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Homoploid hybrid speciation in closely related taxa of wetland plants

Homoploidní hybridní speciace u blízce příbuzných taxonů mokřadních rostlin

Doctoral thesis

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Contents

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Declaration

I declare that I have written this thesis independently using the mentioned references. It has not been submitted elsewhere, in full or in part, to obtain the same or other academic degree.

Prohlašuji, že jsem disertační práci napsala samostatně, s využitím uvedených informačních zdrojů a literatury. Tato práce, ani žádná její část nebyla předložena k získání stejného nebo jiného akademického titulu.

Soňa Píšová

Author contributions

Paper I. **Píšová S.** & Fér T. (submitted): Homoploid hybrid speciation in *Sparganium erectum*: molecular, genome size and morphometric analyses.

Field work; molecular, genome size and morphometric analyses, including data analyses; writing paper – 90%

Paper II. Píšová S., Hroudová Z., Chumová Z. & Fér T. (2017): Ecological hybrid speciation in central-European species of *Bolboschoenus*: genetic and morphological evaluation. – Preslia. 89: 17–39.

Field work; molecular and morphometric analyses of achenes, including data analyses; writing paper – 80%

Paper III. Píšová S., Hroudová Z., Chumová Z., Schmickl R. & Fér T. (manuscript): Convergent evolution, migration and homoploid hybridization in the genus *Bolboschoenus* (Cyperaceae) on a worldwide scale.

Field and garden work; molecular and morphometric analyses of achenes, including data analyses; writing paper – 70%

Summary

Wetland plants share several common characters, such as clonality, wind pollination and self-compatibility that facilitate hybridization, especially in complexes of closely related taxa. In this thesis, a *Sparganium erectum* complex of four subspecies and 14 species of the genus *Bolboschoenus* were investigated to detect hybridization and verify the origins of putative hybrids.

The first part of the thesis is dedicated to an introduction to hybridization, a process of great evolutionary impact, and its several general consequences, which are broadly discussed with numerous examples. In addition, an introduction to the studied taxa is provided as well as the main results of three papers that are presented and discussed herein.

The second part of the thesis consists of three papers on hybridization within the *Sparganium erectum* aggregate (Paper I) and in the genus *Bolboschoenus* (Paper II, central European species; Paper III all 14 species worldwide). AFLP molecular marker analysis, sequencing of nuclear and chloroplast DNA, and genome size and morphometric analyses were applied to elucidate the genetic relationships among taxa and to confirm the suitability of morphometric characters for taxa and hybrid delimitation.

The results clearly present the differentiation of individual taxa and their stable hybrids with intermediate morphology and intermediate genetic information. These hybrids were proven to inhabit divergent and/or disturbed habitats, unlike their parents. Moreover, several recent hybrids were detected; this finding indicates ongoing introgression, which might be adaptive in at least one recent hybrid.

In addition, the convergent morphology of *Bolboschoenus* species among continents appears to be the result of the migration or long-distance dispersal of already-differentiated morphotypes to North America and Australia. True convergence may only be observed in the independent hybrid origin of *B. novae-angliae* from North America, whereas Asian and Australian hybrids might have polytopic origins.

To conclude, in this thesis, the hybrid origins of several putative homoploid hybrid taxa with intermediate characters were confirmed, and younger hybrids indicating recent hybridization were detected.

Souhrn

Mokřadní rostliny sdílí několik společných vlastností jako je klonalita, opylování větrem a samoopylování, které usnadňují hybridizaci, zvláště u komplexů blízce příbuzných taxonů. V této práci byl zkoumán komplex čtyř poddruhů *Sparganium erectum* a komplex 14 druhů rodu *Bolboschoenus* za účelem odhalení hybridizace a ověření původu předpokládaných hybridů.

První část práce se zabývá všeobecným úvodem k hybridizaci, procesu s velkým evolučním dopadem, a několika jejími obecnými důsledky, které jsou zde široce diskutovány a doplněny konkrétními příklady. Zároveň jsou zde představeny studované taxony stejně jako hlavní výsledky tří článků, které jsou následně diskutovány.

Druhá část práce obsahuje tři články o hybridizaci v komplexu *Sparganium erectum* (první článek) a v rodě *Bolboschoenus* (druhý článek, středoevropské druhy; třetí článek, 14 druhů z celého světa). K objasnění fylogenetických vztahů mezi taxony a ověření spolehlivosti morfologických znaků odlišujících taxony a jejich hybridy bylo použito AFLP (polymorfismus délek amplifikovaných fragmentů), sekvenování jaderné a chloroplastové DNA, analýza velikosti genomu a morfologické analýzy.

Ve výsledcích bylo jasně ukázáno odlišení jednotlivých taxonů a jejich stabilních hybridů s přechodnými morfologickými znaky a sdílenou genetickou informací. U těchto hybridů bylo prokázáno, že se na rozdíl od svých rodičů vyskytují na odlišných a/nebo narušených stanovištích. Kromě toho bylo zjištěno několik současných hybridů, což ukazuje na stále probíhající introgresi, která by mohla být adaptivní minimálně u jednoho z nich.

Potvrzená konvergentní morfologie druhů rodu *Bolboschoenus* mezi kontinenty se ukazuje spíše jako výsledek migrace nebo šíření na dlouhé vzdálenosti již odlišených morfotypů do Severní Ameriky a Austrálie. Za skutečnou konvergenci může být považován jen nezávislý hybridní původ druhu *B. novae-angliae* v Severní Americe, zatímco hybridní taxony z Asie a Austrálie mohly vzniknout polytopně.

