river below the bridge of Zur Örtze road	52°50'41.9"N, 10°6'00.7"E	52		2012-08-03 JP & ZK	
B12-062	398	*R. peltatus*	5	1	1	2.043	6.905	Germany; Niedersachsen; Garstedt: Luhe river below bridge of Vierhöfener Straße	53°16'47.1"N, 10°10'59.7"E	16		2012-08-03 JP & ZK	
B14-099	399	*R. peltatus*	1	1	0	1.959	6.621	Hungary; Hajdú-Bihar County; distr. Balmazújváros; Balmazújváros: ditch in Hortobágyi Nemzeti Park ca 10.4 km WNW of railway station in town	47°38'47.4"N, 21°13'11.1"E	85		2014 Lukács B.	
B14-002	400	*R. peltatus*	1	1	1	1.979	6.689	Hungary; Vas County; Csesztreg: Holt-Kerka-rivulet in the village	46°42'59"N, 16°30'46"E	180		2014-04-24 Mesterházy A.	
K14-03	401	*R. peltatus*	3	3	0	1.983 ± 0.006	6.701 ± 0.022	Poland; Śląskie voivodeship (province); Kobiór: right-side tributary to Korzeniec brook, ca 2.5 km W of the village	50°03'42"N, 18°54'05"E	260		2014-05-31 Hlisnikovský D.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B12-095	402	*R. peltatus*	1	1	0	2.063	6.973	United Kingdom; England; North Yorkshire; Craven district; Skipton: ditch along road ca 6.8 km NNE of Skipton castle	54°1'4.1"N, 1°57'51.6"W	300		2012-08-02 Chudáčková H.	
K10-06	273	*R. peltatus* (5x?)	1	1	1	2.472	8.309	Czech Republic; distr. Jihlava; Popice: Horní Okrouhlík fishpond, ca 1.4 km NE of the village	49°21'28"N, 15°33'12"E	565	*R. peltatus* (4x)	2010-05-30 PK	
K15-19	361	*R. peltatus* (5x?)	2	2	2	2.528 ± 0.001	8.543 ± 0.002	Czech Republic; distr. Strakonice; Horní Poříčí: small fishpond W of Pohodnice settlement, SE of the village	49°16'48"N, 13°48'00"E	410	*R. peltatus* (4x)	2015-06-23 PK	
B15-053	371	*R. peltatus* (6x?)	1	1	1	2.997	10.130	Czech Republic; distr. Svitavy; Modřec: Modřecký fishpond, exposed bottom	49°41'33.0"N, 16°17'46.5"E	595	*R. peltatus* (4x)	2015-10-08 JP	single sterile plant
K15-23-2	403	*R. peltatus* (aneuploid)	1	1	0	2.102	7.105	Czech Republic; distr Prachatice; Borová Lada, Knížecí Pláně: a small fishpond ca 0.65 km NW of the ruin of the church in the former village	48°57'21"N, 13°36'35"E	1015	*R. peltatus*	2015-06-27 PK	single plant with slightly higher genome size than the rest of the population
K11-12-12	216	*R. peltatus* (aneuploid)	1	1	1	1.960	6.588	Czech Republic; distr. České Budějovice; Branišov: Návesný fishpond, 1 km N of the village	48°59'16"N, 14°23'48"E	390	*R. peltatus*, *R. trichophyllus* A, *R. peltatus* × *trichophyllus* A (various hybrids)	2011-06-07 PK	lower genome size than the rest of the population, confirmed with double-peak in simultaneous FCM analysis

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B14-080	302	*R. peltatus* (aneuploid)	1	1	1	1.887	6.378	Czech Republic; distr. Karlovy Vary; Bražec: Javorenský fishpond, exposed bottom	50°10'37.4"N, 13°3'50.9"E	733	*R. peltatus*, *R. trichophyllus* A	2014-07-02 JP	single plant with reduced fertility
K17-32	208	*R. peltatus* × *R. trichophyllus* A (F1)	1	1	0	2.298	7.767	Austria; Niederösterreich (Lower Austria); Rudmanns (distr. Zwettl): exposed bottom of Rudmannser Teich fishpond, SSE of the village	48°35'28"N, 15°21'52"E	590	*R. peltatus, R. trichophyllus* A	2017-06-06 PK	
B16-047	404	*R. peltatus* × *R. trichophyllus* A (F1)	5	5	5	2.328 ± 0.002	7.870 ± 0.006	Czech Republic; distr. České Budějovice; Čejkovice: storage ponds "Ostrov" below Mlýnský fishpond, pond no. 1, exposed bottom	49°0'26"N, 14°22'50"E	390		2016-09-14 Šumberová K.	
K17-91	405	*R. peltatus* × *R. trichophyllus* A (F1)	3	1	0	2.308	7.801	Czech Republic; distr. České Budějovice; Lišov: Čekal fishpond, ca 1.3 km SW of the church in the village	49°00'30"N, 14°35'30"E	550		2017-09-18 Šumberová K.	
K17-35	232	*R. peltatus* × *R. trichophyllus* A (F1)	3	3	0	2.224 ± 0.120	7.516 ± 0.407	Czech Republic; distr. Český Krumlov; Bukovsko: Čirý fishpond, ca 1.4 km W of the centre of the village	48°40'23"N, 14°31'19"E	655	*R. peltatus*	2017-06-08 Šumberová K.	markedly variable population
K16-16	406	*R. peltatus* × *R. trichophyllus* A (F1)	7	3	3	2.343 ± 0.009	7.918 ± 0.030	Czech Republic; distr. Český Krumlov; Křemže: Křemžský potok brook, at the S edge of the town, ca 400 m SE of the church	48°54'08"N, 14°18'30"E	495		2016-07-30 PK	
B14-058	407	*R. peltatus* × *R. trichophyllus* A (F1)	16	2	1	2.360 ± 0.037	7.975 ± 0.127	Czech Republic; distr. Cheb; Mnichov: Mnichovský stream W of village	50°2'16.5"N, 12°46'17.9"E	690		2013-06-06 Čertner M.; 2014-06-19 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B17-014	268	*R. peltatus* × *R. trichophyllus* A (F1)	2	2	2	2.361 ± 0.022	7.978 ± 0.073	Czech Republic; distr. Jeseník; Zlaté Hory: Zlaté jezero reservoir ca 2.0 km NNE of railway station	50°16'35.0"N, 17°23'41.0"E	381	*R. peltatus*	2017-10-15 JP	
B14-077	301	*R. peltatus* × *R. trichophyllus* A (F1)	1	1	1	2.322	7.848	Czech Republic; distr. Karlovy Vary; Bražec: "Bražecké hliňáky" ponds WSW of village, easternmost pond	50°10'19.0"N, 13°2'22.0"E	690	*R. peltatus*, *R. trichophyllus* A	2014-07-02 JP & Melichar V.	no floating leaves
B14-078	408	*R. peltatus* × *R. trichophyllus* A (F1)	1	1	1	2.313	7.818	Czech Republic; distr. Karlovy Vary; Bražec: fishpond near NE edge of village	50°10'36.9"N, 13°3'6.1"E	712		2014-07-02 Rydlo Jar.	no floating leaves
B14-055	321	*R. peltatus* × *R. trichophyllus* A (F1)	1	1	1	2.382	8.051	Czech Republic; distr. Klatovy; Chanovice: small fishpond NE of Starý fishpond	49°24'49.2"N, 13°43'43.9"E	520	*R. peltatus*, *R. trichophyllus* A	2014-06-18 JP	
K14-07	409	*R. peltatus* × *R. trichophyllus* A (F1)	20	9	7	2.335 ± 0.004	7.892 ± 0.014	Czech Republic; distr. Pelhřimov; Benešov: Nevilák fishpond, W of the village	49°20'18"N, 14°59'46"E	650		2013-06 Lepš J.; 2014-06-01 PK	
B16-016	353	*R. peltatus* × *R. trichophyllus* A (F1)	4	4	4	2.343 ± 0.006	7.919 ± 0.021	Czech Republic; distr. Příbram; Hvoždany: Raputovský fishpond	49°31'55"N, 13°50'33"E	580	*R. peltatus*, *R. trichophyllus* A, *R. peltatus* × *R. trichophyllus* A (various hybrids)	2016-06-10 JP & ZK	
B13-051	355	*R. peltatus* × *R. trichophyllus* A (F1)	18	18	3	2.320 ± 0.004	7.842 ± 0.013	Czech Republic; distr. Příbram; Mokrovraty: Dolní fishpond NW of village	49°48'25.8"N, 14°14'3.7"E	377	*R. peltatus*, *R. peltatus* × *trichophyllus* A (various hybrids)	2013-06-29 JP & Nunvářová Kabátová K.	both heterophyllous and homophyllous plants

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K14-36	363	*R. peltatus* × *R. trichophyllus* A (F1)	2	2	0	2.348 ± 0.032	7.936 ± 0.108	Czech Republic; distr. Strakonice; Leskovice: Malduchy fishpond SW of the village, sandy souther bank of the eastern part of the fishpond	49°19'12"N, 13°54'51"E	470	*R. peltatus*, *R. trichopyllus* A	2014-07-10 PK	
K13-23d	410	*R. peltatus* × *R. trichophyllus* A (F1)	1	1	0	2.309	7.804	Czech Republic; distr. Tábor; Vlkov: "Vlkovská pískovna" sand pit, ca 1.8 km NNW of the railway station	49°09'43"N, 14°42'55"E	408	*R. trichophyllus* A, various hybrids of *R. peltatus* and *R. trichophyllus* A		
K14-40	411	*R. peltatus* × *R. trichophyllus* A (F1)	1	1	0	2.380	8.044	Czech Republic; distr. Žďár nad Sázavou; Koroužné: Svratka river, ca 1 km NNW of the church	49°32'12"N, 16°20'41"E	365		2014-07-16 Ekrt L.	
B14-008	412	*R. peltatus* × *R. trichophyllus* A (F1)	6	3	3	2.324 ± 0.008	7.855 ± 0.026	Czech Republic; distr. Žďár nad Sázavou; Švařec: Svratka river in S part of village	49°31'03.0"N, 16°20'31.5"E	350	*R. peltatus* hybr.	2013-06-20 JP & ZK; 2014-05-19 JP	
K11-12-13	216	*R. peltatus* × *R. trichophyllus* A (other hybrids)	1	1	1	2.130	7.160	Czech Republic; distr. České Budějovice; Branišov: Návesný fishpond, 1 km N of the village	48°59'16"N, 14°23'48"E	390	*R. peltatus*, *R. peltatus* aneuploid, *R. trichophyllus* A	2011-06-07 PK	
K15-03	413	*R. peltatus* × *R. trichophyllus* A (other hybrids)	1	1	1	2.083	7.041	Czech Republic; distr. Český Krumlov; Černá v Pošumaví: Lukavický brook ca 0.6 km S of Pláničský fishpond, ca 4.2 km SE of the town	48°42'57"N, 14°09'29"E	745		2015-05-28 Štech M. & Schaabová V.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B17-005-03	285	*R. peltatus* × *R. trichophyllus* A (other hybrids)	1	1	1	2.076	7.017	Czech Republic; distr. Jindřichův Hradec; Hatín: Zedník fishpond ca 1.25 km NW of chapel in village	49°7'0.9"N, 14°53'55.1"E	459	*R. peltatus*	2017-07-04 JP, M. Lučanová & J. Hanzlíčková	
B15-017	336	*R. peltatus* × *R. trichophyllus* A (other hybrids)	2	2	1	2.119 ± 0.020	7.162 ± 0.068	Czech Republic; distr. Ostrava-město; Polanka nad Odrou: Kačírek fishpond, ca 1.3 km SSW of the railway station	49°46'0.9"N, 18°11'9.0"E	218	*R. peltatus*	2013-06-11 PK; 2015-05-26 Rydlo Jar.	
B16-015-01	353	*R. peltatus* × *R. trichophyllus* A (other hybrids)	1	1	1	2.093	7.074	Czech Republic; distr. Příbram; Hvoždany: Raputovský fishpond	49°31'55"N, 13°50'33"E	580	*R. peltatus*, *R. trichophyllus* A, *R. peltatus* × *trichophyllus* A	2016-06-10 JP & ZK	double-peak with the rest of *R. peltatus* population
B13-006	355	*R. peltatus* × *R. trichophyllus* A (other hybrids)	2	2	2	2.140 ± 0.055	7.233 ± 0.186	Czech Republic; distr. Příbram; Mokrovraty: Dolní fishpond NW of village	49°48'25.8"N, 14°14'3.7"E	377	*R. peltatus*, *R. peltatus* × *trichophyllus* A (F1)	2013-05-05 Nunvářová Kabátová K.	
B10-034	373	*R. peltatus* × *R. trichophyllus* A (other hybrids)	1	1	1	2.258	7.632	Czech Republic; distr. Tábor; Drahov: exposed bottom of Smíchov II fishpond, ca 2.6 km SE of the church	49°9'36.7"N, 14°46'41.7"E	419	*R. peltatus* (2014), *R. trichophyllus* A (2014)	2010-09-20 Píšová S.	
K13-23b	410	*R. peltatus* × *R. trichophyllus* A (other hybrids)	10	10	10	2.227 ± 0.006	7.527 ± 0.021	Czech Republic; distr. Tábor; Vlkov: "Vlkovská pískovna" sand pit, ca 1.8 km NNW of the railway station	49°09'43"N, 14°42'55"E	408	*R. trichophyllus* A, various hybrids of *R. peltatus* and *R. trichophyllus* A	2012-08-20 Kubátová B.; 2013-06-22 PK	

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K13-23c	410	*R. peltatus* × *R. trichophyllus* A (other hybrids)	5	5	5	2.492 ± 0.008	8.423 ± 0.028	Czech Republic; distr. Tábor; Vlkov: "Vlkovská pískovna" sand pit, ca 1.8 km NNW of the railway station	49°09'43"N, 14°42'55"E	408	*R. trichophyllus* A, various hybrids of *R. peltatus* and *R. trichophyllus* A	2013-06-22 PK	
K17-42	414	*R. peltatus* hybr.	7	7	5	2.046 ± 0.005	6.916 ± 0.015	Austria; Oberösterreich (Upper Austria), Aigen-Schlägl: Große Mühl river, ca 2.9 km SE of the centre of the village	48°37'33"N, 13°59'47"E	530		2017-06-13 PK & Lučanová M.	
K13-22	415	*R. peltatus* hybr.	12	12	12	2.014 ± 0.011	6.802 ± 0.036	Czech Republic; distr. České Budějovice; Dubné: Velký Hájský fishpond, ca 0.75 km SSW of the centre of the village	48°58'11"N, 14°21'21"E	430		2010-06-12 PK; 2013-06-15 PK	variable, some individuals *R. peltatus*-like but some with relatively small flowers and/or lacking floating leaves, probably backcrosses with *R. trichophyllus*
K17-43	416	*R. peltatus* hybr.	5	5	3	1.971 ± 0.003	6.662 ± 0.009	Czech Republic; distr. České Budějovice; Třebeč: Stropnice river ca 1 km ENE of the village	48°52'37"N, 14°41'21"E	450		2017-06-15 PK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K10-32	417	*R. peltatus* hybr.	7	5	5	1.996 ± 0.006	6.732 ± 0.011	Czech Republic; distr. Český Krumlov; Rychnov nad Malší: Malše river, by the bridge at the SW edge of the village	48°40'04"N, 14°28'50"E	595		2010-08-26 PK	
B13-085	18	*R. peltatus* hybr.	1	1	1	1.995	6.743	Czech Republic; distr. Hradec Králové; Hradec Králové: pool in "Na Plachtě" area on SE edge of town	50°11'19"N, 15°51'37"E	239	*R. aquatilis*	2013-05-27 Rydlo Jan	morphologically strange individual with medium-sized flowers, without floating leaves, probably affected by introgression
K16-06	418	*R. peltatus* hybr.	5	5	5	1.990 ± 0.011	6.728 ± 0.037	Czech Republic; distr. Jindřichův Hradec; Halámky: Lužnice river upstream from the bridge ca 0.4 km WSW from the village	48°51'46"N, 14°53'56"E	480		2016-06-27 PK	some individuals of *R. peltatus* appearence, other look like hybrids
K16-08	419	*R. peltatus* hybr.	14	10	7	2.003 ± 0.007	6.769 ± 0.023	Czech Republic; distr. Jindřichův Hradec; Majdalena: Lužnice river just above "Rozvodí" weir, ca 2.65 km NNW of the church in the village	48°59'13"N, 14°50'55"E	435		2016-06-27 PK	some individuals of *R. peltatus* appearence, other look like hybrids
K16-07	420	*R. peltatus* hybr.	7	7	4	2.010 ± 0.007	6.794 ± 0.024	Czech Republic; distr. Jindřichův Hradec; Suchdol nad Lužnicí: Lužnice river by the road bridge in the S part of the village (the road to Halámky)	48°53'31"N, 14°53'09"E	450		2016-06-27 PK	some individuals of *R. peltatus* appearence, other look like hybrids

Ref. no.	**Locality no.**	**Taxon / Cytotype**	**N**	**N (GS)**	**N repeats**	**Ratio to *Bellis***	**2C-value (pg)**	**Locality**	**Coordinates (WGS 84)**	**Elevation (m)**	**Other taxa**	**Collected**	**Notes**
B13-046	421	*R. peltatus* hybr.	22	2	2	1.998 ± 0.010	6.753 ± 0.034	Czech Republic; distr. Olomouc; Litovel: water race along Čihadlo street in town	49°41'57.7"N, 17°04'41.7"E	239		2013-06-21 JP & ZK	morphologically strange plants, possibly introgresants
B13-045	422	*R. peltatus* hybr.	8	1	1	2.033	6.872	Czech Republic; distr. Olomouc; Nové Zámky: oxbow of Morava river W–WSW of chateau	49°43'14.8"N, 17°01'01.4"E	238		2013-06-21 JP & ZK	morphologically strange plants, possibly introgresants
K15-09	423	*R. peltatus* hybr.	5	3	3	2.018 ± 0.005	6.821 ± 0.015	Czech Republic; distr. Opava; Zálužné: Moravice river, NW of the village	49°49'23"N, 17°42'32"E	380		2015-06-09 PK	
K13-23a	410	*R. peltatus* hybr.	10	10	4	2.010 ± 0.005	6.783 ± 0.015	Czech Republic; distr. Tábor; Vlkov: "Vlkovská pískovna" sand pit, ca 1.8 km NNW of the railway station	49°09'43"N, 14°42'55"E	408	*R. trichophyllus* A, *R. peltatus* × *trichophyllus* A (various hybrids)	2010-05-22 PK; 2012-08-20 Kubátová B.; 2013-06-22 PK	
B14-007	412	*R. peltatus* hybr.	13	13	1	1.994 ± 0.004	6.739 ± 0.012	Czech Republic; distr. Žďár nad Sázavou; Švařec: Svratka river in S part of village	49°31'03.0"N, 16°20'31.5"E	350	*R. peltatus* × *trichophylus* A	2014-05-19 JP	possibly introgresants (all individuals of hybrid appearance)

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B12-060	424	*R. peltatus* hybr.	1	1	1	2.028	6.855	Denmark; Jutland; reg. Midtjylland; Tarm: Sonderå river WNW of town	55°55'06.4"N, 08°28'58.0"E	1		2012-08-03 JP & ZK	forming only intermediate leaves when flowering
K14-32	425	*R. peltatus* hybr. (upper Vltava river)	5	0	0			Czech Republic; distr Prachatice; Volary: Teplá Vltava river, above the confluence with Studená Vltava river, S of the town, ca 2.65 km E of Černý kříž railway station	48°51'40"N, 13°53'50"E	743		2014-06-21 PK & JP	
K15-17	426	*R. peltatus* hybr. (upper Vltava river)	3	3	3	2.861 ± 0.004	9.670 ± 0.014	Czech Republic; distr. Český Krumlov; Bližní Lhota: Hamerský brook, ca 300 m SW of Zadní Hamry settlement, S of the village	48°44'23"N, 14°00'48"E	730		2015-06-13 Štech M. & Štechová T.	
B13-063	427	*R. peltatus* hybr. (upper Vltava river)	15	7	0	2.902 ± 0.029	9.810 ± 0.099	Czech Republic; distr. Prachatice; Dobrá: Teplá Vltava river below footbridge	48°53'54"N, 13°50'19"E	741		2013-08-18 Rydlo Jar.; 2014-06-21 PK & JP	
B12-068	428	*R. peltatus* hybr. (upper Vltava river)	6	6	1	2.844 ± 0.014	9.612 ± 0.048	Czech Republic; distr. Prachatice; Lenora: Teplá Vltava river, SE of the village, by the "Soumarský most" bridge	48°54'33.7"N, 13°49'25.2"E	746		2012-08-14 Hrdinová M.; 2014-06-21 PK & JP	
K14-28	429	*R. peltatus* hybr. (upper Vltava river)	5	0	0			Czech Republic; distr. Prachatice; Lenora: Teplá Vltava river, SE of the village, ca 700 m NW of the Soumarský most railway station	48°54'43"N, 13°49'16"E	750		2014-06-21 PK & JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B12-069	430	*R. peltatus* hybr. (upper Vltava river)	1	1	1	2.916	9.856	Czech Republic; distr. Prachatice; Ovesná: Teplá Vltava river ESE of railway station	48°48'18.5"N, 13°56'47.2"E	723		2012-08-15 Hrdinová M.	
K14-33	431	*R. peltatus* hybr. (upper Vltava river)	5	5	3	2.898 ± 0.030	9.795 ± 0.103	Czech Republic; distr. Prachatice; Pěkná: Vltava river, ca 1.05 km WSE of the church	48°50'49"N, 13°55'16"E	742		2014-06-21 PK & JP	
K14-31	432	*R. peltatus* hybr. (upper Vltava river)	5	5	0	2.842 ± 0.012	9.607 ± 0.041	Czech Republic; distr. Prachatice; Volary: Teplá Vltava river, above the confluence with Volarský brook, S of the town, ca 2.35 km NE of the Černý kříž railway station	48°52'31"N, 13°53'03"E	744		2014-06-21 PK & JP	
K14-34	433	*R. peltatus* hybr. (upper Vltava river)	5	0	0			Czech Republic; distr. Prachatice; Želnava: Vltava river, ca 1.7 km NW of the church	48°49'11"N, 13°56'37"E	740		2014-06-21 PK & JP	
K15-30	434	*R. peltatus* hybr. (Váh river)	5	3	3	2.679 ± 0.005	9.054 ± 0.017	Slovakia; distr. Liptovský Mikuláš; Liptovský Mikuláš: Váh river (slowly floating side channel by the right bank of the main channel) at the S edge of the town, ca 1.8 km SE of the St. Mikuláš church (in the town centre)	49°04'12"N, 19°37'40"E	590		2015-07-15 PK	
K15-29	435	*R. peltatus* hybr. (Váh river)	1	1	1	2.692	9.099	Slovakia; distr. Ružomberok; Ružomberok: confluence of Váh and Revúca rivers in the town	49°04'59"N, 19°18'26"E	460		2015-07-10 Rydlo J.	
B10-021	436	*R. peltatus-penicillatus* A	9	9	8	2.035 ± 0.003	6.877 ± 0.011	Czech Republic; distr. Cheb; Mostov: Ohře river near NW edge of village	50°7'1.2"N, 12°29'21.6"E	416		2010-07-19 JP & ZK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B13-103	437	*R. peltatus-penicillatus* A	1	1	0	2.002	6.767	Czech Republic; distr. Cheb; Nebanice: Ohře river below bridge S of village	50°6'35.3"N, 12°28'19.4"E	419		2013-08-24 Rydlo Jan & Rydlo Jar.	
K12-14	438	*R. peltatus-penicillatus* A	7	7	7	1.991 ± 0.003	6.691 ± 0.009	Czech Republic; distr. Cheb; Odrava: Odrava river, by the bridge at the W edge of the village	50°06'09"N, 12°28'56"E	420		2012-06-22 PK	
B14-102	439	*R. peltatus-penicillatus* A	1	1	0	2.021	6.831	Czech Republic; distr. Cheb; Vokov: Ohře river near S edge of settlement	50°6'35.6"N, 12°25'51.6"E	422		2013-08-24 Rydlo Jan & Rydlo Jar.	
K10-21	440	*R. peltatus-penicillatus* A	9	9	0	2.034 ± 0.008	6.854 ± 0.025	Czech Republic; distr. Chomutov; Klášterec nad Ohří: Ohře river, by the railway bridge at the S edge of the town	50°23'02"N, 13°10'53"E	290		2010-06-22 PK; 2011-04-21 JP	
B15-039	441	*R. peltatus-penicillatus* A	5	5	4	2.084 ± 0.014	7.044 ± 0.047	Czech Republic; distr. Chomutov; Okounov: Ohře river NW of village	50°21'56.2"N, 13°05'56.6"E	304		2015-07-24 JP & PK	
K10-25	442	*R. peltatus-penicillatus* A	2	2	0	2.040 ± 0.025	6.855 ± 0.082	Czech Republic; distr. Chomutov; Perštejn: Ohře river by the village	50°22'10"N, 13°06'43"E	305		2010-07 Blažek P.	
B13-108	443	*R. peltatus-penicillatus* A	1	1	0	2.090	7.064	Czech Republic; distr. Karlovy Vary; Dalovice: Ohře river near village	50°14'28.6"N, 12°53'47.6"E	365		2013-08-29 Rydlo Jan & Rydlo Jar.	
B15-034	444	*R. peltatus-penicillatus* A	5	5	5	2.035 ± 0.004	6.879 ± 0.012	Czech Republic; distr. Karlovy Vary; Karlovy Vary: town distr. Drahovice, Ohře river NE of road no. 6 bridge	50°14'30.7"N, 12°53'26.5"E	366		2015-07-22 JP & PK	
B15-036	445	*R. peltatus-penicillatus* A	5	5	5	2.087 ± 0.006	7.055 ± 0.019	Czech Republic; distr. Karlovy Vary; Kyselka: Ohře river in village	50°16'4.4"N, 12°59'28.3"E	342		2015-07-23 JP & PK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B13-109	446	*R. peltatus-penicillatus* A	1	1	0	2.070	6.997	Czech Republic; distr. Karlovy Vary; Sedlečko: Ohře river WNW of village	50°14'20.6"N, 12°56'44.1"E	359		2013-08-29 Rydlo Jan & Rydlo Jar.	
B15-035	447	*R. peltatus-penicillatus* A	5	5	4	2.064 ± 0.013	6.978 ± 0.044	Czech Republic; distr. Karlovy Vary; Sedlečko: Ohře river WSW of village	50°13'59.5"N, 12°56'8.3"E	359		2015-07-23 JP & PK	
B12-083	448	*R. peltatus-penicillatus* A	1	1	0	2.040	6.895	Czech Republic; distr. Karlovy Vary; Stráž nad Ohří: Ohře river in village	50°20'21.9"N, 13°3'16.3"E	318		2012-06-17 A. Potůčková	
B15-038	449	*R. peltatus-penicillatus* A	5	5	5	2.078 ± 0.006	7.025 ± 0.019	Czech Republic; distr. Karlovy Vary; Stráž nad Ohří: Ohře river SW of village	50°19'52.5"N, 13°2'9.7"E	320		2015-07-24 JP & PK	
B13-111	450	*R. peltatus-penicillatus* A	1	1	0	2.086	7.051	Czech Republic; distr. Karlovy Vary; Šemnice: Ohře river near bridge NW of village	50°14'38.7"N, 12°58'11.9"E	354		2013-08-29 Rydlo Jan & Rydlo Jar.	
K10-23	451	*R. peltatus-penicillatus* A	3	3	0	2.042 ± 0.018	6.865 ± 0.062	Czech Republic; distr. Karlovy Vary; Vojkovice: Ohře river by the bridge in the village	50°18'11"N, 13°01'04"E	330		2010-06-25 PK	
B15-037	452	*R. peltatus-penicillatus* A	5	5	5	2.062 ± 0.009	6.969 ± 0.030	Czech Republic; distr. Karlovy Vary; Vojkovice: Ohře river near settlement Hradiště	50°17'34.5"N, 13°00'54.9"E	329		2015-07-23 JP & PK	
K15-24	453	*R. peltatus-penicillatus* A	20	20	0	2.069 ± 0.009	6.994 ± 0.029	Czech Republic; distr. Louny; Libočany: Ohře river, under a weir ca 0.8 km SE of the castle	50°19'46"N, 13°31'20"E	200		2011-04-21 JP; 2015-06-30 Štech M. & Piherová J.	
K15-25	454	*R. peltatus-penicillatus* A	3	3	0	2.124 ± 0.019	7.179 ± 0.064	Czech Republic; distr. Louny; Postoloprty: Ohře river, by the bridge ca 400 m SSE of the castle	50°21'22"N, 13°42'15"E	185		2015-06-30 Štech M. & Piherová J.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected
K14-42	455	*R. peltatus-penicillatus* A	3	3	0	2.042 ± 0.012	6.901 ± 0.040	Czech Republic; distr. Louny; Žatec: Ohře river, by the railway bridge at the E edge of the town	50°20'03"N, 13°33'44"E	200		2014-07-20 Litvín R.
K10-22	456	*R. peltatus-penicillatus* A	2	2	0	1.994 ± 0.004	6.702 ± 0.013	Czech Republic; distr. Sokolov; Citice: Ohře river; upstream from the bridge (road to Tisová) at the E edge of the village	50°09'36"N, 12°36'51"E	385		2010-06-25 PK
B13-105	457	*R. peltatus-penicillatus* A	1	1	0	1.981	6.696	Czech Republic; distr. Sokolov; Dasnice: Ohře river below weir in village	50°8'44.2"N, 12°33'55.8"E	412		2013-08-25 Rydlo Jan & Rydlo Jar.
B10-020	458	*R. peltatus-penicillatus* A	4	1	1	2.028	6.855	Czech Republic; distr. Sokolov; Chotíkov: Ohře river on S edge of village	50°6'58.0"N, 12°30'18.2"E	414		2010-07-19 JP & ZK
B13-104	459	*R. peltatus-penicillatus* A	1	1	0	2.021	6.831	Czech Republic; distr. Sokolov; Kynšperk nad Ohří: Ohře river in town	50°7'32.5"N, 12°31'46.3"E	414		2013-08-24 Rydlo Jan & Rydlo Jar.
B15-033	460	*R. peltatus-penicillatus* A	5	5	5	2.060 ± 0.010	6.963 ± 0.035	Czech Republic; distr. Sokolov; Loket: Ohře river above Svatošské skály rock formation	50°11'36.8"N, 12°48'39.4"E	377		2015-07-22 JP & PK
B15-032	461	*R. peltatus-penicillatus* A	5	5	3	2.082 ± 0.008	7.037 ± 0.029	Czech Republic; distr. Sokolov; Loket: Ohře river below camp NE of town	50°11'45.9"N, 12°46'6.7"E	383		2015-07-22 JP & PK
B14-149	462	*R. peltatus-penicillatus* A	2	2	1	2.077 ± 0.025	7.019 ± 0.083	Czech Republic; distr. Sokolov; Sokolov: Ohře river in town centre	50°11'0.5"N, 12°38'34.3"E	397		2013-08-25 Rydlo Jan & Rydlo Jar.; 2014-08-14 Rydlo Jan & Rydlo Jar.

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B16-042	463	*R. peltatus-penicillatus* A	3	3	3	2.072 ± 0.015	7.003 ± 0.052	Czech Republic; distr. Sokolov; Šabina: Ohře river below weir on NW edge of village	50°8'10"N, 12°34'50"E	410		2016-09-05 Brabec J. & Šumberová K.	
B13-106	464	*R. peltatus-penicillatus* A	1	1	0	2.035	6.878	Czech Republic; distr. Sokolov; Šabina: Ohře river near footbridge in village	50°8'5.3"N, 12°34'45.2"E	408		2013-08-25 Rydlo Jan & Rydlo Jar.	
B14-031	465	*R. penicillatus* A	10	3	3	2.163 ± 0.008	7.311 ± 0.028	Czech Republic; distr. Česká Lípa; Mimoň: Panenský stream near brige of Lužická street in town	50°39'41.1"N, 14°43'28.5"E	277		2014-05-31 JP	
B16-041	251	*R. penicillatus* A	11	11	5	2.187 ± 0.010	7.391 ± 0.034	Czech Republic; distr. Děčín; Šluknov: Spréva (Spree) stream near state border in former village Fukov	51°2'38"N, 14°30'15"E	305	*R. peltatus* (2010, 2012)	2016-08-11 Dvořák V.	
ZK2367	466	*R. penicillatus* A	1	1	1	2.138	7.226	Czech Republic; distr. Havlíčkův Brod; Smrčná: Sázava river near bridge between village and railway station	49°39'35.1"N, 15°20'56.2"E	378		2011-06-08 ZK	
ZK3112	467	*R. penicillatus* A	1	1	1	2.112	7.139	Czech Republic; distr. Chrudim; Horní Bradlo: Chrudimka river above E bridge in village	49°48'10.4"N, 15°44'45.2"E	509		2014-06-21 ZK	
ZK2365	468	*R. penicillatus* A	1	1	0	2.172	7.341	Czech Republic; distr. Kutná Hora; Vlastějovice: Sázava river above bridge	49°43'40.5"N, 15°10'26.4"E	335		2011-06-08 ZK	
K10-31	469	*R. penicillatus* A	12	5	3	2.130 ± 0.009	7.176 ± 0.040	Czech Republic; distr. Opava; Děhylov: Opava river, E of Štěpán fishpond, ca 2.4 km ESE of the village	49°51'43"N, 18°11'56"E	215		2010-08-20 PK; 2012-07-14 JP & Pachmanová L.; 2015-05-28 Rydlo Jar.	no floating leaves

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B10-014	470	*R. penicillatus* A	5	1	0	2.112	7.139	Germany; Bayern (Bavaria); Pfreimd: Pfreimd river in settlement Stein	49°30'37.9"N, 12°13'30.4"E	375		2010-07-09 Kolář F.	
K15-22	471	*R. penicillatus* A	3	3	2	2.155 ± 0.010	7.285 ± 0.034	Germany; Sachsen (Saxony), distr. Leipzig; Sermuth: Freiberger Mulde river, ca 1.9 km ESE of the village, by the bridge 100 m SW of the guest house "Zur Eintracht"	51°09'27"N, 12°48'05"E	130		2015-06-24 Štech M. & Piherová J.	no floating leaves
K14-43	472	*R. penicillatus* A	5	3	3	2.133 ± 0.016	7.210 ± 0.053	Germany; Sachsen (Saxony), distr. Mittelsachsen; Erdmannsdorf: Zschopau river ca asi 160 m N of the bridge in hte village	50°49'19"N, 13°04'59"E	280		2014-07-28 Štech M.	no floating leaves
B15-049	473	*R. penicillatus* A	1	1	0	2.165	7.318	Germany; Sachsen (Saxony); Lohsdorf: Schwarzbach stream SSE of village	50°57'59"N, 14°10'54"E	240		2015-09-17 Rydlo Jan & Rydlo Jar.	
PT10-01	474	*R. penicillatus* B	4	2	1	2.477 ± 0.029	8.372 ± 0.098	Czech Republic; distr. Ústí nad Orlicí; Vysoké Mýto: Loučná river near settlement Jangelec E of town	49°57'13.4"N, 16°11'20.8"E	269		2010-05-15 PT; 2015-07-17 ZK	no floating leaves
PT10-02	475	*R. penicillatus* B	4	2	2	2.464 ± 0.000	8.328 ± 0.000	Czech Republic; distr. Ústí nad Orlicí; Vysoké Mýto: Mlýnský stream (race of Loučná river) in NE part of town	49°57'16.5"N, 16°9'54.0"E	266		2010-05-15 PT; 2015-07-17 ZK	no floating leaves
PT10-03	476	*R. penicillatus* B	1	1	1	2.470	8.349	Czech Republic; distr. Ústí nad Orlicí; Vysoké Mýto: race of Loučná river ("Postulánek") NE of town	49°57'28.1"N, 16°10'6.2"E	266		2010-05-15 PT	no floating leaves
B17-002	477	*R. penicillatus* B	3	3	3	2.444 ± 0.011	8.260 ± 0.038	Czech Republic; distr. Ústí nad Orlicí; Zámrsk: Loučná river below bridge in village	49°59'45.2"N, 16°6'57.7"E	254		2017-06-21 Šumberová K.	no floating leaves

Ref. no.	Loca-lity no.	Taxon / Cytotype	N	N (GS)	N rep-eats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Other taxa	Collected	Notes
B12-071	478	*R. penicillatus* B	3	1	0	2.515	8.501	Poland; Warmian-Masuria Voivodeship; Pisz County; Bogumiły: Pisz river near bridge SW of village	53°31'55.4"N, 21°50'51.7"E	105		2012-08-31 PT & Kubátová B.	no floating leaves
B12-047	479	*R. penicillatus* C	2	1	1	2.679	9.055	Denmark; Jutland; reg. Midtjylland; Langå: Lilleå stream near mouth to Gudenå river	56°22'47.9"N, 09°53'37.1"E	2		2012-07-31 JP & ZK	no floating leaves
B12-050	480	*R. penicillatus* D	1	1	1	2.849	9.630	Denmark; Jutland; reg. Midtjylland; Skivum: Sønderup Å stream near bridge NE of village	56°52'49.9"N, 09°36'45.1"E	6		2012-08-01 JP & ZK	no floating leaves
B12-094	481	*R. penicillatus* E	1	1	1	3.214	10.863	United Kingdom; England; Derbyshire; distr. High Peak; Wormhill: river Wye ca 0.75 km SSE of church in village	53°15'28.6"N, 1°48'42.2"W	236		2012-07-07 Chudáčková H.	no floating leaves
K15-12	482	*R. penicillatus* F	8	8	4	4.108 ± 0.011	13.885 ± 0.037	Austria; Oberösterreich, Innviertel; Bogenhofen: a stream in the southern part of the village	48°16'00"N, 13°06'42"E	350	*R. trichophyllus* B, *R. penicillatus* F × *R. trichophyllus* B (F1)	2015-06-13 PK & JP & Hohla M.	no floating leaves
K15-12B	482	*R. penicillatus* F (F1)	1	1	1	2.062	6.970	Austria; Oberösterreich, Innviertel; Bogenhofen: a stream in the southern part of the village	48°16'00"N, 13°06'42"E	350	*R. trichophyllus* B, *R. penicillatus* F × *R. trichophyllus* B (F1)	2015-06-13 PK & JP & Hohla M.	F1 hybrid of the presumed parental taxa or polyhaploid individual
K15-13	482	*R. penicillatus* F × *R. trichophyllus* B (F1)	1	1	1	3.544	11.979	Austria; Oberösterreich, Innviertel; Bogenhofen: a stream in the southern part of the village	48°16'00"N, 13°06'42"E	350	*R. penicillatus* F, *R. trichophyllus* B	2015-06-13 PK & JP & Hohla M.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B14-037	36	*R. rionii*	1	1	1	1.559	5.269	Austria; Burgenland; Sankt Andrä am Zicksee: Seewinkelhauptkanal canal near mouth to Zicksee lake	47°47'49"N, 16°54'6"E	119	*R. baudotii*	2014-05-23 Mesterházy A.	
K17-95	483	*R. rionii*	4	1	0	1.558	5.266	Austria; Burgenland; Seewinkel, Apetlon: NE part of Östliche Wörtenlacke, c. 5.35 km NE of Apetlon church	47°46'45"N, 16°52'46"E	119		2017-09-23 Pachschwöll C.	collections held under the permit no. A4/NN.AB-10056-5-2016 to C. Pachschwöll
K17-18	484	*R. rionii*	2	2	2	1.581 ± 0.006	5.342 ± 0.019	Austria; Burgenland; Tadten: groundwater channel “Herrschaftsgraben”, SSE of the village	47°42'48"N, 17°02'12"E	114	*R. trichophyllus* B	2017-06-05 PK & Englmaier P.	
K17-12	485	*R. rionii*	7	3	3	1.604 ± 0.007	5.422 ± 0.024	Austria; Niederösterreich (Lower Austria); Markthof an der March: ancient stretch of river Morava named „Lüssarm“ at the bridge of the cyclists route between Schlosshof and Devinska Nova Ves	48°12'42"N, 16°57'38"E	139	*R. trichophyllus* B	2017-06-05 PK & Englmaier P.	
B16-003	74	*R. rionii*	5	3	1	1.600 ± 0.002	5.408 ± 0.007	Czech Republic; distr Jičín; Jičíněves: fishpond near football field in village	50°22'15"N, 15°20'23"E	246	*R. circinatus*	2016-05-26 JP & Hanzlíčková J.	
B16-039	486	*R. rionii*	1	1	1	1.629	5.506	Czech Republic; distr. Brno-město; Brno: city distr. Sadová, small fishpond on Zaječí stream, 2nd southernmost pond	49°14'11"N, 16°36'22"E	276		2016-07-08 Šumberová K.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B14-012	3	*R. rionii*	3	3	1	1.592 ± 0.010	5.381 ± 0.033	Czech Republic; distr. Brno-venkov; Holasice: "Ludmila" oxbow	49°4'1.6"N, 16°36'23.9"E	185	*R. aquatilis, R. trichophyllus* B, *R. aquatilis* × *trichophyllus* B (F1)	2014-05-19 JP	
B14-105	487	*R. rionii*	24	6	6	1.614 ± 0.003	5.455 ± 0.011	Czech Republic; distr. Břeclav; Hlohovec: storage ponds W of village (ponds "Jan", "Antonín", "Jaroslav", "Bohumír")	48°46'46.3"N, 16°45'16.8"E	168		2010-06-07 PT; 2014-07-20 JP; 2015-05-31 ZK	
B14-027	488	*R. rionii*	6	1	0	1.606	5.428	Czech Republic; distr. Břeclav; Lanžhot: pool (canal) on Košarské louky meadow complex SSW of town	48°38'49"N, 16°55'59.5"E	151		2014-05-21 Rydlo Jan	
K14-16	12	*R. rionii*	2	2	0	1.650 ± 0.009	5.577 ± 0.030	Czech Republic; distr. Břeclav; Lanžhot: Soutok area, ca 10.1 km S of the church, abandoned sand pit "Dědova louka"	48°37'59"N, 16°57'34"E	150	*R. aquatilis*	2014-06-07 PK & ZK	
B11-018	489	*R. rionii*	5	1	1	1.629	5.506	Czech Republic; distr. Břeclav; Lednice: Prostřední fishpond, exposed bottom	48°46'58.9"N, 16°47'31.7"E	165		2011-06-29 JP	
K16-03	490	*R. rionii*	3	1	0	1.630	5.509	Czech Republic; distr. Břeclav; Rakvice: artifical pond in the area of subhalophytic vegetation "Trkmanský dvůr", ca 2.4 km ENE of the church in the village	48°51'58"N, 16°50'35"E	165	*R. trichophyllus* B	2016-06-11 Lepšová O.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K12-06	491	*R. rionii*	22	7	7	1.594 ± 0.008	5.366 ± 0.025	Czech Republic; distr. Břeclav; Sedlec: Dolní Mušlovský fishpond, ca 1.9 km NW of the church	48°47'41"N, 16°41'03"E	201		2012-05-30 Štech M. & Kúr P.; 2010-06-07 PT	
PT10-13	492	*R. rionii*	3	1	1	1.610	5.442	Czech Republic; distr. Břeclav; Sedlec: Nesyt fishpond, NW part	48°46'49.2"N, 16°42'28.5"E	173		2010-06-07 PT	
K12-07	493	*R. rionii*	12	7	7	1.616 ± 0.007	5.439 ± 0.021	Czech Republic; distr. Břeclav; Úvaly: Úvalský fishpond, NE of the village	48°44'50"N, 16°42'51"E	200		2012-05-30 Štech M. & Kúr P.; 2017-06-20 Šumberová K.	
K14-10	494	*R. rionii*	3	2	0	1.550 ± 0.009	5.237 ± 0.032	Czech Republic; distr. Hodonín; Bzenec: pond in the N part of "Vlčí hrdlo" wetland, ca 3.5 km SE of the town	48°57'19"N, 17°18'29"E	170		2014-06-06 PK	
B14-016	495	*R. rionii*	10	1	1	1.591	5.378	Czech Republic; distr. Hodonín; Milotice: Milotický fishpond	48°57'48.7"N, 17°8'59.7"E	183	*R. trichophyllus* B	2014-05-20 JP	
K14-11	496	*R. rionii*	4	3	0	1.589 ± 0.012	5.372 ± 0.040	Czech Republic; distr. Hodonín; Moravský Písek: artificial pond in the restored wetland "Vypálenky", ca 2.0 km SSW of the church	48°58'24"N, 17°19'28"E	170		2014-06-06 PK	
K14-18	497	*R. rionii*	1	1	0	1.547	5.229	Czech Republic; distr. Hodonín; Mutěnice: Mutěnické fishponds, triangular fishpond in the northern part of the fishpond complex, ca 2.35 km NE of the church	48°54'29"N, 17°03'20"E	185		2014-06-08 Kúr P. & Píšová S.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K11-14	498	*R. rionii*	20	5	5	1.592 ± 0.004	5.353 ± 0.014	Czech Republic; distr. Hodonín; Tvarožná Lhota: shallow estern part of Lučina dam, ca 3.4 km SE of the church	48°51'47"N, 17°23'42"E	295		2011-06-09 Štech M.	
B14-097	43	*R. rionii*	1	1	1	1.567	5.296	Czech Republic; distr. Chomutov; Dobřenec: fishpond 1 km E of village	50°14'43.0"N, 13°16'08.2"E	425	*R. baudotii*	2014-07-03 ZK	
ZK2529	499	*R. rionii*	1	1	0	1.597	5.398	Czech Republic; distr. Jičín; Budčeves; inflow canal to fishpond Zrcadlo	50°18'22.3"N, 15°14'33.7"E	209		2012-06-10 ZK	
ZK2526	500	*R. rionii*	1	1	0	1.584	5.354	Czech Republic; distr. Jičín; Ledkov; fishpond on Dědek stream	50°20'57.5"N, 15°13'44.3"E	219		2012-06-10 ZK	
B12-003	501	*R. rionii*	9	1	1	1.588	5.367	Czech Republic; distr. Kladno; Kamenné Žehrovice: pool in 2nd lowest drained sludge lagoon W of village	50°7'36.2"N, 14°0'19.9"E	402		2012-05-17 JP	
B12-004	502	*R. rionii*	10	1	1	1.595	5.391	Czech Republic; distr. Kladno; Kamenné Žehrovice: pool in lowest drained sludge lagoon W of village	50°7'35.0"N, 14°0'33.3"E	399		2012-05-17 JP	
B16-035	140	*R. rionii*	4	4	4	1.594 ± 0.004	5.389 ± 0.014	Czech Republic; distr. Kroměříž; Hulín: flooded sand-pit Hulínská pískovna, S bank	49°17'47"N, 17°26'56"E	185	*R. circinatus* × *R. rionii* (F1)	2016-06-18 Trávníček B.	also pure *R. circinatus* proved to occur on the locality (photo and several herbarium specimens B. Trávníček, OL)