Závěrem lze konstatovat, že v této práci byl potvrzen hybridní původ několika domnělých homoploidních hybridních taxonů s přechodnými morfologickými znaky. Zároveň byla prokázána existence recentních hybridů naznačující nedávnou hybridizaci.

Introduction

Hybridization

The term "hybridization" has been broadly used, with definitions ranging from the crossing of genetically distinct individuals of the same taxon to the crossing of individuals from different taxa, i.e., interspecific hybridization (Harrison 1993, Arnold 1997). Estimates of hybridization among plant species in the literature vary depending on the methodology applied, the studied flora and the authors. To date, hybridization has been considered to be ubiquitous (Stebbins 1959, Whitham et al. 1991). However, flora surveys suggest that the percentage of plant species that hybridize ranges from 3% of angiosperms (the Concord Flora, Massachusetts) to 25% of angiosperms and pteridophytes (the flora of the British Isles, Mallet 2005). However, this process is unevenly distributed, with some plant groups having a higher propensity for hybridization than that of others (e.g., Cyperaceae; Ellstrand et al. 1996). Interspecific hybrids occur in 40% of families and 16% of genera of vascular plants, with an overall percentage of approximately 10% (Whitney et al. 2010a). Furthermore, the frequency of hybridization is significantly higher in perennial, clonal and/or closely related species than that in annual, sexually reproducing and/or distantly related ones (Rieseberg 1997, Mallet 2007). Interspecific hybrids are also common in crop plants and invasive species (Ellstrand & Schierenbeck 2000, Ellstrand 2003). Remarkably, percentages of hybridization up to 63% of hydrophyte monocots genera have been reported (Les & Philbrick 1993).

Natural hybridization plays important roles in plant speciation. It may have evolutionary consequences, e.g., an increase in genetic diversity (Anderson 1948), the origin of adaptations and their transfer (Stebbins 1959), the origin of new taxa (Grant 1981), and the reinforcement or breakdown of reproductive isolation (Levin et al. 1996). Hybrid speciation may occur at the same ploidy level (homoploid hybrid speciation) or at the polyploid level after chromosome doubling (allopolyploid speciation, Soltis & Soltis 2009). In comparison to homoploid hybrid speciation, allopolyploidy is much more common (11% of plant species, Barker et al. 2016), partly due to the creation of a reproductive barrier that reduces backcrossing efficiency with parental taxa. However, gene flow may occur between ploidy levels, either through triploids or unreduced gametes (Stebbins 1971, Soltis et al. 2010). Homoploid hybrid

formations tend to occur between closely related taxa (Darlington 1937), whereas allopolyploidy is more common at higher levels of parental divergence (Paun et al. 2009). Newly formed polyploids must compete with their parents for reproductive opportunities. As polyploids commonly possess only post-zygotic reproductive isolation (poor endosperm development or decreased fitness of hybrids due to failures in meiosis, Baack et al. 2015), their gametes (2n) are much less frequent than are those of diploids (1n), and sterile triploids are mostly formed. This phenomenon is known as minority cytotype exclusion and was described by Levin (1975). The establishment of allopolyploids may be facilitated by increased self-fertilization ability, hybrid vigour, increased frequency of polyploid individuals, increased unreduced gamete formation in diploids and slight niche differentiation (Soltis et al. 2010).

Unlike polyploids, homoploid hybrids are not immediately reproductively isolated from their parents. A chromosomal or sterility barrier is required to decrease gene flow between species that coexist in sympatry (Yakimowski & Rieseberg 2014). A speciation process that involves chromosomal rearrangements or gene incompatibilities was described as recombinational speciation by Grant (1981). Furthermore, geographic or ecological isolation has also been found to be essential for the establishment of a homoploid hybrid species, even in the absence of a chromosomal or genetic sterility barrier (described below; Abbott et al. 2010, Abbott & Rieseberg 2012). Habitat specialization of hybrid species relative to the habitats of their parental taxa has been reported in several studies (Wang et al. 2001, Donovan et al. 2010, Hroudová et al. 2014). Examples of ecological divergence of some homoploid hybrids have been summarized by Gross and Rieseberg (2005). In a study of the diploid hybrid species *Pinus densata* Masters (*P. tabuliformis* Carrière × *P. yunnanensis* Franch.), which occurs in an extreme habitat (at high elevation), niche modelling was used to detect both spatial and ecological separation from its parent species (Wang et al. 2001, Mao & Wang 2011). A preference for extreme habitats was also reported in two Hawaiian homoploid hybrids, *Scaevola kilaueae* O.Deg. (*S. coriacea* Nutt. × *S. chamissoniana* Gaudich.) and *S. procera* Hillebr. (*S. gaudichaudii* Hook. & Arn. × *S. mollis* Hook. & Arn.). The hybrid *S. kilaueae* inhabits novel habitats of young lava flows, and *S. procera* occurs in wetter forests and at higher elevations than its parents do (Howarth & Baum 2005).

Hybrid zones and adaptive introgression

A hybrid zone is a narrow area where genetically different populations meet and hybridize (Barton & Hewitt 1985). In the absence of an open habitat, the formation of stable hybrid zones is more common than is adaptive introgression or homoploid hybrid speciation, but a combination of these hybridization outcomes is possible (Buerkle et al. 2000). Hybrid zones occur at boundaries between different habitats where taxa meet and form offspring of mixed ancestry (Harrison 1993). Such zones are often considered as valuable opportunities for studying evolutionary processes such as speciation (Harrison 1990). These zones can be very narrow (e.g., a few hundred metres wide) and long (e.g., several hundreds of kilometres) in the case of secondary contact between two allopatric species, or they may be in the form of local hybrid swarms within the distributions of two sympatric taxa (primary contact). The zones may contain a vast variety of genotypes that result from hundreds or thousands of generations of recombination (Rieseberg 1999b). Most hybrid zones are maintained through a stable balance between dispersal and selection against hybrids (Barton & Hewitt 1985). This scenario was observed in a hybrid zone of *Senecio aethnensis* Jan ex DC. and *S. chrysanthemifolius* Poiret on Mount Etna, Sicily, where selection against hybrids maintains the hybrid zone, and species distinctiveness remains despite gene flow between the parent species (Brennan et al. 2009).

Reproduction of natural hybrids is often limited to backcrossing with parental species due to the insufficient spatial separation of hybrids as a consequence of a lack of intermediate habitat for hybrids (Anderson 1948). It has been repeatedly suggested that habitat disturbance may promote hybrid formation through the breakdown of ecological barriers and the creation of intermediate niches for hybrids (Stebbins 1959, Rieseberg et al. 1999b, Howard et al. 2004). A well-known example is the occurrence of hybrid zones between two North American sunflowers, *Helianthus annuus* L. and *H. petiolaris* Nutt. Three hybrid zones between these species were examined, each of which occurred in human-disturbed sites (Rieseberg 1999b). However, the first generation of hybrids was highly sterile, with fertility regained in later generations. Thus, backcrossing of the hybrids with their parental species facilitated introgression that increased the ability of the two parental species to inhabit new areas (Heiser 1947).

Moreover, genetic mapping experiments revealed that these species are divergent chromosomally and that these chromosomal rearrangements contribute to reproductive isolation by reducing pollen viability in hybrids (Rieseberg et al. 1999b).

Through hybrid zones and hybrid backcrosses, genetic material can be transferred between taxa. Although this introgression is often seen as maladaptive, adaptive introgression is being reported in an increasing number of studies (wherein beneficial alleles from one species are incorporated into the gene pool of another; Anderson 1953, Harrison & Larson 2014, Schmickl et al. 2017). One well-documented case is interspecific gene exchange in *Senecio*. After the introduction and dispersal of the diploid hybrid species *S. squalidus* L. (its origin is described below) throughout the United Kingdom, it started crossing with the native allotetraploid *S. vulgaris* L. Whereas *S. squalidus* is animal-pollinated and has ray florets, *S. vulgaris* is self-pollinated and possesses only central disk flowers. Introgression of the radiate trait from *S. squalidus* to *S. vulgaris* led to floral asymmetry and the development of ray flowers in *S. vulgaris*. The introduction of ray traits into *S. vulgaris* could facilitate a switch to outcrossing and even rescue it from potential extinction (Kim et al. 2008, Rieseberg 2009).

Another example of adaptive introgression is provided by Louisiana irises. Transplantation experiments of *Iris fulva* Ker Gawl. (flood tolerant, occurring in swamps), *I. brevicaulis* Raf. (occurring in drier hardwood forest) and their hybrids confirmed expected scenario that backcrosses to *I. fulva* are flood tolerant and that backcrosses to *I. brevicaulis* show increased survivorship in flood conditions (Arnold 2004, Martin et al. 2006).

Introgression through hybrid individuals can thus play key roles in adaptive evolution (Anderson 1953, Lewontin & Birch 1966, Minder et al. 2007). However, introgression can also cause phylogenetic discordance, especially in closely related taxa (lineage reticulation, Mallet et al. 2016).

Homoploid hybrid speciation

Tracing the origin of a new hybrid that lacks a change in chromosome number from the parent species (e.g., by chromosomal rearrangement) is difficult because reproductive isolation has to be developed in sympatry (Rieseberg 1997). This process is known as a homoploid hybrid speciation. Until recently, homoploid hybrid speciation was thought to be rare (Abbott et al. 2013), but evidence of this phenomenon is increasing (Yakimowski & Rieseberg 2014, Nieto Feliner et al. 2017). The establishment of a new homoploid hybrid was described by chromosomal models (Stebbins 1959, Grant 1981) as follows: First, the two parental taxa should be distinguished by two or more separable chromosomal rearrangements. Their hybrids are typically partially or highly sterile, and this sterility serves as a barrier to gene flow from the parent species. Subsequently, through segregation and recombination, new homozygous recombinants are produced (Rieseberg 1997). Hybrid segregates can be further stabilized by either pre-zygotic or post-zygotic barriers to gene flow. However, a new hybrid can arise even in the absence of a post-zygotic barrier, such as hybrid sterility, when gene flow between the parental species is absent due to pre-zygotic isolation (e.g., ecological or geographical divergence, Rieseberg 1997). Pre-zygotic barriers are very important in limiting gene flow between wind-pollinated plant species that lack post-zygotic barriers to reproduction (Soltis & Soltis 2009). However, the establishment of hybrids may be prevented after interspecies mating by post-zygotic barriers such as endosperm failure, hybrid necrosis and hybrid sterility (Baack et al. 2015). Isolation is further facilitated by hybrid sterility, which is often caused by genetic or chromosomal barriers, and by a low abundance of hybrids. In addition, Templeton (1981) emphasized the availability of suitable habitat as crucial factor for hybrid establishment.