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B15-008	503	*R. rionii*	20	1	1	1.604	5.422	Czech Republic; distr. Kroměříž; Záhlinice: pool in pool complex NNW of village	49°17'47.3"N, 17°29'0.2"E	189		2015-05-18 JP	
B14-093	504	*R. rionii*	10	1	1	1.568	5.300	Czech Republic; distr. Louny; Kněžice: Dolní Kněžický fishpond	50°17'47.8"N, 13°26'19.8"E	239		2014-07-04 Nunvářová Kabátová K. & Rydlo Jar.	
B14-082	505	*R. rionii*	1	1	1	1.565	5.290	Czech Republic; distr. Louny; Krásný Dvůr: pool on left side of Leska stream SW of chateau	50°14'59.6"N, 13°21'58.0"E	294		2014-07-02 Kolář F.	
K14-21	506	*R. rionii*	4	2	0	1.571 ± 0.000	5.310 ± 0.000	Czech Republic; distr. Louny; Lenešice: small fishpond N of the road Lenešice-Břvany, ca 1.9 km NE of the church	50°23'22"N, 13°44'33"E	190		2014-06-12 Hauer T.	
B15-024	507	*R. rionii*	10	10	9	1.599 ± 0.004	5.405 ± 0.013	Czech Republic; distr. Louny; Lenešice: small pond in fishpond complex NE of village	50°22'47.8"N, 13°46'53.1"E	184		2015-06-18 JP	
B13-024	508	*R. rionii*	10	1	1	1.585	5.357	Czech Republic; distr. Mladá Boleslav; Chudíř: Dubnický fishpond, exposed bottom	50°18'7.5"N, 15°0'16.5"E	222	*R. trichophyllus* A	2013-06-03 JP	
B12-012b	509	*R. rionii*	7	7	0	1.556 ± 0.009	5.260 ± 0.029	Czech Republic; distr. Mladá Boleslav; Žehrov: Dolní fishpond	50°32'0.6"N, 15°5'15.7"E	239	*R. trichophyllus* A	2012-06-14 JP & PT	
B12-007	510	*R. rionii*	12	2	1	1.603 ± 0.002	5.418 ± 0.007	Czech Republic; distr. Most; Mariánské Radčice: flooded mining area NE of village	50°34'33.3"N, 13°40'15.1"E	251		2012-05-27 JP; 2014-05-23 Hroudová Z.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B12-008	511	*R. rionii*	3	1	0	1.614	5.455	Czech Republic; distr. Most; Mariánské Radčice: pool in drained SW tip of reservoir in mining area ESE of village	50°34'15.6"N, 13°41'11.4"E	234		2012-05-27 JP	
B10-005	512	*R. rionii*	3	1	1	1.601	5.411	Czech Republic; distr. Most; Most: pond near church of Nanebevzetí Panny Marie	50°31'4.5"N, 13°38'39.4"E	224		2010-06-02 JP	
B11-004	513	*R. rionii*	28	4	3	1.556 ± 0.010	5.259 ± 0.033	Czech Republic; distr. Nymburk; Loučeň: Nový fishpond NNW of village	50°18'3.0"N, 15°0'52.2"E	228		2011-05-30 JP; 2012-06-10 ZK	
B10-003	514	*R. rionii*	5	1	1	1.563	5.283	Czech Republic; distr. Praha; Praha: city distr. Řeporyje, flooded mining area near historical settlement Řepora	50°2'22.1"N, 14°18'27.1"E	321		2010-05-20 JP	
B13-008	515	*R. rionii*	50	7	6	1.575 ± 0.005	5.323 ± 0.018	Czech Republic; distr. Praha-západ; Hostivice: Kala fishpond	50°4'12.5"N, 14°15'20.3"E	351		2013-05-19 JP	
B13-007	516	*R. rionii*	11	1	0	1.619	5.472	Czech Republic; distr. Praha-západ; Chýně: Bašta fishpond	50°3'50.6"N, 14°13'16.5"E	365		2013-05-19 JP	
B13-040	118	*R. rionii*	4	1	1	1.574	5.320	Czech Republic; distr. Svitavy; Třebařov: Rohlíček fishpond	49°50'40.5"N, 16°40'35.1"E	339	*R. circinatus*, *R. peltatus*	2013-06-20 JP & ZK	
B13-043	517	*R. rionii*	5	2	1	1.570 ± 0.003	5.307 ± 0.010	Czech Republic; distr. Svitavy; Třebařov: Velký Třebařovský fishpond	49°50'37.1"N, 16°41'30.9"E	332		2013-06-20 JP & ZK	
B15-003	55	*R. rionii*	5	1	1	1.570	5.307	Czech Republic; distr. Uherské Hradiště; Ostrožská Nová Ves: NE tip of big sand-pit W of village	49°0'53.3"N, 17°25'25.2"E	172	*R. baudotii*	2015-05-17 JP	
B15-005	56	*R. rionii*	3	1	1	1.600	5.408	Czech Republic; distr. Uherské Hradiště; Ostrožská Nová Ves: S tip of sand-pit WNW of village	49°0'59.4"N, 17°25'31.7"E	172	*R. baudotii*	2015-05-17 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B15-051	518	*R. rionii*	10	3	0	1.588 ± 0.010	5.367 ± 0.032	Czech Republic; distr. Ústí nad Orlicí; Vračovice-Orlov: hatchery pond near SE tip of Prostřední Vračovický fishpond	49°57'7.9"N, 16°14'48.7"E	291		2015-10-08 JP	
B15-006	126	*R. rionii*	5	1	1	1.598	5.401	Czech Republic; distr. Zlín; Spytihněv: big flooded sand-pit E of village	49°8'06.8"N, 17°31'5.3"E	178	*R. circinatus*	2015-05-17 JP	
B10-009	519	*R. rionii*	1	1	1	1.590	5.374	Czech Republic; distr. Zlín; Zlín: city distr. Chlum, Chlumské rybníky fishponds, uppermost fishpond, exposed bottom	49°13'41.3"N, 17°36'49.1"E	219		2010-06-30 JP	
K14-26	520	*R. rionii*	3	1	0	1.583	5.351	Czech Republic; distr. Znojmo; Vrbovec: Vrbovecký fishpond, ca 2.8 km ESE of the village	48°47'30"N, 16°08'18"E	205		2014-06-13 PK	
B14-001	521	*R. rionii*	1	1	1	1.609	5.438	Hungary; Jász-Nagykun-Szolnok County; Jászapáti: small ditch NNE of village	47°29'11"N, 20°13'24"E	84		2014-04-23 Mesterházy A.	
K17-52	522	*R. rionii*	1	1	0	1.593	5.384	Moldova: distr. Rîşcani; Avrămeni: lake ca 2.5 km NE of the village	47°48'17''N, 27°17'00''E	140		2017-06-29 PK	
K12-13	523	*R. rionii*	5	5	5	1.627 ± 0.011	5.468 ± 0.038	Romania; distr. Iaşi; Horleşti: ca 2.5 km SE of the village, shallow pond in a salt marsh	47°14'27"N, 27°26'25"E	65		2012-06-14 PK	
B12-019	524	*R. rionii*	1	1	0	1.573	5.317	Slovakia; distr. Bratislava; Bratislava: city distr. Devín, big pool of Morava river above confluence with Dunaj river	48°10'30.9"N, 16°58'35.3"E	137		2012-07-01 JP & Píšová S.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K17-44	525	*R. rionii*	6	2	0	1.624 ± 0.011	5.487 ± 0.035	Slovakia; distr. Komárno: Komárno: partly dried out oxbow of Váh river, on its right bank ca 0.5 km N of the town part Nová Osada (N of the town)	47°47'52"N, 18°06'04"E	109	*R. trichophyllus* B	2017-06-15 ZK	
B14-133	526	*R. rionii*	4	4	0	1.598 ± 0.005	5.400 ± 0.016	Slovakia; distr. Michalovce; Kaluža: Zemplínská Šírava reservoir in village	48°48'22.7"N, 22°0'32.3"E	120	*R. trichophyllus* A, *R. trichophyllus* B	2014-07-26 Nunvářová Kabátová K.	
ZK2540	527	*R. rionii*	1	1	0	1.596	5.394	Slovakia; distr. Nitra; Malý Cetín: flooded gravel-pit SW of village	48°13'48.0"N, 18°09'58.2"E	125		2012-07-06 ZK	
K17-46	528	*R. rionii*	3	3	0	1.633 ± 0.014	5.521 ± 0.048	Slovakia; distr. Nové Zámky; Chľaba: abandoned sand pit S of the railway station, 350 m S of the SE margin of the village	47°49'26"N, 18°49'48"E	120		2017-06-15 ZK	
B13-019	529	*R. rionii*	17	5	5	1.600 ± 0.007	5.407 ± 0.023	Slovakia; distr. Nové Zámky; Komoča: pond on NE edge of settlement Pačérok	47°56'9.9"N, 18°1'21.4"E	110	*R. trichophyllus* B	2013-06-05 JP & Hrdinová M.	
B16-004-05	530	*R. rionii* × *R. trichophyllus* A (F1)	1	1	1	2.140	7.233	Czech Republic; distr. Jičín; Kovač: Strašidlo pond	50°23'41.8"N, 15°27'15.2"E	259	*R. trichophyllus* A	2016-05-26 JP & Hanzlíčková J.	single young small-flowered plant, achens not yet developed
K17-80	531	*R. trichophyllus* A	2	1	0	2.659	8.987	Austria; Niederösterreich (Lower Austria); Hoheneich (distr. Gmünd): Mitterteich fishpond; ca 1.3 km E of the centre of the town	48°46'18"N, 15°02'38"E	520		2017-08-12 Pachschwöll C.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K17-31	208	*R. trichophyllus* A	5	5	0	2.616 ± 0.017	8.842 ± 0.057	Austria; Niederösterreich (Lower Austria); Rudmanns (distr. Zwettl): exposed bottom of Rudmannser Teich fishpond, SSE of the village	48°35'28"N, 15°21'52"E	590	*R. peltatus, R. peltatus* × *R. trichophyllus* A	2017-06-06 PK	
K16-19	531	*R. trichophyllus* A	5	3	3	2.609 ± 0.007	8.819 ± 0.024	Austria; Steiermark, distr. Murau; Krakaudorf: NE part of Etrachsee lake, ca 6 km NNW of the church	47°13'39"N, 13°58'37"E	1370		2016-09-04 PK	not rooting at nodes, relatively stout (not the subsp. *eradicatus*)
K13-11	532	*R. trichophyllus* A	10	5	5	2.661 ± 0.003	8.995 ± 0.011	Czech Republic; distr. Bruntál; Andělská Hora: fishpond at the NW edge of the village	50°03'51"N, 17°23'38"E	660		2013-06-09 PK	
K13-10	533	*R. trichophyllus* A	10	5	5	2.679 ± 0.016	9.054 ± 0.054	Czech Republic; distr. Bruntál; Krnov: WNW of the town, S of Chomýž settlement, fishpond "Rusák" (Chomýž I)	50°06'29"N, 17°38'45"E	350		2013-06-09 PK	
K12-18	534	*R. trichophyllus* A	4	3	3	2.654 ± 0.002	8.920 ± 0.008	Czech Republic; distr. Bruntál; Město Albrechtice: the northern of the two fishponds by the road to Třemešná, N of the town	50°10'46"N, 17°34'53"E	395		2012-07-10 PK	
B14-032	78	*R. trichophyllus* A	14	1	1	2.705	9.143	Czech Republic; distr. Česká Lípa; Bukovany: Kachní fishpond	50°42'40.0"N, 14°35'4.4"E	265	*R. circinatus*	2014-05-30 JP	
B14-034	535	*R. trichophyllus* A	15	1	1	2.641	8.927	Czech Republic; distr. Česká Lípa; Volfartice: Černý fishpond W of village	50°43'53.0"N, 14°25'29.5"E	344		2014-06-01 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K11-13	216	*R. trichophyllus* A	20	5	5	2.666 ± 0.008	8.962 ± 0.026	Czech Republic; distr. České Budějovice; Branišov: Návesný fishpond, 1 km N of the village	48°59'16"N, 14°23'48"E	390	*R. peltatus*, *R. peltatus* (aneuploid), *R. peltatus* × *trichophyllus* A (various hybrids)	2011-06-07 PK	
K11-11	536	*R. trichophyllus* A	16	5	5	2.683 ± 0.006	9.018 ± 0.019	Czech Republic; distr. České Budějovice; Čakovec: Podvesný fishpond, ca 0.4 km NNE	48°58'20"N, 14°19'22"E	425		2011-06-04 PK	
K15-27	219	*R. trichophyllus* A	5	3	3	2.664 ± 0.022	9.003 ± 0.075	Czech Republic; distr. České Budějovice; České Budějovice: Velký Vávrovský fishpond, at the NW edge of the town	48°59'35"N, 14°26'16"E	385	*R. peltatus*	2015-07-01 PK	
K10-07	537	*R. trichophyllus* A	1	1	0	2.657	8.931	Czech Republic; distr. České Budějovice; Dívčice: "Březovečky" fish storage ponds, S of Březovec fishpond, E of the village	49°06'30"N, 14°18'55"E	400		2010-06 Kúr P.	
K12-25	538	*R. trichophyllus* A	2	2	0	2.609 ± 0.008	8.770 ± 0.027	Czech Republic; distr. České Budějovice; Novosedly: Dolní fishpond, at the S edge of the village	49°05'25"N, 14°16'51"E	390		2012-08-09 PK	
K16-18	229	*R. trichophyllus* A	1	1	1	2.621	8.859	Czech Republic; distr. České Budějovice; Strýčice: fishpond ca 0.9 km SW of the church	49°02'27"N, 14°15'21"E	430	*R. peltatus*	2016-08-15 Šumberová K.	
B14-035	539	*R. trichophyllus* A	20	1	1	2.641	8.927	Czech Republic; distr. Děčín; Krásná Lípa: small pond on N edge of town	50°55'17.5"N, 14°31'8.7"E	446		2014-06-01 JP	
B16-045	81	*R. trichophyllus* A	2	2	2	2.666 ± 0.011	9.009 ± 0.035	Czech Republic; distr. Havlíčkův Brod; Krucemburk: Řeka fishpond, SW tip	49°40'13"N, 15°50'51"E	550	*R. circinatus*	2016-09-08 Šumberová K.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B10-037	540	*R. trichophyllus* A	1	1	1	2.665	9.008	Czech Republic; distr. Cheb; Mnichov: small fishpond SW of village	50°2'2.9"N, 12°46'50.9"E	733		2010-07-13 Čertner M.	
K11-25	541	*R. trichophyllus* A	15	5	5	2.674 ± 0.008	8.990 ± 0.025	Czech Republic; distr. Chrudim; Běstvina: Hlubošský fishpond, 1.15 km WSW of the church	49°49'59"N, 15°35'02"E	325		2011-06-27 PK	
B15-014	542	*R. trichophyllus* A	1	1	1	2.630	8.889	Czech Republic; distr. Chrudim; Bítovany: pool near NW bank of Farář fishpond	49°53'21.7"N, 15°51'48.6"E	289		2015-05-17 Nunvářová Kabátová K.	
ZK2371	82	*R. trichophyllus* A	1	1	0	2.683	9.069	Czech Republic; distr. Chrudim; Podlažice: Horecký fishpond	49°53'20.5"N, 15°56'36.8"E	276	*R. circinatus*	2011-06-08 ZK	
B16-004	530	*R. trichophyllus* A	4	4	0	2.632 ± 0.005	8.895 ± 0.016	Czech Republic; distr. Jičín; Kovač: Strašidlo pond	50°23'41.8"N, 15°27'15.2"E	259	*R. rionii* × *trichophyllus* A (F1)	2016-05-26 JP & Hanzlíčková J.	
K11-06	543	*R. trichophyllus* A	5	5	5	2.646 ± 0.003	8.893 ± 0.011	Czech Republic; distr. Jihlava; Telč: small fishpond at the forest edge E of the northermost part of Roštejnský fishpond, NW of the town	49°12'00"N, 15°26'52"E	540		2011-06 Ekrt L.	
B13-128	277	*R. trichophyllus* A	10	1	0	2.665	9.008	Czech Republic; distr. Jindřichův Hradec; Červená Lhota: Dub fishpond	49°14'52.2"N, 14°52'18.9"E	483	*R. peltatus*, *R. trichophyllus* hybr.?	2013-06-02 Lučanová M.	
K12-03	544	*R. trichophyllus* A	3	2	2	2.688 ± 0.017	9.035 ± 0.057	Czech Republic; distr. Jindřichův Hradec; Červená Lhota: Vlčiny fishpond, ca 1 km WNW of the castle	49°15'05"N, 14°52'12"E	490		2012-05-26 PK	
B13-094	545	*R. trichophyllus* A	1	1	0	2.652	8.964	Czech Republic; distr. Jindřichův Hradec; Hospříz: Kopeček fishpond	49°07'42.4"N, 15°05'59.2"E	549		2013-06-27 ZK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B12-086	297	*R. trichophyllus* A	3	3	0	2.658 ± 0.003	8.983 ± 0.011	Czech Republic; distr. Jindřichův Hradec; Přeseka: Velký Tisý Fishpond, Na Lůsech peninsula	49°03'12.7''N, 14°43'40.1''E	424	*R. peltatus*	2011-07-26 Píšová S.	
B17-008	546	*R. trichophyllus* A	1	1	1	2.655	8.974	Czech Republic; distr. Jindřichův Hradec; Rodvínov: Davidův fishpond ca 2.4 km ESE of railway station	49°9'27.8"N, 15°4'57.1"E	500		2017-07-05 Hanzlíčková J.	
B17-020	547	*R. trichophyllus* A	1	1	1	2.676	9.045	Czech Republic; distr. Jindřichův Hradec; Rodvínov: Horní Bláhů fishpond ca 1.9 km ESE of railway station	49°9'37.6"N, 15°4'34.0"E	493		2017-07-05 Hanzlíčková J.	
K11-09	548	*R. trichophyllus* A	6	2	2	2.627 ± 0.011	8.829 ± 0.039	Czech Republic; distr. Jindřichův Hradec; Střížovice: Vosecký fishpond, ca 2 km WSW of the village	49°08'06"N, 15°07'56"E	535		2011-06 Kúr P.	
B14-076	301	*R. trichophyllus* A	1	1	1	2.659	8.987	Czech Republic; distr. Karlovy Vary; Bražec: "Bražecké hliňáky" ponds WSW of village, easternmost pond	50°10'19.0"N, 13°2'22.0"E	690	*R. peltatus*, *R. peltatus* × *R. trichophyllus* A	2014-07-02 JP & Melichar V.	
B14-081	302	*R. trichophyllus* A	6	1	1	2.640	8.923	Czech Republic; distr. Karlovy Vary; Bražec: Javorenský fishpond, exposed bottom	50°10'37.4"N, 13°3'50.9"E	733	*R. peltatus*, *R. peltatus* (aneuploid)	2014-07-02 JP	
B14-086	85	*R. trichophyllus* A	1	1	1	2.606	8.808	Czech Republic; distr. Karlovy Vary; military training area Hradiště: small pond near settlement Hájovna	50°12'5.0"N, 13°12'5.9"E	674	*R. circinatus*, *R. peltatus*	2014-07-03 Kolář F.	
K15-20	549	*R. trichophyllus* A	5	3	3	2.680 ± 0.002	9.058 ± 0.007	Czech Republic; distr. Klatovy; Hradešice: Strašný fishpond, ca 1.6 km WSW of the church	49°19'02"N, 13°34'33"E	490		2015-06-23 PK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B16-034	320	*R. trichophyllus* A	1	1	1	2.664	9.004	Czech Republic; distr. Klatovy; Chanovice: Hladoměř fishpond SW of Újezd u Chanovic village	49°23'49"N, 13°41'19"E	504	*R. peltatus*	2016-06-11 JP, ZK, Hanzlíčková J. & Rybka V.	
B16-032	550	*R. trichophyllus* A	1	1	0	2.686	9.079	Czech Republic; distr. Klatovy; Chanovice: Prostřední Újezd fishpond SW of Újezd u Chanovic village	49°23'42"N, 13°41'26"E	500		2016-06-11 JP, ZK & Hanzlíčková J.	
B14-054	321	*R. trichophyllus* A	10	1	1	2.641	8.927	Czech Republic; distr. Klatovy; Chanovice: small fishpond NE of Starý fishpond	49°24'49.2"N, 13°43'43.9"E	520	*R. peltatus*, *R. peltatus* × *trichophyllus* A (F1)	2014-06-18 JP	
B16-024	322	*R. trichophyllus* A	3	3	3	2.667 ± 0.006	9.014 ± 0.019	Czech Republic; distr. Klatovy; Kvášňovice: Jámský fishpond	49°24'27"N, 13°39'4"E	523	*R. peltatus*	2016-06-11 JP, ZK & Hanzlíčková J.	
B16-026	323	*R. trichophyllus* A	1	1	1	2.681	9.062	Czech Republic; distr. Klatovy; Pačejov: Buxín fishpond near railway station Pačejov, NW tip	49°23'41"N, 13°38'18"E	522	*R. peltatus*	2016-06-11 JP, ZK & Hanzlíčková J.	
B16-028	551	*R. trichophyllus* A	5	3	3	2.693 ± 0.004	9.102 ± 0.012	Czech Republic; distr. Klatovy; Pačejov: Velký Blýskota fishpond, SE edge	49°22'54"N, 13°38'9"E	505		2016-06-11 JP, ZK, Hanzlíčková J.	
B16-029	552	*R. trichophyllus* A	1	1	0	2.678	9.052	Czech Republic; distr. Klatovy; Velký Bor: Benátka fishpond NW of village	49°22'7"N, 13°41'4"E	462		2016-06-11 JP, ZK, Hanzlíčková J.	
B12-077	329	*R. trichophyllus* A	2	2	2	2.702 ± 0.001	9.133 ± 0.003	Czech Republic; distr. Liberec; Pertoltice: Šálkův fishpond	50°59'01.5"N, 15°06'12.5"E	319	*R. peltatus* (2013)	2012-10-11 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B12-010	553	*R. trichophyllus* A	5	5	0	2.670 ± 0.008	9.026 ± 0.027	Czech Republic; distr. Mladá Boleslav; Březina: Oběšenec fishpond, exposed bottom	50°32'20.2"N, 15°3'36.4"E	240		2012-06-14 JP & PT	
B13-025	508	*R. trichophyllus* A	10	1	1	2.636	8.910	Czech Republic; distr. Mladá Boleslav; Chudíř: Dubnický fishpond, exposed bottom	50°18'7.5"N, 15°0'16.5"E	222	*R. rionii*	2013-06-03 JP	
B12-012a	509	*R. trichophyllus* A	5	5	0	2.578 ± 0.006	8.714 ± 0.022	Czech Republic; distr. Mladá Boleslav; Žehrov: Dolní fishpond	50°32'0.6"N, 15°5'15.7"E	239	*R. rionii*	2012-06-14 JP & PT	
B12-034	102	*R. trichophyllus* A	10	1	0	2.713	9.170	Czech Republic; distr. Opava; Děhylov: Štěpán fishpond	49°51'36.3"N, 18°11'40.5"E	212	*R. circinatus*	2012-07-14 JP & Pachmanová L.	
B16-008	104	*R. trichophyllus* A	3	3	3	2.649 ± 0.007	8.954 ± 0.024	Czech Republic; distr. Pardubice; Lázně Bohdaneč: Bohdanečský fishpond, shallow lagoones near E bank	50°5'42"N, 15°41'13"E	220	*R. circinatus*, *R. peltatus*	2016-05-26 JP & Hanzlíčková J.	
B16-030	554	*R. trichophyllus* A	1	1	1	2.658	8.984	Czech Republic; distr. Plzeň-jih; Nepomuk: Písařovský fishpond ca 2,2 km W of náměstí Augustina Němejce square in town	49°29'14"N, 13°33'3"E	453		2016-06-11 JP, ZK & Hanzlíčková J.	
B16-022	555	*R. trichophyllus* A	5	3	3	2.667 ± 0.006	9.014 ± 0.020	Czech Republic; distr. Plzeň-jih; Oselce: Kutinův fishpond	49°25'46"N, 13°41'13"E	562		2016-06-11 JP	
B16-020	106	*R. trichophyllus* A	1	1	1	2.662	8.998	Czech Republic; distr. Plzeň-jih; Oselce: Pihovatý fishpond	49°25'56"N, 13°41'12"E	559	*R. circinatus*	2016-06-11 JP & ZK	
K12-23	351	*R. trichophyllus* A	20	5	5	2.656 ± 0.009	8.926 ± 0.030	Czech Republic; distr. Prachatice; Volary: Volarský brook, 0-500 m W of the bridge by Planerův Dvůr settlement, S of the town	48°53'24"N, 13°53'35"E	740	*R. peltatus*	2012-07-17 PK	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B16-017	353	*R. trichophyllus* A	1	1	1	2.645	8.940	Czech Republic; distr. Příbram; Hvoždany: Raputovský fishpond	49°31'55"N, 13°50'33"E	580	*R. peltatus*, *R. peltatus* × *trichophyllus* A (various hybrids)	2016-06-10 JP & ZK	
B12-016	556	*R. trichophyllus* A	6	2	1	2.670 ± 0.030	9.000 ± 0.126	Czech Republic; distr. Semily; Borek: Rokytnický fishpond, ca 0.8 km SW of the village	50°31'50.7"N, 15°13'18.7"E	268		2010-06 Štech M.; 2012-06-14 JP & PT	
B16-014	114	*R. trichophyllus* A	1	1	0	2.684	9.072	Czech Republic; distr. Strakonice; Březí: Kozor fishpond	49°29'39"N, 13°49'0"E	514	*R. circinatus*	2016-06-10 JP & ZK	
K14-38	363	*R. trichophyllus* A	12	7	1	2.647 ± 0.012	8.934 ± 0.047	Czech Republic; distr. Strakonice; Leskovice: Malduchy fishpond SW of the village, exposed sandy souther bank of the eastern part of the fishpond	49°19'12"N, 13°54'51"E	470	*R. peltatus*, *R. peltatus* × *R. trichophyllus* A	2012-06 Kúr P.; 2014-07-10 PK	
K11-22	557	*R. trichophyllus* A	10	5	5	2.671 ± 0.004	8.977 ± 0.012	Czech Republic; distr. Strakonice; Záboří: Hůrka fishpond, 1.7 km SE of the church	49°22'21"N, 13°50'45"E	530		2011-06-21 PK	
B12-080	558	*R. trichophyllus* A	1	1	0	2.675	9.042	Czech Republic; distr. Svitavy; Jedlová: Pulec fishpond	49°39'37.5"N, 16°20'15.2"E	584		2012-10-16 JP	
B12-081	370	*R. trichophyllus* A	1	1	0	2.684	9.072	Czech Republic; distr. Svitavy; Jedlová: Ráček I pond	49°40'7.0"N, 16°19'59.5"E	595	*R. peltatus* (2013)	2012-10-16 JP	
B12-082	559	*R. trichophyllus* A	3	1	1	2.662	8.998	Czech Republic; distr. Svitavy; Jedlová: Ráček II pond	49°40'14.3"N, 16°19'52.5"E	594		2012-10-16 JP	
B15-055	560	*R. trichophyllus* A	6	3	3	2.637 ± 0.004	8.914 ± 0.014	Czech Republic; distr. Svitavy; Stašov: U Vodárny fishpond	49°40'44.5"N, 16°21'25.5"E	606		2015-10-08 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K14-09	373	*R. trichophyllus* A	2	2	0	2.680 ± 0.002	9.058 ± 0.007	Czech Republic; distr. Tábor; Drahov: exposed bottom of Smíchov II fishpond, ca 2.6 km SE of the church	49°09'37"N, 14°46'41"E	425	*R. peltatus*, *R. peltatus* × *trichophyllus* A (various hybrids; 2010)	2014-06-02 PK	
K12-04	374	*R. trichophyllus* A	15	5	5	2.669 ± 0.011	8.970 ± 0.038	Czech Republic; distr. Tábor; Hamr: Hluboký u Hamru fishpond, SE part, ca 1,05 km ENE of the town	49°09'34"N, 14°46'21"E	415	*R. peltatus*	2012-05-26 PK	
B16-010	375	*R. trichophyllus* A	1	1	0	2.699	9.123	Czech Republic; distr. Tábor; Tábor: city distr. Zahrádka, unnamed fishpond W of Pochytil fishpond	49°25'11"N, 14°37'17"E	466	*R. peltatus*	2016-06-02 JP	
B16-011	561	*R. trichophyllus* A	1	1	0	2.695	9.109	Czech Republic; distr. Tábor; Tábor: city distr. Zahrádka, Zahrádecký fishpond	49°25'18"N, 14°37'29"E	471		2016-06-02 JP	
K13-24	410	*R. trichophyllus* A	6	6	6	2.629 ± 0.008	8.886 ± 0.026	Czech Republic; distr. Tábor; Vlkov: "Vlkovská pískovna" sand pit, ca 1.8 km NW of the railway station	49°09'43"N, 14°42'55"E	408	*R. trichophyllus* A, various hybrids of *R. peltatus* and *R. trichophyllus* A	2013-06-22 PK	
B14-108	378	*R. trichophyllus* A	1	1	0	2.668	9.018	Czech Republic; distr. Tachov; Kyjov: fishpond near road Kyjov – Planá SE of village	49°52'51.7"N, 12°41'45.2"E	504	*R. peltatus*	2014-07-19 Čertner M.	
K17-37	384	*R. trichophyllus* A	1	1	0	2.648	8.950	Czech Republic; distr. Třebíč; Studenec: exposed bottom of Hlad fishpond, E bank; ca 0.9 km WNW of the railway station	49°13'27"N, 16°03'26"E	435	*R. peltatus* (2011)	2017-05-25 Šumberová K.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K17-40	383	*R. trichophyllus* A	3	1	0	2.640	8.923	Czech Republic; distr. Třebíč; Studenec: exposed bottom of Štičí fishpond, along its N coast, ca 1.95 km NW of the railway station Studenec	49°13'48"N, 16°02'42"E	450	*R. peltatus*	2017-05-25 Šumberová K.	
B16-043	562	*R. trichophyllus* A	5	5	5	2.668 ± 0.002	9.019 ± 0.007	Czech Republic; distr. Žďár nad Sázavou: Malé Dářko fishpond S of village	49°39'58"N, 15°52'48"E	630		2016-09-08 Šumberová K.	
K17-38	563	*R. trichophyllus* A	4	1	0	2.581	8.724	Czech Republic; Studenec (distr. Třebíč): rybník Štěpánek (t. č. na nízké vodě), jihozápadní pobřeží ca 2,15 km WNW of the railway station	49°13'23"N, 16°02'23"E	440		2017-05-25 Šumberová K.	
B12-070	564	*R. trichophyllus* A	3	3	3	2.626 ± 0.022	8.875 ± 0.074	Poland; Podlaskie Voivodeship; Suwałki County; Bakałarzewo: inlet of Rospuda river to Sumowo lake	54°5'31.5"N, 22°38'43.0"E	152		2012-08-30 PT & Kubátová B.	
B14-131	526	*R. trichophyllus* A	2	2	0	2.663 ± 0.008	8.999 ± 0.029	Slovakia; distr. Michalovce; Kaluža: Zemplínská Šírava reservoir in village	48°48'22.7"N, 22°0'32.3"E	120	*R. rionii*, *R. trichophyllus* B	2014-07-26 Nunvářová Kabátová K.	
B11-012	565	*R. trichophyllus* A	3	1	0	2.655	8.974	Slovakia; distr. Námestovo; Zubrohlava: Orava reservoir S of village, exposed bottom	49°25'06.4"N, 19°30'50.9"E	595		2011-06-24 JP	
B12-030	566	*R. trichophyllus* A	10	1	0	2.658	8.984	Slovakia; distr. Zlaté Moravce; Jelenec: Jelenec fishpond on N edge of settlement Remitáž, exposed bottom	48°24'4.1"N, 18°12'13.6"E	234		2012-07-04 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K17-17	484	*R. trichophyllus* B	3	3	2	2.945 ± 0.021	9.954 ± 0.069	Austria; Burgenland; Tadten: groundwater channel “Herrschaftsgraben”, SSE of the village	47°42'48"N, 17°02'12"E	114	*R. rionii*	2017-06-05 PK & Englmaier P.	
K17-16	567	*R. trichophyllus* B	3	3	2	2.980 ± 0.009	10.074 ± 0.031	Austria; Burgenland; Tadten: groundwater channel SSE of the village	47°43'29"N, 17°00'51"E	114		2017-06-05 PK & Englmaier P.	
K17-14	1	*R. trichophyllus* B	6	3	3	2.980 ± 0.010	10.071 ± 0.033	Austria; Niederösterreich (Lower Austria); Marchegg: Mühlbach brook (the ancient lower reach of the Weidenbach), west of Marchegg center, at the bridge of the federal road B49	48°16'39"N, 16°53'44"E	143	*R. aquatilis*	2017-06-05 PK & Englmaier P.	
K17-13	485	*R. trichophyllus* B	2	2	0	2.966 ± 0.004	10.025 ± 0.014	Austria; Niederösterreich (Lower Austria); Markthof an der March: ancient stretch of river Morava named „Lüssarm“ at the bridge of the cyclists route between Schlosshof and Devinska Nova Ves	48°12'42"N, 16°57'38"E	139	*R. rionii*	2017-06-05 PK & Englmaier P.	
K15-14	482	*R. trichophyllus* B	1	1	0	2.977	10.062	Austria; Oberösterreich, Innviertel; Bogenhofen: a stream in the southern part of the village	48°16'00"N, 13°06'42"E	350	*R. penicillatus* F, *R. penicillatus* F × *R. trichophyllus* B (F1)	2015-06-13 PK & JP & Hohla M.	
K15-15	568	*R. trichophyllus* B	5	3	3	2.901 ± 0.016	9.804 ± 0.053	Austria; Oberösterreich, Innviertel; Mattighofen: Kühbach brook in the village	48°06'11"N, 13°08'53"E	450		2015-06-13 PK & JP & Hohla M.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B14-122	569	*R. trichophyllus* B	2	2	1	2.922 ± 0.029	9.876 ± 0.098	Austria; Oberösterreich; Grünau im Almtal: Almsee lake	47°44'50.0"N, 13°57'02.0"E	589	*R. trichophyllus* subsp. *eradicatus*	2014-07-27 ZK	
B14-119	73	*R. trichophyllus* B	1	2	2	3.037 ± 0.003	10.265 ± 0.010	Austria; Oberösterreich; Hallstatt: Hallstättersee lake	47°36'40.8"N, 13°37'58.0"E	508	*R. circinatus*, *R. circinatus* × *trichophyllus* A (F1)	2014-07-27 ZK	
B11-025	570	*R. trichophyllus* B	1	1	1	2.954	9.985	Austria; Steiermark; Tragöß-Oberort: Grüner See lake	47°32'34.7"N, 15°3'26.8"E	776		2011-07-22 Kolář F.	
B14-166	571	*R. trichophyllus* B	1	1	1	2.895	9.785	Austria; Vorarlberg; Altach: Koblacher Kanal canal near bridge of Rheinstraße street	47°21'52.1"N, 9°38'48.0"E	409		2014-10-05 Jäger D.	
B14-011	3	*R. trichophyllus* B	7	7	1	2.952 ± 0.009	9.979 ± 0.030	Czech Republic; distr. Brno-venkov; Holasice: "Ludmila" oxbow	49°4'1.6"N, 16°36'23.9"E	185	*R. aquatilis*, *R. rionii*, *R. aquatilis* × *trichophyllus* B (F1)	2014-05-19 JP	
B14-014	572	*R. trichophyllus* B	8	1	1	2.944	9.951	Czech Republic; distr. Brno-venkov; Nosislav: pool in Knížecí les forest SW of village	49°0'0.2"N, 16°38'16.0"E	176		2014-05-19 JP	
B11-013	5	*R. trichophyllus* B	12	2	0	2.947 ± 0.007	9.962 ± 0.022	Czech Republic; distr. Břeclav; Hlohovec: Aloch III pond ESE of village	48°46'7.4"N, 16°47'47.4"E	175	*R. trichophyllus* B, *R. aquatilis* × *R. trichophyllus* B (F1)	2011-06-27 JP; 2010-06-07 PT	
B12-084	573	*R. trichophyllus* B	1	1	0	2.920	9.870	Czech Republic; distr. Břeclav; Hlohovec: Aloch ponds, uppermost pond SE of village	48°45'51.7"N, 16°47'14.4"E	188		2011-08-04 Píšová S.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
K14-19	574	*R. trichophyllus* B	6	6	0	2.912 ± 0.011	9.843 ± 0.037	Czech Republic; distr. Břeclav; Novosedly: small pond at the N edge of the village and periodic pools in the adjacent halophilic meadow	48°50'28"N, 16°29'50"E	175		2014-06-09 PK	
B11-016	490	*R. trichophyllus* B	4	2	0	2.910 ± 0.011	9.834 ± 0.039	Czech Republic; distr. Břeclav; Rakvice: flooded depressions and artificial ponds in the area of subhalophytic vegetation "Trkmanský dvůr", ca 1.8 km ENE of the church in the village	48°51'50"N, 16°50'27"E	162	*R. rionii*	2011-06-28 JP; 2016-06-11 Lepšová O.	
B14-017	495	*R. trichophyllus* B	10	1	1	2.920	9.870	Czech Republic; distr. Hodonín; Milotice: Milotický fishpond	48°57'48.7"N, 17°8'59.7"E	183	*R. rionii*	2014-05-20 JP	
B13-012	575	*R. trichophyllus* B	7	1	1	2.938	9.930	Czech Republic; distr. Kolín; Choťovice: pool in slope above railway station	50°08'51.7"N, 15°19'46.0"E	226		2013-05-29 JP	
B13-009	576	*R. trichophyllus* B	23	2	1	2.977 ± 0.023	10.063 ± 0.079	Czech Republic; distr. Kolín; Klipec: artificial pool in protected area V Jezírkách WNW of village	50°04'58.0"N, 15°06'44.7"E	193		2013-05-27 JP; 2012-05-30 ZK; 2017-05-23 Hauer T.	
B16-001	577	*R. trichophyllus* B	5	3	3	2.961 ± 0.010	10.007 ± 0.032	Czech Republic; distr. Kolín; Radovesnice II: forest ditch ca 1,75 km W(–WSW) of municipal office in village	50°06'18"N, 15°20'37"E	226		2016-05-20 JP & Hanzlíčková J.	
B15-010	578	*R. trichophyllus* B	13	4	1	2.920 ± 0.019	9.868 ± 0.065	Czech Republic; distr. Kutná Hora; Hořany: small pond SW of village	49°58'10.8"N, 15°13'52.4"E	289		2014-09-15 Rydlo Jan & Rydlo Jar.; 2015-05-18 JP	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B12-005	579	*R. trichophyllus* B	37	4	2	2.976 ± 0.018	10.058 ± 0.060	Czech Republic; distr. Litoměřice; Litoměřice: town distr. Pokratice, fishpond near Štampův Mlýn mill	50°33'18.5"N, 14°7'44.2"E	258		2011-05-28 Čertner M.; 2012-05-23 JP; 2013-07-07 JP	
K14-22	580	*R. trichophyllus* B	6	4	0	2.939 ± 0.016	9.932 ± 0.055	Czech Republic; distr. Most; Horní Jiřetín: pond on the "Hornojiřetínská" coal mine deposit, ca 2,0 km ENE of the church	50°34'43"N, 13°34'33"E	280		2014-06-12 Hauer T.	
K15-10	581	*R. trichophyllus* B	3	3	3	2.930 ± 0.009	9.905 ± 0.030	Czech Republic; distr. Nový Jičín; Pustějov: oxbow lake ca 1.9 km SE of the church	49°14'05"N, 18°01'17"E	230		2015-06-10 PK	
B13-013	582	*R. trichophyllus* B	8	1	1	2.909	9.832	Czech Republic; distr. Nymburk; Kněžičky: pond NNW of hotel in deer-park Kněžičky	50°09'12.6"N, 15°20'07.2"E	247		2013-05-29 JP	
B12-018	583	*R. trichophyllus* B	6	6	0	2.977 ± 0.014	10.063 ± 0.046	Czech Republic; distr. Nymburk; Lysá nad Labem: pool on S edge of Hrabanovská černava fen	50°12'48.8"N, 14°49'43.6"E	187		2012-06-14 JP & PT; 2017-05-23 Hauer T.	
B14-050	584	*R. trichophyllus* B	10	2	1	2.903 ± 0.004	9.810 ± 0.012	Czech Republic; distr. Nymburk; Veleliby: unnamed stream near mouth to Liduška stream W of village	50°12'41.9"N, 15°0'59.5"E	188		2013-08-31 Nunvářová Kabátová K. & Rydlo Jar.; 2014-06-14 JP	
B14-156	585	*R. trichophyllus* B	8	1	1	2.889	9.765	Czech Republic; distr. Pardubice; Hrobice: Baroch fishpond	50°05'53.7"N, 15°46'56.2"E	224		2014-08-29 ZK	

Ref. no.	**Loca-lity no.**	**Taxon / Cytotype**	**N**	**N (GS)**	**N rep-eats**	**Ratio to *Bellis***	**2C-value (pg)**	**Locality**	**Coordinates (WGS 84)**	**Eleva-tion (m)**	**Other taxa**	**Collected**	**Notes**
B13-062	586	*R. trichophyllus* B	7	1	1	2.939	9.934	Czech Republic; distr. Semily; Turnov: middle pond below castle Hrubý Rohozec	50°35'56.9"N, 15°9'34.2"E	249		2013-08-19 JP & Nunvářová Kabátová K.	
K15-21	587	*R. trichophyllus* B	5	5	4	2.959 ± 0.021	10.002 ± 0.070	Czech Republic; distr. Strakonice; Makarov: fishpond 0.45 km SE of the village	49°14'33"N, 13°48'43"E	480		2015-06-23 PK	
K14-39	588	*R. trichophyllus* B	10	5	0	2.934 ± 0.027	9.916 ± 0.093	Czech Republic; distr. Strakonice; Radomyšl: fishpond E of the outdoor swimming pool, ca 1.3 km S of the church	49°18'20"N, 13°55'47"E	440		2014-07-10 PK	
B12-049	589	*R. trichophyllus* B	5	1	1	2.957	9.995	Denmark; Jutland; reg. Midtjylland: Sønderup: Sønderup Å stream near bridge of Sønderhedevej road NW of village	56°49'38.9"N, 09°37'15.7"E	19		2012-08-01 JP & ZK	
B12-051	590	*R. trichophyllus* B	4	1	1	2.964	10.018	Denmark; Jutland; reg. Nordjylland; Bislev: Binderupå stream near the bridge of Jordemodervej road	56°56'14.8"N, 09°38'15.7"E	22		2012-08-01 JP & ZK	
K16-15	591	*R. trichophyllus* B	1	1	0	2.912	9.843	Germany; Bayern (Bavaria), distr. Traunstein; Ruhpolding: Förchensee lake, ca 6.5 km SSW of the centre of the village	47°42'29"N, 12°37'11"E	740		2016-07-23 Kotilínek M.	
B14-069	592	*R. trichophyllus* B	10	1	1	2.881	9.738	Hungary; Bács-Kiskun County; Fülöpszállás: channel near crossing with road no. 52, ENE of village	46°50'25"N 19°16'06"E	94		2014-06 Nunvářová Kabátová K.	
B14-101	64	*R. trichophyllus* B	1	1	0	2.907	9.826	Hungary; Bács-Kiskun County; Szabadszállás: small swamp SSW of village	46°50'13"N, 19°12'21"E	92	*R. baudotii*	2014-06-19 Mesterházy A.	

Ref. no.	Loca-lity no.	Taxon / Cytotype	N	N (GS)	N rep-eats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Other taxa	Collected	Notes
B14-004	593	*R. trichophyllus* B	1	1	1	2.874	9.714	Hungary; Vas County; Nemeskocs: small ditch E of village	47°12'13"N, 17°11'56"E	123		2014-04-24 Mesterházy A.	
B14-005	594	*R. trichophyllus* B	1	1	1	2.929	9.900	Hungary; Veszprém County; Adorjánháza: "Hunyor-stream" W of village	47°14'25"N, 17°13'41"E	123		2014-04-25 Mesterházy A.	
ZK3494	595	*R. trichophyllus* B	1	1	1	2.936	9.924	Hungary; Veszprém County; Szentimrefalva: pool in tall-sedge stand on meadows 2.1 km SSE of village	47°03'10.0"N, 17°17'56.0"E	150		2017-06-21 ZK & A. Mesterházy	
B14-003	596	*R. trichophyllus* B	1	1	1	2.938	9.930	Hungary; Zala county; Zalaszentmihály: small pool on the pasture near E bank of Öreg-tó lake	46°42'00"N, 16°56'37"E	141		2014-04-24 Mesterházy A.	
K17-45	525	*R. trichophyllus* B	1	1	0	2.978	10.066	Slovakia; distr. Komárno: Komárno: partly dried out oxbow of Váh river, on its right bank ca 0.5 km N of the town part Nová Osada (N of the town)	47°47'52"N, 18°06'04"E	109	*R. rionii*	2017-06-15 ZK	
B14-132	526	*R. trichophyllus* B	2	2	0	2.932 ± 0.029	9.910 ± 0.098	Slovakia; distr. Michalovce; Kaluža: Zemplínská Šírava reservoir in village	48°48'22.7"N, 22°0'32.3"E	120	*R. rionii, R. trichophyllus* A	2014-07-26 Nunvářová Kabátová K.	
B13-018	529	*R. trichophyllus* B	7	2	2	2.958 ± 0.004	9.998 ± 0.014	Slovakia; distr. Nové Zámky; Komoča: pond on NE edge of settlement Pačérok	47°56'9.9"N, 18°1'21.4"E	110	*R. rionii*	2013-06-05 JP & Hrdinová M.	
B15-011	597	*R. trichophyllus* B	1	1	1	2.934	9.917	Slovenia; Cerknica: reg. Littoral–Inner Carniola, intermittent lake Cerkniško jezero near settlement Otok	45°44'26.5"N, 14°22'12.9"E	550		2015-05-12 Šumberová K.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B13-127	277	*R. trichophyllus* hybr.	1	1	1	2.823	9.542	Czech Republic; distr. Jindřichův Hradec; Červená Lhota: Dub fishpond	49°14'52.2"N, 14°52'18.9"E	483	*R. peltatus*, *R. trichophyllus* A	2013-06-02 Lučanová M.	
B11-026	598	*R. trichophyllus* hybr.	1	1	1	2.969	10.035	Czech Republic; distr. Tábor; Vlkov: S tip of sand-pit, ca 1.6 km WNW of the railway station	49°9'13.6"N, 14°42'30.0"E	408		2011-07-13 Kolář F.	
B14-121	599	*R. trichophyllus* subsp. *eradicatus*	7	7	0	2.657 ± 0.006	8.979 ± 0.022	Austria; Oberösterreich; Grünau im Almtal: Almsee lake	47°44'50.0"N, 13°57'02.0"E	589	*R. trichophyllus* B	2014-07-27 ZK	
K15-31	600	*R. trichophyllus* subsp. *eradicatus*	1	1	1	2.599	8.785	Austria; Salzburg state, distr. Tamsweg; Wald: Riedingtal valley, Ilgsee mountain lake ca 7 km W of the village	47°11'32"N, 13°20'50"E	2100		2015-07-27 Lepšová O.	
B14-163	601	*R. trichophyllus* subsp. *eradicatus*	1	1	1	2.615	8.839	Austria; Salzburg; Heiligenblut: Fuscher Lacke lake	47°6'39.4"N, 12°49'56.6"E	2262		2014-10-05 Jäger D.	
K17-82	602	*R. trichophyllus* subsp. *eradicatus*	5	3	3	2.630 ± 0.001	8.889 ± 0.003	Austria; Steiermark (Styria); Totes Gebirge, Altausee: Wildensee lake, ca 10.4 km NE of the centre of the village	47°42'44"N, 13°51'14"E	1540		2017-08-27 Lučanová M.	
K17-81	603	*R. trichophyllus* subsp. *eradicatus*	6	5	5	2.641 ± 0.004	8.926 ± 0.013	Austria; Steiermark (Styria); Totes Gebirge, Grundlsee: Henar-See lake, ca 8.2 km NNE of the church in the village	47°41'42"N, 13°52'53"E	1700		2017-08-27 Lučanová M.	
K17-51	604	*R. trichophyllus* subsp. *eradicatus*	2	2	2	2.669 ± 0.004	9.020 ± 0.015	Austria; Steiermark (Styria); Totes Gebirge, Tauplitz: Tauplitzalm, Steirersee lake, S coast, ca 4,3 km NNE of the village	47°35'54"N, 14°01'57"E	1450		2017-07-09 Lepš J. & Lepšová O.	

Ref. no.	Locality no.	Taxon / Cytotype	N	N (GS)	N repeats	Ratio to *Bellis*	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Other taxa	Collected	Notes
B14-162	605	*R. trichophyllus* subsp. *eradicatus*	1	1	1	2.617	8.845	Austria; Vorlarlberg; Baad: Hochalpsee lake	47°16'50.4"N, 10°7'16.6"E	1969		2014-10-05 Jäger D.	
B14-159	606	*R. trichophyllus* subsp. *eradicatus*	1	1	1	2.614	8.835	Austria; Vorlarlberg; Gerlos: Langer See lake	47°15'57.0"N, 11°59'8.4"E	1937		2014-10-05 Jäger D.	
B14-161	607	*R. trichophyllus* subsp. *eradicatus*	1	1	1	2.633	8.900	Austria; Vorlarlberg; Lech: Monzabonsee lake	47°11'49.6"N, 10°10'15.8"E	2226		2014-10-05 Jäger D.	
B14-160	608	*R. trichophyllus* subsp. *eradicatus*	1	1	1	2.597	8.778	Austria; Vorlarlberg; Tschagguns: Tilisunasee lake	47°1'35.6"N, 9°52'53.5"E	2105		2014-10-05 Jäger D.	
B14-165	609	*R. trichophyllus* subsp. *eradicatus*	1	1	1	2.597	8.778	Austria; Vorlarlberg; Warth: Seebachsee lake	47°15'26.1"N, 10°11'7.3"E	1480		2014-10-05 Jäger D.	
K14-41	610	*R. trichophyllus* subsp. *eradicatus*	5	3	0	2.653 ± 0.009	8.966 ± 0.029	Slovenia; Upper Carniolia region; Julian Alps, Trenta: Spodnje Kriško jezero lake, ca 4.1 km NE of the village	46°23'56"N, 13°48'07"E	1900		2014-07-19 PK	
B14-164	611	*R. trichophyllus* subsp. *eradicatus*	1	1	1	2.606	8.808	Switzerland; Graubünden Canton; Sankt Antönien: Partnunsee lake	47°0'31.5"N, 9°51'34.9"E	1869		2014-10-05 Jäger D.	
K15-34	612	*R. trichophyllus* subsp. *eradicatus*	1	1	0	2.655	8.974	Switzerland; Valais district (canton); Zermatt: Schwarzsee mountain lake, SW of the village	45°59'27"N, 07°42'24"E	2570		2015-08-29 Lepšová O.	
			3354	1707	1032								

ELECTRONIC APPENDIX 2. Niche differentiation: input data (A); results of canonical discriminant analyses demonstrating the most informative parameters (B)

A – Input environmental parameters:

Alt = Altitude (m)
BIO1 = Annual Mean Temperature
BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3 = Isothermality (BIO2/BIO7) (* 100)
BIO4 = Temperature Seasonality (standard deviation *100)
BIO5 = Max Temperature of Warmest Month
BIO6 = Min Temperature of Coldest Month
BIO7 = Temperature Annual Range (BIO5-BIO6)
BIO8 = Mean Temperature of Wettest Quarter
BIO9 = Mean Temperature of Driest Quarter
BIO10 = Mean Temperature of Warmest Quarter
BIO11 = Mean Temperature of Coldest Quarter
BIO12 = Annual Precipitation
BIO13 = Precipitation of Wettest Month
BIO14 = Precipitation of Driest Month
BIO15 = Precipitation Seasonality (Coefficient of Variation)
BIO16 = Precipitation of Wettest Quarter
BIO17 = Precipitation of Driest Quarter
BIO18 = Precipitation of Warmest Quarter
BIO19 = Precipitation of Coldest Quarter

B – Contributions of individual environmental parameters to the canonical axis in the canonical discriminant analyses for taxa couples *R. aquatilis* – *R. peltatus* and *R. trichophyllus* A – *R. trichophyllus* B; characters with the highest absolute loadings are presented in bold. Variable ‘BIO7’, a simple linear combination of ‘BIO5’ and ‘BIO6’, was excluded prior the analysis.

R. aquatilis + *R. peltatus*:

Variable	Loadings on CCA axis
Alt	**-0.5589**
BIO1	**0.6032**
BIO2	0.3210
BIO3	-0.2063
BIO4	**0.5587**
BIO5	**0.6546**
BIO6	-0.0290
BIO8	0.4558
BIO9	-0.0835
BIO10	**0.6827**
BIO11	0.1499
BIO12	-0.4261
BIO13	-0.3264
BIO14	-0.4533
BIO15	0.4146
BIO16	-0.3431
BIO17	-0.4676

PAPER III

Prančl J., Fehrer J., Caklová P., Bambasová V., Lučanová M. & Kaplan Z.: Intricate evolutionary history of *Callitriche* (Plantaginaceae) taxa elucidated by a combination of DNA sequencing and flow cytometry. – Manuscript, accepted for Taxon.

A newly recognized cryptic taxon within *Callitriche truncata*. Photo J. Prančl, Katochi, Greece.

Intricate evolutionary history of *Callitriche* (Plantaginaceae) taxa elucidated by a combination of DNA sequencing and genome size

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ABSTRACT

The widespread aquatic plant genus *Callitriche* is taxonomically very challenging, but noteworthy in many evolutionary aspects including a high overall diversity, extensive phenotypic plasticity, remarkable reproductive systems and a large variation in ploidy levels and chromosome numbers. We conducted a multi-level systematic study on 344 individuals of 25 taxa from 21 mostly European countries. Flow cytometric estimation of genome size, chromosome counting and direct sequencing of ITS and *trn*T–*trn*L DNA markers combined with RFLPs of the ITS region were applied in order to unravel the phylogenetic relationships among *Callitriche* taxa and to clarify the origin of polyploid species and hybrids. Additionally, ITS sequences from a recent worldwide phylogenetic study of the genus were included for comparison. We demonstrate that most of the traditionally recognized European *Callitriche* taxa are well defined by a combination of genome size and molecular markers. Several species showed remarkable intraspecific genetic variation; previously unknown cryptic taxa were revealed within *C. truncata*, *C. stagnalis* and *C. heterophylla*. The origin of selected polyploid taxa was investigated in detail. Diploid *C. cophocarpa* was confirmed to be the parental species of tetraploid *C. platycarpa*, but we did not find direct evidence for the putative allopolyploid origin of this species. The complex of *C. brutia* included three taxa; of these, *C. hamulata* is probably an allooctoploid derivative of *C. brutia* var. *brutia* and *C. cophocarpa*/*C. platycarpa*. The third member, *C. brutia* var. *naftolskyi*, was newly reclassified at the subspecies level; for the first time, chromosome numbers are provided for this poorly known taxon. For a single triploid sample, our results suggested an autopolyploid origin from *C. stagnalis*. Four *Callitriche* hybrids were revealed, two of which are newly described and validated here as *C.* ×*nyrensis* and *C. brutia* nothosubsp. *neglecta*. A tentative concept of two intrageneric sections (*Callitriche*, *Pseudocallitriche*) is adopted, with the need for a more detailed evaluation in the future.

KEYWORDS: diversity; hybridization; molecular identification; NeighborNet analysis; phylogenetic analysis; polyploidy

SUPPORTING INFORMATION may be found online in the Supporting Information section at the end of the article.

INTRODUCTION

Virtually all fields of biology rely on a solid framework of systematic classification, based primarily on phylogenetic relationships among organisms and their morphological and genetic differentiation. A detailed knowledge of the living objects we work with is essential for drawing any scientific hypotheses and conclusions, allowing to explore nature in a broader ecological context (Guerra-García & al., 2008; Ruggiero & al., 2015). Phylogenetic research enables us to better understand the evolutionary mechanisms responsible for the origin of the observed variation and the emergence of new species (e.g. Alix & al., 2017). Nevertheless, the existing, genetically-determined biodiversity often remains neglected, being not reflected in conspicuous morphological characters. Despite the difficult detection of such cryptic taxa, studying them in an integrative research approach can bear substantial implications for evolutionary theories, biogeography, as well as for nature conservation (Bickford & al., 2007).

Among angiosperms, the genus *Callitriche* L. (water-starwort; Plantaginaceaee Juss. sensu Albach & al., 2005) is exceptional in a number of evolutionary aspects. With ca 75 recognized species (Hassemer & Lansdown, 2018), it is one of the most diversified genera of aquatic plants. Water-starworts are considered taxonomically extremely challenging, which is mainly due to their reduced morphology (Schotsman, 1967; Lansdown, 2008), an extensive phenotypic plasticity (Schotsman, 1954; Jones, 1955; Martinsson, 1996) and the complex evolutionary history of particular taxa (e.g. Philbrick & Les, 2000; Demars & Gornall, 2003; Lansdown, 2006a; Ito & al., 2017). In total, eleven different chromosome numbers ranging from 2n=6 to 2n=40 are currently reported in the genus, including five ploidy levels (summarized in Prančl & al., 2014). The genus is also remarkable for its highly diversified pollination strategies including anemogamy (the dispersal of pollen by the wind), epihydrogamy (the spread of pollen across the water surface) and hypohydrogamy (underwater pollination through wettable exine-reduced pollen), combined with various modes of selfing (in fact, geitonogamy;

Schotsman, 1982; Philbrick & Anderson, 1992; Philbrick & Bernardello, 1992; Martinsson, 1996).

The genus has been thoroughly studied using morphology (predominantly based on minute fruit and floral characters) and chromosome counting (e.g. Fasset, 1951; Mason, 1959; Schotsman, 1967, 1977; Philbrick, 1994; Lansdown, 2006b, 2008; Bean, 2007). However, molecular or cytogenetic approaches were rarely employed to examine the evolutionary relationships of Callitriche taxa. Two larger phylogenetic studies of the genus are available (Philbrick & Les, 2000; Ito & al., 2017); the former included 20 taxa from Europe and North America, using the rbcL plastid gene marker. However, the relationships among some taxa included in the study remained largely unresolved. The latter study involved 22 taxa from six continents, applying nuclear (ITS) and plastid (matK, rbcL) DNA regions. That study outlined basic phylogenetic relationships within Callitriche, but did not solve any formal intrageneric classification nor did the authors provide a taxonomic evaluation of the ascertained intraspecific genetic variation. Although polyploidy was detected in 19 of 35 taxa for which the chromosome numbers are known (Prančl & al., 2014), the evolutionary origins of particular polyploid taxa have remained entirely unknown. The only exception is the European species *C. platycarpa* Kütz., which has been repeatedly confirmed to be an allotetraploid derivative of the diploid parental species *C. cophocarpa* Sendtn. and *C. stagnalis* Scop. (Bączkiewicz & al., 2007; Schwarzacher & al., 2017). Also the impact of hybridization on the overall *Callitriche* diversity is poorly known. To date, only one interspecific hybrid has been formally described (*C. ×vigens* K.Martinsson, i.e. the primary triploid hybrid of *C. cophocarpa* and *C. platycarpa*; Martinsson, 1991). Although hybridization appeared to be relatively rare in *Callitriche*, direct evidence of this assumption using molecular markers was still lacking.

Recently, flow cytometry has been successfully utilized to distinguish Central-European *Callitriche* taxa, manifesting genome size as a suitable independent character, which can serve as a basic marker to recognize taxonomic entities within the genus (Prančl & al., 2014). That study also revealed a previously unknown hybrid of the putative parents *C. hamulata* Kütz. ex W.D.J.Koch and *C. cophocarpa*, indicating that hybridization in this genus could be more frequent than hitherto assumed, but had remained elusive using the traditional morphological approach.

In Europe, 14–15 native and three rare introduced species are reported (Lansdown, 2006a, 2008; Table 1). Water-starworts occur in almost all types of aquatic habitats, but prefer shallow waters including small temporary wetlands such as puddles on forest paths or various vernal pools. While most aquatic plants generally show relatively wide range of distribution, limited taxonomic differentiation, and low infra-specific genetic variation (Santamaría, 2002), many *Callitriche* taxa are endemics of relatively small geographic regions (see Table 1). Intraspecific taxa have been described within three European species, including both diploids (*C. hermaphroditica* L., *C. truncata* Guss.) and polyploids (the complex of *C. brutia* consisting of hexaploid *C. brutia* Petagna var. *brutia*, *C. brutia* var. *naftolskyi* (Warb. & Eig) Lansdown with unknown chromosome number [until recently classified at the species level or treated as an unresolved taxon] and octoploid *C. hamulata*, recently re-evaluated as *C. brutia* var. *hamulata* (Kütz. ex W.D.J.Koch) Lansdown; Lansdown, 2006; Lansdown & al., 2017). There is also indication that some species show wide morphological variation in some parts of Europe and may contain several cryptic taxa (e.g. *C. stagnalis* in Spain and *C. hermaphroditica* in Russia; Lansdown, 2008). Recently, many new records of *Callitriche* taxa had been reported, especially in the Mediterranean area, which is considered as a species diversity centre of the genus in Europe (Lansdown & Strid, 2011; Lansdown & al., 2016, 2017). All aforementioned facts illustrate the need to investigate the evolution of *Callitriche* species in more detail and suggest an indisputable potential for elucidating the processes that shape the evolution of aquatic plants as well as of angiosperms in general.

This article provides a molecular and cytogenetic study of European *Callitriche* taxa using flow cytometry and chromosome counting combined with direct sequencing of nuclear ribosomal (ITS) and plastid (*trn*T–*trn*L) DNA regions, complemented by RFLPs of ITS to clarify the origin of several hybrid taxa. We investigated the genetic variation among and within particular species, specifically focusing on hybridization processes and phylogenetic relationships among neglected and morphologically poorly characterized taxa. We compared our ITS data with the results of a recent worldwide phylogenetic study on *Callitriche* (Ito & al., 2017). In addition, we discuss the evolutionary origins of polyploids in *Callitriche* and newly describe two previously undetected hybrids.

TABLE 1. List of native European *Callitriche* taxa. Distribution data were adopted mainly from Lansdown (2008) and Lansdown & al. (2017), with additional information from Mason (1959), Morita & Lee (1998), Philbrick & al. (1998), Bean (2007), Hassemer & O'Leary (2018) and Volkova & al. (2020). Diagnostic characters are based on the most relevant taxonomic studies (Schotsman, 1967; Lansdown, 2008; Lansdown & al., 2017) and our observations. Ploidy levels associated with particular chromosome counts: 2x (2n=6,8,10), 4x (2n=20), 6x (2n=28), 8x (2n=38). Contacter – pollination takes place through the contact between stigma and anther (details in the text). * – the chromosome number is only known for *C. truncata* subsp. *occidentalis*. ** – the chromosome number is only known for *C. brutia* var. *brutia*. *** – *C. hamulata* is sometimes classified as *C. brutia* var. *hamulata* (Kütz. ex W. D. J. Koch) Lansdown, see Discussion for details.

Section	Taxon	2n	Distribution	Growth habit	Pollination	Key morphological characters
Pseudocallitriche	*C. hermaphroditica* L.	6	boreal Europe and Asia, boreal and temperate areas of North America (subsp. *macrocarpa* is more abundant in the northern part of the range)	submersed	submerged, hypohydrogamy, contacter	leaves translucent, lingulate, 1-veined, leaf rosettes absent; peltate scales absent; bracts absent; pollen grains colourless; fruits broadly winged, 1.2–1.7 × 1.1–1.7 mm (subsp. *hermaphroditica*) or 1.5–2.4 × 1.6–2.8 mm (subsp. *macrocarpa*)
	C. transvolgensis Tzvelev	?	Russia (Volgograd region)	submersed	submerged, hypohydrogamy	leaves translucent, lingulate, 1-veined, leaf rosettes absent; peltate scales absent; bracts absent; pollen grains colourless; fruits longer than wide, 2.2–2.4 × 1.6–1.8 mm, winged only or mainly at apex
	C. truncata Guss.	6*	coastal areas of W Europe and Mediterranean (subsp. *occidentalis*); coastal areas of middle and E Mediterranean, introduced in Chile and Argentina (subsp. *truncata*); Volgograd region of Russia (subsp. *fimbriata*)	usually submersed	submerged, hypohydrogamy	leaves translucent, lingulate, 1-veined, leaf rosettes absent; bracts absent, peltate scales absent; pollen grains colourless; fruits wider than long, 0.9–1.5 × 1.1–1.9 mm, subsessile or shortly pedunculate, narrowly winged (subsp. *truncata*), wings absent (subsp. *occidentalis*) or wings composed of a fringe of whitish fibrils (subsp. *fimbriata*)
	C. pulchra Schotsman	8	Greece (island of Gavdos), Cyprus, N Libya	submersed	submerged, hypohydrogamy	leaves translucent, lingulate, 1-veined, leaf rosettes absent; peltate scales absent; bracts absent; pollen grains colourless; fruits wider than long, 1.4–1.8 × 1.6–2.2 mm, all shortly pedunculate, ± broadly winged
	C. lusitanica Schotsman	8	Iberian Peninsula, Sardinia, Sicily, Greece (island of Lesvos), Israel, NW Africa	amphibious	aerial/ epihydrogamy/ submerged, contacter	leaves translucent, lingulate, leaf rosettes with irregular venation sometimes present; stem scales of 7–9 cells; bracts absent; pollen grains whitish to pale yellow; fruits 1–1.4 × 1.2–1.9 mm, narrrowly to broadly winged

Section	Taxon	2n	Distribution	Growth habit	Polli-nation	Key morphological characters
Callitriche	*C. cribrosa* Schotsman	8	Iberian Peninsula, central Italy, NW Africa	amphibious	aerial/ epihydrogamy	leaves up to 11.7 mm wide, often more than 11-veined, lingulate leaves usually absent; stem scales of 3–4 cells; bracts present, often forked; pollen grains yellow, filaments up to 9.4 mm; fruits 1.4–1.7 × 1.4–1.8 mm, ± broadly winged, grayish
	C. cophocarpa Sendtn.	10	central, N and E Europe	amphibious	aerial/ epihydrogamy	leaves up to 6 mm wide, 1–5-veined, often lingulate; stem scales of 6–10 cells (most often 8); bracts present; pollen grains yellow, filaments up to 8.3(–12) mm, female and male flowers generally separated on different branches; fruits 0.9–1.2 × 0.9–1.1 mm, unwinged or narrowly winged, brown
	C. lenisulca Clavaud	10	European & Asian Mediterranean, NE coast of the Black Sea	amphibious	aerial/ epihydrogamy/ submerged, contacter	leaves up to 4.0 mm wide, 1–3-veined, often lingulate; stem scales of 8–16 cells; bracts present; male and female flowers generally in alternating pairs along stem, pollen grains yellow, filaments up to 2.3 mm, anthers small, <0.5 mm in diameter; fruits 1.1–1.4 × 1.3–1.6 mm, unwinged or very narrowly winged, pale brown to brown, generally occurring in every second pair of axils
	C. obtusangula Le Gall	10	W and S Europe, NW Africa	amphibious	aerial/ epihydrogamy	leaves up to 7 mm wide, 1–5(–7)-veined, often lingulate, wider leaves often rhombic; stem scales of 6–10 cells; bracts present; pollen grains yellow, elongate-ellipsoid and curved, filaments up to 7.6(–12.3) mm long; fruits 1.1–1.8 × 1.1–1.7 mm, ellipsoid, usually longer than wide, unwinged (without even a ridge), pale brown
	C. regis-jubae Schotsman	10	Iberian Peninsula, Sardinia (?), NW Africa	amphibious	aerial/ epihydrogamy, contacter	leaves up to 4.1 mm wide, 1–5-veined, lingulate leaves sometimes present; stem scales of 7–10 cells; bracts present; pollen grains yellow, filaments up to 1.5 mm, anthers small, <0.6 mm in diameter; fruits 1–1.4 × 1.2–1.6 mm, wider than long, pedunculate, pale brown to pale maroon
	C. stagnalis Scop.	10	most of Europe, NW Africa and Macaronesia, Middle East (?); introduced in North America, Japan, Australia, New Caledonia and New Zealand	amphibious	aerial/ epihydrogamy	leaves up to 9 mm wide, 1–7-veined, fresh-green, lingulate leaves usually absent; stem scales of 7–10 cells (most often 8); bracts present; pollen grains yellow, filaments up to 5.3(–8.5) mm; fruits 1.2–1.6 × 1.2–1.7 mm, broadly winged, pale brown to grayish

Section	Taxon	2n	Distribution	Growth habit	Pollination	Key morphological characters
Callitriche	*C. palustris* L.	20	Europe (predominantly Central, N and E), Asia & North America (predominantly boreal and temperate); introduced in Australia	amphibious	aerial/ epihydrogamy/ submerged, internal geitonogamy	leaves up to 4.5 mm wide, 1–5-veined, fresh-green, lingulate leaves often present; stem scales of 8–16 cells; bracts often absent; pollen grains yellow, filaments up to 2.9(–3.8) mm, often <1 mm with aborted anthers, also styles often aborted; fruits 0.9–1.4 × 0.7–1 mm, obovate, longer than wide, brown-black, often without rests of styles on the top
	C. platycarpa Kütz.	20	NW Europe, NW Spain, S Italy, Aegean Islands	amphibious	aerial/ epihydrogamy	leaves up to 9 mm wide, 1–5(–7)-veined, ± deep green, lingulate leaves sometimes present; stem scales of 7–10 cells (most often 8); bracts present; pollen grains yellow to bright yellow, ellipsoid to bluntly triangular, filaments up to 7.8(–15.5) mm; fruits 1.2–1.7 × 1.2–1.6 mm, narrowly winged, brown
	C. brutia Petagna	28**	W, NW and SW Europe, NW Africa, Middle East (?), introduced in Australia and New Zealand (var. *brutia*); Sardinia, Sicily, Capraia Island, Aegean Islands, Israel, Syria, N Africa (var. *naftolskyi*)	amphibious	submerged, hypohydrogamy, contacter	leaves up to 3.8 mm wide, 1–3(–5)-veined, lingulate leaves often present, usually not expanded on notched apices; stem scales of 7–19 cells; bracts caducous; pollen grains colourless, with rudimentary exine, filaments <1.2 mm, styles strongly reflexed; fruits 1–1.5 × 1–1.6 mm, with rests of styles appressed to side of fruit, ± orbicular, shiny, narrowly winged, sessile when submerged and with long peduncles up to 12 mm when terrestrial (var. *brutia*) or ± wider than long, matt, narrowly to broadly winged with undulate margin, always pedunculate (var. *naftolskyi*)
	C. hamulata Kütz. ex W.D.J. Koch***	38	W, N & Central Europe, Greenland, Kamchatka; introduced on the W coast of North America	amphibious	submerged, hypohydrogamy, contacter	leaves up to 5.4 mm wide, 1–5-veined, lingulate leaves often present, often expanded on notched apices; stem scales of 9–19 cells; bracts caducous; pollen grains colourless, with rudimentary exine, filaments <1.2 mm, styles strongly reflexed; fruits 1–1.5 × 1–1.4 mm, with rests of styles appressed to side of fruit, ± orbicular, shiny, narrowly winged, sessile or very rarely on peduncles up to 2.6 mm

MATERIALS AND METHODS

Field sampling

Plant samples were collected in 19 European countries and include all native European taxa except of *C. transvolgensis* Tzvelev and *C. truncata* subsp. *fimbriata* Schotsman, which are extremely rare and restricted to a small area of the Volga river delta. In addition, we included eight samples of European species that were collected in other continents, i.e. *C. stagnalis* from Australia and USA (introduced), *C. hamulata* from USA (introduced), *C. palustris* L. from USA (native to both Eurasia and North America), four samples of *C. heterophylla* Pursh from USA (considered to be closely related to *C. palustris*; Philbrick, & Les 2000; Ito & al., 2017), and *C. muelleri* Sond. from Australia, (regarded as a sister to the remaining *Callitriche* taxa, being possibly the most ancestral water-starwort species; Ito & al., 2017). The initial determination of the samples followed the taxonomic treatments of Lansdown (Lansdown, 2008; Lansdown & al., 2017; with the exception of *C. hamulata*, see below) and Bean (2007). The subspecies of *C. heterophylla* were identified on the basis of the width of the ripe fruits (cf. Lansdown, 2009), if these were available. The sample of *C. heterophylla* var. *bolanderi* (Hegelm.) Fassett (C14-144) was collected out of the known distribution range of that subspecies (cf. Fassett, 1951). As the determination of that sample was not entirely clear, we refer to it as 'cf. bolanderi'.

Two samples of *Hippuris vulgaris* L. were included as an outgroup; this genus is sister to *Callitriche* (Albach & al., 2005) and was also used as an outgroup in previous phylogenetic analyses of water-starworts (Philbrick & Les, 2000; Ito & al., 2017).

The sampling was carried out to embrace materials from the widest possible range of aquatic habitats and covering a wide range of morphological variation. If necessary, multiple individuals were collected from several populations, especially when the presence of multiple species or hybrids was suspected. In total, 344 *Callitriche* individuals from 180 localities were obtained (for locality details, see Fig. 1 and Suppl. Table S1). Voucher specimens are preserved in the herbarium of Charles University, Prague (acronym PRC).

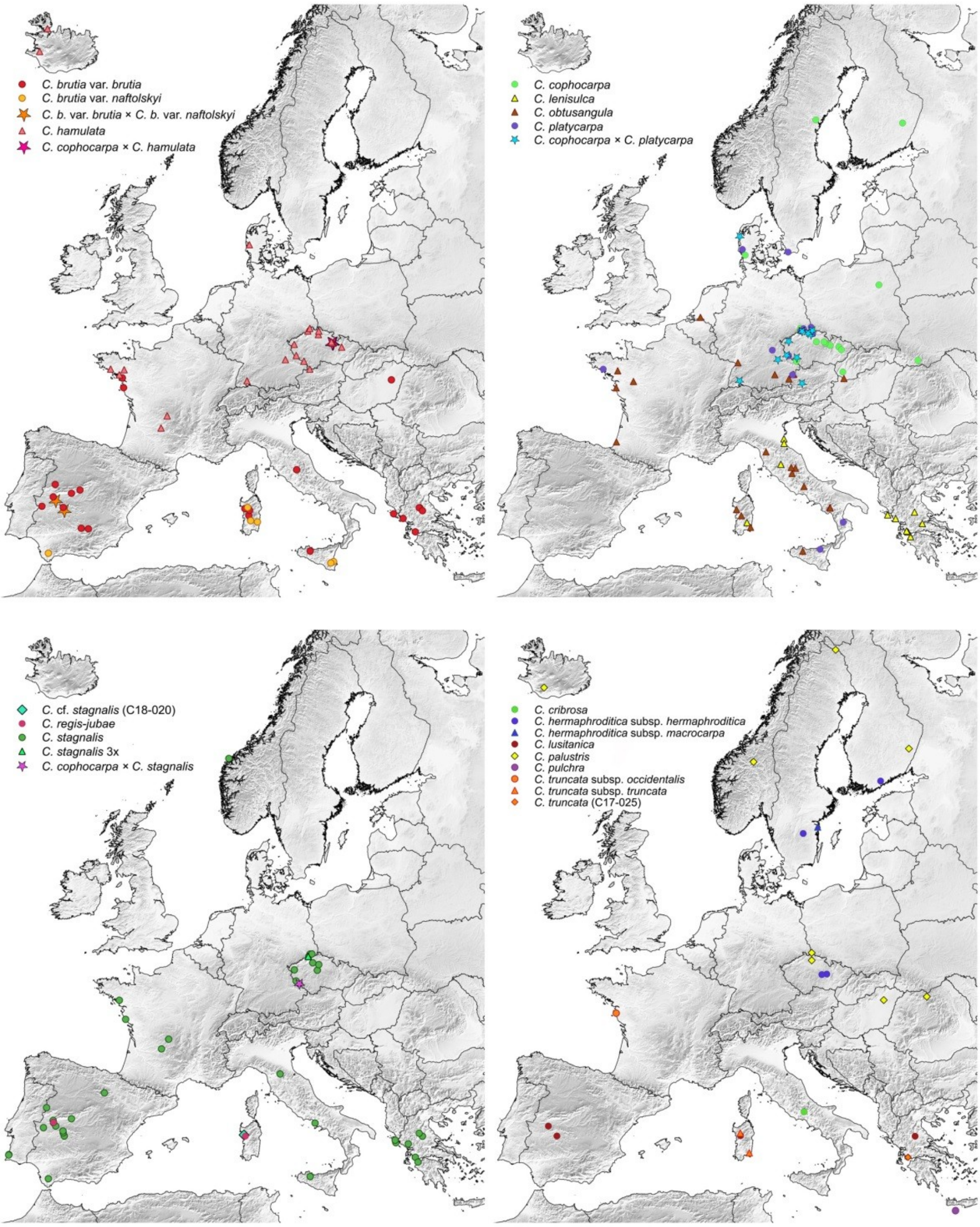


FIG. 1. Maps showing the locations of the *Callitriche* samples. Samples collected in the USA and Australia are not included. Due to numerous overlapping localities, individual taxa are depicted in four separate maps.

Flow cytometry

Genome size was estimated for 330 of 344 plants using flow cytometry (FCM). Of these, genome sizes of 149 individuals were taken from our previous cytometric study (Prančl & al., 2014) and 181 samples were newly analyzed from fresh plant material (see Suppl. Table S1) using the identical procedure and laboratory equipment. Fresh material was not available for the remaining 14 samples. The sample preparation followed the simplified two-step procedure described by Doležel & al. (2007). Samples were analyzed individually, using propidium iodide (PI) as a fluorescent stain. Additionally, a simultaneous analysis of *C. brutia* var. *brutia* and *C. brutia* var. *naftolskyi* (bulked sample of two individuals in a single run) was performed in order to confirm differences between the genome sizes of both taxa. In this case, the sample was stained using 4,6-diamidino-2-phenylindole (DAPI) to achieve a higher resolution of peaks.

If possible, each sample was analysed 2–3 times on different days to account for random measurement error; if the range of variation of the repeated measurements exceeded a 2% threshold, the outlying value was discarded and the sample re-analysed. Histograms were evaluated using the FloMax software v.2.4d (Partec GmbH) or FlowJo 10 (TreeStar Inc.). In total, exact genome size (i.e. calculated as the mean of the repeated measurements) was estimated for 195 individuals, for which repeated analyses of appropriate quality were available (147 newly analyzed and 48 taken from the previous study). Only these repeatedly measured individuals were used for the calculation of the genome size statistics of particular taxa (see below).

The genome size was expressed as the ratio of the mean fluorescence of the sample and the internal standard. *Bellis perennis* L. was selected as a primary reference standard as it has a similar, but non-overlapping genome size with the majority of the samples studied (2C = 3.96 pg, Leong-Škorničková & al., 2007; because several different genome size values are reported for *Bellis perennis*, we adopt 2C-value that was calibrated via simultaneous analyses of *Bellis* with the second standard used in this study, *Glycine max*). *Glycine max* (L.) Merr. ‘Polanka’ (2C = 2.50 pg; Doležel & al., 2007) served as a reference standard for *C. heterophylla*, *C. obtusangula* Le Gall and *C. palustris*, because the genome sizes of these taxa overlapped with that of *Bellis perennis*. The extent of the total variation in intraspecific genome size was calculated as a percentage of the difference between the highest and lowest genome size value and expressed as a percentage of the minimum.

In order to compare the recent results with our previous genome size estimations and to gain the most accurate genome size values, we extended the dataset for genome size statistics with 132 additional samples from our previous study (Prančl & al., 2014; Suppl. Table S1). These samples, mostly originating from Central Europe, are not formally included in the present paper (as they were not sequenced), but their mean genome sizes estimated from the repeated measurements have been used. In total, genome size statistics of particular taxa were calculated using the combined dataset of 327 samples (including 147 newly analyzed samples and 180 genome size values published in the previous study).

Chromosome counts

Selected plants were cultivated in a garden tank until they formed adventive roots on their stems, which were used for chromosome counting. Alternatively, plants were cultivated on wet mud in pots in a greenhouse and chromosomes were counted using shoot apical meristem and the youngest leaves emerging in the centre of the leaf rosettes.

The meristematic tissue was pre-treated in a saturated aqueous solution of p-dichlorobenzene at room temperature for approximately three hours, then fixed in a freshly prepared 3:1 mixture of 96% ethanol and acetic acid and stored at -20°C until further processing. Before chromosome preparation, the material was macerated in a 1:1 mixture of ethanol and hydrochloric acid for 10 s, then transferred onto a microscope slide. Non-meristematic tissues were removed, and the meristem was stained in a drop of lacto-propionic orcein, covered with a coverslip and squashed. The preparations were examined under an Olympus BX 51 microscope equipped with a DP-71 Olympus digital camera with the DP Controller imaging software v.3.1 (Olympus Corp.). Only slides on which at least five mitoses were found were considered.

Our previous study provided chromosome counts for eight *Callitriche* taxa growing in Central Europe (Prančl & al., 2014). In this study, we determined chromosome numbers for additional eight samples belonging to seven taxa, which were not included in the previous study. For the remaining species included in this study, we were not able to obtain/cultivate usable material.

Molecular procedures

In total, 224 individuals including samples from 180 populations were subjected to molecular analyses. A single sample was sequenced from the majority of populations that were homogenous morphologically and also proved to be invariable in genome size. Several samples were processed from populations that were assumed to be mixed on the basis of genome sizes and/or morphology, and also for some populations that included individuals of putative hybrid origin. Total genomic DNA was extracted from silica gel-dried leaf tissue according to a sorbitol extraction method (Štorchová & al., 2000). The internal transcribed spacer region of nuclear ribosomal DNA (containing ITS1, 5.8S rDNA and ITS2) was amplified using primers ITS F (King & al., 2001) and ITS 4 (White & al., 1990); the *trn*T-*trn*L plastid intergenic spacer was amplified using primers a and b (Taberlet & al., 1991). The ITS region was amplified as described in Kaplan & Fehrer (2004); PCR conditions for the *trn*T-*trn*L region follow Fehrer & al. (2007) except that *Taq* DNA polymerase and PCR Blue buffer from Top-Bio (Vestec, Czech Republic) were used. PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced at GATC Biotech (Konstanz, Germany) / Eurofins Genomics (Ebersberg, Germany) using the PCR primers in one or both directions depending on read quality.

Sequences of the ITS region of several samples showed polymorphisms, i.e. superimposed peaks and occasionally shifts. This was especially true for several samples of putative hybrids (namely *C. cophocarpa* × *C. stagnalis*, *C. brutia* var. *brutia* × *C. brutia* var. *naftolskyi* and *C. hamulata* × *C. cophocarpa/platycarpa*) and for some samples of *C. hamulata*. For these samples, cloning and RFLP analysis were applied to make final identifications and documentations of hybrid identity. In the case of the first two aforementioned hybrids, multiple individuals showed the same patterns of polymorphisms, therefore only one sample of each hybrid was selected (C15-084-03 and C16-013, respectively) and cloned as described in Fehrer & al. (2009). Eight clones were sequenced for each sample, and the parental copies were identified. For *C. hamulata* and the putative hybrid *C. hamulata* × *C. cophocarpa/platycarpa*, the peaks corresponding to the polymorphisms were very small so that a too high number of clones would have to be sequenced to retrieve the underrepresented copies. Therefore these samples were subjected to RFLP analysis. Based on the putative parental sequences, diagnostic restriction sites were identified that distinguished all species except *C. platycarpa* and *C. cophocarpa* whose sequences were identical. RFLPs of ITS were performed using a double digest with *Bam*HI (G'GATC_C) and *Bsi*WI (C'GTAC_G) enzymes (Fisher Scientific, Pardubice, Czech Republic). *Bam*HI cuts only *C. cophocarpa* and *C. platycarpa* once; *Bsi*WI cuts only *C. brutia* once, and both cut *C. brutia* var. *naftolskyi* once resulting in three fragments of distinguishable size. Restriction digests were performed with 10 units of *Bam*HI and 3 units of *Bsi*WI according to the manufacturer's instructions using approx. 250 ng of PCR product in overnight digests. Products were separated on 2% agarose gels with 200 ng of DNA size standard. In total, 18 samples of six taxa were subjected to RFLPs, covering all putative parental species and samples representing the majority of the observed intraspecific genetic variation.

All sequences were submitted to GenBank (accession numbers MN091382–MN091622 [ITS], MN091980–MN092205 [*trn*T-*trn*L]). For a detailed list, see Suppl. Table S1.

Molecular data analyses

Sequence electropherograms were edited manually using Chromas v. 1.45 (Technelysium Pty Ltd., Australia) and aligned by hand in Bioedit v. 7.0.9.0 (Hall, 1999; for alignments, see Suppl. Appendices S1, S2). Additive nucleotide polymorphisms in the ITS region were coded using the IUPAC nucleotide ambiguity codes. For the ITS dataset, available sequences from the study of Ito & al. (2017) were retrieved from GenBank and added to the alignment (see Suppl. Appendix S1). Additionally, the individual ITS variants of the hybrids resulting from cloning were included. Before performing phylogenetic analyses, the number of samples for both ITS and *trn*T-*trn*L datasets were reduced in an effort to cover the whole molecular variation and a representative geographic range for all taxa. Samples containing nucleotide polymorphisms were excluded from the ITS dataset in order to prevent branch collapses (with a few exceptions such as *C. hamulata* samples, of which all sequences showed at least some polymorphisms). The final ITS dataset consisted of 73 of our samples (including eight clones) and 35 sequences from GenBank (Suppl. Appendix S3). The final *trn*T-*trn*L dataset included of 90 accessions (Suppl. Appendix S4); no corresponding data of this region were available in GenBank. All our samples included in the ITS dataset were also included in the *trn*T-*trn*L dataset. Since both trees were mostly congruent (see below), we also analyzed a concatenated dataset, consisting of 65 accessions that were included in both ITS and *trn*T-*trn*L trees.

Indel coding for both datasets was performed with FastGap v. 1.2 (Borchsenius, 2009) based on the simple method of Simmons & Ochoterena (2000). Phylogenetic relationships were estimated using Maximum Likelihood (ML) and Bayesian analyses (BA). Prior to analyses, the model of molecular evolution best fitting the data was determined for all datasets with Modeltest 3.5 (Posada & Crandall 1998). For ITS and the concatenated dataset, a TrN+Γ model was found in Hierarchical Likelihood Ratio Tests (hLRTs). ML analysis was performed with MEGA v. X (Kumar & al., 2018) using a Tamura-Nei model and gamma distribution with 5 discrete rate categories. All sites, extensive subtree-pruning-regrafting and a very strong branch swap filter were used. Bootstrap support was computed using 1000 replicates. Bayesian analyses were conducted with MrBayes v. 3.2.6 (Ronquist & al., 2012), six substitution rates and gamma distribution as priors. Analyses were run with the default settings for 2.5 million generations (ITS) or 1 million generations (for the smaller concatenated dataset), sampling every 1000th tree. All indicators suggested that convergence between the different runs was achieved. The first 25% of trees were discarded as burn-in and the rest of the trees were summarized. For *trn*T-*trn*L, a TVM+Γ model was found to best represent the data. A transversion model is not implemented in MEGA, it was replaced by the most similar one, a general time reversible model. For BA, 1.5 million generations were needed to reach convergence. Other parameters were the same as before.

In order to visualize the reticulate relationships among the species studied, two datasets (ITS and the concatenated dataset of ITS and *trn*T-*trn*L) were subjected to NeighborNet analysis performed with SplitsTree4 v. 4.14.8 (Huson & Bryant, 2006), applying uncorrected *p* distances with ambiguities handled as average. Bootstrap support was calculated with 1000 replicates. For these datasets, all 224 of own sequenced samples were included, but the sequences from Ito & al. (2017) were omitted, because polymorphisms were obviously not scored and evaluated in that study, and *trn*T-*trn*L was examined only in our study.

Results

Genome size and chromosome counts

Genome size was determined for all species included in this study except *C. pulchra* Schotsman, for which we did not have living plants. In total, 24 taxa of *Callitriche* were analyzed (Table 2, Fig. 2). The majority of species differs clearly in nuclear DNA content. The differences in genome sizes are

Table 2. Summary of flow cytometric genome size estimations. Taxa, for which the genome size is estimated here for the first time, are in bold. **2n** – chromosome number; values in bold indicate taxa, for which the chromosomes were counted in this study or in Prančl & al. (2014); values indicated by "?" were estimated on the basis of 2C-values, chromosome numbers for these taxa are unknown. **2C ± SD** – mean genome size (2C value) in pg of DNA ± standard deviation. **2C range** – minimum and maximum 2C values. **Var (%)** – difference between minimum and maximum expressed as % of the minimum. **1Cx** – monoploid genome size in pg of DNA calculated from the mean 2C value and the ploidy level; if the ploidy level is only estimated, the values are in italics; for some taxa, the 1Cx value cannot be meaningfully calculated due to aneuploid chromosome counts. **Mean chromosome size** – theoretical value calculated from the mean 2C value and the chromosome number. **Standard** – internal standard (B = *Bellis perennis*, G = *Glycine max* 'Polanka').

Taxon	2n	Ploidy	N	2C ± SD	2C range	Var (%)	1Cx	Mean chromo-zome size	Stan-dard
***C. truncata* (Greece)**	6?	2x-4	1	1.88	–	–	*0.94*	*0.31*	B
C. truncata* subsp. *truncata	6	2x-4	1	2.14	–	–	1.07	0.36	B
C. truncata* subsp. *occidentalis	**6**	2x-4	1	2.45	–	–	1.23	0.41	B
C. hermaphroditica subsp. *hermaphroditica*	6	2x-4	7	1.96 ± 0.03	1.92 – 2.01	4.69	0.98	0.33	B
C. hermaphroditica* subsp. *macrocarpa	**6**	2x-4	1	2.01	–	–	1.01	0.34	B
C. lusitanica	**8**	2x-2	3	1.83 ± 0.01	1.82 – 1.84	1.10	0.92	0.23	B
C. cribrosa	8	2x-2	1	3.62	–	–	1.81	0.45	B
C. muelleri	**10**	2x	2	1.21 ± 0.01	1.20 –1.21	0.83	0.61	0.12	B
C. regis-jubae	**10**	2x	2	2.99 ± 0.02	2.97 – 3.01	1.35	1.50	0.30	B

Taxon	2n	Ploidy	N	2C ± SD	2C range	Var (%)	1Cx	Mean chromozome size	Standard
C. cophocarpa × _C. stagnalis_	10?	2x	14	3.12 ± 0.01	3.10 – 3.14	1.29	*1.56*	*0.31*	B
C. cophocarpa	**10**	2x	39	3.20 ± 0.04	3.11 – 3.26	4.82	1.60	0.32	B
C. lenisulca	**10**	2x	10	3.63 ± 0.03	3.58 – 3.69	3.07	1.82	0.36	B
C. obtusangula	**10**	2x	20	3.86 ± 0.06	3.71 – 3.93	5.93	1.93	0.39	G
autotriploid *C. stagnalis*	15?	3x	1	4.55	–	–	*1.52*	*0.30*	B
C. ×vigens [*C. cophocarpa* × *C. platycarpa*]	**15**	3x	19	4.66 ± 0.04	4.62 – 4.72	2.16	1.55	0.31	B
C. palustris	**20**	4x	24	3.90 ± 0.05	3.75 – 3.96	5.60	0.98	0.20	G
C. heterophylla var. cf. _bolanderi_	**20**	4x	1	4.05	–	–	2.03	0.20	G
C. platycarpa	**20**	4x	27	6.19 ± 0.06	6.06 – 6.33	4.46	1.55	0.31	B
C. brutia var. _brutia_	**28**	6x-2	17	5.86 ± 0.04	5.81 – 5.96	2.58	–	0.21	B
C. brutia var. _brutia_ × _C. brutia_ var. _naftolskyi_	**28**	6x-2	2	5.96 ± 0.02	5.94 – 5.98	0.67	–	0.21	B
C. brutia var. _naftolskyi_	**28**	6x-2	5	6.13 ± 0.03	6.10 – 6.19	1.48	–	0.22	B
C. cophocarpa × *C. hamulata*	**29**	6x-1	16	7.63 ± 0.06	7.56 – 7.78	2.91	–	0.26	B
C. hamulata	**38**	8x-2	56	8.90 ± 0.09	8.73 – 9.15	4.81	–	0.23	B

insignificant only for the pairs of *C. regis-jubae* Schotsman – *C. stagnalis*, *C. obtusangula* – *C. palustris* and *C. brutia* var. *naftolskyi* – *C. platycarpa*. The detected 2C-values varied 7.36-fold from 1.21 pg in the Australian species *C. muelleri* up to 8.90 pg in *C. hamulata* (Fig. 2). Monoploid genome sizes (1Cx-values) were also highly variable, ranging 3.16-fold from 0.61 pg in *C. muelleri* to 1.93 pg in *C. obtusangula*. Flow cytometry was for the first time applied to estimate the genome size of five European and two non-European species (namely *C. brutia*, *C. cribrosa* Schotsman, *C. lusitanica* Schotsman, *C. regis-jubae*, *C. truncata*, *C. heterophylla* and *C. muelleri*). Additionally, cytotype variation was detected within *C. brutia* and *C. truncata*. In *C. brutia*, two cytotypes with similar, but non-overlapping cytotypes correspond well with two subordinate taxa, *C. brutia* var. *brutia* (lower genome size) and *C. brutia* var. *naftolskyi* (larger genome size; difference between means 4.6%). The simultaneous analysis of these two taxa confirmed the difference, resulting in a bifurcated peak. The case of *C. truncata* is more complicated, because three clearly different cytotypes were revealed among plants that fit morphologically to this species. From these, the cytotype with the largest genome size corresponds to *C. truncata* subsp. *occidentalis* (Rouy) Schotsman, the second to subsp. *truncata* and the third with the lowest DNA content,

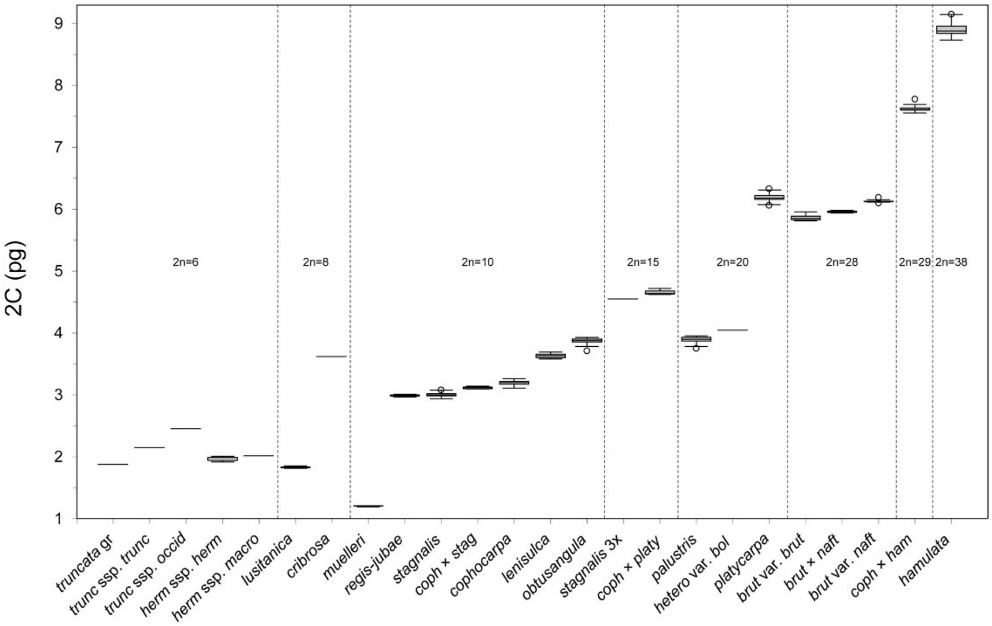


FIG. 2. Box-and-whisker plots showing the genome size variation (2C-values) of 24 *Callitriche* taxa. Taxa abbrevitions: *truncata* gr = *C. truncata* from Greece (C17-025); *trunc* ssp. *trunc* = *C. truncata* subsp. *truncata*; *trunc* ssp. *occid* = *C. truncata* subsp. *occidentalis*; *herm* ssp. *herm* = *C. hermaphroditica* subsp. *hermaphroditica*; *herm* ssp. *macro* = *C. hermaphroditica* subsp. *macrocarpa*; *coph* × *stag* = putative hybrid *C. cophocarpa* × *C. stagnalis*; *C. stagnalis* 3x = putative autotriploid *C. stagnalis*; *coph* × *platy* = *C. cophocarpa* × *C. platycarpa* [*C.* ×*vigens*]; *hetero* var. *bol* = *C. heterophylla* var. *bolanderi*; *brut* var. *brut* = *C. brutia* var. *brutia*; *brut* × *naft* = putative hybrid *C. brutia* var. *brutia* × *C. brutia* var. *naftolskyi*; *coph* × *ham* = putative hybrid *C. cophocarpa* × *C. hamulata*.

represented by one population from Greece (C17-025), is not clearly attributable to any subspecies (see Discussion). The mean genome sizes differed by 14.5% (subsp. *truncata* – subsp. *occidentalis*), 13.8% (Greek *truncata* – subsp. *truncata*) and even by 30.3% (Greek *truncata* – subsp. *occidentalis*). We managed to count the chromosome number only for subsp. *occidentalis* (2n=6). In contrast, two subspecies recognized within *C. hermaphroditica*, i.e. subsp. *hermaphroditica* and subsp. *macrocarpa* (Hegelm.) Lansdown, are indistinguishable using FCM. Two previously unknown taxa of putative hybrid origin were revealed. The first one (C15-084) was found at a single locality in the Czech Republic, co-occurring with *C. stagnalis*. Individuals from this flowering, but non-fertile population showed intermediate genome size between *C. stagnalis* and *C. cophocarpa* and were therefore assumed to be the hybrid of these species. The identity of this hybrid was later confirmed by molecular analyses (see below). The other hybrid was found in two streams in Spain (C16-009, C16-013). These plants show genome sizes at the upper end of the range of *C. brutia* var. *brutia*, but both possess mostly underdeveloped fruits. These samples were assigned to *C. brutia* var. *brutia* × *C. brutia* var. *naftolskyi* based on results of the molecular analyses (see below).

For eleven taxa, genome sizes were published previously (Prančl & al., 2014). The current FCM data correspond well to those previously published. The only exception is *C. obtusangula*, for which two cytotypes with slightly different genome sizes were reported in the previous study, one including plants from Italy and the second represented by plants from north-western Europe. Our new data, including more samples of this species, suggest that although the genome size variation of the Italian samples is higher in comparison with samples from the rest of Europe, the genome size range of this species is rather continuous. Therefore we consider all samples of *C. obtusangula* as belonging to a single cytotype.

Chromosome numbers quoted in published sources are confirmed in all seven taxa studied (Fig. 3, Table 2). For *C. brutia* var. *naftolskyi* (2n=28), the chromosome number is determined here for the first time.

Molecular phylogenetic analyses

Phylogenetic trees reconstructed on the basis of the plastid *trn*T-*trn*L region show with strong support that the Australian species *C. muelleri* is sister to the remaining *Callitriche* taxa together with the outgroup *Hippuris vulgaris*

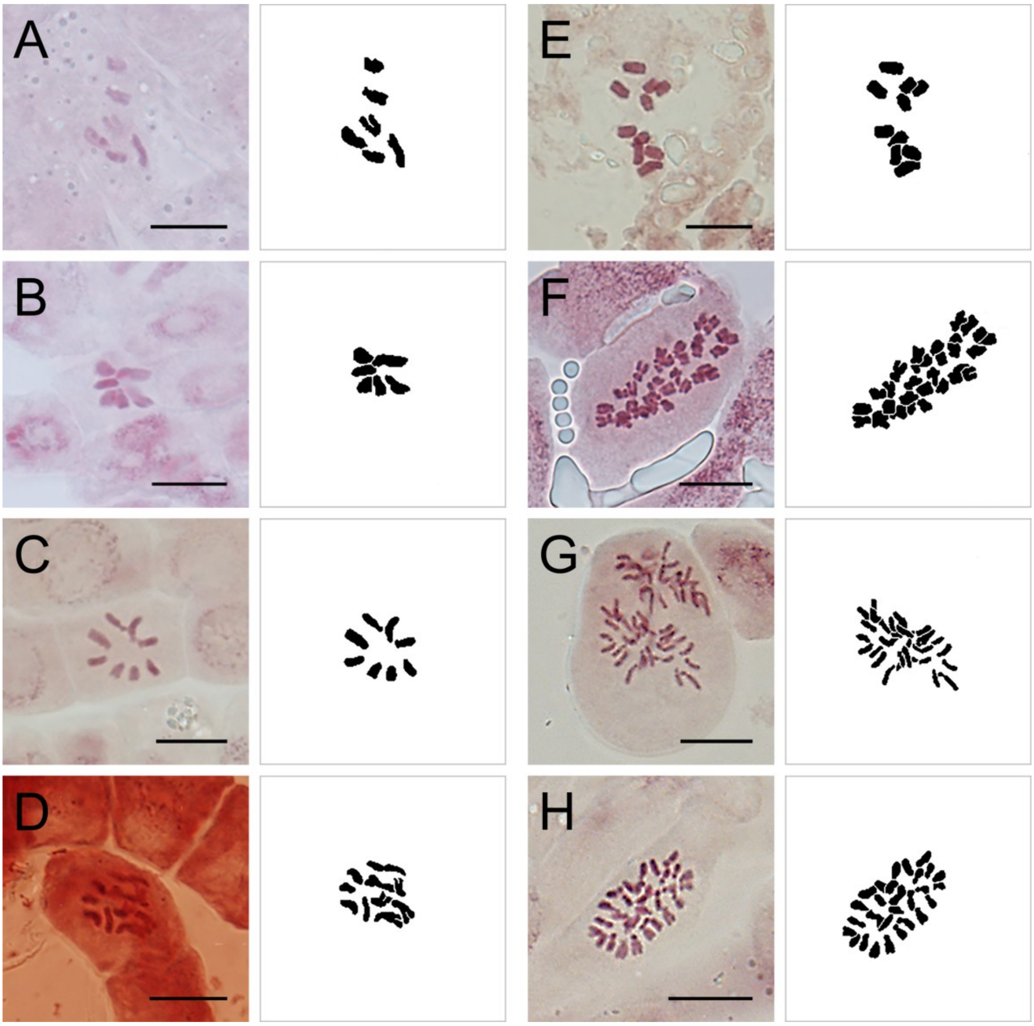
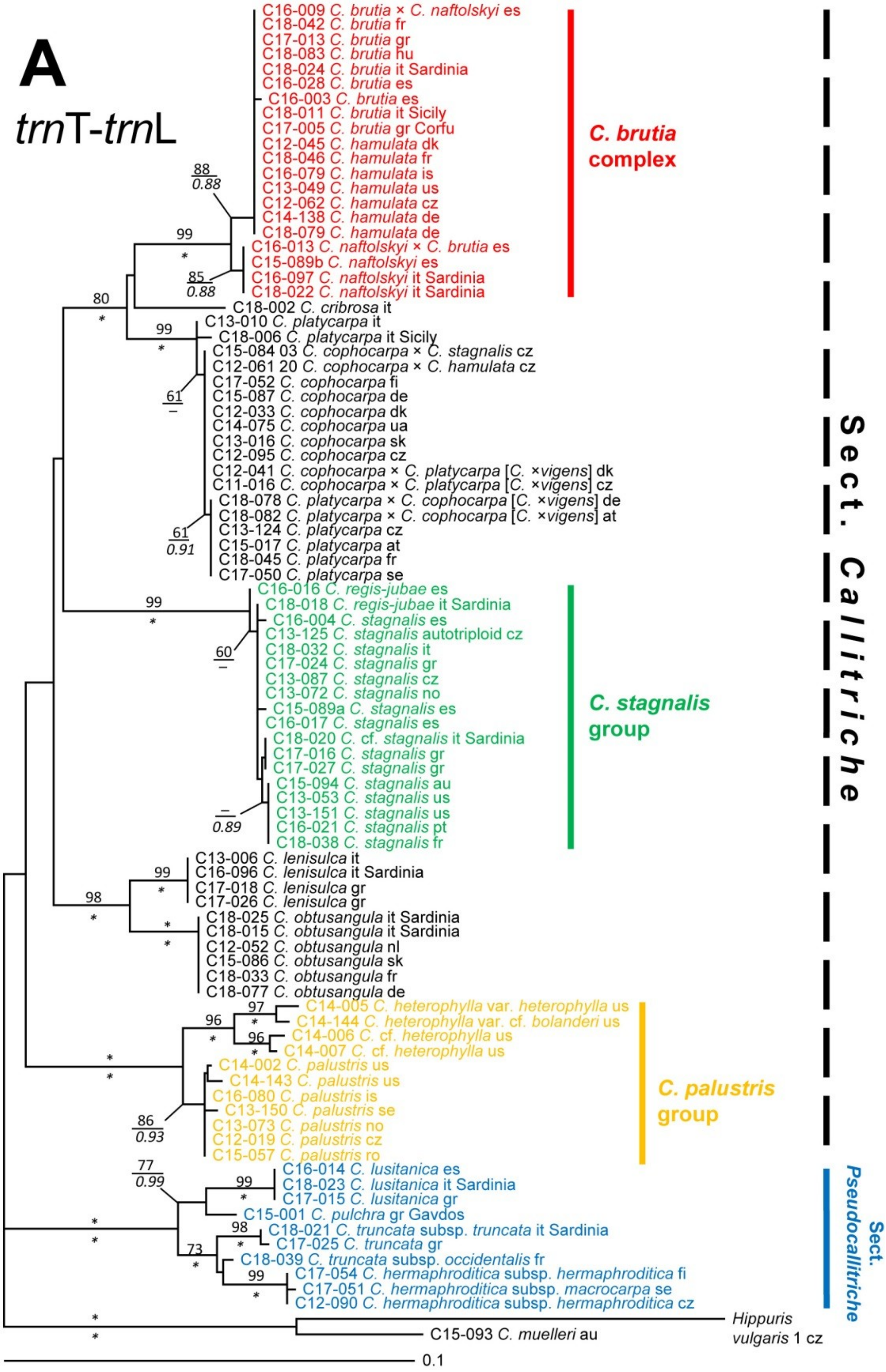


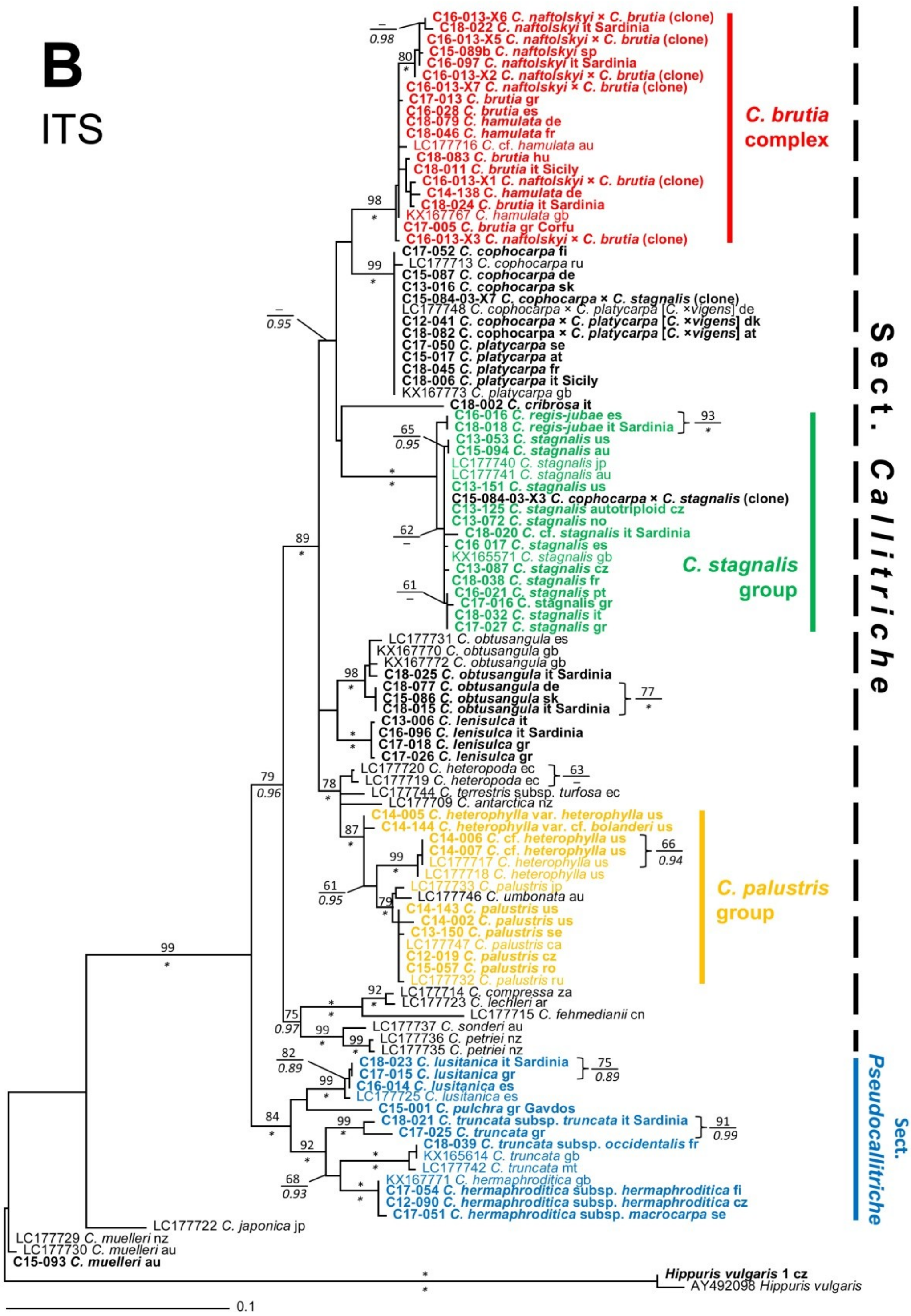
FIG. 3. Chromosomes (photograph of cytological preparation on the left with its interpretation on the right in each pair) of seven *Callitriche* taxa at mitotic metaphase in somatic cells, arranged according to increasing chromosome number: A – *C. hermaphroditica* subsp. *macrocarpa*, sample C17-051 (Sweden), 2n=6; B – *C. truncata* subsp. *occidentalis*, sample C18-039 (France), 2n=6; C – *C. lusitanica*, sample C17-015 (Greece), 2n=8; D – *C. muelleri*, sample C15-093 (Australia), 2n=10; E – *C. regis-jubae*, sample C16-016 (Spain), 2n=10; F – *C. brutia* var. *naftolskyi*, sample C16-097 (Sardinia), 2n=28; G – *C. brutia* var. *brutia*, sample C16-098 (Sardinia), 2n=28; H – *C. brutia* var. *brutia*, sample C17-012 (Greece), 2n=28. Scale bar = 10 μm.