Hybrids have often lower fitness than their parents do, but there are several examples in which the fitness of some hybrid genotypes were equivalent or higher than those of the parents (Heiser 1947, Arnold & Hodges 1995). Increased fitness was observed in the North American hybrid *Typha* x *glauca* [Godr.] (*T. angustifolia* L. x *T. latifolia* L., Snow et al. 2010). Despite the many isolating mechanisms, many plant species can hybridize, and new hybrid taxa can be established by even a slight change in phenology, pollination or by a chromosomal mutation that increases fertility (Soltis & Soltis 2009). These phenomena are a common case, especially in plants that occupy

disturbed habitats or that have been introduced to novel habitats through long-distance dispersal events via agents such as humans or birds. The introduction of hybrid material to the British Isles from a hybrid zone between *Senecio aethnensis* and *S. chrysanthemifolius* on Mount Etna (Sicily, Italy) resulted in the recent hybrid *S. squalidus*. However, the recent hybrids, with morphology intermediate between the parental morphologies, differ in morphology from the hybrids on Mount Etna (James & Abbott 2005).

Morphological intermediacy can be expected in homoploid hybrids, but a range of outcomes is possible, from characters resembling one of the parents, to intermediate characters, to novel traits, with intergradations among these conditions (Rieseberg 1995, Soltis & Soltis 2009). Similarly, hybrid genotypes are not always intermediate between the parental genotypes, and they can vary from the genotype of one parent to that of the other. Strictly intermediate genotypes are expected in hybrid species with asexual reproduction (clonally and/or by apomixis) or in F1 hybrids; otherwise, genetic admixture should occur in hybrids, with genotypes ranging from one parental genotype to the other. Moreover, through transgressive segregation, new combinations of alleles from the parents can give rise to extreme phenotypes, which in some cases may confer fitness that exceeds that of the parents or that increases adaptation to the habitat. In a survey, approximately 58% of 579 plant traits were reported to be transgressive traits (38% among crosses between wild populations and 92% between inbred domesticated lines (Rieseberg et al. 1999a).

The recombinational model of homoploid hybrid speciation was examined in detail in North American sunflowers by Rieseberg and his collaborators (Rieseberg 1991, Lexer et al. 2003, Baack et al. 2005, Lai et al. 2005, Rieseberg et al. 2007). Molecular phylogenetic studies identified three stabilized homoploid hybrids, *Helianthus anomalus* Blake, *H. deserticola* Heiser and *H. paradoxus* Heiser, that were derived independently from the same two parent species, *H. annuus* and *H. petiolaris*. Genetic mapping has suggested that these hybrids differ by 7–12 chromosomal rearrangements from their parents and are strongly isolated from each other by a sterility barrier (Lai et al. 2005). Moreover, all three hybrids are ecologically and geographically divergent from the parental species and occupy extreme habitats. *H. anomalus* occurs on sand dunes, *H. deserticola* occurs on the desert floor (both in the Great Basin desert), and *H. paradoxus* is endemic to saline brackish marshes in western Texas. In contrast to the narrow distributions of the hybrids, their

parental species are widespread throughout the central and western United States; *H. annuus* occurs in mesic soils, and *H. petiolaris* occurs in dry, sandy soils (Heiser et al. 1969). The morphological and ecophysiological traits of the hybrid are intermediate between those of the parents, parent-like or extreme. Transgressive segregation proved to be crucial in this hybrid speciation, with 39% of the studied traits found to be transgressive (Rosenthal et al. 2002).

Clonal reproduction facilitates the stabilization of highly heterozygous hybrid genotypes, increases the number of flowering ramets per genet, increases the survival of rare genotypes and, consequently, facilitates self-fertilization, which is crucial for homoploid hybrid speciation (Grant 1981, Burke et al. 2000). A computer simulation confirmed that the probability of recombinational speciation increases with the selfing rate (McCarthy et al. 1995). In a case study of the Louisiana irises, vegetative reproduction appears to have influenced hybrid populations. Burke et al. (2000) hypothesized that along with parental habitat divergence, clonality played a role in the population establishment of the tri-hybrid *Iris nelsonii* Randolph. According to genetic analyses of nuclear and chloroplast DNA, this hybrid originated from *I. brevicaulis*, *I. fulva* and *I. hexagona* Walter (Arnold 1993). Moreover, all three parental species and the hybrid have divergent habitats: *I. fulva* occupies shady habitats, such as the banks of bayous of the Mississippi River; *I. hexagona* occupies open, freshwater marshes; and *I. brevicaulis* occupies drier oak forests and pastures. In contrast, their hybrid has invaded a novel habitat: deeply shaded freshwater swamps with high water levels (Randolph 1966). However, all three parental taxa co-occur in sympatry in southern Louisiana, where several hybrid populations are found. A highly clonal population ("Young´s Coulee") of plants that were genetically identical as and phenotypically similar to plants of *Iris nelsonii* was suggested to have given rise to the hybrid (Arnold 1993).

Homoploid hybrids have also been reported among aquatic and wetland plants that frequently share evolutionarily important features such as clonal reproduction, selfing, wind pollination, long-distance dispersal and a cosmopolitan distribution that facilitate hybrid speciation. One example is found in the genus *Carex* L., with the homoploid hybrids *Carex rostrata* Muhl. ex Willd. var. *borealis* Kük. (*Carex rostrata* Muhl. ex Willd. × *C. rotundata* Wahlenb.) and *C. stenolepis* Less. (*C. vesicaria* L. × *C. saxatilis* L.) being the results of recurrent hybridization. Both hybrids have an AFLP pattern intermediate of those of the parents but are more similar to one of the parental species in morphology (Pedersen et al. 2016). In addition, morphologically

intermediate hybrids were found in the Japanese hybrid *Nuphar* × *hokkaiensis* Shiga & Kadono (*N. japonica* DC. × *N. pumila* (Timm) DC.) (Shiga & Kadono 2007). Furthermore, investigation of the hybrid *Typha* × *glauca* (*T. angustifolia* × *T. latifolia*) in North America revealed that the F_1 hybrids were morphologically intermediate, whereas the backcrosses showed morphologies overlapping with the hybrids and the parent species (Snow et al. 2010). Several other hybrids have also been suggested based on plants of intermediate morphology in the genus *Bolboschoenus* (Asch.) Palla and *Sparganium* L. (Cook & Nicholls 1987, Browning & Gordon-Gray 2000).