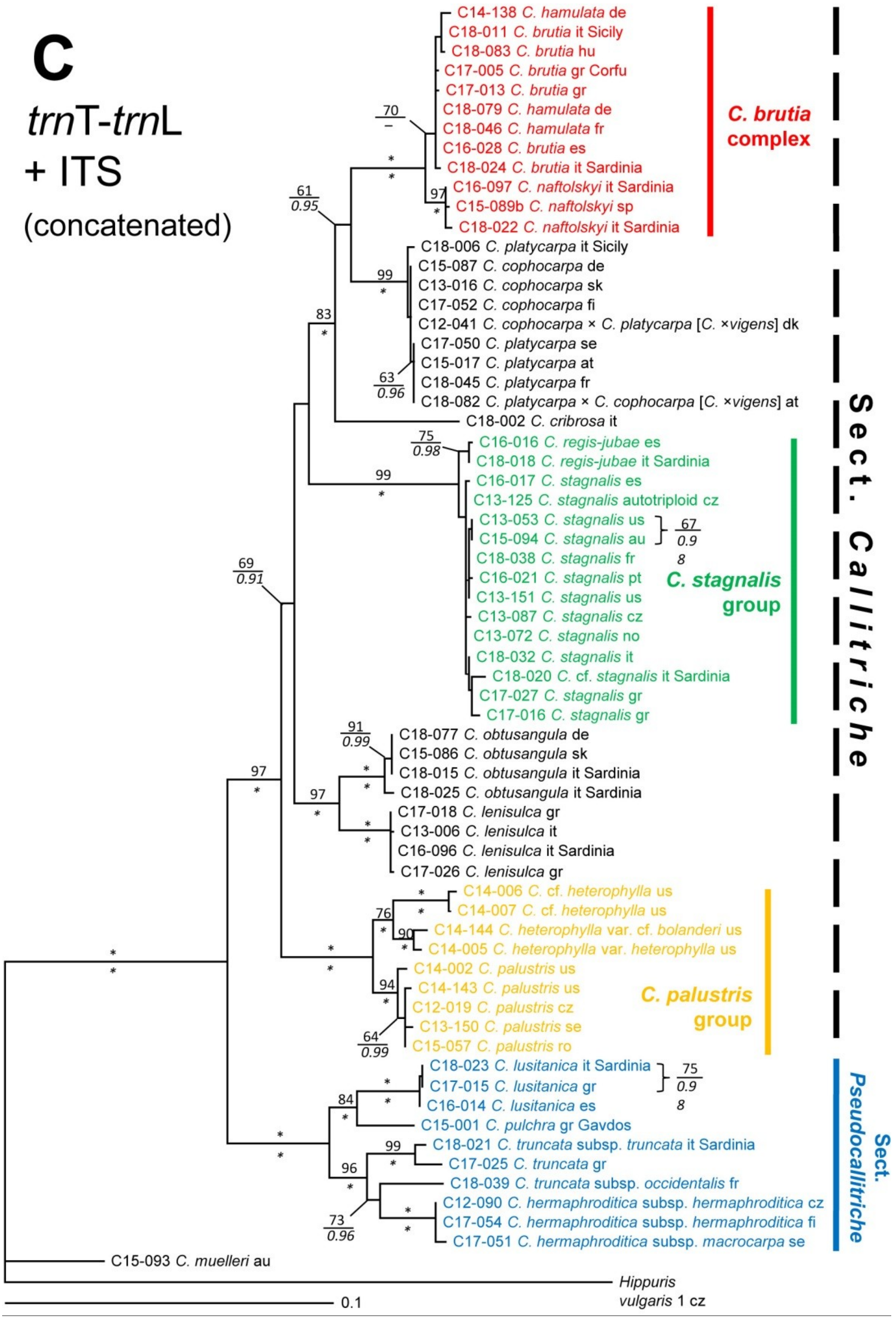
(Fig. 4A). Also in the ITS tree, in which also samples from the study of Ito & al. (2017) are included, *C. muelleri* results as the most basally branching *Callitriche* species, followed by *C. japonica* Engelm. ex Hegelm. forming a second branch, which is sister to the clade consisting of the rest of the genus, the latter is also strongly supported (Fig. 4B). In all datasets, including the tree reconstructed on the basis of concatenated data (*trn*T-*trn*L + ITS; Fig. 4C), the clade corresponding to the traditionally recognized sect. *Pseudocallitriche* (Hegelmaier, 1864; Philbrick & Les, 2000) is also well-supported. Other smaller groups having high support in all trees are the complex of *C. brutia*, the group of *C. cophocarpa*, *C. platycarpa* and *C. ×vigens* and the species pairs *C. truncata* + *C. hermaphroditica*, *C. palustris* + *C. heterophylla* (*C. palustris* group; also including *C. umbonata* Hegelm. in the ITS dataset) and *C. stagnalis* + *C. regis-jubae*. The clade of *C. lenisulca* Clavaud and *C. obtusangula* possesses a high support in the *trn*T-*trn*L and concatenated trees, but it is not significantly supported in the ITS dataset. *Callitriche cribrosa* forms an isolated lineage with unclear relationships in all trees. The ITS dataset also contains some well-supported groups of species that were not included in the other trees such as clades of *C. compressa* N.E.Br. + *C. lechleri* (Hegelm.) Fassett + *C. fehmedianii* Majeed Kak & Javeid or *C. sonderi* Hegelm. + *C. petriei* R.Mason. The Southern Hemisphere taxa *C. terrestris* subsp. *turfosa* (Bertero ex Hegelm.) Bacigalupo, *C. antarctica* Engelm. ex Hegelm. and *C. heteropoda* Engelm. ex Hegelm. end up as sister to the *C. palustris* group (Fig. 4B).

Most of the traditionally recognized *Callitriche* species are well separated and supported in all trees, with several exceptions. The samples of *C. cophocarpa* and *C. platycarpa* share mostly identical ITS ribotypes (Fig. 4B). Plastid sequences of these two species differ only in one site except of two Italian accessions of *C. platycarpa*, which show slight differences (Fig. 4A). Likewise, *C. brutia* var. *brutia* and *C. hamulata* share an identical haplotype and are also indistinguishable on the basis of ITS sequences (Fig. 4A,B,C). Finally, both samples of *C. regis-jubae* are significantly supported as sister to *C. stagnalis* with ITS and in the concatenated tree, but the genetic distance between both species is very low, and only one sample of *C. regis-jubae* has also a slightly distinct *trn*T-*trn*L haplotype.

In general, plastid and ITS trees (Figs 4A,B) are fairly congruent, resulting in high support of most main branches in the concatenated tree (Fig. 4C).


A
trnT-trnL
C16-009 C. brutia × C. naftolskyi es
C18-042 C. brutia fr
C17-013 C. brutia gr
C18-083 C. brutia hu
C18-024 C. brutia it Sardinia
C16-028 C. brutia es
C16-003 C. brutia es
C18-011 C. brutia it Sicily
C17-005 C. brutia gr Corfu
C12-045 C. hamulata dk
C18-046 C. hamulata fr
C16-079 C. hamulata is
C13-049 C. hamulata us
C12-062 C. hamulata cz
C14-138 C. hamulata de
C18-079 C. hamulata de
C16-013 C. naftolskyi × C. brutia es
C15-089b C. naftolskyi es
C16-097 C. naftolskyi it Sardinia
C18-022 C. naftolskyi it Sardinia
C18-002 C. cribrosa it
C13-010 C. platycarpa it
C18-006 C. platycarpa it Sicily
C15-084 03 C. cophocarpa × C. stagnalis cz
C12-061 20 C. cophocarpa × C. hamulata cz
C17-052 C. cophocarpa fi
C15-087 C. cophocarpa de
C12-033 C. cophocarpa dk
C14-075 C. cophocarpa ua
C13-016 C. cophocarpa sk
C12-095 C. cophocarpa cz
C12-041 C. cophocarpa × C. platycarpa [C. ×vigens] dk
C11-016 C. cophocarpa × C. platycarpa [C. ×vigens] cz
C18-078 C. platycarpa × C. cophocarpa [C. ×vigens] de
C18-082 C. platycarpa × C. cophocarpa [C. ×vigens] at
C13-124 C. platycarpa cz
C15-017 C. platycarpa at
C18-045 C. platycarpa fr
C17-050 C. platycarpa se
C16-016 C. regis-jubae es
C18-018 C. regis-jubae it Sardinia
C16-004 C. stagnalis es
C13-125 C. stagnalis autotriploid cz
C18-032 C. stagnalis it
C17-024 C. stagnalis gr
C13-087 C. stagnalis cz
C13-072 C. stagnalis no
C15-089a C. stagnalis es
C16-017 C. stagnalis es
C18-020 C. cf. stagnalis it Sardinia
C17-016 C. stagnalis gr
C17-027 C. stagnalis gr
C15-094 C. stagnalis au
C13-053 C. stagnalis us
C13-151 C. stagnalis us
C16-021 C. stagnalis pt
C18-038 C. stagnalis fr
C13-006 C. lenisulca it
C16-096 C. lenisulca it Sardinia
C17-018 C. lenisulca gr
C17-026 C. lenisulca gr
C18-025 C. obtusangula it Sardinia
C18-015 C. obtusangula it Sardinia
C12-052 C. obtusangula nl
C15-086 C. obtusangula sk
C18-033 C. obtusangula fr
C18-077 C. obtusangula de
C14-005 C. heterophylla var. heterophylla us
C14-144 C. heterophylla var. cf. bolanderi us
C14-006 C. cf. heterophylla us
C14-007 C. cf. heterophylla us
C14-002 C. palustris us
C14-143 C. palustris us
C16-080 C. palustris is
C13-150 C. palustris se
C13-073 C. palustris no
C12-019 C. palustris cz
C15-057 C. palustris ro
C16-014 C. lusitanica es
C18-023 C. lusitanica it Sardinia
C17-015 C. lusitanica gr
C15-001 C. pulchra gr Gavdos
C18-021 C. truncata subsp. truncata it Sardinia
C17-025 C. truncata gr
C18-039 C. truncata subsp. occidentalis fr
C17-054 C. hermaphroditica subsp. hermaphroditica fi
C17-051 C. hermaphroditica subsp. macrocarpa se
C12-090 C. hermaphroditica subsp. hermaphroditica cz
Hippuris vulgaris 1 cz
C15-093 C. muelleri au
0.1
88
0.88
99
*
85
0.88
80
*
99
*
61
–
61
0.91
99
*
60
–
–
0.89
99
*
98
*
*
*
97
*
96
*
96
*
*
*
86
0.93
77
0.99
99
*
98
*
73
*
99
*
*
*
*
*
C. brutia complex
C. stagnalis group
C. palustris group
Sect. Callitriche
Sect. Pseudocallitriche


B
ITS
–
0.98
C16-013-X6 C. naftolskyi × C. brutia (clone)
C18-022 C. naftolskyi it Sardinia
C16-013-X5 C. naftolskyi × C. brutia (clone)
80
*
C15-089b C. naftolskyi sp
C16-097 C. naftolskyi it Sardinia
C16-013-X2 C. naftolskyi × C. brutia (clone)
C16-013-X7 C. naftolskyi × C. brutia (clone)
C17-013 C. brutia gr
C16-028 C. brutia es
C18-079 C. hamulata de
C18-046 C. hamulata fr
LC177716 C. cf. hamulata au
C18-083 C. brutia hu
C18-011 C. brutia it Sicily
C16-013-X1 C. naftolskyi × C. brutia (clone)
C14-138 C. hamulata de
C18-024 C. brutia it Sardinia
KX167767 C. hamulata gb
C17-005 C. brutia gr Corfu
C16-013-X3 C. naftolskyi × C. brutia (clone)
98
*
C. brutia complex
C17-052 C. cophocarpa fi
99
*
LC177713 C. cophocarpa ru
C15-087 C. cophocarpa de
C13-016 C. cophocarpa sk
C15-084-03-X7 C. cophocarpa × C. stagnalis (clone)
LC177748 C. cophocarpa × C. platycarpa [C. ×vigens] de
–
0.95
C12-041 C. cophocarpa × C. platycarpa [C. ×vigens] dk
C18-082 C. cophocarpa × C. platycarpa [C. ×vigens] at
C17-050 C. platycarpa se
C15-017 C. platycarpa at
C18-045 C. platycarpa fr
C18-006 C. platycarpa it Sicily
KX167773 C. platycarpa gb
C18-002 C. cribrosa it
C16-016 C. regis-jubae es
C18-018 C. regis-jubae it Sardinia
93
*
65
0.95
C13-053 C. stagnalis us
C15-094 C. stagnalis au
*
*
LC177740 C. stagnalis jp
LC177741 C. stagnalis au
C13-151 C. stagnalis us
C15-084-03-X3 C. cophocarpa × C. stagnalis (clone)
C13-125 C. stagnalis autotriploid cz
C13-072 C. stagnalis no
62
–
C18-020 C. cf. stagnalis it Sardinia
89
*
C16 017 C. stagnalis es
KX165571 C. stagnalis gb
C13-087 C. stagnalis cz
C18-038 C. stagnalis fr
61
–
C16-021 C. stagnalis pt
C17-016 C. stagnalis gr
C18-032 C. stagnalis it
C17-027 C. stagnalis gr
C. stagnalis group
Sect. Callitriche
LC177731 C. obtusangula es
KX167770 C. obtusangula gb
KX167772 C. obtusangula gb
98
*
C18-025 C. obtusangula it Sardinia
C18-077 C. obtusangula de
C15-086 C. obtusangula sk
C18-015 C. obtusangula it Sardinia
77
*
C13-006 C. lenisulca it
*
*
C16-096 C. lenisulca it Sardinia
C17-018 C. lenisulca gr
C17-026 C. lenisulca gr
LC177720 C. heteropoda ec
LC177719 C. heteropoda ec
63
–
79
0.96
78
*
LC177744 C. terrestris subsp. turfosa ec
LC177709 C. antarctica nz
C14-005 C. heterophylla var. heterophylla us
87
*
C14-144 C. heterophylla var. cf. bolanderi us
C14-006 C. cf. heterophylla us
C14-007 C. cf. heterophylla us
66
0.94
99
*
LC177717 C. heterophylla us
LC177718 C. heterophylla us
61
0.95
LC177733 C. palustris jp
LC177746 C. umbonata au
79
*
C14-143 C. palustris us
C14-002 C. palustris us
C13-150 C. palustris se
LC177747 C. palustris ca
C12-019 C. palustris cz
C15-057 C. palustris ro
LC177732 C. palustris ru
C. palustris group
99
*
92
LC177714 C. compressa za
*
*
LC177723 C. lechleri ar
75
0.97
LC177715 C. fehmedianii cn
99
*
LC177737 C. sonderi au
99
*
LC177736 C. petriei nz
LC177735 C. petriei nz
82
0.89
C18-023 C. lusitanica it Sardinia
C17-015 C. lusitanica gr
75
0.89
99
*
C16-014 C. lusitanica es
LC177725 C. lusitanica es
C15-001 C. pulchra gr Gavdos
84
*
99
*
C18-021 C. truncata subsp. truncata it Sardinia
C17-025 C. truncata gr
91
0.99
92
*
C18-039 C. truncata subsp. occidentalis fr
*
*
KX165614 C. truncata gb
LC177742 C. truncata mt
68
0.93
KX167771 C. hermaphroditica gb
*
*
C17-054 C. hermaphroditica subsp. hermaphroditica fi
C12-090 C. hermaphroditica subsp. hermaphroditica cz
C17-051 C. hermaphroditica subsp. macrocarpa se
Sect. Pseudocallitriche
LC177722 C. japonica jp
LC177729 C. muelleri nz
LC177730 C. muelleri au
C15-093 C. muelleri au
*
*
Hippuris vulgaris 1 cz
AY492098 Hippuris vulgaris
0.1


C
trnT-trnL
+ ITS
(concatenated)
C14-138 C. hamulata de
C18-011 C. brutia it Sicily
C18-083 C. brutia hu
C17-005 C. brutia gr Corfu
C17-013 C. brutia gr
C18-079 C. hamulata de
C18-046 C. hamulata fr
C16-028 C. brutia es
C18-024 C. brutia it Sardinia
C16-097 C. naftolskyi it Sardinia
C15-089b C. naftolskyi sp
C18-022 C. naftolskyi it Sardinia
C. brutia complex
70 –
* *
97 *
61 0.95
C18-006 C. platycarpa it Sicily
C15-087 C. cophocarpa de
C13-016 C. cophocarpa sk
C17-052 C. cophocarpa fi
C12-041 C. cophocarpa × C. platycarpa [C. ×vigens] dk
C17-050 C. platycarpa se
C15-017 C. platycarpa at
C18-045 C. platycarpa fr
C18-082 C. platycarpa × C. cophocarpa [C. ×vigens] at
C18-002 C. cribrosa it
99 *
63 0.96
83 *
C16-016 C. regis-jubae es
C18-018 C. regis-jubae it Sardinia
C16-017 C. stagnalis es
C13-125 C. stagnalis autotriploid cz
C13-053 C. stagnalis us
C15-094 C. stagnalis au
C18-038 C. stagnalis fr
C16-021 C. stagnalis pt
C13-151 C. stagnalis us
C13-087 C. stagnalis cz
C13-072 C. stagnalis no
C18-032 C. stagnalis it
C18-020 C. cf. stagnalis it Sardinia
C17-027 C. stagnalis gr
C17-016 C. stagnalis gr
C. stagnalis group
75 0.98
99 *
67 0.9 8
69 0.91
C18-077 C. obtusangula de
C15-086 C. obtusangula sk
C18-015 C. obtusangula it Sardinia
C18-025 C. obtusangula it Sardinia
C17-018 C. lenisulca gr
C13-006 C. lenisulca it
C16-096 C. lenisulca it Sardinia
C17-026 C. lenisulca gr
91 0.99
* *
* *
97 *
97 *
C14-006 C. cf. heterophylla us
C14-007 C. cf. heterophylla us
C14-144 C. heterophylla var. cf. bolanderi us
C14-005 C. heterophylla var. heterophylla us
C14-002 C. palustris us
C14-143 C. palustris us
C12-019 C. palustris cz
C13-150 C. palustris se
C15-057 C. palustris ro
C. palustris group
* *
76 *
90 *
* *
94 *
64 0.99
Sect. Callitriche
C18-023 C. lusitanica it Sardinia
C17-015 C. lusitanica gr
C16-014 C. lusitanica es
C15-001 C. pulchra gr Gavdos
C18-021 C. truncata subsp. truncata it Sardinia
C17-025 C. truncata gr
C18-039 C. truncata subsp. occidentalis fr
C12-090 C. hermaphroditica subsp. hermaphroditica cz
C17-054 C. hermaphroditica subsp. hermaphroditica fi
C17-051 C. hermaphroditica subsp. macrocarpa se
75 0.9 8
* *
84 *
99 *
96 *
73 0.96
* *
* *
Sect. Pseudocallitriche
* *
C15-093 C. muelleri au
Hippuris vulgaris 1 cz
0.1

◀ **FIG. 4.** Maximum likelihood (ML) trees of *Callitriche* species based on *trn*T–*trn*L **(A)**, ITS **(B)** and on the concatenated dataset of ITS and *trn*T–*trn*L sequences **(C)**. Bootstrap support values are indicated above branches, posterior probabilities from Bayesian analysis are given below branches (in italics). Only bootstrap support values >60% and posterior probabilities >0.85 are shown, confidence values of 100% / 1.00 are indicated by asterisks (*). For simplification, *C. brutia* var. *brutia* and *C. b.* var. *naftolskyi* are listed as *C. brutia* and *C. naftolskyi*. In the ITS dataset, samples from this study are in bold, those from Ito & al. (2017) are in normal font. The sample LC177716-1 from Australia was originally listed as *C. brutia* var. *hamulata* by Ito & al., but according to Bean (2007), this taxon does not occur in that country; therefore it is labelled here as *C.* cf. *hamulata*. The sample LC177744 was originally listed as *C. turfosa*, but classified here as *C. terrestris* subsp. *turfosa*, following the recent treatment in Flora Argentina (Hassemer & O'Leary 2018). For *C. cophocarpa* × *C. stagnalis* and *C. brutia* var. *brutia* × *C. brutia* var. *naftolskyi*, the ITS tree (B) includes cloned sequences of the respective parents.

Intraspecific variation

The majority of species show very little or no intraspecific genetic variation. On the other hand, molecular analyses confirmed differencies between some previously known intraspecific taxa. In the complex of *C. brutia*, *C. brutia* var. *naftolskyi* is clearly distinguished from *C. brutia* / *C. hamulata* (Fig. 4A,B,C). Genetic differences, although slight, were revealed also between two recognized subspecies of *C. hermaphroditica*.

In *C. truncata*, three distinct genotypes were distinguished in all datasets (Figs 4A,B,C), corresponding to the three groups revealed via flow cytometry (see above). Two of them from Sardinia and Greece form well-supported branches in both trees while the branch including *C. truncata* subsp. *occidentalis* is sister to *C. hermaphroditica*, albeit with low support. Additional ITS sequences from Ito & al. (2017) group with *C. truncata* subsp. *occidentalis* with high support (Fig. 4B). The North American species *C. heterophylla* is another taxon in which surprisingly high genetic variation was revealed, forming two well-supported clusters in the *trn*T-*trn*L tree as well as in the concatenated tree (Fig. 4A,C). The topology of the ITS tree even suggests that this species is paraphyletic (Fig. 4B).

Hybridization

While most ITS sequences show occasional polymorphic sites (small additional peaks) that appear to be singlets or are without any particular pattern, sequences of several samples show nucleotide polymorphisms that are additive for particular species pairs indicating hybridization (Suppl. Appendix S1). Several species possess no polymorphisms (e.g. the diploid species *C. stagnalis*, *C. hermaphroditica*, *C. lusitanica*) or only sporadically (e.g. diploids *C. cophocarpa*, *C. lenisulca*, tetraploid *C. platycarpa*) whereas other species show numerous polymorphic sites in most sequences (diploid *C. obtusangula*, hexaploid *C. brutia*, octoploid *C. hamulata*). Three out of four putative hybrids (*C. cophocarpa* × *C. stagnalis*, *C. cophocarpa* × *C. hamulata*, *C. brutia* var. *brutia* × *C. brutia* var. *naftolskyi*) show clearly additive patterns (Fig. 5A,B). The remaining hybrid *C.* ×*vigens* shared an identical ITS sequence with both putative parents, *C. cophocarpa* and *C. platycarpa*, without any visible polymorphisms (Fig. 4B, 5A). Regarding plastid sequences, the hybrids *C. cophocarpa* × *C. stagnalis* and *C. cophocarpa* × *C. hamulata* show the haplotype of *C. cophocarpa* indicating that this species is the maternal parent (Fig. 4A). From 12 samples of *C.* ×*vigens*, nine possess a haplotype identical with Central and Western European samples of *C. platycarpa* whereas three samples have the same haplotype as *C. cophocarpa* (see Suppl. Appendix S2); thus, this hybrid apparently is a result of reciprocal crosses. Similarly, one sample of *C. brutia* var. *brutia* × *C. brutia* var. *naftolskyi* (C16-009) shares the haplotype of *C. brutia* var. *brutia* whereas the second (C16-013) shows the same haplotype as *C. brutia* var. *naftolskyi* (Fig. 4A).

Cloning of the hybrid *C. cophocarpa* × *C. stagnalis* retrieved ribotypes corresponding to each putative parent, whereas six ribotypes were revealed within *C. brutia* var. *brutia* × *C. brutia* var. *naftolskyi*, three clustering with var. *naftolskyi* in ITS trees and three with the rest of the clade including *C. brutia* var. *brutia* and *C. hamulata* (Fig. 4B).

Closer inspection of the ITS electropherograms showed some very small additional peaks in sequences of *C. hamulata* that suggested a contribution from *C. cophocarpa*/*C. platycarpa* according to some readable diagnostic single nucleotide polymorphisms (SNPs) and one diagnostic 1 bp-indel leading to a frameshift. These small peaks were readable only in some samples of *C. hamulata* while lacking in *C. brutia*. In most samples of *C. hamulata*, only a part of the expected polymorphic sites was visible, but all predicted hybrid sites were present in at least some samples (Suppl. Appendix S1). Additionally, three variable sites were revealed, shared by both *C. brutia* var. *brutia* and *C. hamulata*, in which most samples were hybridogenous. This pattern leads to a complex reticulate structure between *C. cophocarpa* and taxa of the *C. brutia* complex in the NeighborNet diagrams (Fig. 5A,B). The somewhat intermediate positions of the octoploid species *C. hamulata* along with heavily skewed ratios of peaks at polymorphic sites did not recommend a cloning approach; therefore the *C. brutia* complex was additionally subjected to discriminating restriction digests.

RFLP analysis shows that the putative hybrid *C. cophocarpa* × *C. hamulata* exhibits a clearly additive pattern, combining bands from *C. hamulata* and *C. cophocarpa/platycarpa* (Fig. 6). One sample of *C. platycarpa* from Sicily (C18-006) shows a partial loss of the single restriction site, which is also detectable in all accessions of *C. hamulata* and their hybrid. The contribution of *C. cophocarpa/platycarpa* to the hybrid is more pronounced than that of *C. hamulata*. All samples of *C. hamulata* show a complex pattern suggesting the same origin of all samples with the strongest contribution from *C. brutia* var. *brutia*, but also additivity of bands with *C. brutia* var. *naftolskyi* and *C. cophocarpa/platycarpa* including a partially undigested band as in *C. platycarpa* (C18-006). This octoploid therefore shows an allopolyploid origin with detectable traces of three different taxa. However, *C. brutia* var. *brutia* shares all three bands characteristic for *C. brutia* var. *naftolskyi*, although two of them are weak and not clearly visible. Therefore the involvement of *C. brutia* var. *naftolskyi* in the emergence of *C. hamulata* is not clear.

DISCUSSION

Divergence among and within Callitriche taxa

Despite the general morphological similarity of water-starworts, most European *Callitriche* species are well-defined by the combination of genome size, ITS and *trn*T-*trn*L markers. The only species that are difficult or even impossible to distinguish on the basis of direct sequences are the couples *C. cophocarpa* – *C. platycarpa* and *C. brutia* var. *brutia* – *C. hamulata* (see below). Our results also indicate that the western Mediterranean species *C. regis-jubae* is closely related to broadly distributed *C. stagnalis*. Both species are indistinguishable by genome size, they are clearly separated only with ITS but not with *trn*T-*trn*L (Figs 4A,B,C, 5A,B) and the genetic distance between the sister taxa is small. Based on these findings, *C. regis-jubae* is probably a recently diverged taxon, and one possible solution would be to reclassify it as a subspecies of *C. stagnalis*. At the current state of knowledge,

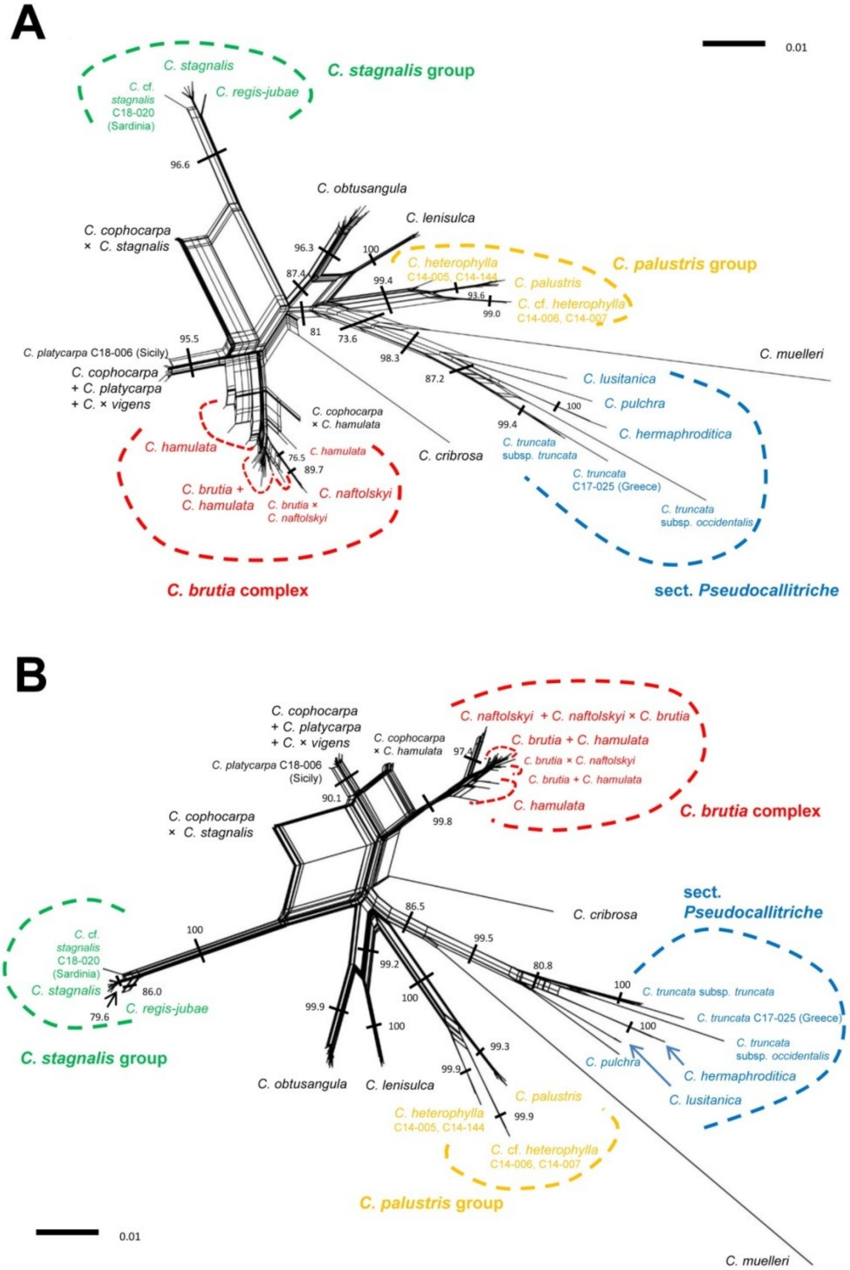


FIG. 5. NeighborNet analysis of *Callitriche* samples based on ITS sequences **(A)** and on the concatenated dataset of ITS and *trn*T–*trn*L **(B)**. Bootstrap support for clusters is indicated next to the respective cluster delimitation, only values >70% are shown for main clusters.

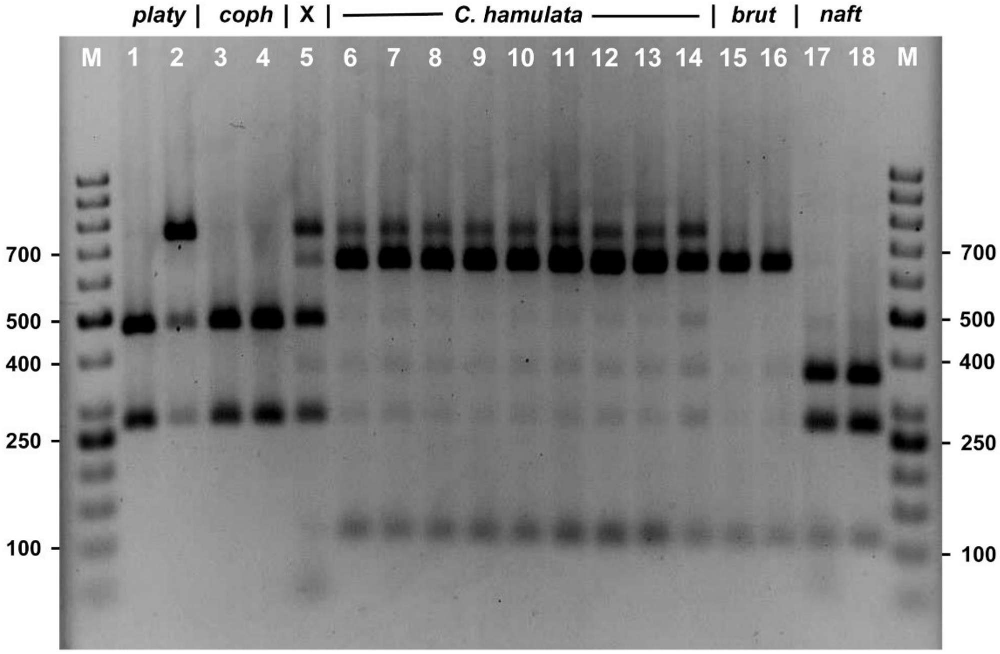


FIG. 6. RFLP analysis of 18 *Callitriche* samples. M = molecular size standard, *platy* = *C. platycarpa*, *coph* = *C. cophocarpa*, X = putative hybrid *C. cophocarpa* × *C. hamulata*, *brut* = *C. brutia* var. *brutia*, *naft* = *C. brutia* var. *naftolskyi*. Samples: 1 – C14-139 (Germany), 2 – C18-006 (Sicily), 3 – C17-052 (Finland), 4 – C12-063 (Czech Republic), 5 – C12-061-20 (Czech Republic), 6 – C12-062 (Czech Republic), 7 – C13-077 (Czech Republic), 8 – C13-132b (Germany), 9 – C14-138 (Germany), 10 – C14-077 (Austria), 11 – C18-046 (France), 12 – C12-045 (Denmark), 13 – C16-079 (Iceland), 14 – C13-049 (USA), 15 – C15-091a (Spain), 16 – C17-022 (Greece), 17 – C15-089b (Spain), 18 – C18-022 (Sardinia).

we propose to keep *C. regis-jubae* at the species level, because it is morphologically well distinguishable from *C. stagnalis* (see Table 1). Pollination modes also seem to be different for both taxa: in *C. regis- jubae*, pollination is referred to be obligatory geitonogamous, taking place through the direct contact between stigmata and anthers ("contacter"), whereas in *C. stagnalis* the contact between male and female flowers does not occur ("non-contacter"; Schotsman, 1982). Both species occur sympatrically, therefore the switch of *C. regis-jubae* to an autogamous (in fact, strictly geitonogamous) strategy could be indicative of reproductive isolation and may be one of the main reasons of their divergence. Nevertheless, further research is necessary to accurately assess the overall variation in the entire *C. stagnalis* group (see also below).

Within *C. truncata*, we revealed a surprisingly large genetic variation. The Western European *C. truncata* subsp. *occidentalis* is so divergent (and even paraphyletic) in phylogenetic analyses based on plastid and nuclear markers as well as in genome size that it deserves to be classified at the species level (Fig. 4A,B,C). Two other samples of *C. truncata* from the study of Ito & al. (2017) also fall within into this well-supported clade with little or no variation between accessions. The type subspecies has been described from Calabria, Italy (Gussone, 1826), and it is reported also from the Middle and Eastern Mediterranean (Lansdown, 2008). In this study, we included two samples of *C. truncata* from Sardinia, genetically and cytometrically virtually identical (Suppl. Appendix S1, S2, Suppl. Table S1). One of them (C18-021) is fertile and shows typical characters of *C. truncata* subsp. *truncata*. Additionally, we collected a single sample in south-western Greece (C17-025), which is genetically and cytometrically clearly different from the Sardinian plants (Table 2, Figs 2, 4A,B,C). The Greek plants, unlike the typical *C. truncata* subsp. *truncata*, have fruits with very narrow wings, which are often not apparent on dried material. This population obviously represents a hitherto unknown cryptic taxon. It is clear that the entire *C. truncata* requires taxonomic revision and very probably also a reassessment of the nomenclature in connection with changes of taxonomic ranks. However, it would not be sensible to make any taxonomic changes until larger comparative material from a wider area can be investigated.

Two North American species included in our study (*C. palustris*, *C. heterophylla*) also show noticeable intraspecific variation. This is particularly evident in *C. heterophylla*, which clustered in two distinct groups in all datasets (Figs 4A,C, 5B) and is not monophyletic with ITS (Figs 4B, 5A). This species deserves a thorough taxonomic revision throughout its distribution area, since it probably contains several cryptic taxa.

In Europe, terrestrial plants commonly have diversity hotspots in the Mediterranean area and in high mountain ranges, especially the Alps (Myers & al., 2000; Väre & al., 2003). In contrast, there is typically no conspicuous variation among the numbers of aquatic plants reported from different parts of Europe (Chappuis & al., 2012). Despite this general view, our results suggest that the genetic diversity centre of *Callitriche* in Europe is situated in the Mediterranean area. Also, additional cryptic taxa may occur in the

Mediterranean: a single sample of *C. stagnalis* from Sardinia (C18-020) is genetically similarly distant from the rest of *C. stagnalis* as *C. regis-jubae* (Figs 4C, 5B). These plants were collected young and without ripe fruits, yet it is apparent that at least some fruits are pedunculate, unlike all other samples of *C. stagnalis*. However, it is not appropriate to draw any conclusions on the basis of a single sample. Our study shows a good agreement with that from Ito & al. (2017), because all species included in both studies clustered together (Fig. 4B) without any exceptions. The phylogenetic positions of other species from the study of Ito & al. (2017), which were not covered by our sampling, are difficult to assess. All ITS sequences from that study do not contain any additive polymorphisms, contrary to our data including numerous polymorphic sites. The phylogenetic positions of some species are rather surprising, namely the very close relationship between South African *C. compressa* and South American *C. lechleri*, as well as between Australian *C. umbonata* and the sample of *C. palustris* from Japan (Fig. 4B). Their relative genetic divergences, when compared with that of the remaining taxa, correspond rather to the subspecies than the species level.

In accordance with Ito & al. (2017), we propose to distinguish only one particular clade as sect. *Pseudocallitriche* and the main clade as a broadly defined sect. *Callitriche* (Figs 4A,B,C, 5A,B). On the other hand, we leave the basally branching clades, including *C. muelleri*, *C. japonica* and a branch containing *C.compressa*, *C. lechleri*, *C. fehmedianii*, *C. petriei* and *C. sonderi*, without a formal assignment to taxonomic units. More species (especially from America, Africa and Asia) will need to be included to better resolve the classification of ancestral *Callitriche* species.

Polyploid origin of Callitriche species

Four polyploid species are recognized in Europe (Tables 1, 2). From these, the evolutionary origin has been studied only in tetraploid (2n=20) *C. platycarpa*. According to Philbrick & Les (2000), *C. platycarpa* shares an identical *rbc*L haplotype with *C. stagnalis*, contrary to the results of Ito & al. (2017) who suggested that *C. cophocarpa* is the maternal parent of *C. platycarpa*. Bączkiewicz & al.'s (2007) isozyme study on plant materials from north-western Poland and Schwarzacher & al.'s (2017) genomic in situ hybridization (GISH) on a plant material from England consistently concluded that *C. platycarpa* is an allotetraploid formed by the diploid parental species

C. cophocarpa and *C. stagnalis*. According to the Polish study, *C. stagnalis* is a maternal parent of *C. platycarpa*. Contrary to that study, we revealed that the plastid haplotype of all included samples of *C. platycarpa* is very similar (although not entirely identical) to that of *C. cophocarpa* (Fig. 4A). The ITS sequences of *C. platycarpa* are identical with those of *C. cophocarpa*, without any visible polymorphisms (Fig. 4B, 5A, Suppl. Appendix S1). The only exception is a single sample from Sicily (C18-006), showing three additional polymorphisms corresponding to SNPs characteristic for both *C. cophocarpa* and *C. stagnalis*, but no visible polymorphisms on additional ca 37 positions distinguishing these two species from each other. Two possible evolutionary scenarios can be inferred: a) all samples of *C. platycarpa* included in our study are autotetraploids derived from *C. cophocarpa* and the discrepancy to previous studies may be due to different material or different methods of inference; b) at least some (if not all) samples are allotetraploids, but the contribution of *C. stagnalis* is not visible in electropherograms due to the process of concerted evolution in the ITS sequences (Arnheim, 1983; Elder & Turner, 1995). The latter scenario is also supported by flow cytometric results, because the monoploid genome size (1Cx-value) of *C. platycarpa* is exactly intermediate between the values determined for *C. cophocarpa* and *C. stagnalis* (Table 2). However, we cannot rule out that some lineages of *C. platycarpa* can have different origins or arose recurrently from independent hybridization events, as is documented in many polyploid plant species (e.g., Soltis & Soltis, 1999). This may explain why plastid DNA of all 13 accessions of *C. platycarpa* included in our study corresponds to that of *C. cophocarpa* but none to *C. stagnalis*, in contrast to the findings of Philbrick & Les (2000) and Bączkiewicz & al. (2007). In the latter study, non-fertile plant material of three species (*C. cophocarpa*, *C. platycarpa* and *C. stagnalis*) was identified using chromosome counting (however, both *C. cophocarpa* and *C. stagnalis* have 2n=10) and sequencing of the *rbc*L plastid gene; the Polish sequences were subsequently compared with *rbc*L data published by the former study and the corresponding samples identified to fit the sequences. Therefore, it is worth noting that the correctness of the results of Bączkiewicz & al. (2007) is entirely dependent on the species identifications made by Philbrick & Les (2000).

The complex of *C. brutia* is taxonomically the most challenging polyploid complex among European *Callitriche*. Here we found that the hitherto poorly known Mediterranean taxon *C. brutia* var. *naftolskyi* is hexaploid (2n=28) like *C. brutia* var. *brutia* (Fig. 3), and that it significantly differs from both *C. brutia* var. *brutia* and *C. hamulata* in genome size as well as in ITS and plastid DNA molecular analyses (Table 2, Fig. 4A,B,C). Recently, it was published that *C. b.* var. *brutia* and *C. b.* var. *naftolskyi* possess the same genome size (Prančl in Lansdown & al., 2017), but this information was reported by mistake, caused by confusion of seeds of both taxa, from which the genome size was established. The extent of the genetic divergence between *C. b.* var. *naftolskyi* and the rest of the group suggests it would be more appropriate to classify this taxon at a higher taxonomic rank. It is also worth mentioning that two hybrid samples between *C. b.* var. *naftolskyi* and *C. b.* var. *brutia* revealed by this study, show significantly reduced fertility (see below). However, both taxa are morphologically very similar. Although *C. b.* var. *naftolskyi* differs from *C. b.* var. *brutia* in a number of features (Table 1), these are rather insignificant compared to the characters separating most species within the genus (Lansdown & al., 2017). For the above-mentioned reasons, we recommend to classify both taxa at the subspecies level and designate the name *C. brutia* subsp. *naftolskyi* here as a new combination (see below). The remaining taxon of the aggregate, *C. hamulata*, differs from *C. brutia* by its octoploid chromosome number (2n=38). We revealed that *C. brutia* subsp. *brutia* and *C. hamulata* share an identical plastid haplotype (Fig. 4A) and are also indistinguishable on the basis of ITS ribotypes (Fig. 4B). However, ITS sequences of some samples of *C. hamulata* show the weak admixture of another ribotype from *C. cophocarpa*/*C. platycarpa* (Fig. 5A,B). RFLP results indicate that the restriction pattern of *C. hamulata* can be that of a triple hybrid, showing bands of *C. brutia* subsp. *brutia*, *C. brutia* subsp. *naftolskyi* and *C. platycarpa* in all samples even though this contribution was hardly or not at all detectable in ITS sequences (Fig. 6). Because all accessions of *C. hamulata* share an identical plastid DNA and also show very low variation in ITS and no variation in RFLP, it is likely that this species arose from a single polyploidization event. However, the exact evolutionary origin of particular taxa within *C. brutia* complex remains a question for further research. With certainty, *C. brutia* subsp. *brutia* (2n=28) is the maternal parent of *C. hamulata*. Both *C. cophocarpa* and *C. platycarpa* (2n=10 or 20, respectively) can represent the second parental species, as the ITS sequences of both species are identical (Fig. 4B). On the other side, included samples of *C. cophocarpa* do not show a partial loss of the restriction site, which is visible in RFLP pattern of *C. hamulata* and a single sample of *C. platycarpa* (C18-006; Fig. 6). The contribution of *C. brutia* subsp. *naftolskyi* (2n=28), although suggested by the results of RFLP analysis, is not unequivocal. We should not forget that *C. brutia* is also a putative allopolyploid. The presence of weak bands corresponding to *C. brutia* subsp. *naftolskyi* in the banding patterns of *C. b.* subsp. *brutia* and *C. hamulata* indicate that *C. b.* subsp. *brutia* may contain a genetic contribution of *C. b.* subsp. *naftolskyi*. The partly missing / erased polymorphisms seen in ITS direct sequences of *C. hamulata* and the relatively weak bands corresponding to *C. platycarpa* and *C. brutia* subsp. *naftolskyi* in RFLP analysis suggest that concerted evolution is indeed ongoing in *Callitriche* allopolyploids. In this case the homogenization went into the direction of *C. brutia* subsp. *brutia*. It should be further noted that while the pollen grains of *C. brutia* completely lack the exine (an adaptation for hypohydrogamy, see above), the exine is developed in *C. hamulata*, albeit strongly reduced (Cooper & al., 2000). This pattern also suggests that *C. hamulata* could be a hybridogenous species between *C. brutia* and some other species with normally developed exine (e.g. *C. platycarpa* and *C. cophocarpa*).

Lansdown (2006a) concluded on the basis of a detailed morphological study, that *C. brutia* (subsp. *brutia*) and *C. hamulata* are reliably distinguishable in the field only in the terrestrial state. Under such environmental conditions, *C. brutia* produces long pedunculated fruits whereas the fruits of *C. hamulata* remain subsessile; when growing in water, both taxa are virtually indistinguishable. On the basis of their strong morphological similarity, he re-evaluated *C. hamulata* as a variety of *C. brutia*. In accordance with Lansdown, we did not observe any other reliable characters for distinguishing both taxa. However, we assume that the rank of a variety is not appropriate for distinct allopolyploid taxa with different chromosome numbers. Allopolyploids with different evolutionary origins are usually classified at the species level, even if they share one or more parental species (e.g., Soltis & al., 2004; Kelly & al., 2012; Zou & al., 2015; Barker & al. 2016); in some cases, even the products of independent hybridization with an identical parental combination are being evaluated as separate species (mostly in apomictic genera, but also in allogamous species, e.g. Efimov & al., 2016). We also point out that *C. hamulata* and *C. brutia* subsp. *brutia*, if growing terrestrially, are easily recognizable. Both taxa occur sympatrically in western Europe (Schotsman 1967, Lansdown 2006a), but *C. hamulata* appears to be very rare or absent from the Mediterranean area whereas *C. brutia* subsp. *brutia* is almost completely absent from Central Europe (Kaplan & al., 2018a). With the current state of knowledge, we prefer the classification of *C. hamulata* as a separate species. However, a further in-depth study of the evolutionary relationships within the *C. brutia* complex may consider whether the species or the subspecies rank would be more appropriate.

Inter- and intraspecific hybridization

Interspecific hybridization has so far been considered a rare phenomenon in *Callitriche*. This is mainly explained by the extraordinary differentiation of pollination systems across the genus, including various modes of (obligatory) geitonogamous pollination (Schotsman, 1982; Philbrick & Anderson, 1992; Martinsson, 1996). However, the only recognized and described hybrid, triploid *C.* ×*vigens* (*C. cophocarpa* × *C. platycarpa*), has been reported as relatively abundant in several areas of Europe (Martinsson, 1991; Kaplan & al., 2018a). Triploid plants are easily detectable using genome size (Table 2, Fig. 2), but their identification based on molecular sequences can be more tricky. Both putative parental species share an identical ITS ribotype (corresponding to *C. cophocarpa*), but differ slightly in *trn*T-*trn*L sequences (Figs 4A,B). Most of the triploid samples included in our study have a haplotype identical to tetraploid *C. platycarpa*, which suggests these plants really belong to *C.* ×*vigens*. Three triploid samples (C11-016, C12-041, C13-108) share a haplotype identical to *C. cophocarpa* (Suppl. Appendix S2). They probably represent the same hybrid combination, but we cannot exclude that at least some of these samples may actually be autotriploids of *C. cophocarpa*. Another triploid with different origin was recently found at a single locality in the Czech Republic (Prančl & al., 2014). In molecular analyses, this plant (C13-125) shows a sequence pattern identical to *C. stagnalis* in both *trn*T–*trn*L and ITS trees (Fig. 4A,B,C), which confirmed the original assumption that it is an autotriploid of *C. stagnalis*.

Three previously unknown hybrids were revealed based on additive patterns of ITS ribotypes (Fig. 5). Two of these hybrids, *C. cophocarpa* × *C. stagnalis* and *C. brutia* subsp. *brutia* × *C. brutia* subsp. *naftolskyi*, are newly described here as *C.* ×*nyrensis* and *C. brutia* nothosubsp. *neglecta* (see below; Fig. 7). The remaining hybrid (2n=29), discovered in the Tichá Orlice river, Czech Republic, has been attributed to *C. hamulata* × *C. cophocarpa*, but

C. platycarpa could not be excluded as a putative parental species (Prančl & al., 2014). Our study confirmed that ITS sequences of this hybrid represent a mixture of ribotypes of *C. hamulata*/*C. brutia* subsp. *brutia* and *C. cophocarpa*/*C. platycarpa*, and the haplotype of the hybrid is identical to *C. cophocarpa*, but differing from *C. platycarpa* in only a single nucleotide. Only *C. hamulata* and *C. cophocarpa* were found growing together with the hybrid in the river, therefore these species are indeed the most probable parents. Nevertheless, we consider it more appropriate to postpone the description of this hybrid until the identity of the parents can be confirmed unequivocally.

Besides interspecific hybrids, also ‘pure’ species often possess additive polymorphisms in ITS sequences, indicating intraspecific hybridization among particular, slightly different ribotypes. These polymorphisms were most often recorded in *C. brutia* subsp. *brutia*, *C. brutia* subsp. *naftolskyi*, *C. obtusangula* and *C. palustris*. It is interesting to compare the ITS variation within two widespread diploid species, *C. stagnalis* and *C. obtusangula*. Both species show significant intraspecific variation (Figs 4B, 5A), but while most samples of *C. obtusangula* contain multiple polymorphic sites, no polymorhisms were found in *C. stagnalis* (Suppl. Appendix S1). This may suggest that gene flow is efficiently ongoing among particular genotypes of *C. obtusangula*, whereas intraspecific recombination is rare or not occurring among individual variants of *C. stagnalis*.

New distribution information

Our study contributes to the better understanding of the distribution of some taxa in Europe. During our fieldwork, we found *C. obtusangula* for the first time in Slovakia (C15-086). The discovered locality in the Danubian Lowland is linked to the previously known occurrence in the Lower Austrian Danube basin (Englmaier, 1985). We confirmed *C. brutia* subsp. *brutia* for Hungary (C18-083), which is probably the first unequivocally confirmed occurrence in the Pannonian Basin. The other intraspecific taxon of *C. brutia*, subsp. *naftolskyi*, was for the first time found in Spain (C15-089b). We also discovered *C.* ×*vigens* for the first time for Austria (C18-082) and *C. lusitanica* for continental Greece (C17-015). *Callitriche platycarpa* is a species with a distinctive European sub-Atlantic distribution, but very rarely occurring in the Mediterranean (Lansdown 2006a, 2008; Lansdown & Strid, 2011; Prančl & al., 2014). We confirmed this species for the first time in Sicily (C18-006).

Callitriche lenisulca has been referred to as a lowland species with a maximum elevation of 170 m and with all confirmed records from within 50 km of the sea (Lansdown, 2008). We found this species growing in Greece up to 78 km from the sea coast (C17-018) and at elevations of up to 650 m (C17-019). Finally, we managed to find the first recent occurrence of *C. cribrosa* for Italy (C18-002), where it has been probably last recorded in 1907 (Schotsman 1977), and of *C. regis-jubae* for Sardinia (C18-018), where it has been recorded only once in 1972 (Schotsman 1973).

TAXONOMIC TREATMENT

Callitriche brutia **subsp.** ***naftolskyi*** (Warburg & Eig) Prančl, **stat. nov.** ≡ *Callitriche naftolskyi* Warburg & Eig in Repert. Spec. Nov. Regni Veg. 26: 84. 1929 ≡ *Callitriche brutia* var. *naftolskyi* (Warburg & Eig) Lansdown in Phytotaxa 313: 92. 2017 – Lectotype (designated by Lansdown & al., 2017: 92): Israel, Sharon Plain, north-east of Tel Aviv, 23 Apr 1927, *Naftolsky* 01853 (HUJ).

NOTE. – Morphological description and other details were provided by Lansdown & al. (2017).

Descriptions of new Callitriche hybrids

Callitriche **×*nyrensis*** Prančl, **nothosp. nov.** [*C. cophocarpa* Sendtn. × *C. stagnalis* Scop.] – Holotype: CZECH REPUBLIC; distr. Klatovy; Hamry: Úhlavský luh Nature Reserve, marsh with small pools on left bank of Úhlava river above bridge near settlement Hamerský Dvůr, 920 m N–NNW of church, alt. 529 m, 49°14'15.1"N, 13°09'27.0"E (WGS 84), 26 June 2016, *J. Prančl* C16-051 (holotype: PRC barcode PRC 455760; isotypes: PRC barcode PRC 455761, PR barcode PR 964819, PRA barcode PRA 16236).

Perennial amphibious herbs, producing floating rosettes when reaching the water surface, or semi-terrestrial. Stem much-branched, supported by water or prostrate and creeping when terrestrial, with scales of (6–)7–9 cells. Leaves narrowly oblanceolate to broadly spathulate, less often almost linear, 1–5-veined, up to 25 mm long, 1.1–5.2 mm wide, 2.5–11× longer than wide, narrower leaves shallowly notched at the apex, broader leaves obtuse. Bracts falcate, translucent, appearing whitish, 0.6–1.4 mm long, persistent. Flowers solitary in leaf axils, generally a pair of male flowers or a pair of female flowers in a pair of axils, often flowers of one sex are placed on separate stems or on different parts of the same stem. Styles usually erect, up to 5.6 mm long. Stamens with filaments strongly reduced before dehiscence, appearing sessile, usually completely covered by bracts, sometimes lengthening after anthesis, up to 4.2 mm long, anthers 0.3–0.6 mm wide; pollen bright yellow to sulphur-yellow, generally aborted, of irregular shape. Fruits not developed (plants sterile). Chromosome number probably diploid, 2n=10 (DNA ploidy level = 2x).

ETYMOLOGY. – The epithet *nyrensis* is derived from Nyra, the old name considered a Latin variant of Nýrsko, the town near which the hybrid was found.

KEY CHARACTERS. – The hybrid is intermediate between the parents, forming relatively broad leaves like *C. stagnalis*, but it is also capable of creating forms with narrow lingulate leaves like *C. cophocarpa*. The flower pattern of the hybrid resembles *C. cophocarpa*, generally having flowers of one sex placed on separate stems or on different parts of the same stem, but this pattern is not as regular as in *C. cophocarpa*. Also *C. platycarpa* is very similar, however, this species does not occur in this part of the Czech Republic (Kaplan & al., 2018a). The hybrid can be separated from all three species by malformed pollen and the peculiar appearance of undehisced stamens, which are mostly reduced to a small anther situated directly in the leaf axil, almost completely lacking filament (Fig. 7A). The hybrid also does not set fruits though it flowers abundantly. Nevertheless, the other hybrid *C.* ×*vigens* (*C. cophocarpa* × *C. platycarpa*) possesses virtually the same floral characteristics like *C.* ×*nyrensis* (cf. Martinsson, 1991; Lansdown, 2008) and can only be reliably distinguished from it by the triploid (2n=15) chromosome number. If *C. platycarpa* is allotetraploid with diploid parental species *C. cophocarpa* and *C. stagnalis* (see above), *C.* ×*vigens* would have two chromosome sets corresponding to *C. cophocarpa* and one set of chromosomes corresponding to *C. stagnalis*. Therefore, the genetic composition of both hybrids may be similar.

DISTRIBUTION. – *Callitriche* ×*nyrensis* is only known from a single locality in the Czech Republic. At this site it occurs together with *C. stagnalis* (C15-084-01), which is, however, much rarer there. The second parent, *C. cophocarpa*, was not found at the locality. *Callitriche* ×*nyrensis* is probably a rare hybrid. Both parental species have partly different ecological demands: whereas *C. cophocarpa* prefers permanent waters, *C. stagnalis* is typical for temporary habitats with shallow water (Kaplan & al., 2018a). In our previous cytometric paper (Prančl & al., 2014) we analyzed 150 populations of *C. cophocarpa* and 104 populations of *C. stagnalis* from Central Europe, but only eight of these populations hosted both species. Both species also frequently remained unflowering, especially in deeper or running water or in shaded habitats.

ADDITIONAL SPECIMENS EXAMINED (PARATYPES). – CZECH REPUBLIC; distr. Klatovy; Hamry: Úhlavský luh Nature Reserve, marsh with small pools on the left bank of Úhlava river above the bridge near the settlement Hamerský Dvůr, 920 m N–NNW of the church, alt. 529 m, 49°14'15.1"N, 13°09'27.0"E (WGS 84), 12 Sept 2015, *J. Prančl* C15-068 (PRC barcode PRC 455762), 31 Oct 2015, *J. Prančl* C15-084 (PRC barcodes PRC 455763–455766, PR barcodes PR 964820–964824, PRA barcodes PRA 16237–16241). All paratypes were sampled non-flowering.

Callitriche brutia nothosubsp. ***neglecta*** Prančl, **nothosubsp. nov. [***C. brutia* Petagna subsp. *brutia* × *C. brutia* subsp. *naftolskyi* (Warburg & Eig) Prančl] – Holotype: SPAIN; comm. Extremadura; prov. Cáceres; Jaraicejo: Almonte river below bridge of N-V road (Carretera de Extremadura), 1.7 km SSW of village, alt. 349 m, 39°38'46.7"N, 05°49'04.6"W (WGS 84), 3 May 2016, *J. Prančl, Z. Kaplan & P. Koutecký C16-013* (PRC barcode PRC 455758).

Amphibious herbs, producing floating rosettes when reaching the water surface, or semi-terrestrial. Stem much-branched, with scales of 8–16 cells, often irregular in outline. Leaves narrowly linear to broadly spathulate, 1–3-veined, often with sinuous venation, up to 10 mm long, 0.3–2.6 mm wide, 1.5–25× longer than wide, broader leaves usually very shallowly notched at the apex. Bracts apparently absent. Flowers solitary in leaf axils, generally a male flower opposed by a female. Styles up to 0.5 mm long, initially ± erect but soon becoming strongly reflexed, most styles very short. Stamens with filaments up to 0.4 mm long, anthers ca 0.3 mm wide, appearing whitish. Peduncles 0–16(–30) mm long; fruits mostly undeveloped or underdeveloped, most often pedunculate, less often subsessile, well-developed fruits rare, 0.8–1.1 mm long × 0.8–1.1 mm wide, dark brown when mature, narrowly winged throughout, wing 0.02–0.07 mm wide, rests of styles not visible or apressed to side of fruit. Chromosome number probably 2n=28 (based on flow cytometric genome size analyses).

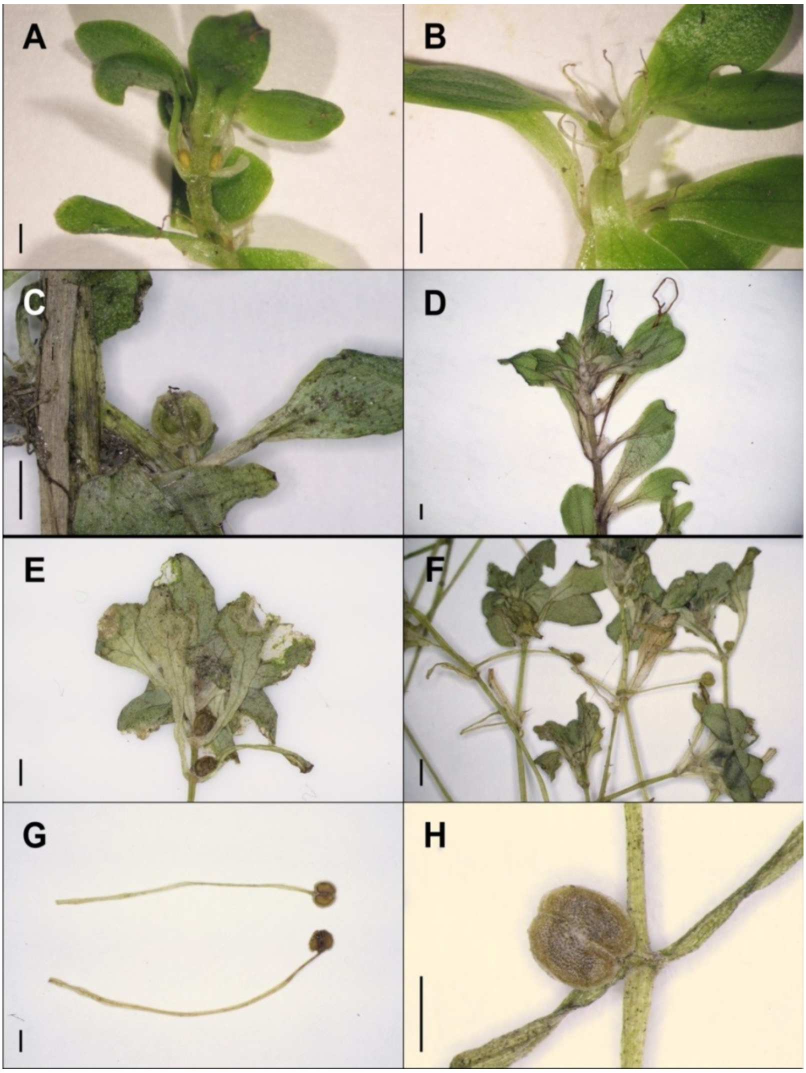


FIG. 7. Diagnostic features of two newly described hybrids. ***Callitriche* ×*nyrensis*** (*C. cophocarpa* × *C. stagnalis*): **A** – leaf rosette with two reduced stamens in a single node, almost completely lacking filaments, surrounded by translucent bracts; **B** – detail of female flowers, composed of two styles and 4-locular (but bicarpellate) ovary; **C** – fruit in the initial stage of development (ripe fruits never develop in this hybrid); **D** – stem with female flowers and a single stamen (on the right in a leaf rosette). ***Callitriche brutia* nothosubsp. *neglecta*** (*C. brutia* subsp. *brutia* × *C. brutia* subsp. *naftolskyi*): **E** – leaf rosette composed of leaves with characteristic sinuous venation; **F** – stems with peduncles bearing under-developed fruits; **G** – typical appearance of under-developed pedunculate fruits; **H** – subsessile fruit lacking rests of styles Scale bar for all figures = 1 mm.

Etymology. – The epithet *neglecta* means "neglected", reflecting the fact that the true identity of this hybrid was not recognized in the field, but revealed on the basis of molecular analyses.

Key characters. – This hybrid differs from the parental subspecies having most fruits undeveloped or small, not filled by well-developed seeds (Fig. 7G). One of the parents, *C. brutia* subsp. *naftolskyi*, always has pedunculate fruits, whereas *C. brutia* subsp. *brutia* forms subsessile fruits when growing in water, but pedunculate fruits when terrestrialised (Lansdown & al., 2017). The hybrid has most often long pedunculate fruits, but also sessile fruits are present one the same individuals. The fertility of the hybrid is not known, but at least some mericarps (although rare) seem to appear normally with fully developed seeds. Thus, it cannot be ruled out that the hybrid could be capable of breeding F2 offsprings or even backcrossing with the parents.

Distribution. – *Callitriche brutia* nothosubsp. *neglecta* is known only from two localities in Spain, both hosting rich aquatic vegetation (*Ranunculus peltatus* s.l., *Callitriche lusitanica*, *C. stagnalis* and many other species). The question is how often this hybrid can arise, because all taxa of the *C. brutia* complex are believed to be strongly geitonogamous and the pollen transfer is usually mediated through the direct contact of anther and stigma in adjacent leaf axils ("contacter", Schotsman 1982). Both localities of the hybrid are situated in streams. While *C. brutia* subsp. *brutia* can grow in rivers and brooks (see the list of localities in Suppl. Table S1), *C. brutia* subsp. *naftolskyi* typically grows in vernal pools and has never been found in running water (Lansdown & al., 2017). On the other hand, rivers and streams often provide shelter for of years through vegetative propagation (e.g., King & al., 2001; Kaplan & Fehrer, 2009, 2011; Kaplan & al., 2018b; Prančl & al., 2018).

Additional specimen examined (paratype). – Spain; comm. Extremadura; prov. Badajoz; Herrera del Duque: Arroyo Pelochejo stream (tributary of Guadiana river) 650 m NNE of town, alt. 420 m, 39°10'40.2"N, 05°02'45.9"W (WGS 84), 2 May 2016, *J. Prančl, Z. Kaplan & P. Koutecký C16-009* (PRC barcode PRC 455759).

AUTHOR CONTRIBUTIONS

JP, ZK and JF made the design of the research; JP and ZK collected the samples; JP made cytometric analyses; VB, PC and JP produced molecular data and alignments; ML performed chromosome counting; JF and JP analyzed the molecular data; JP & ML prepared figures; JP wrote the manuscript, JF and ZK helped with preparing the manuscript. — JP, https://orcid.org/0000-0003-4308-0824; JF, https://orcid.org/0000-0002-0337-5444; ML, https://orcid.org/0000-0002-4612-3693; ZK, https://orcid.org/0000-0003-1707-7461.

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THE ELECTRONIC SUPPLEMENT, TABLE S1 (PART 1). Locality details for 344 *Callitriche* individuals from 180 localities. ***Ref. no.:*** reference number unique for each combination of Taxon and Locality (i.e., if there are more taxa at one site, they have different reference numbers). ***Loc. no.:*** identification number of the locality (the same for all taxa at the site). ***N:*** numbers of individuals analysed using flow cytometry. ***2C-value (pg):*** Mean 2C value calculated for the sample(s) + standard deviation (provided only for the repeatedly analyzed samples); the values written in bold are published for the first time here, the values in normal font were taken from Prančl & al., 2014. ***Collected:*** Date of collection and collector name(s); JP = Jan Prančl, ZK = Zdeněk Kaplan, ML = Magdalena Lučanová. ***GenBank accession numbers:*** The accession numbers under which the sequences individual samples were submitted to GenBank, given separately for ITS and trnT-trnL regions.

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. brutia subsp. *brutia*	C18-042	1	1	**5.96±0.06**	France; reg. Pays de la Loire; dep. Loire-Atlantique; La-Chapelle-des-Marais: Grande Brière area, big pool 1.6 km W of church	47°26'53.6"N, 02°15'44.4"W	1	2018-05-09 JP	MN091382	MN091980	
C. brutia subsp. *brutia*	C18-041	2	1	**5.92±0.11**	France; reg. Pays de la Loire; dep. Vendée; Saint-Urbain: channel (brackish water) between settlements La Ramée and Beauséjour, 1.2 km NNW of village	46°53'22.9"N, 02°1'40.4"W	0	2018-05-08 JP	MN091383	MN091981	
C. brutia subsp. *brutia*	C17-028	3	1	**5.82±0.01**	Greece; reg. Epirus; Igoumenitsa municipality; Morfi: Kalodiki lakes, smaller lake 1.2 km W of village	39°18'37.8"N, 20°27'56.3"E	120	2017-05-08 JP, ZK & P. Koutecký	MN091384	MN091982	
C. brutia subsp. *brutia*	C17-005	4	1	**5.84±0.03**	Greece; reg. Ionian Islands; Kerkyra (Corfu) island; Temploni: eastern part of lake Ghavrolimni, surrounding maquis and grassland, 2.2 km N of church	39°39'33.5"N, 19°48'0.2"E	79	2017-04-07 C. Gilli, M. Hofbauer, D. Reich & R. Sander	MN091385	MN091983	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. brutia subsp. *brutia*	C17-022	5	1	**5.81±0.08**	Greece; reg. Periféreia Dytikis Elládas (Western Greece); Missolonghi municipality; Katochi: drying marshes adjacent to a complex of overgrown reservoirs 1.5 km NNW of village	38°25'41.6"N, 21°14'35.8"E	5	2017-05-07 JP, ZK & P. Koutecký	MN091386	MN091984	
C. brutia subsp. *brutia*	C17-017	6	–	–	Greece; reg. Thessalía (Thessaly); Farkadona municipality; Oichalia: Neochoritis river under bridge 1.6 km SSE of church	39°35'29.0"N, 21°59'10.8"E	100	2017-05-02 JP, ZK & P. Koutecký	MN091387	MN091985	
C. brutia subsp. *brutia*	C17-013	7	1	**5.87±0.06**	Greece; reg. Thessalía (Thessaly); Kalambaka municipality; Flampouresi: small rivulet with puddles crushed by cattle, along road 640 m SE–SSE of village	39°47'47.2"N, 21°46'7.3"E	790	2017-05-01 JP, ZK & P. Koutecký	MN091388	MN091986	
C. brutia subsp. *brutia*	C17-012	8	1	**5.86±0.03**	Greece; reg. Thessalía (Thessaly); Kalambaka municipality; Koniskos: small pond in oak forest 1.2 km NE of village	39°47'49.1"N, 21°49'10.8"E	790	2017-05-01 JP, ZK & P. Koutecký	MN091389	MN091987	2n=28 (this study)
C. brutia subsp. *brutia*	C18-083	9	1	**5.86±0.05**	Hungary; Hajdú-Bihar County; Nádudvar: wet muddy depression disturbed by grazing cattle in puszta 9.3 km WNW of town	47°27'46.7"N, 21°01'47.5"E	83	2018-05-22 ZK & A. Mesterházy	MN091390	MN091988	
C. brutia subsp. *brutia*	C18-026	10	1	**5.85**	Italy; reg. Sardegna (Sardinia); prov. Nuoro; Bolotana: Riu Ilde stream at bridge on basalt plateau 4.5 km WNW of village	40°20'50.7"N, 08°54'23.6"E	1030	2018-04-24 ZK, J. Hanzlíčková & P. Koutecký	MN091391	MN091989	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. brutia subsp. *brutia*	C18-024	11	1	**5.91±0.01**	Italy; reg. Sardegna (Sardinia); prov. Oristano; Busachi: vernal pools in grasslands with scattered scrub on basalt plateau 4.2 km W–WNW of village	40°02'24.8"N, 08°50'47.8"E	218	2018-04-24 ZK, J. Hanzlíčková & P. Koutecký	MN091392	MN091990	
C. brutia subsp. *brutia*	C18-011	12	1	**5.93±0.03**	Italy; reg. Sicilia (Sicily); prov. Palermo; Godrano: area of Bosco della Ficuzza, pool in oak forest 3 km WSW of village	37°53'23.9"N, 13°23'40.9"E	740	2018-04-21 ZK, J. Hanzlíčková & P. Koutecký	MN091393	MN091991	
C. brutia subsp. *brutia*	C18-028	13	–	–	Italy; reg. Umbria; prov. Terni; Acquasparta: pool in sinkhole 3.7 km NE of village	42°42'39.2"N, 12°35'09.1"E	640	2018-04-25 ZK, J. Hanzlíčková & P. Koutecký	MN091394	MN091992	
C. brutia subsp. *brutia*	C16-098	14	1	**5.95±0.00**	Italy; reg. Sardegna (Sardinia); prov. Sassari; Villanova Monteleone: shallow hollow in rocky plateau on Monte Minerva hill, 3.7 km SSW of Monteleone Rocca Doria	40°26'28.1"N, 08°32'24.6"E	642	2016-04-24 R. Lansdown	MN091395	MN091993	cultivated from seeds by J. Prančl; 2n=28 (this study)
C. brutia subsp. *brutia*	C16-025	15	1	**5.82±0.03**	Spain; comm. Andalusía; prov. Jaén; Santa Elena: Río de la Campana stream above JA-7100 road, 2.7 km W–WSW of village	38°20'8.5"N, 03°34'24.6"W	646	2016-05-07 JP, ZK & P. Koutecký	MN091396	MN091994	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. brutia subsp. *brutia*	C16-026	16	1	**5.81**	Spain; comm. Andalusía; prov. Jaén; Venta de los Santos: big vernal pool Laguna de los Perales 2.3 km NE–ENE of village	38°22'40.5"N, 03°2'59.2"W	758	2016-05-07 JP, ZK & P. Koutecký	MN091397	MN091995	
C. brutia subsp. *brutia*	C16-018	17	1	**5.88±0.03**	Spain; comm. Castile and León; prov. Salamanca; Cereceda de la Sierra: stream (headwater of Yeltes river) at bridge 270 m N of church	40°34'7.8"N, 06°5'29.8"W	963	2016-05-04 JP, ZK & P. Koutecký	MN091398	MN091996	
C. brutia subsp. *brutia*	C16-003	18	1	**5.83±0.08**	Spain; comm. Castilla–La Mancha; prov. Toledo; La Iglesuela del Tiétar: Arroyo del Cuadro O de Valdelanguilla stream under bridge of CM-5006 road, 2.3 km N–NNE of church	40°15'12.8"N, 04°44'39.5"W	444	2016-05-01 JP, ZK & P. Koutecký	MN091399	MN091997	
C. brutia subsp. *brutia*	C16-024	20	1	**5.84±0.01**	Spain; comm. Extremadura; prov. Badajoz; La Roca de la Sierra: Lagunas de Mogue, vernal pool 6.7 km NW of village	39°8'48.3"N, 6°45'13.9"W	70	2016-05-04 JP, ZK & P. Koutecký	MN091400	MN091998	
C. brutia subsp. *brutia*	C16-005+006	21	2	**5.85±0.01**	Spain; comm. Extremadura; prov. Cáceres; Alía: Guadalupejo river and surrounding pools, 12.3 km SSE of village	39°20'30.5"N, 05°9'52.3"W	379	2016-05-02 JP, ZK & P. Koutecký	MN091401 (C16-005), MN091402 (C16-006)	MN091999 (C16-005), MN092000 (C16-006)	2 samples (005: pool, standing water; 006: river, running water)

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. brutia subsp. *brutia*	C15-091	22	1	**5.90**	Spain; comm. Extremadura; prov. Cáceres; Torrejón el Rubio: Monfragüe area, wet grassy path margin, ca 100 m NW of the castle ruin Monfragüe	39°49'43.5"N, 06°03'9.2"W	400	2015-05-06 P. Koutecký	MN091403	MN092001	
C. brutia subsp. *brutia*	C16-028	23	1	**5.81±0.06**	Spain; comm. Madrid; prov. Madrid; Zarzalejo: Laguna del Castrejón pool 920 m E of railway station	40°32'17.7"N, 04°8'49.6"W	949	2016-05-01 JP, ZK & P. Koutecký	MN091404	MN092002	
C. brutia subsp. *naftolskyi*	C16-097	25	1	**6.19±0.01**	Italy; reg. Sardegna (Sardinia); prov. Nuoro; Perdasdefogu: ditch near Strada Militare road, 5.3 km E(–ESE) of village	39°40'14.8"N, 09°30'22.5"E	617	2016-04-23 R. Lansdown	MN091405	MN092003	cultivated from seeds by J. Prančl; 2n=28 (this study)
C. brutia subsp. *naftolskyi*	C18-022	26	1	**6.13±0.03**	Italy; reg. Sardegna (Sardinia); prov. Sassari; Torralba: vernal pool in pastures on basalt plateau 0.5 km W of village	40°30'50.6"N, 08°45'12.9"E	510	2018-04-23 ZK, J. Hanzlíčková & P. Koutecký	MN091406	MN092004	
C. brutia subsp. *naftolskyi*	C18-016	27	1	**6.13±0.01**	Italy; reg. Sardegna (Sardinia); prov. Sud Sardegna (South Sardinia); Gesturi: shallow lake on Giara di Gesturi basalt plateau 1.7 km WNW of village	39°44'19.0"N, 08°59'48.2"E	578	2018-04-22 ZK, J. Hanzlíčková & P. Koutecký	MN091407	MN092005	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers ITS	*trn*T-*trn*L	Notes
C. brutia subsp. *naftolskyi*	C18-010	28	1	**6.12±0.00**	Italy; reg. Sicilia (Sicily); prov. Siracusa; Buccheri: vernal pool in pasture on basalt plain 350 m SSE of town	37°07'13.5"N, 14°51'13.1"E	750	2018-04-21 ZK, J. Hanzlíčková & P. Koutecký	MN091408	MN092006	
C. brutia subsp. *naftolskyi*	C18-009	29	1	**6.10±0.02**	Italy; reg. Sicilia (Sicily); prov. Siracusa; Sortino: vernal pool on basalt plain 3.5 NNE of town	37°11'38.1"N, 15°03'40.7"E	377	2018-04-20 ZK, J. Hanzlíčková & P. Koutecký	MN091409	MN092007	
C. brutia subsp. *naftolskyi*	C15-089b	24	1	**6.07**	Spain; comm. Andalusía; prov. Cádiz; Alcalá de los Gazules: muddy place near hiking trail on the SW slope of El Picacho mountain, 9.7 km NE of town	36°31'12.5"N, 05°38'11.6"W	560	2015-05-07 P. Koutecký	MN091410	MN092008	
C. brutia nothosubsp. *neglecta* [*C. b.* subsp. *brutia* × *C. b.* subsp. *naftolskyi*]	C16-009	30	1	**5.98±0.03**	Spain; comm. Extremadura; prov. Badajoz; Herrera del Duque: Arroyo Pelochejo stream (tributary of Guadiana river) 650 m NNE of town	39°10'40.2"N, 05°2'45.9"W	420	2016-05-02 JP, ZK & P. Koutecký	MN091411	MN092009	
C. brutia nothosubsp. *neglecta* [*C. b.* subsp. *brutia* × *C. b.* subsp. *naftolskyi*]	C16-013	31	1	**5.94±0.01**	Spain; comm. Extremadura; prov. Cáceres; Jaraicejo: Almonte river below bridge of N-V road (Carretera de Extremadura), 1.7 km SSW of village	39°38'46.7"N, 05°49'4.6"W	349	2016-05-03 JP, ZK & P. Koutecký	MN091412, MN091413–MN091420 (clones x1–x8)	MN092010	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. cophocarpa	C12-095	32	2	3.18	Czech Republic; distr. Domažlice; Pila: "Louka u Šnajberského rybníka" area, artificial pool 560 m ESE of railway station (*resampled from population C10-064*)	49°24'42.7"N, 12°51'55.8"E	469	2012-10-29 JP	MN091421	MN092011	
C. cophocarpa	C13-011	33	1	3.19	Czech Republic; distr. Hradec Králové; Pamětník: Mlýnská Cidlina stream near bridge in village	50°07'25.9"N, 15°26'51.2"E	210	2013-05-28 JP	MN091422	MN092012	
C. cophocarpa	C13-081	34	6	3.22±0.02	Czech Republic; distr. Jablonec nad Nisou; Bedřichov: Blatný rybník reservoir 2.3 km NE of village	50°48'36.9"N, 15°9'53.8"E	770	2013-08-22 JP & K. Kabátová	MN091423	MN092013	
C. cophocarpa	C13-085	35	1	3.22±0.01	Czech Republic; distr. Klatovy; Horská Kvilda: tributary of Hamerský stream 1.3 km W–WNW of village (*resampled from population C08-025*)	49°3'22.6"N, 13°32'23.6"E	1010	2013-08-25 JP & K. Kabátová	MN091424	MN092014	
C. cophocarpa	C13-001	36	1	3.26	Czech Republic; distr. Olomouc; Jívová: pool in valley of Bystřice stream near settlement Panský Mlýn, 850 m SW of railway station	49°42'21.3"N, 17°25'56.4"E	404	2013-04-29 JP & K. Kabátová	MN091425	MN092015	
C. cophocarpa	C13-030	37	1	3.23	Czech Republic; distr. Přerov; Týn nad Bečvou: pool complex 2.2 km NE of church	49°31'37.2"N, 17°38'33.3"E	234	2013-06-21 JP & ZK	MN091426	MN092016	
C. cophocarpa	C15-061	38	11	**3.21±0.02**	Czech Republic; distr. Rychnov nad Kněžnou; Čermná nad Orlicí: Tichá Orlice river 115 m E of bridge in village	50°4'40.4"N, 16°8'14.0"E	259	2015-09-03 JP	MN091427	MN092017	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. cophocarpa	C13-027	39	1	3.20	Czech Republic; distr. Svitavy; Rychnov na Moravě: Rohlíček fishpond 3.2 km NE of church	49°50'40.5"N, 16°40'35.1"E	340	2013-06-20 JP & ZK	MN091428	MN092018	
C. cophocarpa	C13-119	40	1	3.24±0.04	Czech Republic; distr. Ústí nad Labem; Petrovice: artificial pool 170 m ENE of border crossing Petrovice/Bahratal	50°48'55"N, 13°59'3"E	430	2013-08-27 Jan Rydlo & Jar. Rydlo	MN091429	MN092019	
C. cophocarpa	C12-063	41	1	3.16±0.04	Czech Republic; distr. Ústí nad Orlicí; Brandýs nad Orlicí: Tichá Orlice River below bridge of Žerotínova street	50°0'4.9"N, 16°16'45.1"E	295	2012-08-15 JP	MN091430	MN092020	
C. cophocarpa	C13-095	42	2	3.19±0.03	Czech Republic; distr. Ústí nad Orlicí; Choceň: town distr. Peliny, confluence of outlet of Mariánské jezero oxbow to Tichá Orlice river, 1.15 km NE(–ENE) of railway station	49°59'55.7"N, 16°14'17.4"E	295	2013-09-01 JP & K. Kabátová	MN091431	MN092021	
C. cophocarpa	C12-033	43	5	3.25±0.01	Denmark; reg. Syddanmark (Southern Denmark); Esbjerg municipality; Ribe: estuary of field ditch to Ribeå river NE of settlement Seem, 2.75 km ESE of town	55°18'54.8"N, 08°50'04.0"E	1	2012-07-31 JP & ZK	MN091432	MN092022	
C. cophocarpa	C17-052	44	1	**3.24±0.05**	Finland; reg. Etelä-Savo (Southern Savonia); sub-reg. Pieksämäki; Rantasalmi: forest ditch on westernmost peninsula on S edge of lake Rappunen, 13.1 km SSW of village	61°56'28.4"N, 28°15'52.6"E	100	2017-07-28 JP, P. Koutecký & J. Hanzlíčková	MN091433	MN092023	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. cophocarpa	C15-087	45	1	**3.17±0.04**	Germany; Sachsen (Saxony); distr. Sächsische Schweiz-Osterzgebirge; Kurort Rathen: Amselsee reservoir 620 m N of castle	50°57'51.5"N, 14°4'49.4"E	146	2015-11-15 M. Kačmar, Jan Rydlo & Jar. Rydlo	MN091434	MN092024	
C. cophocarpa	C12-074	46	1	3.12±0.02	Poland; Mazowieckie Voivodeship; Ostrołęka County; Tyszki-Gostery: Ruż stream near bridge of Droga Wojewódzka road 710 m NNE of village	52°59'33.0"N, 21°54'58.4"E	111	2012-08-31 P. Trávníček & B. Kubátová	MN091435	MN092025	
C. cophocarpa	C13-016	47	1	3.24±0.03	Slovakia; distr. Galanta; Pusté Úľany: Nový stream close to road bridge 860 m SSW of church	48°13'35.1"N, 17°33'52.0"E	115	2013-07-05 JP & M. Hrdinová	MN091436	MN092026	
C. cophocarpa	C13-071	48	1	3.26	Sweden, Västernorrland county; Kramfors municipality; Bollstabruk: ditch along road no. 90, 6.2 km NNW of village	63°3'18.0"N, 17°36'49.2"E	98	2013-08-03 Jan Rydlo	MN091437	MN092027	
C. cophocarpa	C14-075	49	1	**3.25±0.01**	Ukraine; Zakarpatska (Zakarpattia) oblast; Rachiv (Rakhiv) raion; Jasiňa (Yasinia): Svydovets Mountains, small shallow lake ca 850 m SE of top of Stig peak (1704), 9.2 km W(–WSW) of railway station	48°14'40.9"N, 24°13'49.2"E	1494	2014-07-29 K. Kabátová	MN091438	MN092028	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers ITS	*trn*T-*trn*L	Notes
C. cophocarpa × *C. hamulata*	C12-061	41	5	7.60±0.02	Czech Republic; distr. Ústí nad Orlicí; Brandýs nad Orlicí, Tichá Orlice river below bridge of Žerotínova street (*also resampled as C13-092*)	50°0'4.9"N, 16°16'45.1"E	295	2012-08-15 JP; 2013-09-01 JP & K. Kabátová (C13-092)	MN091439 (C12-061-04), MN091440 (C12-061-20), MN091441 (C13-092-04)	MN092029 (C12-061-04), MN092030 (C12-061-20), MN092031 (C13-092-04)	
C. cophocarpa × *C. hamulata*	C12-066	50	6	7.64±0.08	Czech Republic; distr. Ústí nad Orlicí; Choceň, millrace of Tichá Orlice river on NW edge of town, 1.65 km NW(–WNW) of railway station	50°0'4.5"N, 16°12'13.2"E	270	2012-08-15 JP	MN091442	MN092032	2n=29 (Prančl & al., 2014)
C. cophocarpa × *C. hamulata*	C12-065	51	1	7.61±0.08	Czech Republic; distr. Ústí nad Orlicí; Choceň, Tichá Orlice river on SW edge of town, 1 km W–WNW of railway station	49°59'43.2"N, 16°12'36.1"E	270	2012-08-15 JP	MN091443	MN092033	
C. cophocarpa × *C. hamulata*	C15-060	52	4	**7.61±0.04**	Czech Republic; distr. Ústí nad Orlicí; Choceň: Tichá Orlice river below bridge of railway siding, 2.5 km NW of railway station	50°0'36.8"N, 16°11'57.8"E	279	2015-09-03 JP	MN091444 (C15-060-12)	MN092034 (C15-060-12)	
C. cribrosa	C18-002	53	1	**3.62±0.01**	Italy; reg. Lazio; prov. Frosinone; Posta Fibreno: inflow to Lago de Posta Fibreno lake near NW edge of village	41°41'45.2"N, 13°41'38.1"E	290	2018-04-16 ZK, J. Hanzlíčková & P. Koutecký	MN091445	MN092035	
C. hamulata	C14-077	54	1	**8.88±0.05**	Austria; Oberösterreich (Upper Austria); distr. Rohrbach; Haslach an der Mühl: Große Mühl river 500 m SW(–WSW) of church	48°34'20.0"N, 14°2'6.5"E	496	2014-08-07 M. Hrdinová	MN091446	MN092036	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. hamulata	C13-086	56	1	8.95	Czech Republic; distr. Cheb; Prameny: Dlouhá stoka channel 1.5 km NE of village	50°03'43.2"N, 12°44'43.7"E	780	2013-08-21 J. Chrtek jr.	MN091448	MN092038	
C. hamulata	C13-084	57	1	8.91	Czech Republic; distr. Klatovy; Horská Kvilda: Hamerský stream 380 m SW–SSW of village	49°3'15.6"N, 13°33'17.7"E	1030	2013-08-25 JP & K. Kabátová	MN091449	MN092039	2n=38 (Prančl & al., 2014)
C. hamulata	C12-073	58	4	8.83	Czech Republic; distr. Liberec; Liberec: Lužická Nisa river near Hrazená street in city centre	50°46'01.1"N, 15°03'10.2"E	350	2012-08-26 JP	MN091450	MN092040	
C. hamulata	C13-077	59	3	8.82±0.00	Czech Republic; distr. Mladá Boleslav; Loukovec: Jizera river 1.4 km W of village (*resampled from population C12-022*)	50°33'45.6"N, 14°59'41.3"E	230	2013-08-19 JP & K. Kabátová	MN091451	MN092041	
C. hamulata	C13-028	60	2	8.91±0.04	Czech Republic; distr. Olomouc; Nové Zámky: "Pod Templem" area, old separated arm of Morava river 850 m W of castle	49°43'14.8"N, 17°01'01.4"E	240	2013-06-21 JP & ZK	MN091452	MN092042	
C. hamulata	C15-062	38	4	**8.84±0.06**	Czech Republic; distr. Rychnov nad Kněžnou; Čermná nad Orlicí: Tichá Orlice river 115 m E of bridge in village	50°4'40.4"N, 16°8'14.0"E	259	2015-09-03 JP	MN091453	MN092043	
C. hamulata	C13-117	61	1	8.92±0.01	Czech Republic; distr. Ústí nad Labem; Tisá: Ostrovský fishpond in settlement Ostrov, 2.5 km NNE of church	50°48'20"N, 14°2'47"E	445	2013-08-28 Jan Rydlo & Jar. Rydlo	MN091454	MN092044	

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C. hamulata	C12-062	41	26	8.85±0.09	Czech Republic; distr. Ústí nad Orlicí; Brandýs nad Orlicí: Tichá Orlice River below bridge of Žerotínova street	50°0'4.9"N, 16°16'45.1"E	295	2012-08-15 JP	MN091455	MN092045	
C. hamulata	C15-059	52	16	**8.85±0.04**	Czech Republic; distr. Ústí nad Orlicí; Choceň: Tichá Orlice river below bridge of railway siding, 2.5 km NW of railway station	50°0'36.8"N, 16°11'57.8"E	279	2015-09-03 JP	MN091456	MN092046	
C. hamulata	C12-045	62	1	9.10±0.09	Denmark; reg. Midtjylland (Central Denmark); Ringkøbing-Skjern municipality; Tarm: Skjern Å river 1.7 km WNW of town	55°55'06.4"N, 08°28'58.0"E	1	2012-08-03 JP & ZK	MN091457	MN092047	
C. hamulata	C18-047	63	1	**8.91±0.12**	France; reg. Bretagne (Brittany); dep. Morbihan; Augan: forest pond Étang de Passonne 3.7 km ENE of village	47°55'56.4"N, 02°13'32.3"W	115	2018-05-10 JP	MN091458	MN092048	
C. hamulata	C18-046	64	1	**8.77±0.03**	France; reg. Bretagne (Brittany); dep. Morbihan; Pont-Scorff: Scorff river between two bridges on E edge of village	47°50'09.1"N, 03°23'39.3"W	4	2018-05-09 JP	MN091459	MN092049	
C. hamulata	C18-043	65	1	**8.95±0.12**	France; reg. Bretagne (Brittany); dep. Morbihan; Surzur: retention basin near N165 highway 5.1 km ENE of church in Surzur	47°35'38.7"N, 02°33'55.9"W	8	2018-05-09 JP	MN091460	MN092050	
C. hamulata	C18-036	66	1	**9.01±0.04**	France; reg. Nouvelle-Aquitaine; dep. Corrèze; Bugeat: Vézère river 210 m WNW of railway station	45°36'05.5"N, 01°55'20.2"E	667	2018-05-07 JP	MN091461	MN092051	
C. hamulata	C18-034	67	1	**8.97±0.12**	France; reg. Occitanie; dep. Lot; Pinsac: right bank of Dordogne river below bridge Pont de Pinsac	44°51'8.4"N, 01°31'31.9"E	88	2018-05-06 JP	MN091462	MN092052	

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									ITS	*trn*T-*trn*L	
C. hamulata	C18-079	68	1	**9.15±0.06**	Germany; Baden-Württemberg; distr. Schwarzwald-Baar; Donaueschingen: confluence of Brigach and Breg rivers (where Donau – Danube river originates) 1.6 km ENE of railway station	47°57'3.6"N, 08°31'12.6"E	676	2018-07-24 JP & J. Hanzlíčková	MN091463	MN092053	
C. hamulata	C13-132 b	69	1	8.81	Germany; Bayern (Bavaria); distr. Neumarkt; Hohenfels: Follerenbach stream near S edge of settlement of Lauf	49°11'7.2"N, 11°54'45.7"E	364	2013-09-07 K. Kabátová	MN091464	MN092054	
C. hamulata	C14-138	70	1	**8.85±0.06**	Germany; Sachsen (Saxony); distr. Sächsische Schweiz-Osterzgebirge; Sebnitz: pond 1.1 km N of church in Lichtenhain	50°57'12"N, 14°14'27"E	305	2014-09-18 Jan Rydlo & Jar. Rydlo	MN091465	MN092055	
C. hamulata	C16-081	71	1	**8.96±0.02**	Iceland; reg. Suðurland (Southern Region); Þingvellir area: pool (cove of Öxará river) 115 m NE of Þingvallakirkja (Þingvellir Church) 28 km N(–NNE) of Hveragerði	64°15'29.4"N, 21°07'10.1"W	102	2016-08-01 JP	MN091466	MN092056	
C. hamulata	C16-079	72	1	**9.02±0.05**	Iceland; reg. Vestfirðir (Westfjorde); Hólmavík: a ditch between road 68 and Húsavíkurkleif (a location known for plant fossils), 6.6 km SSE of village	65°38'35.9"N, 21°38'17.2"W	7	2016-07-24 JP	MN091467	MN092057	
C. hamulata	C13-050	73	1	8.86±0.02	USA; Oregon; Lane county; Goshen: Wild Hog Creek at bridge of Seavey Loop Road 1.7 km NNE of village	44°0'41.8"N, 123°0'19.1"W	139	2013-07-28 JP & T. Kávová	MN091468	MN092058	2n=38 (Prančl & al., 2014)

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									ITS	*trn*T-*trn*L	
C. hamulata	C13-049	74	1	8.86±0.01	USA; Oregon; Multnomah County; Troutdale: Sandy River Delta Park, old oxbow of Delta river 1.3 km NE of bridge of Columbia River Freeway	45°33'13.8"N, 122°22'25.0"W	4	2013-07-25 JP & T. Kávová	MN091469	MN092059	
C. hermaphroditica subsp. *hermaphroditica*	C12-090	75	1	1.93±0.01	Czech Republic; distr. Svitavy; Jedlová: Ráček II pond 2.2 km NE(–ENE) of church	49°40'14.3"N, 16°19'52.5"E	595	2012-08-11 JP	MN091470	MN092060	
C. hermaphroditica subsp. *hermaphroditica*	C16-089	76	1	**2.01±0.02**	Czech Republic; distr. Žďár nad Sázavou; Vojnův Městec: Malé Dářko fishpond 1.6 km SSE of church	49°39'58"N, 15°52'48"E	630	2016-09-08 K. Šumberová	MN091471	MN092061	
C. hermaphroditica subsp. *hermaphroditica*	C17-054	77	1	**1.98±0.01**	Finland; reg. Uusimaa, sub-reg. Helsinki; Nurmijärvi: SW edge of Valkajärvi lake, 8.5 km SW–SSW of town	60°23'37.2"N, 24°42'25.1"E	30	2017-07-29 JP, P. Koutecký & J. Hanzlíčková	MN091472	MN092062	
C. hermaphroditica subsp. *hermaphroditica*	C13-127	78	1	2.00±0.02	Sweden; Östergötland county; Ydre municipality; Hestra: Lake Sommen 3.4 km NE of village, part Torpasjön, Stora Bianäset	57°58'49.4"N, 15°6'55.0"E	153	2013-08-25 A. Svenson	MN091473	MN092063	
C. hermaphroditica subsp. *macrocarpa*	C17-051	79	1	**2.01±0.03**	Sweden; Östergötland county; Valdemarsvik municipality; Valdemarsvik: sea bay near Ekhaga settlement, 12.6 km NE of village	58°17'02.5"N, 016°46'03.0"E	0	2017-07-23 JP, P. Koutecký & J. Hanzlíčková	MN091474	MN092064	2n=6 (this study)
C. heterophylla var. cf. *bolanderi*	C14-144	80	1	**4.05±0.04**	USA; Colorado; Lake county; Twin Lakes: Lily Pond, 3.3 km north of town	39°6'35.6"N, 106°23'9.5"W	3175	2014-09-07 M. Majack	MN091475	MN092065	