Polytopic origin and convergence

Recurrent hybridization may result in various evolutionary outcomes: (1) As exemplified by the North American *Helianthus* taxa, several hybrid species may originate from the crossing of the same two parental taxa. Similar scenarios have been observed in *Saxifraga* L. (Steen et al. 2000) and *Senecio* L. (Kadereit et al. 2006). (2) The same type of hybrid may rise repeatedly in different places and at different times (polytopic origin) as reported in allopolyploids (Dobeš et al. 2004, Soltis et al. 2004, Díaz-Pérez et al. 2014). (3) Alternatively, hybridization of more than one combination of parental species may result in hybrids with similar (convergent) characters, including cases in which one of the parents remains the same. Convergence in wing phenotypes was observed in South American butterflies of the genus *Heliconius* Kluk (Nadeau et al. 2014). Wing colour is under strong selection and maintains narrow hybrid zones between the species *H. erato* L. and *H. melpomene* L. Although these species are distantly related, they meet in parallel hybrid zones, and the hybrids have independently converged to a common colour pattern in each hybrid zone.

Another example of hybrid convergence was described by (Marques et al. 2010) in his study of *Narcissus* L. hybrids. While *N. cavanillesii* Barra & G. Lopéz (bee pollinated) primarily acted as the maternal progenitor of two hybrids (*N.* × *alentejanus* Fern.Casas and *N.* × *perezlarae* Font Quer), two different paternal species (*N. serotinus* L. and *N. miniatus* Donn.-Morg., Koop. & Zonn., both butterfly pollinated) gave rise to these morphologically similar hybrids. The two hybrids are reproductively isolated from their parent species by different pollinators, with both being pollinated by ants (Marques et al. 2016).

Several homoploid hybrids have been suggested in the cosmopolitan wetland monocot genera *Sparganium* and *Bolboschoenus*. However, their hybrid status has not yet been confirmed by molecular analyses. Furthermore, remarkable convergence of morphological characters (achenes shape and anatomy) has been observed worldwide in the genus *Bolboschoenus* in both the suggested parental species and their hybrids, but this morphological pattern has not yet been elucidated. Here, the morphologically variable species *Sparganium erectum*, with four subspecies, and the genus *Bolboschoenus*, comprising approximately 15 species, are selected for the investigation of homoploid hybridization within groups of closely related taxa.

The genus *Sparganium* L.

Approximately 14 species of the genus *Sparganium* (Typhaceae) are currently recognized worldwide. A chromosome number of 2n=30 has been found in all species (Löve & Löve 1948, summarized in Cook & Nicholls (1986). *Sparganium* species are distributed throughout the temperate and arctic regions of the Northern Hemisphere, with isolated occurrences in the tropics (Sumatra, New Guinea) and Southern Hemisphere (Australia and New Zealand). Most species occupy northern boreal zones. *Sparganium* can be found in a wide range of aquatic habitats. Seed germination and seedling establishment take place under water. Species are indistinguishable in the juvenile phase, with typical floating or erect leaves developing later in ontogenesis. Plants are predominantly wind pollinated and self-compatible, and their flowers are gathered in unisexual globose heads (Cook & Nicholls 1986). Long-distance dispersal of achenes (which can remain floating for several months) is provided by water currents or water birds, and recruitment into new habitats is facilitated by vegetative reproduction by rhizomes (Pollux et al. 2007).

On the basis of vegetative characters, many taxa have been described at various taxonomic levels (Graebner 1990). However, these perennial, aquatic macrophytes show high plasticity, and their vegetative organs respond to abiotic changes (e.g., changes in water level). Due to this extreme plasticity, their classification according to the development of erect, floating or submerged leaves was unclear (Ascherson & Graebner 1897). Generative characters are considered to be less variable and more suitable than vegetative characters for species differentiation and delimitation (Cook & Nicholls 1986, 1987). A

phylogenetic study of *Sparganium* species examined the character evolution of habitat and stigma number. It appears that the floating-leaved habit has appeared multiple times in the genus from emergent ancestors. Moreover, morphological similarities between North American and Eurasian species suggest close relationships and divergence due to vicariance and long-distance dispersal (Sulman et al. 2013). Despite some recent research progress on *Sparganium*, no molecular studies have yet supported the current morphological concept of species based on achene morphology.

The phylogenetic complexity of the genus is mainly due to hybridization. Hybridization seems to occur frequently in the genus, with many reports or suggestions of inter- and intraspecific hybrids based on the presence of intermediate characters. However, some of the reports are doubtful (Cook & Nicholls 1986, 1987). Studied putative hybrids are often fertile, with the sterility of fruit heads observed in only some cases.

In central Europe, four species are recognized: *S. angustifolium* Michx., *S. emersum* Rehmann, *S. erectum* L. and *S. natans* L. (Ascherson & Graebner 1897, Hegi 1936, Casper & Krausch 1980). These four species are ecologically divergent and well distinguished by achene morphology (Fig. 1). *S. angustifolium* occupies oligotrophic deep waters, and *S. emersum* often grows near the banks of still or flowing waters, as does *S. erectum*, which is common in the shallow waters of ponds, lakes and river banks. *S. natans* prefers peat substrates and inhabits sheltered bays of lakes or ponds and ditches.