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									ITS	*trn*T-*trn*L	
C. heterophylla var. *heterophylla*	C14-005	81	–	–	USA; New Hampshire; Carroll county; Freedom: Ossipee Lake, Square Brook at the north end of Berry Bay	43°48'29.7"N, 71°04'48.5"E	120	2013-08-29 C. B. Hellquist & L. Callahan	MN091476	MN092066	
C. cf. *heterophylla*	C14-006	82	–	–	USA; New Hampshire; Merrimack county; Tilton: Winnipesaukee River at boat launch off Rt. 140 at Shaker Road	43°27'10.1"N, 71°33'43.0"W	145	2013-08-29 C. B. Hellquist & L. Callahan	MN091477	MN092067	
C. cf. *heterophylla*	C14-007	83	–	–	USA; New York; Columbia county; Austerlitz: Beebe Hill State Forest, 730 m NE of jct County RT 5 and Fog Hill Rd, drained beaver pond (north)	42°20'49.4"N, 73°28'5.3"W	471	2013-08-23 G. Stevens	MN091478	MN092068	
C. lenisulca	C17-020	84	1	3.66±0.02	Greece; Peloponnisos (Peloponnese) peninsula; reg. Periféreia Dytikis Elládas (Western Greece); West Achaea municipality; Metochi: salt marsh 560 m W of village	38°7'29.5"N, 21°23'19.1"E	0	2017-05-06 JP, ZK & P. Koutecký	MN091479	MN092069	
C. lenisulca	C17-029	3	1	3.66±0.01	Greece; reg. Epirus; Igoumenitsa municipality; Morfi: Kalodiki lakes, smaller lake 1.2 km W of village	39°18'37.8"N, 20°27'56.3"E	108	2017-05-08 JP, ZK & P. Koutecký	MN091480	MN092070	
C. lenisulca	C17-003	85	1	3.60±0.06	Greece; reg. Ionian Islands; Kerkyra (Corfu) island; Chrisiida: drainige ditch in spring catchment of small creek towards Halkiopoulou lagoon 1 km WNW of Vlacherna Monastery	39°35'24.3"N, 19°54'14.2"E	0	2017-04-05 D. Reich, C. Gilli, M. Hofbauer & R. Sander	MN091481	MN092071	

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									ITS	*trn*T-*trn*L	
C. lenisulca	C17-023	5	1	3.63±0.02	Greece; reg. Periféreia Dytikis Elládas (Western Greece); Missolonghi municipality; Katochi: drying marshes adjacent to a complex of overgrown reservoirs 1.5 km NNW of village	38°25'41.6"N, 21°14'35.8"E	5	2017-05-07 JP, ZK & P. Koutecký	MN091482	MN092072	
C. lenisulca	C17-026	86	1	3.66±0.02	Greece; reg. Periféreia Dytikis Elládas (Western Greece); Xiromero municipality; Strongylovouni: pool along road 4.9 km SSW of settlement of Manina Vlizianion	38°29'52.1"N, 21°9'37.1"E	5	2017-05-07 JP, ZK & P. Koutecký	MN091483	MN092073	
C. lenisulca	C17-019	87	1	3.64±0.03	Greece; reg. Periféreia Stereás Elládas (Central Greece); Lamia municipality; Gorgopotamos: small pond on mountain ridge above village 1.6 km SE of settlement of Dio Vouna	38°47'19.0"N, 22°23'32.2"E	650	2017-05-03 JP, ZK & P. Koutecký	MN091484	MN092074	
C. lenisulca	C17-018	88	1	3.63	Greece; reg. Thessalía (Thessaly); Palamas municipality; Pedino: pond S of the dyke on the S bank of Pinios River, 900 m NW of village	39°31'27.1"N, 21°56'21.5"E	90	2017-05-03 JP, ZK & P. Koutecký	MN091485	MN092075	
C. lenisulca	C13-005	89	1	3.58±0.04	Italy, reg. Emilia-Romagna, prov. Ravenna, Ravenna: Bardello area, pool E of road SS309, 3.1 km NW–WNW of Marina Romea	44°32'26.2"N, 12°14'19.3"E	0	2013-05-24 P. Trávníček & B. Kubátová	MN091486	MN092076	
C. lenisulca	C13-006	90	1	3.61±0.01	Italy; reg. Emilia-Romagna; prov. Ravenna; Ravenna: ditch in fields near Via Bevano road, 1.9 km NW of Castiglione di Ravenna	44°16'37.2"N, 12°14'29.5"E	1	2013-05-23 P. Trávníček & B. Kubátová	MN091487	MN092077	

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									ITS	*trn*T-*trn*L	
C. lenisulca	C16-096	91	1	**3.69±0.02**	Italy; reg. Sardegna (Sardinia); prov. Sud Sardegna (South Sardinia); Orolli: in a small stream running through scrub in pasture, 6.2 km SSE of village	39°38'8.3"N, 09°16'47.8"E	273	2016-04-26 R. Lansdown	MN091488	MN092078	cultivated from seeds by J. Prančl
C. lenisulca	C13-004	92	1	3.59±0.02	Italy; reg. Toscana (Tuscany); prov. Siena; Chiusi: ditch (Torrente Gragnano) 3.3 km NNW of village	43°2'51.0"N, 11°56'8.4"E	250	2013-05-23 P. Trávníček & B. Kubátová	MN091489	MN092079	
C. lusitanica	C17-015	6	1	**1.83±0.01**	Greece; reg. Thessalía (Thessaly); Farkadona municipality; Oichalia: Neochoritis river under bridge 1.6 km SSE of church	39°35'29.0"N, 21°59'10.8"E	100	2017-05-02 JP, ZK & P. Koutecký	MN091490	MN092080	2n=8 (this study)
C. lusitanica	C18-023	93	1	**1.84±0.01**	Italy; reg. Sardegna (Sardinia); prov. Nuoro; Macomer: Riu Badde Cabriolu stream at railway bridge 8.8 km N of town	40°20'56.2"N, 08°46'38.4"E	637	2018-04-23 ZK, J. Hanzlíčková & P. Koutecký	MN091491	MN092081	
C. lusitanica	C16-010	30	–	–	Spain; comm. Extremadura; prov. Badajoz; Herrera del Duque: Arroyo Pelochejo stream 650 m NNE of town	39°10'40.2"N, 05°2'45.9"W	420	2016-05-02 JP, ZK & P. Koutecký	MN091492	MN092082	
C. lusitanica	C16-014	31	1	**1.82±0.00**	Spain; comm. Extremadura; prov. Cáceres; Jaraicejo: Almonte river below bridge of N-V road, 1.7 km SSW of village	39°38'46.7"N, 05°49'4.6"W	349	2016-05-03 JP, ZK & P. Koutecký	MN091493	MN092083	

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									ITS	*trn*T-*trn*L	
C. muelleri	C15-093	94	1	**1.20±0.01**	Australia; Queensland; reg. Tablelands; Herberton: Atherton Tableland, upper Barron River catchment, Mount Hypipamee, Dinner Falls, moist loamy soil in closed forest 11 km ESE of town	17°25'19"S, 145°29'14"E	880	2015-07-26 R. Jobson	MN091494	MN092084	2n=10 (this study)
C. muelleri	C15-085	95	1	**1.21±0.02**	Australia			2015 R. Jobson	MN091495	MN092085	
C. × *nyraensis* [*C. cophocarpa* × *C. stagnalis*]	C15-084	96	14	**3.12±0.02**	Czech Republic; distr. Klatovy; Hamry: PR Úhlavský luh, marsh with small pools on left bank of Úhlava river above bridge near settlement Hamerský Dvůr, 920 m N–NNW of church	49°14'15.1"N, 13°9'27.0"E	529	2015-10-31 JP	MN091496 (C15-084-02), MN091497 (C15-084-03), MN091498– –MN091504 (clones C15-084-03-x1–x8), MN091505 (C15-084-06), MN091506 (C15-084-07)	MN092086 (C15-084-02), MN092087 (C15-084-03), MN092088 (C15-084-06), MN092089 (C15-084-07)	
C. obtusangula	C15-019	97	1	**3.86±0.02**	Austria; Oberösterreich (Upper Austria); distr. Braunau am Inn; Mining: big pool (arm of Inn river) 190 m W–WNW of Untersunzing	48°17'24.5"N, 13°11'16.0"E	322	2015-06-13 JP, P. Koutecký & M. Hohla	MN091507	MN092090	

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									ITS	***trn*T-*trn*L**	
C. obtusangula	C14-079	98	1	**3.89±0.00**	Austria; Oberösterreich (Upper Austria); distr. Braunau am Inn; Ostermiething: chanell 540 m SW of church	48°2'27.1"N, 12°49'45.4"E	375	2014-08-09 M. Hrdinová	MN091508	MN092091	
C. obtusangula	C14-081	99	1	**3.89±0.01**	Austria; Oberösterreich (Upper Austria); distr. Ried im Innkreis; Kirchdorf am Inn: reservoir by power station near Inn river 215 m W of Ufer	48°17'36.8"N, 13°15'58.2"E	320	2014-08-09 M. Hrdinová	MN091509	MN092092	
C. obtusangula	C18-048	100	1	**3.91±0.01**	France; reg. Bretagne (Brittany); dep. Morbihan; Guer: Ruisseau de Saint-Nicolas brook u osady Saint-Nicolas near N edge of settlement of Saint-Nicolas	47°53'02.8"N, 02°07'31.3"W	10	2018-05-11 JP	MN091510	MN092093	
C. obtusangula	C18-033	101	1	**3.99**	France; reg. Nouvelle-Aquitaine; dep. Landes; Soustons: Ruisseau de Bouyic stream 300 m SSE of its mouth to Étang de Soustons lake, along Allée des Chênes Liège street in village	43°45'33.6"N, 01°20'14.7"W	5	2018-05-05 JP	MN091511	MN092094	
C. obtusangula	C18-049	102	1	**3.93±0.02**	France; reg. Pays de la Loire; dep. Maine-et-Loire; Bouchemaine: Les Basses Brosses, pond 2.6 km SW–WSW of village	47°24'40.7"N, 00°38'25.2"W	42	2018-05-11 JP	MN091512	MN092095	
C. obtusangula	C18-040	2	1	**3.93±0.04**	France; reg. Pays de la Loire; dep. Vendée; Saint-Urbain: channel (brackish water) between settlements La Ramée and Beauséjour, 1.2 km NNW of village	46°53'22.9"N, 02°1'40.4"W	0	2018-05-08 JP	MN091513	MN092096	

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									ITS	*trn*T-*trn*L	
C. obtusangula	C18-080	103	1	**3.90±0.02**	Germany; Baden-Württemberg; distr. Karlsruhe; Karlsruhe: city distr. Mühlburg, Alb stream below bridge of Honsellstraße street	49°0'57.0"N, 08°20'48.4"E	106	2018-07-25 JP & J. Hanzlíčková	MN091514	MN092097	
C. obtusangula	C18-077	104	1	**3.88±0.04**	Germany; Bayern (Bavaria); distr. Freising; Eching: Moosach stream 520 m ESE of Ottenburg	48°18'56.1"N, 11°36'16.5"E	459	2018-07-21 JP & J. Hanzlíčková	MN091515	MN092098	
C. obtusangula	C16-085	105	1	**3.87±0.03**	Italy, reg. Campania; prov. Salerno; Padula: Tanagro river near bridge 3.1 km W–WNW of village	40°19'58.5"N, 15°36'48.1"E	462	2016-04-23 P. Trávníček	MN091516	MN092099	
C. obtusangula	C13-008+009	106	2	3.71±0.01 (C13-008), 3.73±0.02 (C13-009)	Italy; reg. Campania; prov. Salerno; Sala Consilina: ditch near SP11/E road 2.5 km S of town	40°22'5.2"N, 15°35'58.6"E (C13-008), 40°22'1.9"N, 15°35'53.3"E (C13-009)	453	2013-05-21 P. Trávníček & B. Kubátová	MN091517 (C13-008), MN091518 (C13-009)	MN0920100 (C13-008), MN092101 (C13-009)	
C. obtusangula	C18-003	53	1	**3.80±0.00**	Italy; reg. Lazio; prov. Frosinone; Posta Fibreno: inflow to Lago de Posta Fibreno lake near NW edge of village	41°41'45.2"N, 13°41'38.1"E	290	2018-04-16 ZK, J. Hanzlíčková & P. Koutecký	MN091519	MN092102	
C. obtusangula	C13-007	107	1	3.76±0.00	Italy; reg. Lazio; prov. Rieti; Colli sul Velino: race of Velino River (Canale di Santa Susanna) 2.2 km E(–ESE) of church	42°29'30.0"N, 12°48'7.1"E	379	2013-05-23 P. Trávníček & B. Kubátová	MN091520	MN092103	

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									ITS	*trn*T-*trn*L	
C. obtusangula	C18-025	11	1	**3.91±0.02**	Italy; reg. Sardegna (Sardinia); prov. Oristano; Busachi: vernal pools in grasslands with scattered scrub on basalt plateau 4.2 km W–WNW of village	40°02'24.8"N, 08°50'47.8"E	218	2018-04-24 ZK, J. Hanzlíčková & P. Koutecký	MN091521	MN092104	
C. obtusangula	C18-019	108	1	**3.86±0.05**	Italy; reg. Sardegna (Sardinia); prov. Oristano; Montresta: muddy brook of Riu sas Corrogas in meadows at bridge 2.9 NW of village	40°23'43.0"N, 08°28'27.6"E	292	2018-04-23 ZK, J. Hanzlíčková & P. Koutecký	MN091522	MN092105	
C. obtusangula	C18-015	109	1	**3.84±0.01**	Italy; reg. Sardegna (Sardinia); prov. Sud Sardegna (South Sardinia); San Priamo: Rio sa Picocca river at bridge 0.5 km WSW of village	39°21'28.7"N, 09°33'09.1"E	5	2018-04-20 ZK, J. Hanzlíčková & P. Koutecký	MN091523	MN092106	
C. obtusangula	C18-012	12	1	**3.92±0.00**	Italy; reg. Sicilia (Sicily); prov. Palermo; Godrano: area of Bosco della Ficuzza, pool in oak forest 3 km WSW of village	37°53'23.9"N, 13°23'40.9"E	740	2018-04-21 ZK, J. Hanzlíčková & P. Koutecký	MN091524	MN092107	
C. obtusangula	C18-031	110	1	**3.88±0.02**	Italy; reg. Toscana (Tuscany); prov. Pistoia; Ponte Buggianese: Lago delle Docce water reservoir 4.5 km SSE of town	43°47'53.2"N, 10°45'45.8"E	20	2018-04-27 ZK, J. Hanzlíčková & P. Koutecký	MN091525	MN092108	
C. obtusangula	C18-030	111	1	**3.87±0.01**	Italy; reg. Umbria; prov. Perugia; Corciano-Pigge: Fiume Clitunno river at WSW edge of village	42°50'57.2"N, 12°45'00.0"E	218	2018-04-26 ZK, J. Hanzlíčková & P. Koutecký	MN091526	MN092109	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. obtusangula	C18-029	112	1	**3.93±0.06**	Italy; reg. Umbria; prov. Perugia; Norcia: Fiume Sordo stream 1.3 km W of village	42°47'40.8"N, 13°04'27.1"E	552	2018-04-25 ZK, J. Hanzlíčková & P. Koutecký	MN091527	MN092110	
C. obtusangula	C12-052	113	1	3.85±0.04	Netherlands; prov. Noord-Brabant (North Brabant); Breda: Turfvaart canal on SW edge of town, 4.7 km SW of railway station	51°33'50.1"N, 04°44'3.3"E	30	2012-08-01 P. Trávníček & B. Kubátová	MN091528	MN092111	2n=10 (Prančl & al., 2014)
C. obtusangula	C15-086	114	1	**3.88±0.04**	Slovakia; distr. Dunajská Streda; Ňárad: Chotárny channel near bridge of road no. 506 at NNW edge of village, 370 m NNW of church	47°50'23.0"N, 17°36'24.6"E	112	2015-09-28 K. Bubíková	MN091529	MN092112	
C. palustris	C12-081	115	1	3.85±0.01	Czech Republic; distr. Liberec; Pertoltice: Šálkův fishpond 1.7 km E of church	50°59'01.5"N, 15°06'12.5"E	319	2012-10-11 JP	MN091530	MN092113	
C. palustris	C12-019	116	1	3.91	Czech Republic; distr. Mladá Boleslav; Březina: Oběšenec fishpond 1.8 km WSW of railway station Březina nad Jizerou	50°32'20.2"N, 15°3'36.4"E	238	2012-06-14 JP & P. Trávníček	MN091531	MN092114	
C. palustris	C17-053	117	1	**3.96±0.07**	Finland; reg. Etelä-Savo (Southern Savonia); sub-reg. Savonlinna; Savonlinna: lake Hirvasjärvi, N edge of lake 6.8 km ESE of railway station	61°50'17.1"N, 28°59'59.2"E	78	2017-07-28 JP, P. Koutecký & J. Hanzlíčková	MN091532	MN092115	
C. palustris	C18-084	118	1	**3.93±0.02**	Hungary; Borsod-Abaúj-Zemplén County; Újszentmargita: cove of Gelei-halastó fishpond 5 km NW–NNW of village	47°46'08.9"N, 21°04'10.9"E	87	2018-05-22 ZK & A. Mesterházy	MN091533	MN092116	

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C. palustris	C16-080	119	1	**3.88±0.06**	Iceland; reg. Suðurland (Southern Region); Landmannalaugar area: stream in Brennisteinsalda Camping cca 54 km WNW of Kirkjubæjarklaustur	63°59'28.6"N, 19°03'47.7"W	592	2016-07-29 JP	MN091534	MN092117	
C. palustris	C13-073	120	1	3.94±0.04	Norway; Trøndelag County; Oppdal municipality; Driva: Hjerkinn/Kongsvoll/Drivdalen protection area, path near Gåvålivatnet lake, ca 28.7 km S of settlement	62°16'26.3"N, 09°37'29.1"E	941	2013-08-04 K. Kabátová	MN091535	MN092118	
C. palustris	C15-057	121	1	**3.92±0.03**	Romania; Bistrița-Năsăud county; Rodna mountains; Rodna: small lake on the NE slope of Corongis mt. (1987 m), 11.5 km N of town	47°31'33.9"N, 24°47'53.0"E	1677	2015-08-15 K. Kabátová	MN091536	MN092119	
C. palustris	C13-150	122	1	3.93	Sweden; Norrbotten county; Kiruna municipality; Karesuando: Munionjoki river on N edge of village	68°26'33"N, 22°28'54"E	320	2013-08-06 ZK	MN091537	MN092120	
C. palustris	C14-143	123	1	**3.75±0.08**	USA; Colorado; Lake county; Twin Lakes: muddy shoreline of unnamed beaver pond north of Lily Pond, 3.3 km north of town	39°6'42.7"N, 106°23'19.4"W	3175	2014-09-07 M. Majack	MN091538	MN092121	
C. palustris	C14-004	124	–	–	USA; Maine; Aroostock county; Presque Isle: bogan (oxbow) of Aroostock River, 6.7 km N(–NNW) of town centre	46°44'22.8"N, 68°02'10.0"W	127	2013-08-06 C. B. Hellquist	MN091539	MN092122	
C. palustris	C14-002 + C14-003	125	–	–	USA; Maine; Washington county; Cherryfield: Narraguagus river on west side of small island N of Rte. 1A	44°36'7.2"N, 68°49'12.8"W	7	2013-08-05 C. B. Hellquist	MN091540 (C14-002), MN091541 (C14-003)	MN092123 (C14-002), MN092124 (C14-003)	

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C. palustris	C14-008	83	–	–	USA; New York; Columbia county; Austerlitz: Beebe Hill State Forest, 730 m NE of jct County RT 5 and Fog Hill Rd, drained beaver pond (north), on a bottom of small pool	42°20'49.4"N, 73°28'5.3"W	471	2013-08-23 G. Stevens & C. Graham	MN091542	MN092125	
C. platycarpa	C15-017	126	1	**6.38**	Austria; Oberösterreich (Upper Austria); distr. Braunau am Inn; Mining: bank of channel 770 m W–WSW of church	48°16'30.0"N, 13°9'5.8"E	334	2015-06-13 JP, P. Koutecký & M. Hohla	MN091543	MN092126	
C. platycarpa	C14-072	127	1	**6.22±0.10**	Czech Republic; distr. Děčín; Jetřichovice: small pond in valley of Jetřichovický brook, 5.1 km NW of church	50°53'38"N, 14°21'31"E	251	2014-07-24 Jan Rydlo & Jar. Rydlo	MN091544	MN092127	
C. platycarpa	C13-124	128	3	6.10±0.02	Czech Republic; distr. Děčín; Jílové: Oborský fishpond 1.1 km NW of Zadní Ves	50°48'21"N, 14°4'20"E	525	2013-08-28 Jan Rydlo & Jar. Rydlo	MN091545	MN092128	
C. platycarpa	C12-093	129	3	6.12	Czech Republic; distr. Domažlice; Babylon: millrace of Teplá Bystřice stream 80 m SE–SSE of railway station (*resampled from population 10-062*)	49°23'54.4"N, 12°52'9.2"E	483	2012-10-29 JP	MN091546	MN092129	
C. platycarpa	C13-079	130	3	6.11±0.01	Czech Republic; distr. Liberec; Černousy: stream along second uppermost fishpond in pond cascade E of settlement V Poli, 1.25 km SSE of village (*resampled from population C12-084*)	50°59'35.3"N, 15°03'26.4"E	242	2013-08-22 JP & K. Kabátová	MN091547	MN092130	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers ITS	trnT-trnL	Notes
C. platycarpa	C13-074	131	4	6.16±0.06	Czech Republic; distr. Semily; Turnov: small ponds below castle Hrubý Rohozec, 2.0 km NE of railway station (*resampled from population C10-013*)	50°35'58.6"N, 15°9'35.6"E	246	2013-08-19 JP & K. Kabátová	MN091548	MN092131	
C. platycarpa	C12-046	132	2	6.29±0.03	Denmark; reg. Syddanmark (Southern Denmark); Varde municipality; Varde: ditch between Varde Å river and Lundvej Road (12;487) 3.1 km NE of railway station Varde Nord	55°38'41.1"N, 08°32'35.7"E	6	2012-08-03 JP & ZK	MN091549	MN092132	
C. platycarpa	C18-045	64	1	**6.33±0.07**	France; reg. Bretagne (Brittany); dep. Morbihan; Pont-Scorff: Scorff river between two bridges on E edge of village	47°50'09.1"N, 03°23'39.3"W	4	2018-05-09 JP	MN091550	MN092133	
C. platycarpa	C12-077	133	1	6.11	Germany; Bayern (Bavaria); distr. Bayreuth; Pottenstein: Weihersbach stream on N edge of settlement of Schüttersmühle	49°45'5.2"N, 11°25'37.9"E	411	2012-09-10 A. Knotek	MN091551	MN092134	
C. platycarpa	C14-139	134	1	**6.23±0.02**	Germany; Sachsen (Saxony); distr. Sächsische Schweiz-Osterzgebirge; Sebnitz: Räumichtbach stream along Hinteres Räumicht street, 1.25 km NNW of church in Hinterhermsdorf	50°56'2"N, 14°21'3"E	340	2014-07-19 Jan Rydlo & Jar. Rydlo	MN091552	MN092135	
C. platycarpa	C13-010	135	1	6.06±0.01	Italy, reg. Calabria; prov. Cosenza; Acri: ditch by waterlogged meadow near SS 660 road, 12.5 km ESE of town	39°26'18.5"N, 16°31'29.2"E	1207	2013-05-20 P. Trávníček & B. Kubátová	MN091553	MN092136	

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									ITS	*trn*T-*trn*L	
C. platycarpa	C18-006	136	3	**6.18±0.06**	Italy, reg. Sicilia (Sicily); prov. Messina; San Fratello: shallow pools in meadows under dam of Lago Maulazzo in Parco dei Nebrodi 10 km SE of village	37°56'36.6"N, 14°40'21.4"E	1440	2018-04-19 ZK, J. Hanzlíčková & P. Koutecký	MN091554	MN092137	
C. platycarpa	C17-050	137	1	**6.15±0.02**	Sweden; Skåne county; Svedala municipality; Svedala: ditch between pools 5.8 km S(–SSE) of railway station	55°27'18.6"N, 13°14'38.5"E	52	2017-07-20 JP, P. Koutecký & J. Hanzlíčková	MN091555	MN092138	
C. pulchra	C15-003	138	–	–	Greece; reg. Kríti (Crete); island of Gavdos; Agios Ioannis: series of karstic limestone pools on the path to a church 750 m NE(–ENE) of Agios Georgios church	34°51'50"N, 24°04'54.2"E (approx.)	80	2015-05-22 I. Bazos & R. Lansdown	MN091556	MN092139	
C. pulchra	C15-001 +002	139	–	–	Greece; reg. Kríti (Crete); island of Gavdos; Agios Panteleimonas: series of karstic limestone pools 750 m WNW of Agios Paleonto church	34°50'59"N, 24°04'23"E (approx.)	150	2015-05-20 I. Bazos & R. Lansdown	MN091557 (C15-001), MN091558 (C15-002)	MN092140 (C15-001), MN092141 (C15-002)	
C. regis-jubae	C18-018	140	1	**2.97±0.03**	Italy; reg. Sardegna (Sardinia); prov. Oristano; Suni: spring area of Riu Pali Trotto rivulet near SS292 road, with puddles crushed by cattle, 5 km SSW of village	40°19'23.1"N, 08°34'50.2"E	330	2018-04-23 ZK, J. Hanzlíčková & P. Koutecký	MN091559	MN092142	
C. regis-jubae	C16-016	141	1	**3.01±0.04**	Spain; comm. Extremadura; prov. Cáceres; Torrejón el Rubio: Monfragüe area, wet grassy road margin near left bank of Tajo river, 1.5 km E(–ENE) of Monfragüe castle	39°49'46.0"N, 06°2'0.0"W	196	2016-05-03 JP, ZK & P. Koutecký	MN091560	MN092143	2n=10 (this study)

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. stagnalis	C15-094	142	1	**2.96±0.00**	Australia; New South Wales; reg. Armidale Regional Council; Jeogla: East Kunderang Station, rainforest creek 50 km SE of Armidale	30°50'1"S, 152°3'35"E	540	2015-11-11 R. Jobson	MN091561	MN092144	
C. stagnalis	C12-076	143	1	2.97±0.02	Czech Republic; distr. Děčín; Brtníky: puddle on forest path 1.06 km S of railway station	50°56'32"N, 14°26'14.9"E	455	2012-07-13 J. Hadinec & P. Bauer	MN091562	MN092145	
C. stagnalis	C13-114	144	1	3.01	Czech Republic; distr. Děčín; Děčín: town distr. Maxičky, Vlčí jezero fishpond 3.3 km W of village	50°48'35"N, 14°8'0"E	480	2013-08-28 Jan Rydlo & Jar. Rydlo	MN091563	MN092146	
C. stagnalis	C12-092	145	1	3.01	Czech Republic; distr. Domažlice, Babylon: ruts on crossroads of forest paths near settlement Na Pohodnici, 910 m NE of railway station	49°24'18.4"N, 12°52'38.4"E	520	2013-10-29 JP	MN091564	MN092147	
C. stagnalis	C13-087	146	1	3.05	Czech Republic; distr. Cheb; Prameny: puddle on forest path 2.2 km NE–ENE of village	50°03'41.3"N, 12°45'36.3"E	760	2013-08-21 J. Chrtek jr.	MN091565	MN092148	
C. stagnalis	C15-084-01	147	1	**3.03±0.05**	Czech Republic; distr. Klatovy; Hamry: PR Úhlavský luh, marsh with small pools on left bank of Úhlava river above bridge near settlement Hamerský Dvůr, 920 m N–NNW of church	49°14'15.1"N, 13°9'27.0"E	529	2015-10-31 JP	MN091566	MN092149	
C. stagnalis	C13-002	148	2	3.01	Czech Republic; distr. Mělník; Želízy: Želízská svodnice ditch along road I/9 in village	50°25'21.1"N, 14°27'51.2"E	176	2013-05-03 JP	MN091567	MN092150	

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C. stagnalis	C13-018	149	1	3.03	Czech Republic; distr. Nymburk; Loučeň: pool on bottom of long-term drained fishpond Lutovník 1.7 km ESE of castle	50°17'34.6"N, 15°0'9.0"E	234	2013-06-07 JP	MN091568	MN092151	
C. stagnalis	C13-135	150	1	3.03±0.00	Czech Republic; distr. Praha-východ; Jevany: middle forest fishpond in Aldašín deer-park 1.6 km E of village (*resampled from population C08-046*)	49°58'6.6"N, 14°50'21.2"E	385	2013-09-09 JP	MN091569	MN092152	
C. stagnalis	C18-044	65	1	**3.01±0.04**	France; reg. Bretagne (Brittany); dep. Morbihan; Surzur: retention basin near N165 highway 5.1 km ENE of church in Surzur	47°35'38.7"N, 02°33'55.9"W	8	2018-05-09 JP	MN091570	MN092153	
C. stagnalis	C18-035	151	1	**2.97±0.01**	France; reg. Nouvelle-Aquitaine; dep. Corrèze; Brive-la-Gaillarde: pond 380 m WNW of settlement of Marcillac	45°07'22.1"N, 01°31'29.9"E	163	2018-05-06 JP	MN091571	MN092154	
C. stagnalis	C18-037	152	1	**2.97±0.03**	France; reg. Nouvelle-Aquitaine; dep. Corrèze; Peyrelevade, small muddy stream between pastures 450 m SW–WSW of settlement of Malsagne	45°43'58.0"N, 02°1'57.8"E	790	2018-05-07 JP	MN091572	MN092155	
C. stagnalis	C18-038	153	1	**3.01±0.02**	France; reg. Pays de la Loire; dep. Vendée; L'Île-d'Olonne: muddy ditch (brackish water) along road 850 m SE–SSE of church	46°33'16.9"N, 01°46'37.4"W	0	2018-05-08 JP	MN091573	MN092156	
C. stagnalis	C14-141	154	1	**2.96**	Germany; Sachsen (Saxony); distr. Sächsische Schweiz-Osterzgebirge; Sebnitz: puddle on forest path in valley of Knechtsbach stream, 1.5 km ESE church in Lichtenhain	50°56'25"N, 14°15'37"E		2014-09-18 Jan Rydlo & Jar. Rydlo	MN091574	MN092157	

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									ITS	*trn*T-*trn*L	
C. stagnalis	C17-021	84	1	**3.01±0.01**	Greece; Peloponnisos (Peloponnese) peninsula; reg. Periféreia Dytikis Elládas (Western Greece); West Achaea municipality; Metochi: salt marsh 560 m W of village	38°7'29.5"N, 21°23'19.1"E	0	2017-05-06 JP, ZK & P. Koutecký	MN091575	MN092158	
C. stagnalis	C17-027	155	1	**3.07±0.03**	Greece; reg. Epirus; Ziros municipality; Agios Georgios: pool on right bank of Louros river around bridge 400 m NWof village	39°16'16.6"N, 20°50'53.9"E	101	2017-05-06 JP, ZK & P. Koutecký	MN091576	MN092159	
C. stagnalis	C17-008	156	1	**3.04±0.01**	Greece; reg. Ionian Islands; Kerkyra (Corfu) island; Argirades: cultivated land E of Korission Lagoon 3.5 km WNW of church	39°26'22.7"N, 19°56'12.9"E	5	2017-04-09 M. Hofbauer, D. Reich & R. Sander	MN091577	MN092160	
C. stagnalis	C17-004	85	1	**2.99±0.02**	Greece; reg. Ionian Islands; Kerkyra (Corfu) island; Chrisiida: drainage ditch NE of village towards Halkiopoulou lagoon 1 km WNW of Vlacherna Monastery	39°35'31.4"N, 19°54'19.7"E	0	2017-04-05 C. Gilli, M. Hofbauer, D. Reich & R. Sander	MN091578	MN092161	
C. stagnalis	C17-024	5	1	**3.03±0.04**	Greece; reg. Periféreia Dytikis Elládas (Western Greece); Missolonghi municipality; Katochi: drying marshes adjacent to a complex of overgrown reservoirs 1.5 km NNW of village	38°25'41.6"N, 21°14'35.8"E	5	2017-05-07 JP, ZK & P. Koutecký	MN091579	MN092162	
C. stagnalis	C17-016	6	1	**3.03±0.01**	Greece; reg. Thessalía (Thessaly); Farkadona municipality; Oichalia: Neochoritis river under bridge 1.6 km SSE of church	39°35'29.0"N, 21°59'10.8"E	100	2017-05-02 JP, ZK & P. Koutecký	MN091580	MN092163	

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C. stagnalis	C17-014	7	1	**3.00±0.01**	Greece; reg. Thessalía (Thessaly); Kalambaka municipality; Flampouresi: small rivulet with puddles crushed by cattle, along road 640 m SE–SSE of village	39°47'47.2"N, 21°46'7.3"E	790	2017-05-01 JP, ZK & P. Koutecký	MN091581	MN092164	
C. stagnalis	C18-004	157	–	–	Italy; reg. Campania; prov. Caserta; Castel Volturno: shallow pools in Oasi dei Variconi coastal marshes at town	41°01'33.1"N, 13°56'08.4"E	0	2018-04-17 ZK, J. Hanzlíčková & P. Koutecký	MN091582	MN092165	
C. stagnalis	C18-013	12	1	**3.04±0.04**	Italy; reg. Sicilia (Sicily); prov. Palermo; Godrano: area of Bosco della Ficuzza, pool in oak forest 3 km WSW of village	37°53'23.9"N, 13°23'40.9"E	740	2018-04-21 ZK, J. Hanzlíčková & P. Koutecký	MN091583	MN092166	
C. stagnalis (cf.)	C18-020	108	1	**3.05±0.01**	Italy; reg. Sardegna (Sardinia); prov. Oristano; Montresta: muddy brook of Riu sas Corrogas in meadows at bridge 2.9 NW of village	40°23'43.0"N, 08°28'27.6"E	292	2018-04-23 ZK, J. Hanzlíčková & P. Koutecký	MN091584	MN092167	
C. stagnalis	C18-032	158	1	**3.08±0.02**	Italy; reg. Toscana (Tuscany); prov. Firenze; San Piero a Sieve: small pond in oak forest 250 m SE of Convento del Bosco ai Frati (convent) 2.3 km NNW of village	43°59'03.7"N, 11°18'23.8"E	260	2018-04-27 ZK, J. Hanzlíčková & P. Koutecký	MN091585	MN092168	
C. stagnalis	C13-072	159	1	2.99±0.04	Norway; Møre og Romsdal County; Herøy municipality; Runde Island; Goksøyr: wet places on path on NW part of island, 1.3 km WNW of village	62°24'29.0"N, 5°35'47.4"E	246	2013-08-07 K. Kabátová	MN091586	MN092169	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. stagnalis	C16-022	160	1	**3.02±0.03**	Portugal; reg. Algarve; Aljezur municipality; Rogil: flooded depression at channel 1.4 km S(–SSW) of village	37°23'23.6"N, 08°47'37.7"W	87	2016-05-06 JP, ZK & P. Koutecký	MN091587	MN092170	
C. stagnalis	C16-021	161	1	**2.97**	Portugal; reg. Algarve; Aljezur municipality; Rogil: flooded holes near N120 road 400 m S of village	37°21'38.9"N, 08°48'4.2"W	101	2016-05-06 JP, ZK & P. Koutecký	MN091588	MN092171	
C. stagnalis	C15-089a	24	1	**3.02**	Spain; comm. Andalusía; prov. Cádiz; Alcalá de los Gazules: small stream on the SW slope of El Picacho mountain, 9.7 km NE of town	36°31'12.5"N, 05°38'11.6"W	560	2015-05-04 P. Koutecký	MN091589	MN092172	
C. stagnalis	C16-020	162	1	**3.00±0.00**	Spain; comm. Castile and León; prov. Salamanca; Fuentes de Oñoro: Rivera de la Mimbre stream below road N-620, 3.2 km E of village	40°35'17.1"N, 06°46'20.5"W	723	2016-05-04 JP, ZK & P. Koutecký	MN091590	MN092173	
C. stagnalis	C16-004	18	1	**3.00**	Spain; comm. Castilla–La Mancha; prov. Toledo; La Iglesuela del Tiétar: Arroyo del Cuadro O de Valdelanguilla stream under bridge of CM-5006 road, 2.3 km N–NNE of church	40°15'12.8"N, 04°44'39.5"W	444	2016-05-01 JP, ZK & P. Koutecký	MN091591	MN092174	
C. stagnalis	C16-011	30	1	**3.00±0.05**	Spain; comm. Extremadura; prov. Badajoz; Herrera del Duque: Arroyo Pelochejo stream 650 m NNE of town	39°10'40.2"N, 05°2'45.9"W	420	2016-05-02 JP, ZK & P. Koutecký	MN091592	MN092175	
C. stagnalis	C16-007	21	1	**2.99±0.02**	Spain; comm. Extremadura; prov. Cáceres; Alía: Guadalupejo river and surrounding pools, 12.3 km SSE of village; pool, standing water	39°20'30.5"N, 05°9'52.3"W	379	2016-05-02 JP, ZK & P. Koutecký	MN091593	MN092176	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. stagnalis	C16-008	21	1	**2.98**	Spain; comm. Extremadura; prov. Cáceres; Alía: Guadalupejo river and surrounding pools, 12.3 km SSE of village; river, running water	39°20'30.5"N, 05°9'52.3"W	379	2016-05-02 JP, ZK & P. Koutecký	MN091594	MN092177	
C. stagnalis	C16-002	163	1	**2.97**	Spain; comm. Extremadura; prov. Cáceres; Alía: muddy ditch along road EX-102 460 m E(–ENE) of village	39°27'0.1"N, 05°12'36.3"W	561	2016-05-03 JP, ZK & P. Koutecký	MN091595	MN092178	
C. stagnalis	C16-012	164	1	**2.96±0.01**	Spain; comm. Extremadura; prov. Cáceres; Aliseda: pool in macchia 2.3 km SSW of church	39°24'11.0"N, 6°42'15.4"W	409	2016-05-03 JP, ZK & P. Koutecký	MN091596	MN092179	
C. stagnalis	C16-015	31	1	**3.01±0.05**	Spain; comm. Extremadura; prov. Cáceres; Jaraicejo: Almonte river below bridge of N-V road, 1.7 km SSW of village	39°38'46.7"N, 05°49'4.6"W	349	2016-05-03 JP, ZK & P. Koutecký	MN091597	MN092180	
C. stagnalis	C16-017	165	1	**3.01±0.01**	Spain; comm. Extremadura; prov. Cáceres; Malpartida de Plasencia: Arroyo de Barbaón stream and surrounding vernal pools, 960 m NE of railway station Monfragüe	39°56'30.1"N, 06°5'27.8"W	391	2016-05-03 JP, ZK & P. Koutecký	MN091598	MN092181	
C. stagnalis	C16-027	166	1	**3.00±0.00**	Spain; comm. Extremadura; prov. Cáceres; Torrejón el Rubio: Arroyo de la Vid stream below EX-208 road, 3.6 km NNW of village	39°48'11.7"N, 06°1'31.6"W	223	2016-05-03 JP, ZK & P. Koutecký	MN091599	MN092182	
C. stagnalis	C15-090	141	1	**3.05**	Spain; comm. Extremadura; prov. Cáceres; Torrejón el Rubio: Monfragüe area, wet grassy road margin near left bank of Tajo river, 1.6 km E(–ENE) of Monfragüe castle	39°49'47.0"N, 6°1'55.2"W	230	2015-05-06 P. Koutecký	MN091600	MN092183	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. stagnalis	C16-001	167	–	–	Spain; comm. La Rioja; prov. La Rioja; Lumbreras: small pool near Rio de Piqueras stream 1.7 km E of San Andrés settlement	42°5'36.6"N, 02°33'46.7"W	1246	2016-04-29 JP, ZK & P. Koutecký	MN091601	MN092184	
C. stagnalis	C13-053	168	1	2.97±0.01	USA; Oregon; Coos county; Coos Bay: garden pond in Mingus Park in city	43°22'18.5"N, 124°13'26.4"W	3	2013-07-29 JP & T. Kávová	MN091602	MN092185	
C. stagnalis	C13-151	169	1	**2.99±0.04**	USA; Washington; Pacific county; South Bend: pool complex between US 101 highway and estuary of Willapa river, 4.1 km NW of town	46°41'58.4"N, 123°50'49.5"W	1	2013-07-03 JP & T. Kávová	MN091603	MN092186	
C. stagnalis (autotriploid)	C13-125	170	1	4.55±0.03	Czech Republic; distr. Děčín; Jílové: on forest path 1 km NNW of Zadní Ves	50°48'26"N, 14°4'45"E	525	2013-08-28 Jan Rydlo & Jar. Rydlo	MN091604	MN092187	
C. truncata	C17-025	5	1	**1.88±0.01**	Greece; reg. Periféreia Dytikis Elládas (Western Greece); Missolonghi municipality; Katochi: drying marshes adjacent to a complex of overgrown reservoirs 1.5 km NNW of village	38°25'41.6"N, 21°14'35.8"E	5	2017-05-07 JP, ZK & P. Koutecký	MN091605	MN092188	
C. truncata subsp. *occidentalis*	C18-039	2	1	**2.45±0.02**	France; reg. Pays de la Loire; dep. Vendée; Saint-Urbain: channel (brackish water) between settlements La Ramée and Beauséjour, 1.2 km NNW of village	46°53'22.9"N, 02°1'40.4"W	0	2018-05-08 JP	MN091606	MN092189	2n=6 (this study)
C. truncata subsp. *truncata*	C18-021	26	1	**2.14±0.02**	Italy; reg. Sardegna (Sardinia); prov. Sassari; Torralba: vernal pool in pastures on basalt plateau 0.5 km W of village	40°30'50.6"N, 08°45'12.9"E	510	2018-04-23 ZK, J. Hanzlíčková & P. Koutecký	MN091607	MN092190	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
C. truncata subsp. *truncata*	C18-014	171	–	–	Italy; reg. Sardegna (Sardinia); prov. Sud Sardegna (South Sardinia); Burcei: Rio sa Picocca river 7.2 km E of village	39°20'51.7"N, 09°27'12.3"E	110	2018-04-24 ZK, J. Hanzlíčková & P. Koutecký	MN091608	MN092191	
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C18-082	172	1	**4.67±0.04**	Austria, Oberösterreich (Upper Austria); distr. Gmunden: shallows at SW edge of Almsee (oligotrophic lake with cold water) 11.5 km S(–SSW) of Grünau im Almtal	47°44'51"N, 13°57'05"E	589	2018-07-30 ZK, P. Koutecký & M. Lučanová	MN091609	MN092192	
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C13-083	173	3	4.72±0.04	Czech Republic; distr. Česká Lípa; Mimoň: ditch in park 1 km E of railway station (*resampled from population C09-017*)	50°39'26"N, 14°43'44"E	280	2013-08-19 JP & K. Kabátová	MN091610	MN092193	
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C13-115	174	1	4.65±0.06	Czech Republic; distr. Děčín; Děčín: town distr. Maxičky, lowermost fishpond 500 m S of village	50°48'19"N, 14°10'58"E	405	2013-08-28 Jan Rydlo & Jar. Rydlo	MN091611	MN092194	
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C13-116	175	1	4.67±0.05	Czech Republic; distr. Děčín; town distr. Přípeř, fishpond close to Drážďanská street 520 m of railway station Děčín-Přípeř	50°47'13"N, 14°12'10"E	180	2013-08-26 Jan Rydlo & Jar. Rydlo	MN091612	MN092195	
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C13-068	176	3	4.64±0.04	Czech Republic; distr. Domažlice; Nemanice: oxbow of Nemanický stream 800 m SW(–WSW) of church	49°25'48.6"N, 12°42'40.6"E	504	2013-08-10 M. Hrdinová	MN091613	MN092196	
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C13-082	177	7	4.63±0.03	Czech Republic; distr. Jablonec nad Nisou; Bedřichov: water reservoir on N edge of village	50°47'43.1"N, 15°8'34.8"E	733	2013-08-22 JP & K. Kabátová	MN091614	MN092197	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Elevation (m)	Collected	GenBank accession numbers ITS	***trn*T-*trn*L**	Notes
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C13-108	178	2	4.72±0.02	Czech Republic; distr. Karlovy Vary; Sedlečko: oxbow of Ohře river 330 m NNE of settlement Hubertus	50°14'27"N, 12°55'49"E	365	2013-08-29 Jan Rydlo & Jar. Rydlo	MN091615	MN092198	
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C11-016	179	5	4.62±0.03	Czech Republic; distr. Klatovy; Čepice: oxbow on right bank of Otava river 500 m ESE of bridge in village	49°15'57.5"N, 13°36'3.7"E	445	2011-06-17 JP & P. Koutecký	MN091616	MN092199	
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C12-021	59	5	4.64±0.02	Czech Republic; distr. Mladá Boleslav; Loukovec: Jizera river 1.4 km W of village	50°33'45.6"N, 14°59'41.3"E	230	2012-06-14 JP & P. Trávníček	MN091617	MN092200	2n=15 (Prančl & al., 2014)
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C12-041	180	2	4.62±0.02	Denmark; reg. Midtjylland (Central Denmark); Lemvig municipality; Bækmarksbro: Søndersund lake 460 m E of Nees church	56°23'57.0"N, 08°13'51.3"E	0	2012-08-01 JP & ZK	MN091618	MN092201	
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C18-078	68	1	**4.63±0.04**	Germany; Baden-Württemberg; distr. Schwarzwald-Baar; Donaueschingen: confluence of Brigach and Breg rivers (where Donau – Danube river originates) 1.6 km ENE of railway station	47°57'3.6"N, 08°31'12.6"E	676	2018-07-24 JP & J. Hanzlíčková	MN091619	MN092202	
C.× vigens [*C. cophocarpa* × *C. platycarpa*]	C13-132a	69	1	4.74	Germany; Bayern (Bavaria); distr. Neumarkt; Hohenfels: Follerenbach stream near S edge of settlement of Lauf	49°11'7.2"N, 11°54'45.7"E	364	2013-09-07 K. Kabátová	MN091620	MN092203	

Taxon	Ref. no.	Loc. no.	N	2C-value (pg)	Locality	Coordinates (WGS 84)	Eleva-tion (m)	Collected	GenBank accession numbers		Notes
									ITS	*trn*T-*trn*L	
Hippuris vulgaris	Hippuris 1	–	–	–	Czech Republic; distr. Nymburk; Městec Králové: Krčský fishpond 2.0 km NW of town; *cultivated in Experimental garden of Institute of Botany of C.A.S., Průhonice, Czech Republic*	50°13'12.0"N, 15°16'9.7"E	208	unknown	MN091621	MN092204	outgroup
Hippuris vulgaris	Hippuris 2	–	–	–	Czech Republic; distr. Ústí nad Orlicí; Vračovice-Orlov: hatchery pond near SE tip of Prostřední Vračovický fishpond, 330 m SW of church	49°57'7.9"N, 16°14'48.7"E	291	2015-10-08 JP	MN091622	MN092205	outgroup

THE ELECTRONIC SUPPLEMENT, TABLE S1 (PART 2). Additional 132 *Callitriche* samples taken from our previous study (Prančl et al. 2014) and used only for for the calculation of genome sizes (not for sequencing). ***Ref. no.:*** reference number unique for each combination of Taxon and Locality (i.e., if there are more taxa at one site, they have different reference numbers). ***2C-value (pg):*** Mean 2C value calculated for the sample(s) + standard deviation.