Fig. 1. – Achene morphology of individual species/subspecies of *Sparganium* occurring in central Europe. a – *S. erectum* subsp. *erectum*, b – *S. erectum* subsp. *oocarpum*, c – *S. erectum* subsp. *neglectum*, d – *S. erectum* subsp. *microcarpum*, e – *S. angustifolium*, f – *S. emersum*, g – *S. natans*.

***Sparganium erectum* L.**

Most common and variable within the genus *Sparganium* is *S. erectum*, which is characterized by large, erect plants with branched inflorescences and triangular leaves. It occurs throughout Europe from the Arctic Circle to North Africa, in temperate Asia and northwestern North America (Cook & Nicholls 1986).

This species is very tolerant to disturbance and is plastic in vegetative characters. Although several authors have used leaf anatomy to distinguish intraspecific forms (Čelakovský 1896, 1899, Belavskaja 1984), the suitability of these characters is dubious (Cook & Nicholls 1987). High polymorphism in achene shape and colour has been observed in this species. Five intraspecific forms have been described at taxonomic scales ranging from variety to species. Recently, these five forms were recognized as separate subspecies: *S. erectum* subsp. *erectum* L., *S. erectum* subsp. *oocarpum* (Čelak.) Domin., *S. erectum* subsp. *neglectum* (Beeby) K. Richt., *S. erectum* subsp. *microcarpum* (Neumann) Domin. and *S. erectum* subsp. *stoloniferum* (Buch.-Ham. ex Graebn.) H. Hara. With the exception of the last subspecies listed that is not included in this thesis, all of these subspecies occur in central Europe. Only slight geographical and ecological differences have been reported among the four central European subspecies, but further investigation is required to ascertain their differences (Cook 1962, Kaplan et al. 2015). Current intraspecific classification is based on less plastic generative traits: (1) *S. erectum* subsp. *erectum* is characteristic by obpyramidal achenes (3–5 angled in cross section), each with a flattened upper part and a distinct shoulder between the upper and lower parts. (2) *S. erectum* subsp. *microcarpum* has smaller obpyramidal achenes (3–5 angled in cross section), each with a rounded upper part and slightly constricted below the shoulder. (3) *S. erectum* subsp. *neglectum* differs from the other subspecies in having ellipsoidal achenes with an indistinct shoulder between the upper and lower parts (almost non-angled in cross section). An additional characteristic is a style longer than 2 mm. (4) *S. erectum* subsp. *oocarpum* forms ovoid achenes with an indistinct shoulder that are almost circular in cross section (Fig. 1a-d). Sterility of the fruit heads was repeatedly observed in this subspecies, and Cook (1961) later described it as hybrid of *S. e*. subsp. *erectum* and subsp. *neglectum*.

Hybridization between other subspecies has also been observed (Ostenfeld-Hansen 1897, Cook & Nicholls 1987), but neither subsp. *oocarpum* nor other putative hybrids (i.e., *S. erectum* subsp. *erectum* × *S. erectum* subsp. *microcarpum*, Fig. 2) have been examined using molecular analyses. Thus, whether hybrids are well established or whether hybridization is only recent remains unknown.

Fig. 2. – *S. erectum* subsp. *erectum* × *S. erectum* subsp. *microcarpum* on the bank of the pond Blatovský in the Prague district Újezd nad Lesy.

The genus *Bolboschoenus* (Asch.) Palla

Approximately 15 species are recognized in the cosmopolitan wetland monocot genus *Bolboschoenus* (Cyperaceae) (Browning & Gordon-Gray 2000, Tatanov 2007). Nevertheless, delimitation of the species is unclear, and a worldwide revision is needed. Chromosome numbers were counted for central European species, and only counts n=54 or n=55 were found (Jarolímová & Hroudová 1998). Although various chromosome numbers within the genus *Bolboschoenus* have been reported at the global scale (caused mainly by differences in species conception by some authors), no clear evidence for polyploid occurrence has been found, and different ploidy levels are considered unlikely (Roalson 2008).

On the basis of inflorescence characters, two subspecies were formerly recognized in central Europe: *B. maritimus* subsp. *compactus* (Hoffm.) Hejný (head-like inflorescence) and *B. maritimus* subsp. *maritimus* (L.) Palla (branched inflorescence) (Hroudová et al. 1998b). However, this complex of *B. maritimus* (L.) Palla *sensu lato* shows high variability in achene shape and anatomy. Four morphotypes of achenes were reported within this species, which were later used to differentiate the species into four closely related species: *B. yagara* (Ohwi) Y.C.Yang & M. Zhan, *B. laticarpus* Marhold & al., *B. planiculmis* (F. Schmidt) T. V. Egorova and *B. maritimus* (Hroudová et al. 1998a).

On account of its intermediate morphology and two chromosome numbers (n=54 and n=55), *B*. *laticarpus* is suspected to be of hybrid origin, with parentage *B*. *yagara* and *B*. *planiculmis* (Marhold et al. 2004).

All of the central European species have divergent ecologies and distributions (Hroudová et al. 2007a). *B*. *yagara* occupies littoral habitats and is found predominantly in fishpond basins, *B*. *maritimus* inhabits coastal area and inland saline habitats, and *B*. *planiculmis* prefers more terrestrial habitats, such as wet field depressions. The hybrid species *B*. *laticarpus* differs from its parent species ecologically and occurs in freshwater along rivers, although it is also often reported on arable land in coexistence with *B*. *planiculmis* or in littoral habitats in coexistence with *B*. *yagara* (Hroudová et al. 2014).

Fig. 3. – *Bolboschoenus laticarpus* can often be found on arable land as a weed.

A morphological pattern of *Bolboschoenus* achenes similar to that observed in central European species has been found in this genus worldwide. Four identical morphotypes were described independently in Eurasia, Australia, Africa and North America (Fig. 4; Paper III, Fig. 2; Browning & Gordon-Gray 1993, Browning et al. 1995, 1996, 1997). Moreover, similarities were observed even between putative hybrids with intermediate morphology between the morphologies of the parental species on each continent: *B*. *laticarpus* in Eurasia, *B*. *medianus* (V.J. Cook) Soják in Australia and *B*. *novae-angliae* (Britt.) S. G. Sm. in North America. As with members of the genus *Sparganium*, *Bolboschoenus* taxa are perennial, wind pollinated, and self-compatible and can disperse via vegetative reproduction, specifically, by tubers. The taxa occupy wetlands that provide a variety of microhabitats with a range of growing conditions and have attributes that facilitate gene exchange and the survival of the first generation of hybrids. Thus, natural hybridization is very likely in this genus.

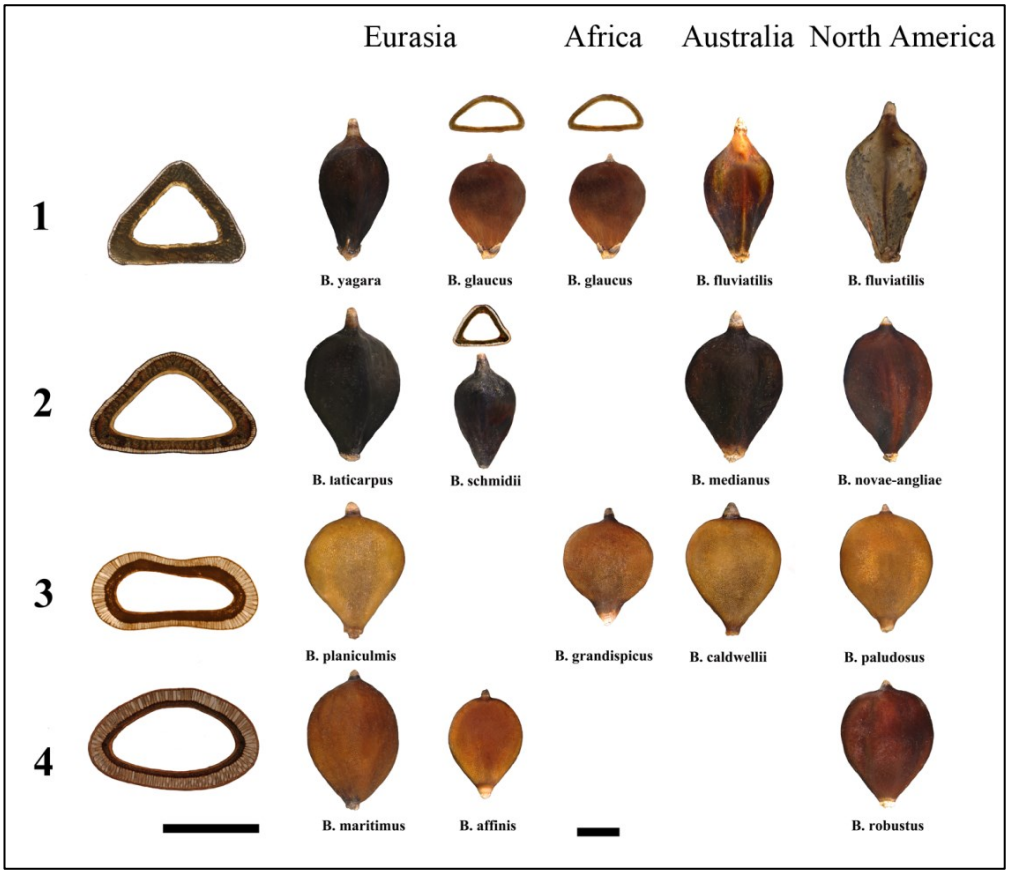

Fig. 4. – Similarities in the morphological and anatomical patterns of achenes in the genus *Bolboschoenus* among continents.

Perhaps not surprisingly, ecological analogies to the Eurasian taxa have been observed in other *Bolboschoenus* species as well. North American *B. fluviatilis* (Torr.) Soják occupies freshwater habitats, *B. robustus* (Pursh) Soják inhabits saline coastal areas, and their hybrid (*B. novae-angliae*) is found only in brackish habitats in Atlantic coastal estuaries, where its parental species occur in sympatry and hybridize (Browning et al. 1995). An analogous situation exists in New Zealand and Australia: *B. fluviatilis* prefers the margins of small lakes or ponds (freshwater), and *B. caldwellii* (V.J. Cook) Soják prefers salt marches near the sea, whereas their putative hybrid, *B. novae-angliae*, occupies adjacent tidal wetlands (Wilcox 2001).

In contrast, different achene morphotype and distribution was observed in Mediterranean *B*. *glaucus* S. G. Sm., which favours freshwater habitats, especially rivers, and temporarily flooded field depressions and can frequently be found as a weed in rice fields (Hroudová et al. 2007b).