Taxon	Reference no.	2C-value (pg) + SD	Locality (country)	Coordinates (WGS 84)
C. cophocarpa	C002-08	3.25±0.03	Czech Republic	50°5'1.0"N, 14°37'30.1"E
C. cophocarpa	C003-08	3.21±0.03	Czech Republic	50°4'59.3"N, 14°35'46.9"E
C. cophocarpa	C010-08	3.18±0.01	Czech Republic	49°48'4.0"N, 15°50'35.4"E
C. cophocarpa	C016-08	3.18±0.03	Czech Republic	50°10'45.8"N, 14°46'2.0"E
C. cophocarpa	C0234-08	3.22±0.03	Czech Republic	48°44'41"N, 16°59'54"E
C. cophocarpa	C025-08	3.22±0.01	Czech Republic	49°3'22.6"N, 13°32'23.6"E
C. cophocarpa	C037-08	3.25±0.03	Czech Republic	49°4'0.2"N, 14°44'9.6"E
C. cophocarpa	C076-08	3.16±0.03	Czech Republic	48°54'52.4"N, 13°51'19.1"E
C. cophocarpa	C101-08	3.21±0.04	Czech Republic	48°51'22"N, 13°50'2"E
C. cophocarpa	C003-09	3.22±0.03	Czech Republic	49°8'3"N, 13°32'14"E
C. cophocarpa	C007-09	3.18±0.03	Czech Republic	50°14'57.6"N, 14°33'43.6"E
C. cophocarpa	C019-09	3.23±0.02	Czech Republic	50°36'39"N, 14°44'29"E
C. cophocarpa	C045-09	3.19±0.02	Czech Republic	49°53'5.8"N, 18°34'3.4"E
C. cophocarpa	C054-09	3.11±0.03	Czech Republic	49°37'19.3"N, 18°2'36.4"E
C. cophocarpa	C094-09b	3.20±0.03	Czech Republic	48°42'27.7"N, 14°41'56.2"E
C. cophocarpa	C096-09	3.20±0.02	Czech Republic	48°43'11.9"N, 14°42'56.6"E
C. cophocarpa	C102-09	3.20±0.01	Czech Republic	50°2'57.1"N, 16°7'54.4"E
C. cophocarpa	C116-09	3.17±0.00	Czech Republic	49°48'58.4"N, 16°28'52.9"E
C. cophocarpa	C004-10	3.19±0.03	Czech Republic	49°38'39.5"N, 17°12'12.8"E
C. cophocarpa	C016-10	3.17±0.03	Czech Republic	50°33'53.8"N, 15°9'23.0"E
C. cophocarpa	C019-10	3.17±0.00	Czech Republic	48°46'46.6"N, 16°45'18.0"E
C. cophocarpa	C022-10	3.18±0.03	Czech Republic	50°22'30.0"N, 15°32'52.3"E
C. cophocarpa	C034-10	3.19±0.02	Czech Republic	49°17'59.1"N, 17°43'26.4"E
C. cophocarpa	C133-10	3.20±0.02	Czech Republic	50°7'50.0"N, 16°7'59.4"E
C. cophocarpa	C002-11	3.13±0.02	Czech Republic	50°05'56.0"N, 14°36'52.2"E
C. cophocarpa	C063-13	3.17±0.05	Czech Republic	50°8'39.8"N, 12°24'49.4"E
C. cophocarpa	C066-13	3.22±0.04	Czech Republic	50°8'19.5"N, 12°24'42.5"E
C. hamulata	C013-08	8.87±0.04	Czech Republic	49°43'52.5"N, 15°55'49.9"E
C. hamulata	C027-08	8.85±0.06	Czech Republic	50°51'50.0"N, 14°19'10.5"E
C. hamulata	C029-08	8.96±0.05	Czech Republic	48°59'17"N, 14°50'54"E
C. hamulata	C044-08	8.88±0.03	Czech Republic	49°58'6.6"N, 14°50'21.2"E
C. hamulata	C004-09	8.83±0.08	Czech Republic	49°9'11.1"N, 13°30'48.4"E

Taxon	**Reference no.**	**2C-value (pg) + SD**	**Locality (country)**	**Coordinates (WGS 84)**
C. hamulata	C018-09	9.02±0.09	Czech Republic	50°37'51.6"N, 14°43'5.7"E
C. hamulata	C046-09	8.85±0.05	Czech Republic	49°53'31.8"N, 18°33'59.5"E
C. hamulata	C064-09	8.88±0.08	Czech Republic	50°59'24.3"N, 14°22'36.4"E
C. hamulata	C093-09	8.88±0.08	Czech Republic	48°42'33.5"N, 14°42'45.2"E
C. hamulata	C099-09	8.87±0.03	Czech Republic	50°2'29.6"N, 16°7'14.4"E
C. hamulata	C109-09	8.99±0.08	Czech Republic	49°50'2.7"N, 16°28'19.4"E
C. hamulata	C001-10	8.83±0.08	Czech Republic	49°46'8.2"N, 14°7'20.4"E
C. hamulata	C007-10	8.96±0.06	Czech Republic	49°59'52.7"N, 14°24'13.0"E
C. hamulata	C012-10	8.91±0.05	Czech Republic	50°30'57.0"N, 13°38'51.2"E
C. hamulata	C014-10	9.06±0.10	Czech Republic	50°35'58.6"N, 15°9'35.6"E
C. hamulata	C021-10	8.84±0.06	Czech Republic	50°22'30.0"N, 15°32'52.3"E
C. hamulata	C052-10	9.02±0.02	Czech Republic	49°37'45.6"N, 12°53'31.3"E
C. hamulata	C139-10	8.97±0.09	Czech Republic	49°47'9.2"N, 15°48'53.5"E
C. hamulata	C145-10	9.04±0.10	Czech Republic	49°44'11.8"N, 15°50'43.5"E
C. hamulata	C147-10	8.89±0.03	Czech Republic	49°36'33.7"N, 15°50'52.6"E
C. hamulata	C030-11	8.77±0.07	Czech Republic	49°17'4.3"N, 13°46'47.7"E
C. hamulata	C045-11	8.88±0.04	Slovakia	49°27'32.7"N, 19°29'50.1"E
C. hamulata	C064-12	8.78±0.08	Czech Republic	49°59'43.2"N, 16°12'36.1"E
C. hamulata	C067-12	8.91±0.05	Czech Republic	50°0'4.5"N, 16°12'13.2"E
C. hamulata	C014-13	8.88±0.06	Czech Republic	48°44'14.0"N, 17°00'21.9"E
C. hamulata	C022-13	8.84±0.05	Czech Republic	49°48'23.7"N, 14°13'50.6"E
C. hamulata	C051-13	8.85±0.07	USA, Oregon	43°55'41.7"N, 124°5'53.3"W
C. hamulata	C058-13	8.73±0.05	Czech Republic	50°9'30.8"N, 12°25'41.5"E
C. hamulata	C065-13	8.81±0.04	Czech Republic	50°8'19.5"N, 12°24'42.5"E
C. hamulata	C080-13	8.89±0.10	Czech Republic	50°48'36.9"N, 15°9'53.8"E
C. hamulata	C090-13	8.80±0.04	Czech Republic	49°59'34.0"N, 16°20'9.5"E
C. hamulata	C091-13	8.82±0.04	Czech Republic	49°59'55.6"N, 16°20'1.2"E
C. hamulata	C14-001	8.67±0.09	Hungary	46°42'59"N, 16°30'46"E
C. hermaphroditica subsp. *hermaphroditica*	C097-09	1.92±0.02	Czech Republic, cultivated	
C. hermaphroditica subsp. *hermaphroditica*	C088-12	1.94±0.01	Czech Republic	49°40'7.0"N, 16°19'59.5"E
C. hermaphroditica subsp. *hermaphroditica*	C089-12	1.94±0.02	Czech Republic	49°40'7.3"N, 16°19'53.7"E
C. obtusangula	C034-12	3.87±0.02	Denmark	55°19'17.5"N, 08°50'09.9"E
C. obtusangula	C048-12	3.88±0.02	Denmark	54°56'16.2"N, 08°41'12.1"E
C. obtusangula	C049-12	3.85±0.02	Germany	53°16'47.1"N, 10°10'59.7"E
C. obtusangula	C053-12	3.88±0.02	Belgium	51°16'38.4"N, 5°9'2.7"E
C. palustris	C008-08	3.86±0.05	Czech Republic	49°50'23.5"N, 15°47'57.6"E
C. palustris	C022-08	3.88±0.03	Czech Republic	48°43'56"N, 16°59'30"E
C. palustris	C036-08	3.90±0.03	Czech Republic	49°3'26.5"N, 14°45'20.8"E
C. palustris	C014-09	3.93±0.03	Czech Republic	50°9'18.1"N, 14°36'3.7"E

Taxon	Reference no.	2C-value (pg) + SD	Locality (country)	Coordinates (WGS 84)
C. palustris	C048-09	3.91±0.03	Czech Republic	49°54'38.5"N, 18°34'9.4"E
C. palustris	C100-09	3.87±0.04	Czech Republic	50°2'29.6"N, 16°7'14.4"E
C. palustris	C105-09	3.89±0.04	Czech Republic	49°50'28.7"N, 16°29'24.2"E
C. palustris	C002-10	3.87±0.04	Czech Republic	49°45'14.2"N, 14°8'48.5"E
C. palustris	C009-10	3.87±0.03	Czech Republic	50°36'51.4"N, 13°43'32.2"E
C. palustris	C015-10	3.90±0.02	Czech Republic	50°35'58.6"N, 15°9'35.6"E
C. palustris	C030-10	3.85±0.05	Czech Republic	49°15'17.6"N, 17°39'29.5"E
C. palustris	C041-10	3.96±0.02	Czech Republic	49°19'39.3"N, 17°43'43.7"E
C. palustris	C158-10	3.95±0.04	Czech Republic	49°9'36.7"N, 14°46'41.7"E
C. palustris	C024-11	3.87±0.03	Czech Republic	49°19'16.1"N, 13°41'29.0"E
C. palustris	C042-11	3.90±0.03	Slovakia	49°25'06.4"N, 19°30'50.9"E
C. palustris	C048-13	3.96±0.00	USA	47°51'33.8"N, 122°5'30.3"W
C. platycarpa	C065-08	6.18±0.02	Czech Republic	50°55'31.4"N, 15°0'44.8"E
C. platycarpa	C067-08	6.16±0.04	Czech Republic	50°57'59.3"N, 15°1'32.3"E
C. platycarpa	C068-08	6.15±0.05	Czech Republic	51°0'6"N, 15°2'16"E
C. platycarpa	C001-09	6.17±0.05	Czech Republic	50°22'21"N, 13°57'48"E
C. platycarpa	C023-09	6.21±0.06	Czech Republic	50°34'43"N, 14°43'42"E
C. platycarpa	C057-09	6.19±0.02	Czech Republic	50°58'50.7"N, 14°20'36.7"E
C. platycarpa	C062-09	6.26±0.06	Czech Republic	50°57'36.7"N, 14°23'28.0"E
C. platycarpa	C063-09	6.18±0.02	Czech Republic	50°58'43.7"N, 14°23'33.4"E
C. platycarpa	C065-09	6.22±0.07	Czech Republic	50°59'24.3"N, 14°22'36.4"E
C. platycarpa	C066-09	6.22±0.05	Czech Republic	51°0'29.3"N, 14°24'50.2"E
C. platycarpa	C070-09	6.18±0.02	Czech Republic	51°0'19.9"N, 14°27'5.7"E
C. platycarpa	C010-10	6.15±0.05	Czech Republic	50°37'5.2"N, 13°43'14.2"E
C. platycarpa	C063-10	6.20±0.03	Czech Republic	49°23'58.4"N, 12°51'53.2"E
C. platycarpa	C097-10	6.27±0.02	Czech Republic	50°45'7.6"N, 14°43'30.6"E
C. platycarpa	C111-10	6.19±0.02	Czech Republic	50°48'0.8"N, 14°21'43.1"E
C. platycarpa	C044-12	6.24±0.04	Denmark	55°55'06.4"N, 08°28'58.0"E
C. platycarpa	C122-13	6.16±0.00	Czech Republic	50°47'51"N, 14°5'0,5"E
C. stagnalis	C005-08	3.02±0.01	Czech Republic	49°58'23"N, 14°22'29"E
C. stagnalis	C007-08	3.02±0.02	Czech Republic	49°54'2"N, 15°47'17.8"E
C. stagnalis	C041-08	2.98±0.03	Czech Republic	49°59'19"N, 14°53'35"E
C. stagnalis	C053-08	3.00±0.01	Czech Republic	50°8'13"N, 12°13'7"E
C. stagnalis	C024-09	3.02±0.03	Czech Republic	50°34'1"N, 14°39'20"E
C. stagnalis	C038-09	2.98±0.01	Czech Republic	50°4'21.8"N, 13°4'22.7"E
C. stagnalis	C053-09	2.99±0.03	Czech Republic	49°37'35.7"N, 18°2'39.0"E
C. stagnalis	C067-09	2.99±0.01	Czech Republic	51°0'39.8"N, 14°24'35.6"E
C. stagnalis	C103-09	3.01±0.01	Czech Republic	50°2'57.1"N, 16°7'54.4"E
C. stagnalis	C017-10	3.00±0.01	Czech Republic	50°33'31.1"N, 15°9'46.5"E
C. stagnalis	C035-10	3.02±0.02	Czech Republic	49°18'23.2"N, 17°43'32.9"E
C. stagnalis	C037-10	2.95±0.01	Czech Republic	49°18'57.8"N, 17°43'32.0"E
C. stagnalis	C107-10	2.99±0.03	Czech Republic	50°48'24.0"N, 14°26'47.5"E

Taxon	**Reference no.**	**2C-value (pg) + SD**	**Locality (country)**	**Coordinates (WGS 84)**
C. stagnalis	C128-10	2.99±0.02	Czech Republic	48°55'29.0"N, 15°54'28.1"E
C. stagnalis	C152-10	2.97±0.03	Czech Republic	49°1'29.1"N, 13°43'57.1"E
C. stagnalis	C007-11	3.00±0.03	Czech Republic	50°17'58.0"N, 15°00'45.6"E
C. stagnalis	C075-12	2.95±0.03	Czech Republic	50°48'45"N, 13°59'45"E
C. stagnalis	C003-13	3.03±0.03	Czech Republic	50°26'12"N, 14°28'13"E
C. stagnalis	C054-13	2.99±0.03	USA	43°1'23.9"N, 124°25'51.8"W
C. stagnalis	C062-13	2.94±0.05	Czech Republic	50°8'57.4"N, 12°25'27.2"E
C. stagnalis	C067-13	2.98±0.03	Czech Republic	50°8'19.5"N, 12°24'42.5"E
C. stagnalis	C089-13	3.04±0.03	Czech Republic	50°10'17.4"N, 16°3'4.8"E
C.× *vigens* [*C. cophocarpa* × *C. platycarpa*]	C058-08	4.69±0.01	Czech Republic	50°8'59.7"N, 12°23'59.3"E
C.× *vigens* [*C. cophocarpa* × *C. platycarpa*]	C026-09	4.72±0.02	Czech Republic	50°38'37"N, 14°30'35"E
C.× *vigens* [*C. cophocarpa* × *C. platycarpa*]	C030-09	4.72±0.05	Czech Republic	50°23'42.6"N 14°05'0.6"E
C.× *vigens* [*C. cophocarpa* × *C. platycarpa*]	C011-10	4.65±0.04	Czech Republic	50°32'31.3"N, 13°37'2.8"E
C.× *vigens* [*C. cophocarpa* × *C. platycarpa*]	C157-10	4.64±0.05	Czech Republic	49°16'20.9"N, 14°5'50.6"E
C.× *vigens* [*C. cophocarpa* × *C. platycarpa*]	C039-11	4.63±0.04	Czech Republic	49°15'30.0"N, 13°55'48.7"E
C.× *vigens* [*C. cophocarpa* × *C. platycarpa*]	C055-13	4.62±0.07	Czech Republic	50°9'21.2"N, 12°24'37.4"E
C.× *vigens* [*C. cophocarpa* × *C. platycarpa*]	C056-13	4.64±0.06	Czech Republic	50°8'53.5"N, 12°24'0.5"E

Distribution papers

Due to the large extent, shortened versions of both distribution papers are presented here, including only the parts relevant for purposes of the thesis (i.e. Introduction, Materials and Methods and distribution maps of taxa belonging to model groups studied).

DISTRIBUTION PAPER I

Kaplan Z., Danihelka J., Chrtek J. Jr., Prančl J., Ducháček M., Ekrt L., Kirschner J., Brabec J., Zázvorka J., Trávníček B., Dřevojan P., Šumberová K., Kocián P., Wild J. & Petřík P. (2018): Distributions of vascular plants in the Czech Republic. Part 7. – Preslia 90: 425–531.

***Callitriche* ×*vigens* [*C. cophocarpa* × *C. platycarpa*], a shoot with female flowers.** Photo J. Prančl, cultivated plant originated from the town of Mimoň, Czech Republic.

Distributions of vascular plants in the Czech Republic. Part 7

Rozšíření cévnatých rostlin v České republice. Část 7

Zdeněk Kaplan[1, 2], Jiří Danihelka[1, 3], Jindřich Chrtek Jr.[1, 2], Jan Prančl[1, 2], Michal Ducháček[4], Libor Ekrt[5], Jan Kirschner[1], Jiří Brabec[6], Jiří Zázvorka[1], Bohumil Trávníček[7], Pavel Dřevojan[3], Kateřina Šumberová[1], Petr Kocián[8], Jan Wild[1] & Petr Petřík[1]

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ABSTRACT

The seventh part of the series on the distributions of vascular plants in the Czech Republic includes grid maps of 104 taxa in the genera *Anthriscus*, *Callitriche*, *Cochlearia*, *Dittrichia*, *Egeria*, *Elodea*, *Elymus*, *Epilobium*, *Gentianella*, *Gnaphalium*, *Gymnocarpium*, *Hordeum*, *Hydrocharis*, *Limonium*, *Najas*, *Phleum*, *Phragmites*, *Polypodium*, *Pseudognaphalium*, *Rubus*, *Sedum*, *Senecio*, *Setaria*, *Stratiotes*, *Trichomanes* and *Woodsia*. These maps were produced by taxonomic experts based on examined herbarium specimens, literature and field records. Many of the studied native species are on the national Red List. The genus most affected by decline in abundance is *Gentianella*, which includes six taxa extirpated from this country and six taxa critically threatened. Another group with a high proportion of endangered species comprises aquatic and wetland plants, which are represented by *Callitriche hermaphroditica*, *Hydrocharis morsusranae*, *Najas minor*, *Pseudognaphalium luteoalbum* and *Stratiotes aloides*. Other ecologically specialized groups include mainly montane wetland plants (*Epilobium anagallidifolium*, *E. nutans* and *Rubus chamaemorus*) and plants of rocky habitats (*Polypodium interjectum*, *Trichomanes speciosum* and *Woodsia ilvensis*). The previously rare *Woodsia alpina* has been extirpated from this country. Alien species mapped in this paper include both archaeophytes and neophytes, mainly from the genera *Anthriscus*, *Cochlearia*, *Elodea*, *Epilobium*, *Hordeum* and *Phleum*. *Cochlearia danica*, *Dittrichia graveolens* and *Limonium gmelinii* have recently colonized habitats along the roads treated by de-icing salt. *Senecio inaequidens* has also spread mainly along motorways. *Epilobium adenocaulon* is another successful neophyte; it is now widespread throughout this country and the most successful hybrid parent within the genus. Neophyte aquatics are represented by *Egeria densa*, *Elodea canadensis* and *E. nuttallii*. Spatial distributions and often also temporal dynamics of individual taxa are shown in maps and documented by records included in the Pladias database and available in electronic appendices. The maps are accompanied by comments that include additional information on the distribution, habitats, taxonomy and biology of the taxa.

KEYWORDS: alien species, central Europe, chorology, Czech Republic, distribution atlas, distribution patterns, endangered species, endemic, flora, grid maps, herbaria, phytogeography, plant record, vascular plants

INTRODUCTION

The mapping of the distributions of plants in the Czech Republic was initiated within the PLADIAS project (www.pladias.org) five years ago. Since then, a new central Pladias database has been established and a web-based mapping interface developed. Based on critically evaluated and sorted records stored in the database, the team of taxonomic experts has produced grid-based distribution maps of 570 vascular plants, which have been published in six papers (Kaplan et al. 2015, 2016a, b, 2017a, b, 2018).

From March to August 2018 the Pladias database increased by about 51,000 new records. Of these, nearly 25,000 resulted from the critical examination of herbarium specimens by taxonomic experts. Maps for a further 104 taxa were finished by the beginning of August 2018 and are included in this paper.

Increasing human impact on the environment is the main source of changes in the flora. Many of the native species are threatened or even on the verge of extirpation (Grulich 2012), while new alien species are being introduced (Pyšek et al. 2012). The Pladias database and the maps resulting from taxonomic revisions reflect these changes. The genus discussed in this paper that is most affected by decline in abundance is *Gentianella*. Among the twelve taxa recorded in the Czech Republic, six have been extirpated and six are critically threatened. Another group with a high proportion of endangered species comprises aquatic and wetland plants. A serious decline is documented here for *Pseudognaphalium luteoalbum*, a century ago a rather widespread species, and *Callitriche hermaphroditica*, *Najas minor* and

Stratiotes aloides are also rare and critically threatened. Some of the montane wetland species, such as *Epilobium anagallidifolium*, *E. nutans* and *Rubus chamaemorus*, and specialists of rocky habitats, such as *Polypodium interjectum*, *Trichomanes speciosum* and *Woodsia ilvensis*, are classified as endangered. The previously rare *Woodsia alpina* has been extirpated from this country.

In contrast, the Czech flora is being enriched by introduced plants. The genera with two or more aliens dealt with in this paper are *Anthriscus*, *Cochlearia*, *Elodea*, *Epilobium*, *Hordeum* and *Phleum*. These include naturalized archaeophytes (e.g. *Anthriscus cerefolium* var. *trichocarpus*, *Hordeum murinum* subsp. *murinum*, *Setaria pumila* and *S. verticillata*), casual archaeophytes (e.g. *Anthriscus caucalis*, *A. cerefolium* var. *cerefolium* and *Setaria italica*), naturalized neophytes (e.g. *Hordeum jubatum*, *Sedum hispanicum* and *Setaria faberi*) and casual neophytes (e.g. *Epilobium brachycarpum*, *E. komarovianum*, *Hordeum brevisubulatum*, *H. secalinum*, *Phleum paniculatum* and *Ph. subulatum*). Three species have been introduced only recently and colonized habitats affected by de-icing salt along roads: while *Cochlearia danica* and *Dittrichia graveolens* have already spread to many sites, *Limonium gmelinii* has been recorded at only four sites to date. Another neophyte, *Senecio inaequidens*, has also spread mainly along motorways. *Epilobium adenocaulon* is another successful neophyte; it is now widespread throughout this country and has become the most frequent hybrid parent within the genus. Neophytes are also represented among aquatics: the casual *Egeria densa* and naturalized *Elodea canadensis* and *E. nuttallii*.

In addition to the flora of the Czech Republic changing, our knowledge of it has increased. Genera that have undergone recent taxonomic revisions include *Callitriche* (Prančl 2012, 2013, Prančl et al. 2014) and *Rubus* (e.g. Krahulcová et al. 2013, Velebil et al. 2016, Király et al. 2017). Maps resulting from these revisions are also included in this paper. The distribution of the species-rich genus *Epilobium*, whose records are often erroneous due to frequent misidentifications, was mapped based mainly on examined herbarium specimens directly for the purpose of this paper; the number of examined specimens amounts to about 18,000.

MATERIALS AND METHODS

Taxonomic scope

The following groups of vascular plants are mapped: native taxa, naturalized aliens, most casuals and certain hybrids. Distribution maps are produced for species and subspecies, and in exceptional cases also for varieties or infrageneric taxa (e.g. sections). Plants of species groups that are difficult to assign to species may be mapped as species aggregates. Field crops and plants deliberately cultivated in gardens and parks are not included in the mapping project. Nomenclature, taxonomic concepts and delimitation of species aggregates mostly follow Danihelka et al. (2012), with differences indicated where necessary. For taxa not included in that checklist, a taxonomic reference is given. Publication of maps does not follow any alphabetical or systematic order, but mainly the maps resulting from recent revisions are included.

Data sources

All relevant floristic data sources are used. Major national herbaria and some local and foreign collections, incl. BRNL, BRNM, BRNU, CB, CBFS, CESK, CHEB, CHOM, FMM, GM, HOMP, HR, KHMS, KM, LIM, LIT, MJ, MMI, MP, MZ, NJM, OH, OL, OLM, OMJ, OMP, OP, OSM, OVMB, PL, PR, PRA, PRC, ROZ, SOB, SOKO, SUM, VM, VYM, W, WA, WU and ZMT (acronyms follow Thiers 2018), were consulted as the main sources of taxonomically examined records. Most records for maps of common and easy-to-identify taxa came from the recently developed Pladias database (hosted at the Institute of Botany, Průhonice), which has integrated all the available records on the distribution of vascular plants in the Czech Republic. Among the most important incorporated databases are: the Database of the Distribution of Vascular Plants in the Czech Republic (FLDOK), the Czech National Phytosociological Database (CNPD), plant records from the Floristic Summer Schools and other activities of the Czech Botanical Society, the Species Occurrence Database of the Nature Conservation Agency of the Czech Republic (NDOP), the Database of Forest Typology of the Forest Management Institute of the Czech Republic (DLT) and the Floristic Database of the South Bohemian Branch of the Czech Botanical Society (JCP CBS). Unpublished field records previously entered into the Pladias database by the maps' authors or regional contributors were also considered.

Mapping procedure

All records used for mapping are entered into the Pladias database and geographically sorted according to the traditionally used CEBA (Central European Basic Area) grid template (Niklfeld 1999) divided into quadrants of 5 × 3 arc minutes (corresponding to approximately 5.5 × 5.9 km). The territory of the Czech Republic is covered by 2551 quadrants, of which 2181 are completely within the borders of this country. Individual records and the whole distribution of each taxon are checked and evaluated by the author of a particular map in a web-based mapping interface of the Pladias database. Maps of taxonomically critical groups are based solely or mainly on herbarium specimens examined by taxonomic experts; these cases are indicated in the text accompanying the particular map. Maps of all other taxa are based on records from databases, literature and herbaria, which were scrutinized by the authors of the respective maps. Records used for producing maps are listed in Electronic Appendices 1–104. In selected maps, native versus introduced occurrences are distinguished, and corresponding records in the database classified accordingly. Draft distribution maps and the background records are released in a web-based review process for scrutiny by field botanists, regional collaborators and members of the Czech Botanical Society. Their comments and additional records are collected in the database and returned to the responsible specialists for consideration before producing the distribution maps.

Final maps and comments

The treatment of each taxon consists of a grid distribution map and accompanying text; the maps' authors, indicated in the figure captions, also had major roles in writing the first drafts of the texts for the subject taxa. Maps are displayed using a spherical Mercator projection (EPSG:3857) in which meridians and parallels appear as straight lines, and the fields of the mapping grid are thus displayed as squares. The background relief was derived from SRTM data (http://www2.jpl.nasa.gov/srtm/, the version provided by http://srtm.csi.cgiar.org) and the river network was adapted from data provided by CENIA (www.cenia.cz). When appropriate, different symbols are used on the maps to distinguish between the following alternative attribute states: (1) recent versus old records; (2) native occurrences versus introductions; and (3) records based on examined herbarium specimens versus all other records. These classifications of records are used only for those taxa where such distinction provides important information and the amount and quality of records are sufficient. The mapping symbols used to indicate the different attributes of the records in particular grid cells are shown in Table 1. Symbols specific to individual maps are explained in their captions. To save space, rare taxa of the genera Cochlearia, Epilobium, Hordeum and Phleum with distinct distributions are shown in maps in groups of two, with symbols and annotations of individual taxa on the maps distinguished using different colours. In the caption for each map, the counts of occupied quadrants are indicated according to the symbols used in the map; uncertain occurrences are not included in the counts. The accompanying text includes the accepted scientific name, a brief outline of the total distribution, information on habitats occupied by the species and a description of its distribution in the Czech Republic. Where appropriate, comments on taxonomy, biology and details of the spatial and temporal dynamics of the distribution are given.

TABLE 1. The symbols used in the distribution maps to indicate the different attributes of occurrence in particular grid cells.

Attribute distinguished	Symbol	Attribute state
None	●	All records
Time	●	Recent occurrence (at least one record since 2000)
	○	Old occurrence (all records before 2000, or demonstrably extirpated from all localities after 2000, or all records undated)
Origin	●	Native (at least one record)
	×	Alien
Source of data	●	Examined herbarium specimen (at least one record)
	▲	All other
All	?	Only record(s) uncertain regarding identification and/or locality

DISTRIBUTION MAPS AND COMMENTS

Callitriche cophocarpa (Fig. 6)

Callitriche cophocarpa occurs in central, northern and eastern Europe. It is distributed from Norway, Denmark, Germany and Switzerland in the west (apparently extirpated from the Netherlands and Belgium) to western Siberia in the east. It extends northwards to the Scandinavian coast of the Arctic Ocean; in the south it occurs as far as northern Italy, Serbia, Bulgaria and Crimea (Schotsman 1967, 1972, Lansdown 2008). In the Czech Republic *C. cophocarpa* has a wide ecological amplitude. It is a perennial species growing in a range of aquatic and wetland habitats, such as pools, oxbows, littoral zones of fishponds and other water reservoirs, ditches, drainage channels, brooks, alder carrs and puddles on forest paths. It prefers shallow (but occasionally up to 1 m deep) waters, often with strongly fluctuating water levels. It tolerates eutrophic and polluted (although not strongly turbid) habitats, often with a thick layer of sapropelic mud on the bottom, but also occurs in oligotrophic or even dystrophic habitats on acidic peat substrates (Prančl 2013). It is frequent throughout this country, being the most common *Callitriche* species in the lowlands and agricultural landscapes and also in some mountains, reaching its elevational maximum at 1260 m in the Jeseníky Mts. It is rare or locally absent from the driest lowlands lacking suitable habitats, and also from the northernmost projections of Bohemia, where it is replaced by *C. platycarpa* and *C. hamulata*. Other gaps on the map are due to under-recording rather than true absences of the species. Because of frequent misidentifications of *Callitriche* species, the distribution map was based solely on examined herbarium specimens and on plants determined using flow cytometry (Prančl et al. 2014).

Callitriche hamulata (Fig. 7)

Callitriche hamulata is mainly a European species with a clearly sub-Atlantic distribution, distributed northwards to Greenland, Iceland and northern Scandinavia. The southern limit of the species' distribution is unclear due to the difficulties in distinguishing it from the closely related Atlantic-Mediterranean species *C. brutia*, which results in frequent classification of *C. hamulata* as a variety of *C. brutia*. *Callitriche hamulata* is probably very rare or absent from the whole of southern Europe and has not yet been reliably proved to occur in the Balkan Peninsula. The eastern limit of this species' range is also poorly known. It has been recorded from Finland, the Baltic countries, Poland, Slovakia and Hungary, but there is also a single specimen from Kamchatka. It has become naturalized on the west coast of North America from Oregon northwards to British Columbia (Schotsman 1967, Lansdown 2008, Prančl et al. 2014). In the Czech Republic *C. hamulata* is a common component of vegetation of running waters, most frequently growing in upper and middle river courses, brooks and millraces with cool, oligotrophic to mesotrophic water and a sandy or less often clayey bottom. It is also frequent in pools, fishponds, ditches and puddles on forest paths, usually in clear water, often in habitats with strongly fluctuating water levels or on exposed wet substrates (Prančl 2012). The species is most frequent in the westernmost and northernmost parts as well as in colder and humid forested areas of Bohemia, including at up to 1040 m a.s.l. in the Šumava Mts. It is scattered to rare in the eastern part of this country, being absent from most of southern Moravia. It is rare in warm lowlands, being confined there mostly to rivers and alluvial pools. Because of frequent misidentifications of *Callitriche* species, the distribution map was based predominantly on examined herbarium specimens and on plants determined using flow cytometry (Prančl et al. 2014).

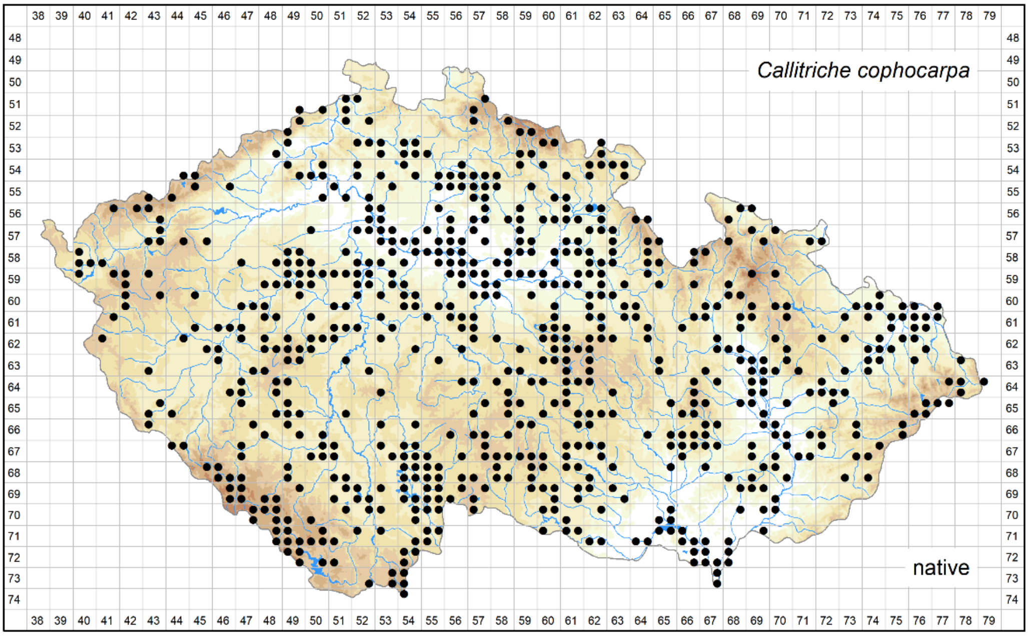

FIG. 6. Distribution of *Callitriche cophocarpa* in the Czech Republic (714 occupied quadrants). Prepared by Jan Prančl.

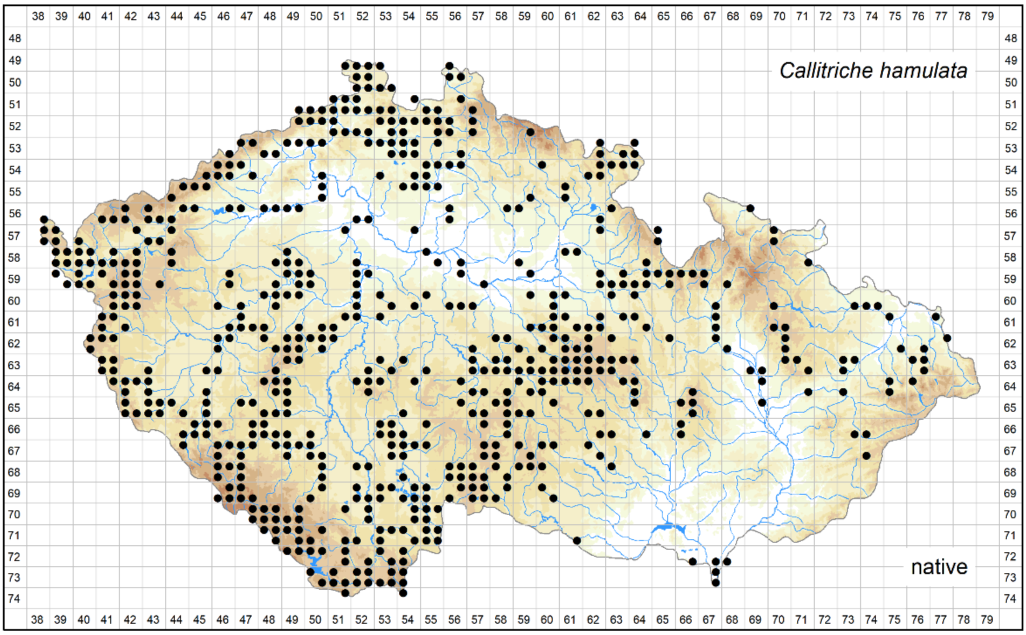

FIG. 7. Distribution of *Callitriche hamulata* in the Czech Republic (632 occupied quadrants). Prepared by Jan Prančl.

Callitriche hermaphroditica (Fig. 8)

Callitriche hermaphroditica has a circumboreal distribution, occurring throughout the Northern Hemisphere, predominantly north of 53°N. The species' distribution includes North America, Greenland, northern Europe, and boreal and mountain areas of Asia eastwards as far as the Chukchi Peninsula. In Europe it is known from Iceland, the British Isles, Scandinavia, and from Denmark and northern Germany eastwards through Poland and the Czech Republic to Ukraine, the Baltic countries and Russia (Hultén & Fries 1986, Lansdown 2008). Two subspecies are recognized, of which only subsp. *hermaphroditica* occurs in central Europe (Lansdown 2008, Prančl et al. 2014). The occurrence of *C. hermaphroditica* in the Czech Republic is isolated, being situated southwards of the species' continuous range. It appears that this species has occurred in this country only in certain periods. It was collected two times in the 19th century (in Prague and eastern Bohemia) and then it was not re-recorded for almost 100 years. However, since 1973, *C. hermaphroditica* has been found again in eastern Bohemia, being recorded reliably from about 20 localities. Unlike other central-European water-starworts, *C. hermaphroditica* is an obligatory submerged, late-flowering species, most developed in the late summer and autumn. In Bohemia it prefers clear, mesotrophic, still waters in the initial stages of succession, such as fishponds with low-intensity management and newly established reservoirs. It has been recorded only twice in rivers. The species is a weak competitor, often forming rich and dense stands but usually disappearing completely after several years (Prančl 2012). At present, *C. hermaphroditica* is critically threatened in the Czech Republic (Grulich 2012). In the last ten years it has been observed only in the Malé Dářko fishpond in the Žďárské vrchy hills and in six small ponds near the town of Polička near the Bohemian-Moravian border.

Callitriche palustris (Fig. 9)

Callitriche palustris is a holarctic species with a remarkably large distribution range covering North America, Europe, and Asia eastwards to Kamchatka, China and Malaysia. It has been also reported from Australia, where it apparently has been introduced. It is distributed across Europe, extending northwards to Iceland and the Scandinavian coast of the Arctic Ocean but is rare in western Europe and almost absent from the British Isles and the Mediterranean area (Mason 1959, Lansdown 2008). In the Czech Republic *C. palustris* appears usually to be an annual species. It is frequent on periodically exposed bottoms of fishponds, where it grows on various substrates from sand to eutrophic sapropelic mud. However, it probably avoids mineral-rich and saline habitats. It often also grows in puddles on forest paths and in other aquatic habitats with fluctuating water levels, including littoral zones of fishponds, wetlands in meadows and arable fields and on sediment accumulations in rivers. It prefers shallow, mesotrophic to eutrophic water that is warm and not shaded in summer. In the Czech Republic it has never been recorded as occurring in running water (Prančl 2012). *Callitriche palustris* is distributed throughout this country, being most frequent in fishpond basins in southern Bohemia and in other fishpond-rich areas, such as the Českomoravská vrchovina highlands. It is also common in forested areas at middle elevations but rather rare in the warmest lowlands and in mountains. The distribution map was based predominantly on examined herbarium specimens. Most of the gaps at middle elevations are due to under-recording rather than true absences.

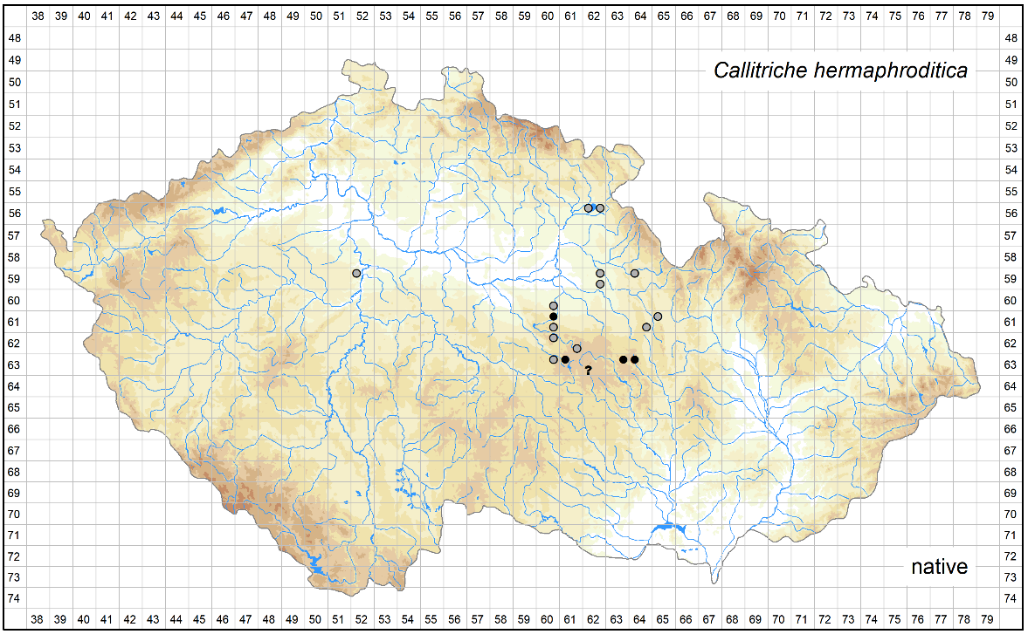

FIG. 8. Distribution of *Callitriche hermaphroditica* in the Czech Republic: ● at least one record in 2000–2018 (4 quadrants), ○ pre 2000 records only (13 quadrants). Prepared by Jan Prančl.

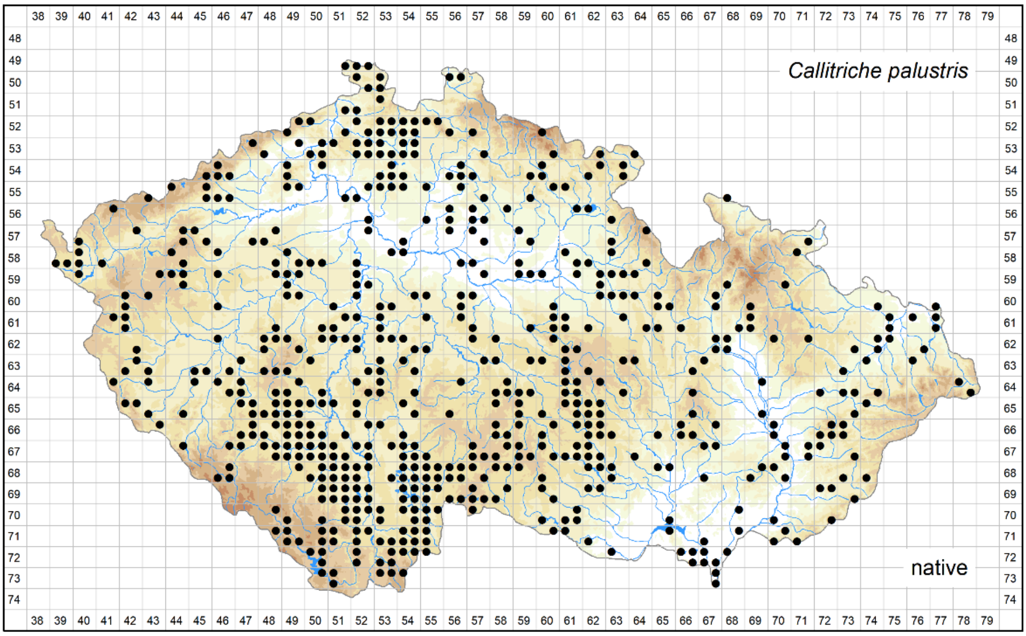

FIG. 9. Distribution of *Callitriche palustris* in the Czech Republic (621 occupied quadrants). Prepared by Jan Prančl.

Callitriche platycarpa (Fig. 10)

Callitriche platycarpa is an allotetraploid species whose diploid parents are *C. cophocarpa* and *C. stagnalis* (Bączkiewicz et al. 2007). It is a European species with a distinctive sub-Atlantic distribution. It occurs from the British Isles and France eastwards to Denmark, southernmost Sweden, western Poland, the Czech Republic and Austria, southwards to the Pyrenees, southern France and Switzerland, with small outposts in north-western Spain, southern Italy and the Aegean Islands (Lansdown 2008, Lansdown & Strid 2011, Prančl et al. 2014). In the Czech Republic *C. platycarpa* reaches the eastern limit of its continuous range, representing a sub-Atlantic floristic element. It is a perennial species occurring in various aquatic and wetland habitats such as brooks and upper courses of rivers (often together with *C. hamulata*), ditches and drainage channels, pools, oxbows, littoral zones of fishponds and puddles on forest paths. It is most common in mesotrophic to eutrophic waters with muddy bottoms but it occasionally grows also in oligotrophic habitats on sandy substrate (Prančl 2013). It is frequent in northernmost Bohemia where it is partially a vicariant of *C. cophocarpa*. It is scattered in the rest of northern Bohemia (eastwards to the vicinity of the town of Turnov) and in the westernmost part of this country. An isolated area of occurrence is situated in the southern part of the Český les hills. It has been recorded at elevations ranging from 155 m (Obrtka stream near the town of Litoměřice) to 820 m (Slavkovský les hills). *Callitriche platycarpa* is classified as vulnerable in the Czech Republic (Grulich 2012). As this species has been recognized only recently in this country, the distribution map is certainly still not comprehensive. Due to frequent misidentifications of this taxonomically challenging species, the map was based solely on examined herbarium specimens and on plants determined using flow cytometry (Prančl et al. 2014).

Callitriche stagnalis (Fig. 11)

Callitriche stagnalis is distributed throughout Europe from the Azores and Iceland in the west to western Russia in the east, but the eastern limit of its range is poorly known. Northwards it extends to the coastal parts of Norway, southern Sweden and the Baltic countries. It is widespread in the Mediterranean area and occurs also in northern Africa (Lansdown 2008). The occurrence in the Pannonian Basin is uncertain due to the absence of reliable records from Hungary (Király 2009), southern Slovakia (Zahradníková 1982) and southern Moravia (see below). The species is also known from North America, Japan, Australia, New Caledonia and New Zealand, having probably been introduced into all these areas (Mason 1959, Morita & Lee 1998, Philbrick et al. 1998). In the Czech Republic *C. stagnalis* is mostly an annual species, largely confined to very shallow muddy waters and similar sub-terrestrial habitats. Most of its occurrences are in puddles on forest paths. It is also frequent in small forest wetlands, muddy depressions, shallow ditches and edges of small forest ponds; less often it grows in brooks, channels and on muddy sediment accumulations in rivers (Prančl 2013). It is common in forested areas, often being the most common representative of the genus there. It is absent from southern Moravia and the lowlands of central Moravia. It may also be locally absent from deforested agricultural landscapes in central Bohemia. Most of the gaps at middle elevations are due to under-recording rather than true absences. The species reaches its elevational maximum at about 1000 m in the Šumava Mts, but it is rather rare in mountains.

Callitriche ×vigens (Fig. 12)

Callitriche ×vigens is a primary triploid sterile hybrid of *C. cophocarpa* and *C. platycarpa*. Its occurrence has been confirmed in southernmost Sweden, Denmark, the Netherlands, Germany, western Switzerland and the Czech Republic (Lansdown 2008, Prančl et al. 2014). It arises in areas where both parental species co-occur but it spreads vegetatively and can survive also in places from which either of the parental species has vanished. In the Czech Republic the hybrid seems to be relatively frequent in some areas of northern Bohemia and scattered in western Bohemia. However, it is also frequent in the alluvial pools of the Otava river in southern Bohemia, i.e. in the area from which *C. platycarpa* is absent. The hybrid is ecologically similar to the parental species, being found in more permanent waters that do not dry out completely in summer and do not freeze to the bottom in winter, such as in streams, ditches, water reservoirs and pools (Prančl 2013). Identification of *C. ×vigens* is very difficult. It has been recognized only recently in the Czech Republic; therefore, the distribution map is certainly incomplete.

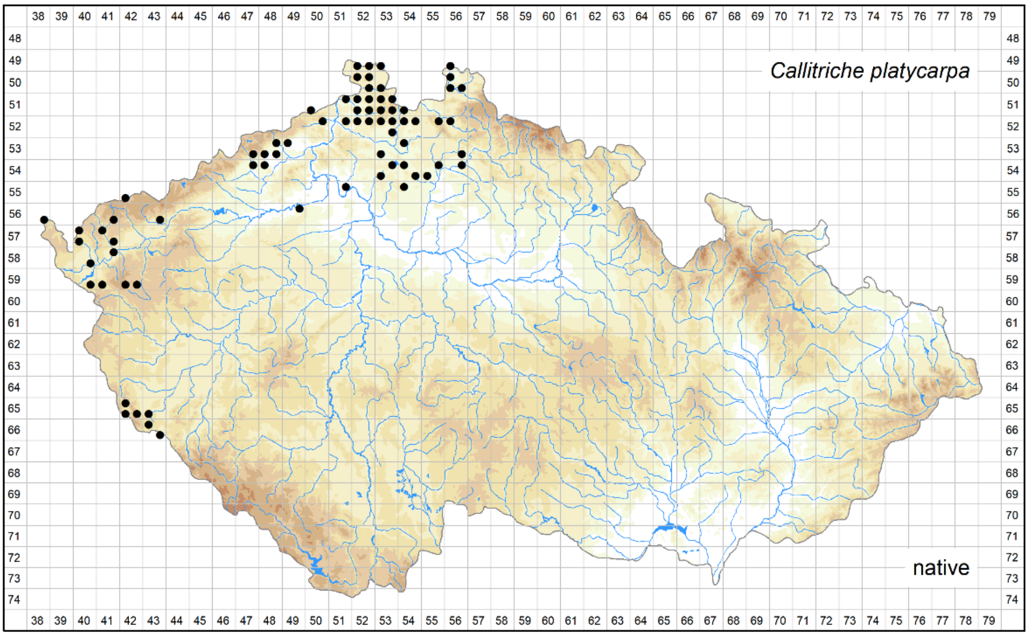

FIG. 10. Distribution of *Callitriche platycarpa* in the Czech Republic (73 occupied quadrants). Prepared by Jan Prančl.

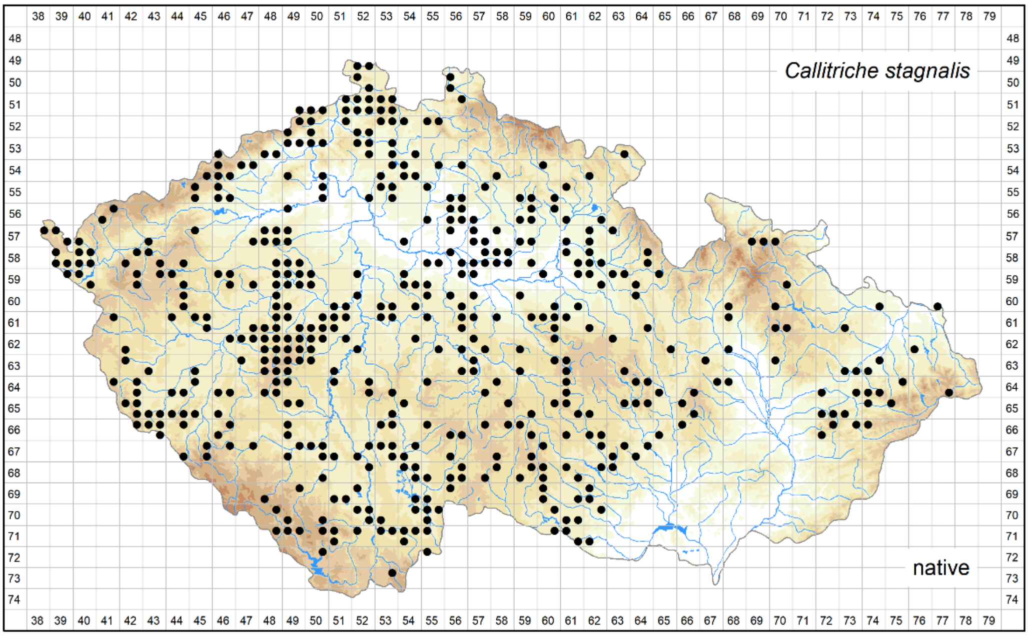

FIG. 11. Distribution of *Callitriche stagnalis* in the Czech Republic (492 occupied quadrants). Prepared by Jan Prančl.

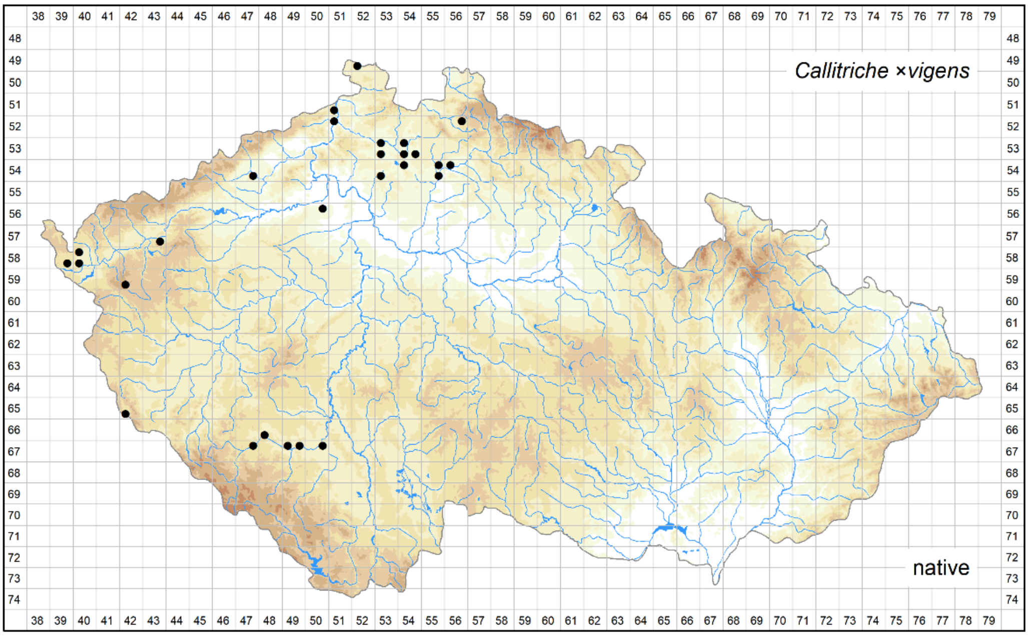

FIG. 12. Distribution of *Callitriche ×vigens* in the Czech Republic (27 occupied quadrants). Prepared by Jan Prančl.

See www.preslia.cz for Electronic Appendices 1–104

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***Ranunculus baudotii*, heterophyllous phenotype.** Photo Z. Kaplan, sandpit near Stará Voda, Czech Republic.

Distributions of vascular plants in the Czech Republic. Part 8

Rozšíření cévnatých rostlin v České republice. Část 8

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ABSTRACT

The eighth part of the series on the distributions of vascular plants in the Czech Republic includes grid maps of 106 taxa in the genera *Abutilon*, *Achillea*, *Arctium*, *Arenaria*, *Arnoseris*, *Carex*, *Chamaecytisus*, *Cornus*, *Diphasiastrum*, *Echinops*, *Galeopsis*, *Galium*, *Huperzia*, *Isoëtes*, *Lycopodiella*, *Lycopodium*, *Moehringia*, *Orobanche*, *Phelipanche*, *Prunus*, *Ranunculus*, *Selaginella*, *Stachys*, *Telekia*, *Typha* and *Zannichellia*. These maps were produced by taxonomic experts based on examined herbarium specimens, literature and field records. Many of the studied native species are on the national Red List. They are represented by plants that are rare in the Czech Republic, in extreme cases confined to single sites (*Arenaria grandiflora*, *Galium austriacum*, *Isoëtes echinospora*, *I. lacustris* and *Orobanche teucrii*), or that have experienced a considerable decline (e.g. *Arnoseris minima*, *Carex hordeistichos*, *C. secalina*, *Diphasiastrum tristachyum* and *Lycopodiella inundata*), or a combination of both (e.g. *Orobanche artemisiae-campestris*, *O. coerulescens*, *Phelipanche arenaria*, *Ph. caesia* and *Stachys germanica*). Three species (*Moehringia muscosa*, *Selaginella helvetica* and *Typha minima*) have been extirpated from this country. Alien species are represented by both archaeophytes (e.g. *Arctium lappa*, *A. tomentosum*, *Orobanche minor*, *Stachys annua* and *S. arvensis*) and neophytes (e.g. *Abutilon theophrasti* and *Typha laxmannii*). Two species have become invasive: *Echinops sphaerocephalus* spreads mainly in dry and disturbed habitats along roads and railways in warm lowlands, whereas *Telekia speciosa* is now locally frequent in various habitats mainly at middle and high elevations. *Echinops bannaticus* is reported here as a new alien species in the Czech Republic that occasionally escapes from cultivation. Spatial distributions and often also temporal dynamics of individual taxa are shown in maps and documented by records included in the Pladias database and available in electronic appendices. The maps are accompanied by comments that include additional information on the distribution, habitats, taxonomy and biology of the taxa.

KEYWORDS: alien species, central Europe, chorology, Czech Republic, distribution atlas, distribution patterns, endangered species, endemic, flora, grid maps, herbaria, phytogeography, plant record, vascular plants

INTRODUCTION

Integration of all available records on the distribution of vascular plants in the Czech Republic into a single central database and initiation of mapping their distributions were two of the aims of the PLADIAS project funded by the Czech Science Foundation in 2014–2018. The plant occurrence module within a new Pladias database (www.pladias.cz) was launched in 2014 and now contains more than 13 million records of almost 5 thousand taxa of various ranks (Wild et al. 2019). This database and an associated web-based mapping interface serve as a basic platform for mapping plant distributions within this country. Based on examined herbarium specimens as well as literature and field records, which were transferred to the database and critically evaluated and sorted by a team of taxonomic experts, grid-based distribution maps of 674 vascular plants were produced and published during the project (Kaplan et al. 2015, 2016a, b, 2017a, b, 2018a, b). Although the PLADIAS project has ended, the database continues to be maintained by the Institute of Botany of the Czech Academy of Sciences in Průhonice. Taxonomic experts collect new records and proceed in evaluating database data with the ultimate aim of producing an atlas of the distribution of vascular plants in the Czech Republic.

Since producing the maps for the seventh part of the series on plant distributions in the Czech Republic in August 2018, the Pladias database has increased by nearly 111,000 new records. Of these, over 41,000 resulted from critical examination of herbarium specimens by taxonomic experts. Maps for a further 106 taxa were finished by the end of July 2019 and are included in this paper.

A second, updated and thoroughly revised edition of the field guide "Key to the Flora of the Czech Republic", another result of the PLADIAS project, was published recently (Kaplan et al. 2019). This new inventory of Czech plant diversity is a result of the cooperation of 51 botanists, many of whose are also involved in this mapping. The Key reflects the considerable progress in plant taxonomy and floristics made since the publication of the first edition (Kubát et al. 2002) and the checklist of vascular plants of the Czech Republic (Danihelka et al. 2012). Many changes in plant systematics resulting from new phylogenetic studies and taxonomic monographs and revisions were adopted, and the novelties in the Czech flora included. This book is therefore used here as a new standard regarding taxonomic delimitations and nomenclature.

Recent taxonomic revisions of taxonomically critical groups have resulted in refining species boundaries and consequently better understanding species distributions. Maps resulting from studies on *Ranunculus* sect. *Batrachium* (Prančl et al. 2018), *Diphasiastrum* (Hanušová et al. 2014) and *Orobanche* (Zázvorka 2010) are included in this paper. Another genus with species difficult to identify is *Carex*, which is represented here by ten species.

Over two-thirds of the studied native species are on the national Red List (Grulich 2012). They represent plants that are rare in the Czech Republic, in extreme cases confined to single sites (*Arenaria grandiflora*, *Galium austriacum*, *Isoëtes echinospora*, *I. lacustris* and *Orobanche teucrii*), or that have experienced considerable declines (e.g. *Arnoseris minima*, *Carex hordeistichos*, *C. secalina*, *Diphasiastrum tristachyum* and *Lycopodiella inundata*) or a combination of both (e.g. *Orobanche artemisiae-campestris*, *O. coerulescens*, *Phelipanche arenaria*, *Ph. caesia* and *Stachys germanica*). Three species (*Moehringia muscosa*, *Selaginella helvetica* and *Typha minima*) have been extirpated from this country.

Alien species comprise both archaeophytes and neophytes. Naturalized archeophytes that are now widespread include *Arctium lappa* and *A. tomentosum*, while *Orobanche minor*, *Stachys annua* and *S. arvensis* are scattered to rare at present, and *Phelipanche ramosa* has vanished. Neophytes that have become naturalized and spread include *Abutilon theophrasti* and *Typha laxmannii*, and two species have even become invasive: *Echinops sphaerocephalus* spreads mainly in dry and disturbed habitats along roads and railways in warm lowlands, whereas *Telekia speciosa* is now locally frequent in various habitats at mainly middle and high elevations. Examination of herbarium specimens of *Echinops* led to the discovery of *E. bannaticus* as a new alien species for this country.

MATERIALS AND METHODS

Taxonomic scope

The following groups of vascular plants are mapped: native taxa, naturalized aliens, most casuals and certain hybrids. Distribution maps are produced for species and subspecies, and in exceptional cases also for varieties or infrageneric taxa (e.g. sections). Plants of species groups that are difficult to assign to species may be mapped as species aggregates. Field crops and plants deliberately cultivated in gardens and parks are not included in the mapping project. Nomenclature, taxonomic concepts and delimitation of species aggregates mostly follow Kaplan et al. (2019), with differences indicated where necessary. For taxa not included in that source, a taxonomic reference is given. Publication of maps does not follow any alphabetical or systematic order, but mainly the maps resulting from recent revisions are included.

Data sources

All relevant floristic data sources are used. Major national herbaria and some local and foreign collections, incl. BRNL, BRNM, BRNU, CB, CBFS, CESK, CHEB, CHOM, FMM, GLM, GM, HOMP, HR, KHMS, LIM, LIT, MJ, MMI, MP, MZ, NJM, OH, OL, OLM, OMJ, OMP, OP, OSM, OVMB, PL, PR, PRA, PRC, ROZ, SOB, SOKO, SUM, VM, VYM, W and ZMT (acronyms follow Thiers 2019), were consulted as the main sources of taxonomically examined records. Most records for maps of common and easyto-identify taxa came from the Pladias database (Wild et al. 2019), which has integrated data from five large national databases, several regional projects and unpublished field records from the maps' authors and regional contributors.

Mapping procedure

All records used for mapping are entered into the Pladias database and geographically sorted according to the traditionally used CEBA (Central European Basic Area) grid template (Niklfeld 1999) divided into quadrants of 5 × 3 arc minutes (corresponding to approximately 5.5 × 5.9 km). The territory of the Czech Republic is covered by 2551 quadrants, of which 2181 are completely within the borders of this country. Individual records and the whole distribution of each taxon are checked and evaluated by the author of a particular map in a web-based mapping interface of the Pladias database. Maps of taxonomically critical groups are based solely or mainly on herbarium specimens examined by taxonomic experts; these cases are indicated in the text accompanying the particular map. Maps of all other taxa are based on records from databases, literature and herbaria, which were scrutinized by the authors of the respective maps. Records used for producing maps are listed in Electronic Appendices 1–106. In selected maps, native versus introduced occurrences are distinguished, and corresponding records in the database classified accordingly. Draft distribution maps and the background records are released in a web-based review process for scrutiny by field botanists, regional collaborators and members of the Czech Botanical Society. Their comments and additional records are collected in the database and returned to the responsible specialists for consideration before producing the distribution maps.

Final maps and comments

The treatment of each taxon consists of a grid distribution map and accompanying text; the maps' authors, indicated in the figure captions, also had major roles in writing the first drafts of the texts for the subject taxa. Maps are displayed using a spherical Mercator projection (EPSG:3857) in which meridians and parallels appear as straight lines, and the fields of the mapping grid are thus displayed as squares. The background relief was derived from SRTM data (http://www2.jpl.nasa.gov/srtm/, the version provided by http://srtm.csi.cgiar.org) and the river network was adapted from data provided by CENIA (www.cenia.cz). When appropriate, different symbols are used on the maps to distinguish between the following alternative attribute states: (1) recent versus old records; (2) native occurrences versus introductions; and (3) records based on examined herbarium specimens versus all other records. These classifications of records are used only for those taxa where such distinction provides important information and the amount and quality of records are sufficient. The mapping symbols used to indicate the different attributes of the records in particular grid cells are shown in Table 1. Symbols specific to individual maps are explained in their captions. To save space, rare taxa of the genera *Echinops*, *Galium*, *Isoëtes*, *Orobanche*, *Phelipanche* and *Typha* with distinct distributions are shown in maps in groups of two, with symbols and annotations of individual taxa on the maps distinguished using different colours. In the caption for each map, the counts of occupied quadrants are indicated according to the symbols used in the map; uncertain occurrences are not included in the counts. The accompanying text includes the accepted scientific name, a brief outline of the total distribution, information on habitats occupied by the species and a description of its distribution in the Czech Republic. Where appropriate, comments on taxonomy, biology and details of the spatial and temporal dynamics of the distribution are given.

TABLE 1. The symbols used in the distribution maps to indicate the different attributes of occurrence in particular grid cells.

Attribute distinguished	**Symbol**	**Attribute state**
None	●	All records
Time	●	Recent occurrence (at least one record since 2000)
	○	Old occurrence (all records before 2000, or demonstrably extirpated from all localities after 2000, or all records undated)
Origin	●	Native (at least one record)
	×	Alien
Source of data	●	Examined herbarium specimen (at least one record)
	▲	All other
All	**?**	Only record(s) uncertain regarding identification and/or locality

DISTRIBUTION MAPS AND COMMENTS

Ranunculus aquatilis (Fig. 74)

Ranunculus aquatilis occurs in most of Europe. It is distributed from southern Scandinavia in the north to the Mediterranean area in the south, extending eastwards to western regions of European Russia. Outside Europe it has been recorded in northern Africa and in the western countries of the Middle East, but the exact eastern boundary is unclear (Cook 1966, Wiegleb et al. 2017). It is also reported to have been introduced into Chile (Lumbreras et al. 2014), but the identity of these plants should be examined. In the Czech Republic *R. aquatilis* grows in still, clear, mesotrophic to naturally eutrophic, mineral-rich aquatic habitats, often with significant fluctuations in water levels during the growing season. It most frequently occurs in pools, oxbows and fishponds in early successional stages or in sites affected by frequent disturbance. In this country it has been only rarely recorded in rivers and streams. It can also grow in periodically dry aquatic habitats, where it produces specific terrestrial forms on exposed bottoms. The species occurs mainly in the lowlands on the base-rich substrates, such as in the Labe river basin, marlstone areas of eastern Bohemia and large river floodplains in southern and central Moravia. It is absent from western and southern Bohemia and appears to have vanished from north-western Bohemia, northern Moravia and Silesia. *Ranunculus aquatilis* was classified as of lower risk – data deficient in the last Red List (Grulich 2012). This species has declined during the last decades due to eutrophication, intensive fishpond management, habitat destruction and draining. We therefore suggest its classification as vulnerable. Until recently, *R. aquatilis* has been considered the most abundant water-crowfoot species in this country; however, most of the earlier records belong to *R. peltatus*, which has not been distinguished in former floras (e.g. Husák et al. 1988). Because of frequent misidentifications, the distribution map of this species is based solely on examined herbarium specimens and on plants examined using flow cytometry (Prančl et al. 2018).

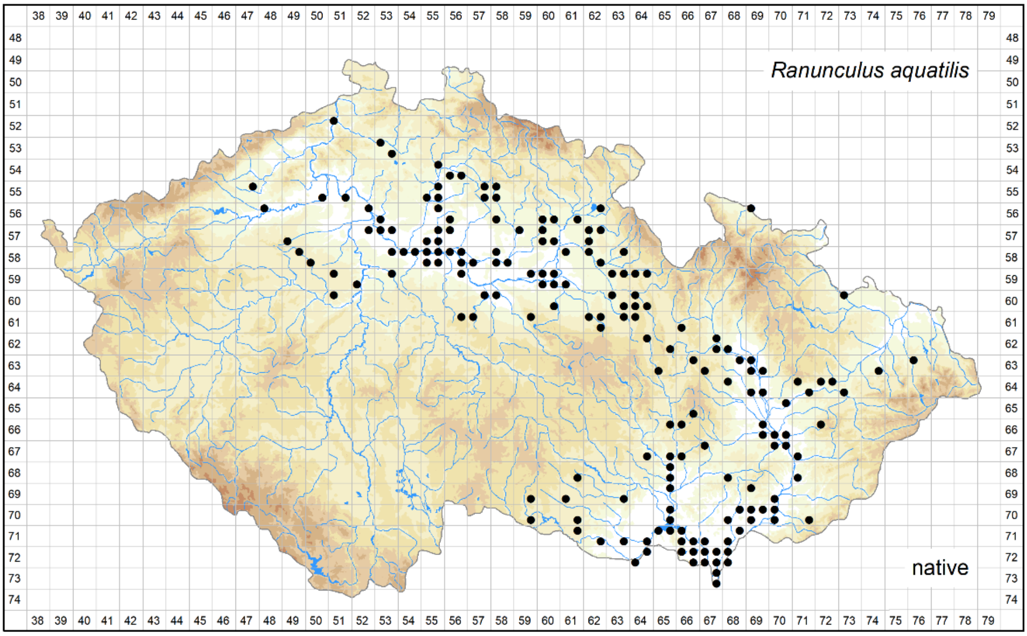

FIG. 74. Distribution of *Ranunculus aquatilis* in the Czech Republic (181 occupied quadrants). Prepared by Jan Prančl, Petr Koutecký & Zdeněk Kaplan.

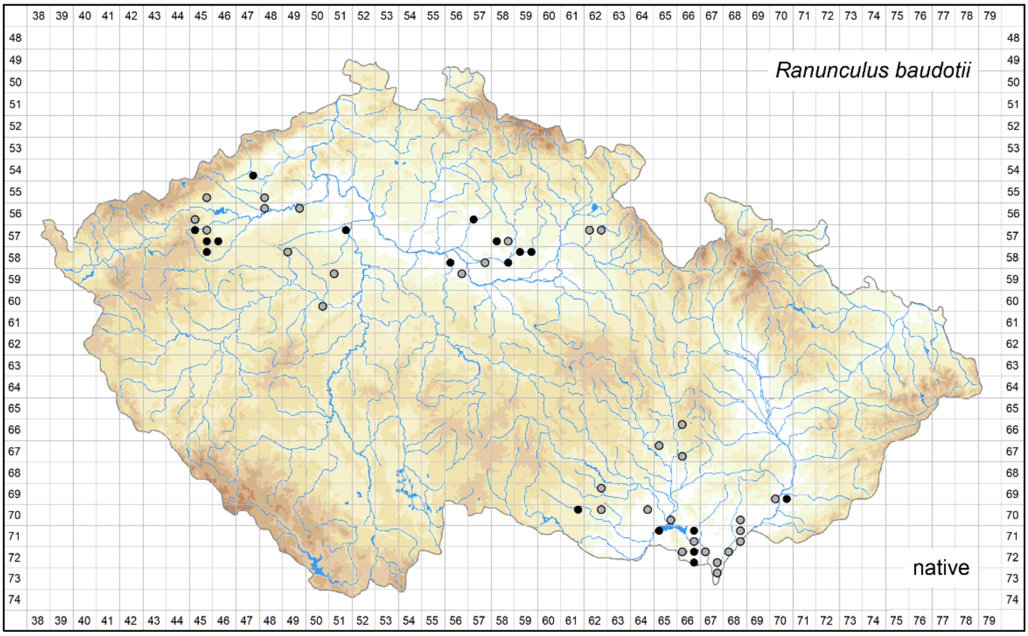

FIG. 75. Distribution of *Ranunculus baudotii* in the Czech Republic: ● at least one record in 2000–2019 (18 quadrants), ○ pre 2000 records only (31 quadrants). Prepared by Jan Prančl, Petr Koutecký & Zdeněk Kaplan.

Ranunculus baudotii (Fig. 75)

Ranunculus baudotii occurs in most of Europe south of 65°N and in northern Africa. It is characteristic of brackish waters in coastal areas across Europe but rarely grows also in inland aquatic habitats. The eastern limit of the species' range is poorly known; it is known from as far east as along the Gulf of Finland, inland areas of Slovakia and Hungary and coastal areas of Slovenia, Croatia and Greece (Cook 1966, Wiegleb et al. 2017, Prančl et al. unpubl.). In the Czech Republic *R. baudotii* grows in eutrophic, mineral-rich, sunny waters, often with high concentrations of chlorides (Šumberová 2011b). It occurs mainly in man-made habitats, such as flooded abandoned quarries or sand and gravel pits on basic substrates (especially kaolinite and calcareous sandstones and sands) and fishponds with naturally eutrophic water. It prefers early successional stages when enough nutrients are available but the water is not yet turbid or affected by massive algal development. It rarely occurs in streams. In this country *R. baudotii* is almost exclusively a lowland species, having most of its sites in southern Moravia, the Labe river basin and the kaolin mining area in the vicinity of the town of

Podbořany. The species is threatened by its overall rarity, strong eutrophication and succession in flooded quarries. It is classified as critically endangered (Grulich 2012). The map is based solely on examined herbarium specimens and on fresh plants examined by flow cytometry (Prančl et al. 2018).

Ranunculus circinatus (Fig. 76)

Ranunculus circinatus is a Eurasian species. In Europe it is restricted mainly to its temperate zone. The species' distribution reaches from Ireland, Scotland and southern Scandinavia in the north to central France, northern Italy and Slovenia southwards. The south-eastern limit of the European distribution is poorly known. In Asia the species has been reported from large areas of the temperate and boreal zones as far as easternmost Siberia (Cook 1966). It is also reported from northern Africa and south-western Asia (Wiegleb et al. 2017) but these occurrences require revision considering the absence of this species in the Mediterranean part of Europe. It occurs in mesotrophic to naturally eutrophic, base-rich sunny waters; it is also capable of growing in brackish habitats. It prefers permanent water bodies that do not dry out, and it often persists only vegetatively in deep water. In the Czech Republic *R. circinatus* occurs mainly in fishponds, alluvial pools and oxbows, abandoned flooded quarries, and sand and gravel pits in early stages of terrestrialization. It avoids organic-rich substrates such as sapropelic mud (Šumberová 2011b). Unlike elsewhere in Europe, it is only rarely found in running water. *Ranunculus circinatus* is distributed in rather warm areas in the northern half of Bohemia, with an outpost in the fishpond-rich areas of south-western Bohemia (but it is absent from the Třeboňská pánev basin), and in large river floodplains in Moravia and Silesia, being rare elsewhere. It is classified as vulnerable (Grulich 2012). The map is based on examined herbarium specimens, flow cytometric data (Prančl et al. 2018) and selected literature and database records.

Ranunculus fluitans (Fig. 77)

Ranunculus fluitans is a European species with a sub-Atlantic distribution, being most common in north-western Europe. In the west it extends to Northern Ireland, France and northernmost Spain, in the east it occurs as far as in Lithuania, Poland, Slovakia and Hungary, northwards it reaches Denmark and southernmost Sweden, southwards it is distributed as far as southern France, Switzerland, Austria and possibly also northernmost Italy (Cook 1966, Englmaier 2016, Wiegleb et al. 2017). *Ranunculus fluitans* is an obligatory running-water species characteristic of fast-running rivers and streams with a stony, gravelly or sandy bottom. In the Czech Republic it most often grows in middle reaches of rivers with cool, clear, nutrient-poor water (Šumberová 2011a), occasionally also in artificial channels and millraces. In large regulated rivers with turbid water it occurs predominantly in rapidly flowing sections below weirs. The distribution of *R. fluitans* is restricted to several dozen streams at middle and low elevations in Bohemia and south-western Moravia. Formerly this species occurred also in the Morava river in central Moravia, from which it has vanished. *Ranunculus fluitans* has somewhat declined due to river regulation, pollution and construction of water reservoirs. It is therefore classified as vulnerable (Grulich 2012). The map is based on examined herbarium specimens, flow cytometric data (Prančl et al. 2018), and selected literature and database records, mainly from those rivers where this species is also documented in herbaria. However, we rejected many non-herbarium records as the name *Batrachium fluitans* has often been erroneously used for any *Ranunculus* sect. *Batrachium* species growing in running waters.

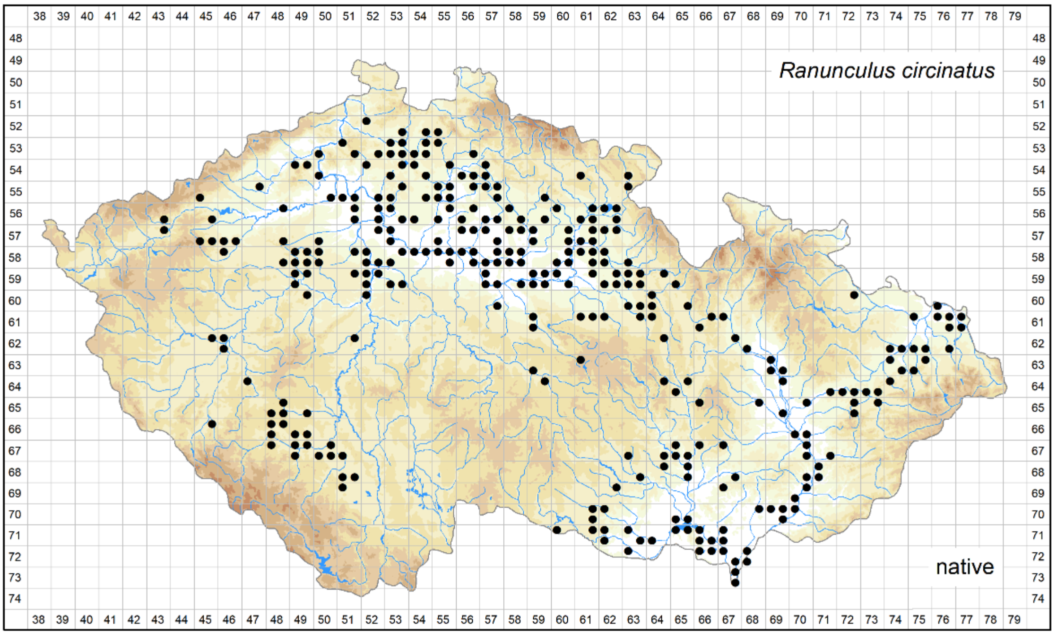

Fig. 76. Distribution of *Ranunculus circinatus* in the Czech Republic (314 occupied quadrants). Prepared by Jan Prančl, Petr Koutecký & Zdeněk Kaplan.

Ranunculus peltatus (Fig. 78)

Ranunculus peltatus is mainly a European species that includes several infraspecific taxa and different ploidy levels (Cook 1966). It is reported from the whole of the continent except the northernmost parts and also occurs in northern Africa and south-western Asia. However, the exact distribution limits in the middle and eastern Mediterranean area are unknown due to the confusion with similar taxa (e.g. *R. saniculifolius* and *R. sphaerospermus*). Also, the northern distribution limits in Scandinavia and Russia are poorly known due to the confusion with *R. schmalhausenii*. In the east, the confirmed species' distribution reaches the Baltic countries, Belorussia and Ukraine (Wiegleb et al. 2017). In the Czech Republic only tetraploid plants occur (Prančl et al. 2018). This species grows in fishponds as well as on their exposed bottoms, in various other types of pools, in rivers and brooks, often at sites with significant fluctuations in water level during the growing season. It has a relatively wide ecological amplitude and occurs in oligotrophic to eutrophic (but not highly turbid) water with sandy or organic substrate, but mostly in areas on acidic bedrock. *Ranunculus peltatus* grows throughout this country but it is rare in the lowlands. This is probably caused by the fact that the warm lowlands in the Czech Republic are composed mainly of mineral-rich sediments. *Ranunculus peltatus* is the most common water-crowfoot species in this country; however, it has somewhat declined as a result of intensive fish farming, eutrophication, river regulation and pollution. The map is based on examined herbarium specimens, flow cytometric data (Prančl et al. 2018), and selected literature and database records.

Ranunculus penicillatus agg. (Fig. 79)

The *Ranunculus penicillatus* agg. consists of various allopolyploids arising from hybridization of *R. fluitans* with several other species (*R. peltatus*, *R. aquatilis*, *R. trichophyllus* and perhaps also *R. circinatus* and *R. baudotii*; e.g. Cook 1966, Zalewska-Gałosz et al. 2014). Some phenotypes are recognized at the species or subspecies level (*R. penicillatus* s. str., *R. pseudofluitans*, *R. vertumnus*; Wiegleb et al. 2017), but the current taxonomic concept does not correspond with the actual diversity, which remains largely unrecognized (Prančl et al. 2018). The taxa of the *R. penicillatus* agg. are obligatory runningwater plants growing in rivers and streams with gravelly, sandy and muddy bottoms, both on alkaline and acidic bedrock. They are reported across Europe, with the highest concentration of localities in the western part of the continent. Northwards they reach Ireland, England, Denmark and the Baltic countries, southwards they are distributed in the western half of the Mediterranean area. The eastern limit of distribution is poorly known; however, the individual taxa have been reported from Belorussia, Ukraine and the Black Sea area (Wiegleb et al. 2017). In the Czech Republic *R. penicillatus* agg. is rare. Two tetraploid cytotypes, apparently of different origin, are reported from this country (cytotypes A and B sensu Prančl et al. 2018). Cytotype A was recorded in the Opava and Odra rivers in Silesia and in a few rivers in Bohemia, namely Sázava and Chrudimka in the Českomoravská vrchovina highlands, and Ploučnice and Spréva (Spree) in northern Bohemia, and formerly also in the Vltava river. Cytotype B has been revealed in the baserich course of the Loučná river in eastern Bohemia. *Ranunculus penicillatus* is classified as endangered (Grulich 2012). It is threatened by river regulation, pollution and eutrophication. The map is based on examined herbarium specimens, flow cytometric data (Prančl et al. 2018), and selected literature and database records.

In the Ohře river and its tributaries in north-western Bohemia, a large hybrid swarm between *R. penicillatus* agg. (cytotype A) and *R. peltatus* occurs. Some plants are morphologically intermediate while others resemble the parental species, although genome size data suggest that all analysed populations (irrespective of their morphology) are intermediate (Prančl et al. 2018). The distribution in the Ohře river basin was therefore mapped including intermediate plants of putative hybrid origin. In most of the mapping grid quadrants, the intermediates (morphology and genome size) are present. In a few quadrants, only herbarium specimens of morphology more or less typical of *R. penicillatus* agg. were available; these quadrants are mapped accordingly as pure *R. penicillatus* agg. although the specimens may represent extremes of morphological variation of the hybrid, whose occurrence in that section of the river is highly probable anyway.

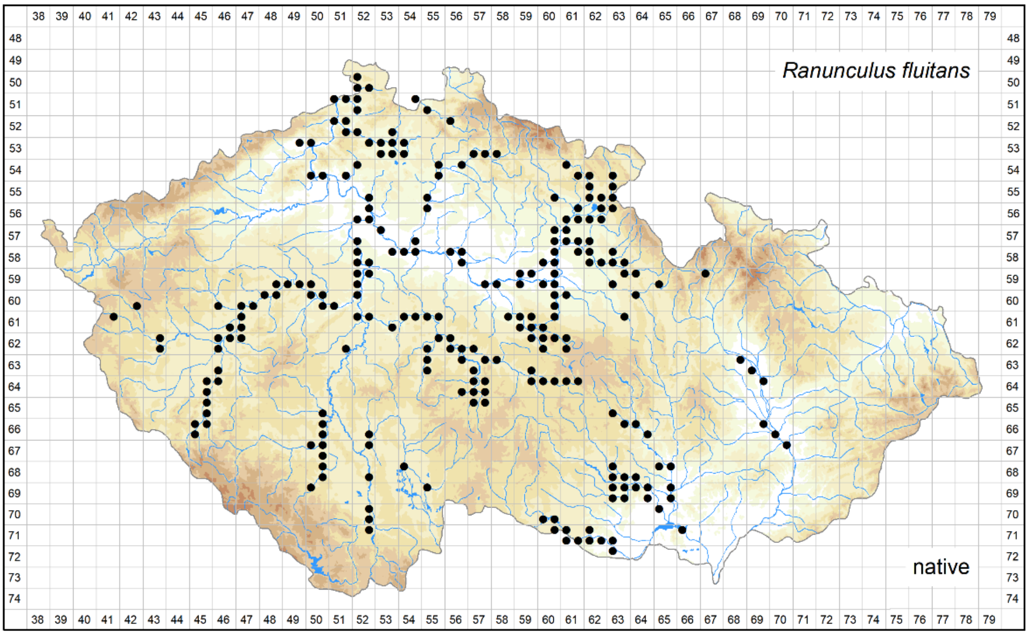

Fig. 77. Distribution of *Ranunculus fluitans* in the Czech Republic (241 occupied quadrants). Prepared by Jan Prančl, Petr Koutecký & Zdeněk Kaplan.

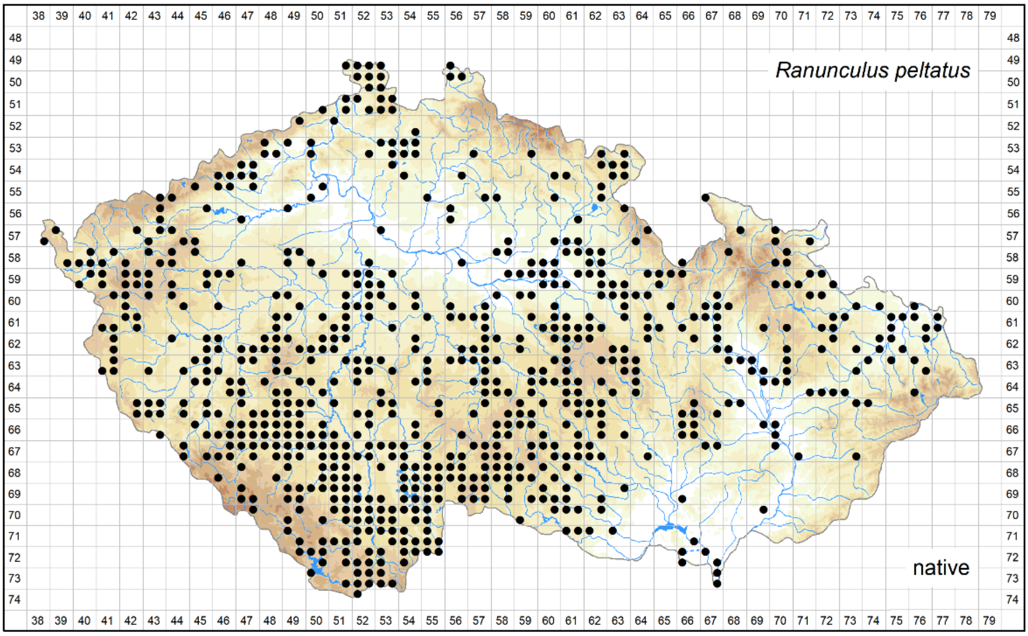

Fig. 78. Distribution of *Ranunculus peltatus* in the Czech Republic (739 occupied quadrants). Prepared by Jan Prančl, Petr Koutecký & Zdeněk Kaplan.

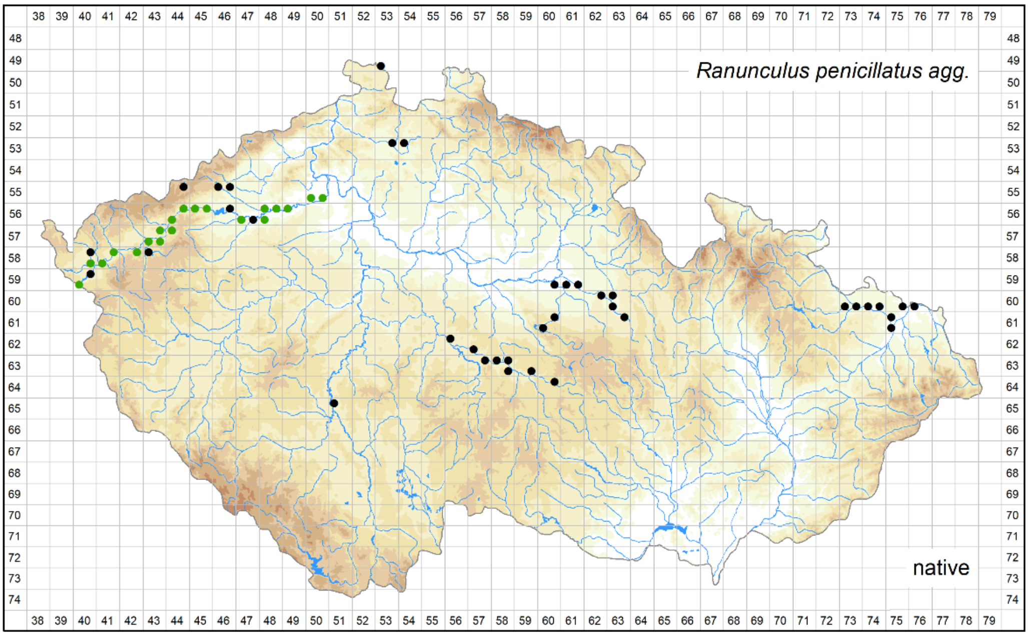

FIG. 79. Distribution of *Ranunculus penicillatus* agg. (●, 37 occupied quadrants) and the hybrid swarm of *R. peltatus* × *R. penicillatus* agg. (●, 20 occupied quadrants) in the Czech Republic. Prepared by Jan Prančl, Petr Koutecký & Zdeněk Kaplan.

Ranunculus rionii (Fig. 80)

Ranunculus rionii is distributed in Europe, western and central Asia eastwards to China. In Europe it is distributed mainly in the south-eastern part of the continent, reaching easternmost France in the west and central Germany, the Czech Republic, Slovakia and Ukraine in the north (Cook 1966, Wolff 1989, Wiegleb et al. 2017). In the Czech Republic *R. rionii* grows in still, eutrophic, mineral-rich sunny aquatic habitats, often with elevated salt concentration, mostly in the lowlands. It is characteristic of habitats in early successional stages, but also tolerates polluted and turbid waters. It mainly occurs in shallow, warm fishpond margins and on exposed bottoms of fishponds, in flooded abandoned quarries and sand and gravel pits on base-rich substrates and occasionally also in alluvial pools. It appears to be an annual species, disappearing during summer. In deep water it often flowers and set fruits completely submerged. In this country *R. rionii* is most frequent in southern Moravia, while in central and northern Moravia it is rare. In Bohemia, the species occurs in four main areas, including the warmest parts of north-western Bohemia, the area west and south-west of Prague, the mildly undulating landscape between the towns of Turnov and Golčův Jeníkov and in marlstone areas of eastern Bohemia. *Ranunculus rionii* is classified as endangered (Grulich 2012). It is locally threatened by intensive fish farming. However, it is also under-recorded due to its inconspicuous appearance and confusion with *R. trichophyllus*. The species was collected for the first time in 1900 in southern Moravia, and the earliest find in Bohemia was not until 1956. In addition, most of the occurrences in this country have been recorded since the 1990s, which indicates that this species has been spreading during the past decades, probably due to the continuing eutrophication, fertilizing and liming of fishponds and perhaps also climatic change. Currently it is probably the most common species of *Ranunculus* sect. *Batrachium* in southern Moravia. The map is based on examined herbarium specimens, flow cytometric data (Prančl et al. 2018), and the limited number of literature and database records.

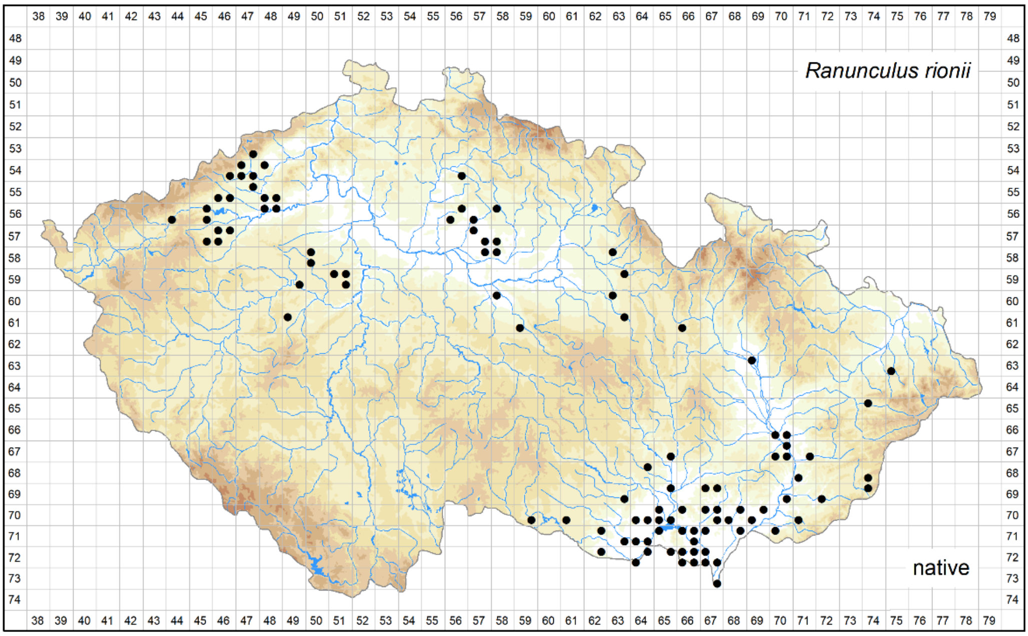

FIG. 80. Distribution of *Ranunculus rionii* in the Czech Republic (103 occupied quadrants). Prepared by Jan Prančl, Petr Koutecký & Zdeněk Kaplan.

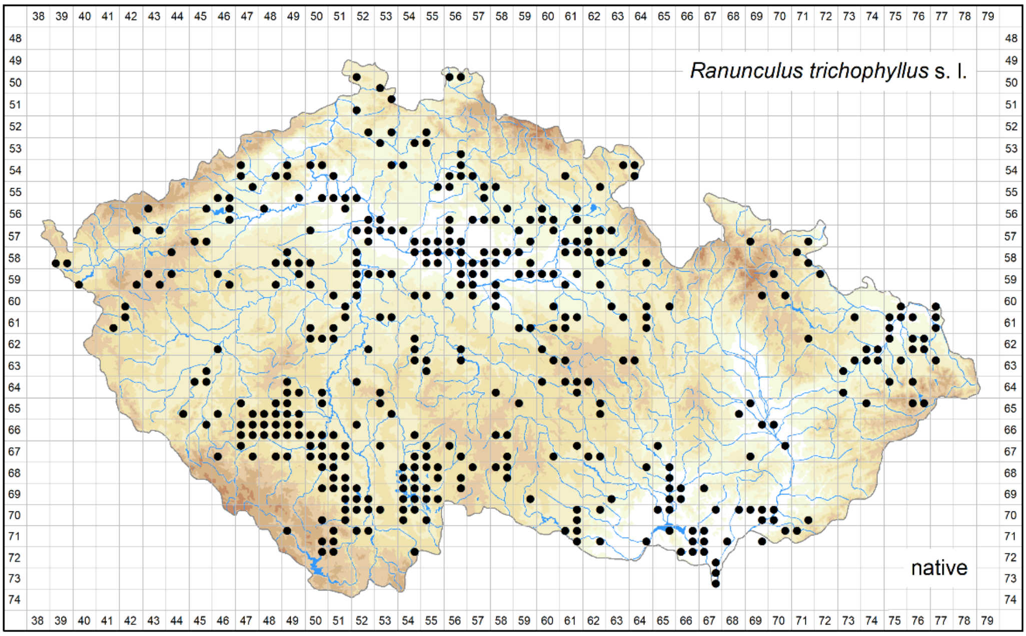

FIG. 81. Distribution of *Ranunculus trichophyllus* s. l. in the Czech Republic (410 occupied quadrants). Prepared by Jan Prančl, Petr Koutecký & Zdeněk Kaplan.

Ranunculus trichophyllus s. l. (Fig. 81)

Ranunculus trichophyllus s. l. has a sub-cosmopolitan distribution, being reported from Eurasia except for Japan, North and South America, Greenland, northern and southern Africa, Australia, Tasmania and New Zealand (Wiegleb et al. 2017). The group includes several cryptic taxa with unresolved taxonomy. In the Czech Republic two tetraploid cytotypes of somewhat different genome size were revealed (cytotypes A and B sensu Prančl et al. 2018). Based on flow cytometric data, the cytotypes significantly differ in their distributions and ecological preferences. Cytotype A occurs in mesotrophic to eutrophic water, usually on acidic substrates. It mainly grows in fishponds, water reservoirs and their exposed bottoms, occasionally also in adjacent pools. It appears to be usually annual. Most localities are situated in the fishpond basins in southern Bohemia. Cytotype B grows mostly in naturally eutrophic, base-rich water, such as in fishponds and alluvial wetlands; formerly it was also frequent in pools in lowland calcareous fens. It is mainly distributed in the lowlands, including the Labe river basin, warm areas of southern Moravia and the Morava river floodplain in central Moravia. In areas with basic bedrock it occurs also at middle elevations, such as in the vicinity of the town of Strakonice in southern Bohemia and in the marlstone areas of eastern Bohemia. Both cytotypes have been rarely found in streams. When growing in deep water, they often flower and set fruits completely submerged. Despite considerable differences, it is not always possible to assign herbarium specimens to a particular cytotype with certainty, and the map is thus prepared only for the collective species. *Ranunculus trichophyllus* is classified as of lower risk – near threatened (Grulich 2012). It has declined as a result of strong eutrophication, intensive fish farming and habitat destruction. It is also frequently overlooked due to its inconspicuous appearance and lack of prominent diagnostic characters; cytotype B may sometimes be confused with *R. aquatilis*.

Ranunculus peltatus × *R. trichophyllus* s. l. (Fig. 82)

This hybrid is known from Great Britain, Germany, Austria and the Czech Republic (Wiegleb et al. 2017, Prančl et al. 2018). It is reported also from the Aegean Islands (Dahlgren 1991), but this record requires revision. Of at least six hybrid combinations of water-crowfoots recorded in the Czech Republic (Prančl et al. 2018), this is the only hybrid that is relatively common. It occurs at sites where parental species co-occur, such as fishponds and streams, but can also persist at localities from which one or both parental species have vanished. It grows mainly in mesotrophic clear waters on sandy or organic substrates, preferring soft waters on acidic bedrock. In the Czech Republic this hybrid has an uneven frequency, with most of its localities situated in the southern Bohemian fishpond basins and in the Českomoravská vrchovina highlands (having the most plentiful localities in the upper course of Svratka river). This hybrid most often represents the F1 generation of a cross between *R. peltatus* and *R. trichophyllus* cytotype A (sensu Prančl et al. 2018; see comment on *R. trichophyllus*) but is also capable of backcrossing with both parental species. The backcrosses, which occur primarily towards *R. peltatus* and rarely towards *R. trichophyllus*, were revealed by flow cytometry (Prančl et al. 2018) and are often difficult to be recognized by morphology; remarkably, backcrossed plants are frequent in several rivers, such as Lužnice, Stropnice and Malše in southern Bohemia and Moravice in northern Moravia (Prančl et al. 2018). This hybrid is often misidentified as the morphologically similar but unrelated *R. aquatilis*. The map is based solely on examined herbarium specimens and flow cytometric data (Prančl et al. 2018).

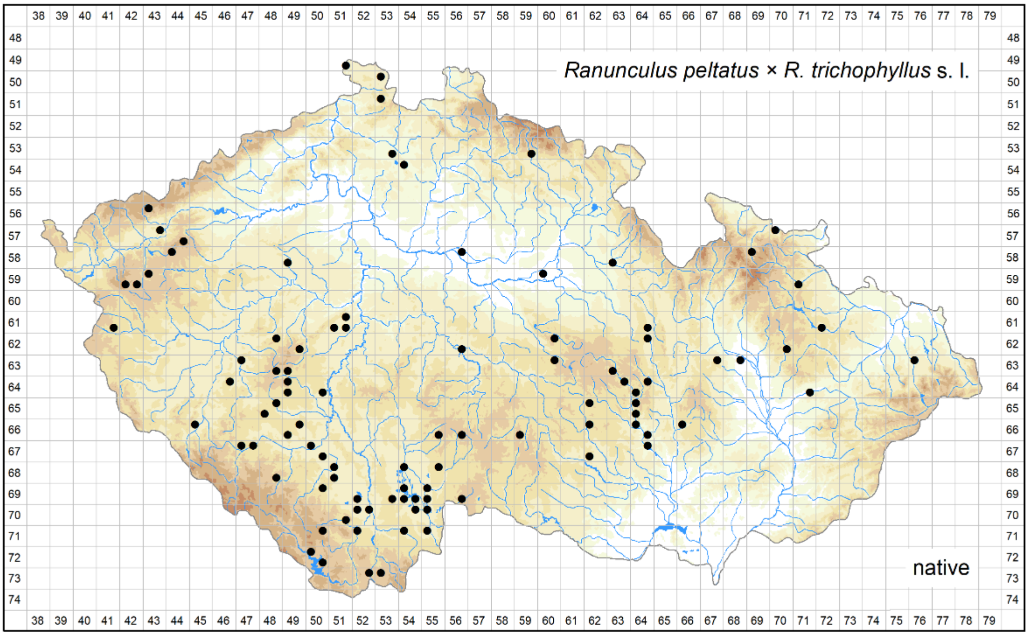

FIG. 82. Distribution of *Ranunculus peltatus* × *R. trichophyllus* s. l. in the Czech Republic (96 occupied quadrants). Prepared by Jan Prančl, Petr Koutecký & Zdeněk Kaplan.

See www.preslia.cz for Electronic Appendices 1–106

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APPENDIX: *CURRICULUM VITAE*

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* 21. 3. 1986, Prague, Czech Republic

EDUCATION:

Since 2011: PhD study in Botany, Department of Botany, Faculty of Science, Charles University (supervisor Z. Kaplan)
2008–2011: MSc study in Vascular Plant Botany, Department of Botany, Faculty of Science, Charles University (supervisor Z. Kaplan)
2005–2008: BSc study in Biology, Department of Botany, Faculty of Science, Charles University (supervisor Z. Kaplan)

RESEARCH INTERESTS:

Plant taxonomy and systematics, microevolution, breeding behaviour, flow cytometry, phytogeography, floristics

EMPLOYMENT:

Since 2012: Department of Taxonomy, Institute of Botany of the Czech Academy of Sciences
2015–2017: Department of Botany, Faculty of Science, Charles University
2012–2015: Biotechnological Centre, Department of Plant Production and Agroecology, Faculty of Agriculture, University of South Bohemia in České Budějovice (participation in the project)

TEACHING:

Evolution and diversity of vascular plants (Faculty of Science, Charles University, 2017, 2019)
Plant morphology, practical classes (Faculty of Science, Charles University, 2011–2012, 2015–2018)
Phylogeny and morphology of vascular plants, practical classes (Faculty of Science, Charles University, 2011–2017)
Field courses of ecological floristics (Faculty of Science, Charles University, since 2012)
Field course in botany (Faculty of Science, Charles University, 2014–2017)
Summer school of field botany (Czech Botanical Society, leading excursions since 2015)

SUPERVISION:

Johana Hanzlíčková (Department of Botany, Faculty of Science, Charles University, Msc study in Vascular Plant Botany since 2017, Bsc study in Biology 2016–2017)

GRANT PROJECTS:

Misunderstood patterns of cryptic variation in aquatic plants caused by hybridization and polyploidization events (GAČR 17-06825S, 2017–2019, team member)

Plant diversity analysis and synthesis centre (PLADIAS) (GAČR 14-36079G, 2014–2018, team member)

Microevolutionary processes and cytotaxonomic structure of water crowfoots (*Ranunculus* subgen. *Batrachium*) in Central Europe (GAUK 744213, 2013–2015, project leader)

Is invasiveness of aquatic plants triggered by polyploidization? Story of *Myriophyllum* species (MŠMT LH12099, 2012–2015, team member)

SCI PUBLICATIONS:

Kaplan Z., Danihelka J., Chrtek J. Jr, Zázvorka J., Koutecký P., Ekrt L., Řepka R., Štěpánková J., Jelínek B., Grulich V., **Prančl J.** & Wild J. (2019): Distributions of vascular plants in the Czech Republic. Part 8. – Preslia 91: 257–368.

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Prančl J. (2015): Lakušníky – výkladní skříň evoluce skrytá v našich vodách [Water crowfoots: a showcase of evolution hidden in our water bodies]. – Živa 63: 12–15.

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