The distribution of *B*. *glaucus* is confined mainly to the Mediterranean region (South Europe, near East and Middle Asia, and Africa; Browning et al. 1998). However, its introduction to North America has been reported (Browning et al. 1995, Smith 2002). The Asian species *B*. *schmidii* (Raymond) Holub resembles *B*. *yagara* in its triangular achenes and the hybrid morphotype in its wider exocarp. The origin of this species has not been clarified; it might be a hybrid species. The species occurs along streams or in channels (Amini Rad & Hroudová 2007, Amini Rad et al. 2010). An achene type similar to that of *B*. *maritimus* was observed in another Asian species, *B*. *affinis* (Roth) Drobow (Egorova 1967), which also shares similar habitats as *B*. *maritimus*, i.e., saline lakeshores and riversides (Amini Rad et al. 2010). In addition, some authors found no morphological differences between the Asian *B*. *affinis* and *B*. *grandispicus* (Steud.) Lewej. & Lobin from Senegal (Africa) and suggested that they be treated as the same species (Browning & Gordon-Gray 2000). However, little is known about the distribution of *Bolboschoenus* species in South America, where to date only *B*. *paludosus* (A. Nelson) Soó and *B*. *robustus* have been reported.

Similarities in achene shape and anatomy within this genus may be caused by either the common origin of morphotypes followed by migration between continents or the convergent evolution of species. The similarity between North American *B*. *fluviatilis* and Asian *B*. *yagara* has raised questions about their non-conspecific status. Some authors have treated them as subspecies or varieties of *B*. *fluviatilis* and suggested possible floristic links between America and Japan in the past (Koyama 1980, Browning et al. 1997).

Aims of the thesis

The classification of wetland plants is often difficult due to widespread phenotypic plasticity that makes species delimitation in some groups a challenge. Taxonomical treatment might be further complicated by frequent hybridization and the existence of complexes of closely related taxa. A crucial prerequisite for the examination of such complexes and the hybrids within is a solid and comprehensive taxonomic study based on reliable morphological characters used for taxa delimitation. Despite the fact that several putative hybrid taxa have been reported in both the genus *Bolboschoenus* and the *Sparganium erectum* species complex, these putative hybrids have not been confirmed by molecular analyses. Thus, it remains unknown whether they can be treated as long-standing, stable taxa of hybrid origin or as the products of recent hybridization. In this thesis, the reliability of morphological characters for both taxa and hybrid delimitation was verified, and the origins of the putative hybrids were determined using a combination of morphometric and molecular analyses.

To examine intraspecific genetic and morphological variation and differences in genome size of *Sparganium erectum*, the following questions were addressed:

- Are genetic variation and variation in genome size correlated with morphological variation? What morphological characters are the most correlated and can be used to differentiate intraspecific taxa?
- Is *Sparganium erectum* subsp. *oocarpum* of hybrid origin? What subspecies are its putative parents?
- Should the subspecies *neglectum* and *microcarpum* be merged into a single taxon or remain distinguished as two distinct taxa, and what characters can be used for their identification?

To elucidate interspecific relationships, hybridization and the convergent morphology of achenes in the genus *Bolboschoenus*, the following questions were addressed:

- What is the overall pattern of genetic variation among *Bolboschoenus* species of the studied morphotypes worldwide?
- Does the present distribution pattern reflect separate phylogenetic lineages with convergent morphology or past migration between continents?
- Does morphological variation among the species correspond to the genetic variation? What morphological and anatomical characters can be used to differentiate particular species?
- What is the role of hybridization in speciation in this group? Are *B. laticarpus*, *B. medianus* and *B. novae-angliae* of hybrid origin, and if so, what are their parental species?

Materials and methods

Sampling design

As the members of both *Sparganium* and *Bolboschoenus* are clonal plants and the determination of single individuals is difficult, their leaves and inflorescences were collected from individuals at least 10 m apart from each other to minimize repeat sampling of the same clone. In the case of *Bolboschoenus*, samples from natural populations were complemented with samples of plants cultivated in the experimental garden of the Institute of Botany of the CAS in Průhonice from seeds sent by collaborators from all over the world. More details about the sampling design are provided in each paper. Lists of the sampled populations are presented in Appendices.

Molecular analyses

AFLP (amplified fragment length polymorphism, Vos et al. 1995) marker was used as molecular markers because it has been demonstrated to be suitable tools for the examination of genetic variation of closely related taxa and the detection of their putative hybrids (Gobert et al. 2002, Guo et al. 2005, Ciotir et al. 2017). For samples of *Sparganium erectum*, three primer combinations were used for selective amplification: *Eco*RI-ACT-(6-FAM)/*Mse*I-CAT, *Eco*RI-AAG-(HEX)/*Mse*I-CTC and *Eco*RI-ACC-(NED)/*Mse*I-CAT (Fér & Pfosser submitted). The *Bolboschoenus* samples were amplified with four pairs of selective primers: *Eco*RI-ATC-(6-FAM)/*Mse*I-CAA, *Eco*RI-AAG-(VIC)/*Mse*I-CTC, *Eco*RI-AAC-(NED)/*Mse*I-CAG, and *Eco*RI-ACA-(PET)/*Mse*I-CAT. All of the AFLP data sets were subsequently scored with the software GeneMarker v1.8 (SoftGenetics LLC, PA, USA). The resulting matrixes were analysed with STRUCTURE 2.3.2.1 (Pritchard et al. 2000) to obtain the best distribution of samples into genetic groups. All of the samples used in further analyses were colour coded or divided into groups in accordance with the STRUCTURE results. Furthermore, the AFLP data were analysed using principal coordinate analysis (PCoA), a neighbour-network analysis and AMOVA.

In addition, sequencing of one nuclear (ITS) and two plastid regions (*trn*H-*psb*A and *trn*C-*psb*MR) was performed to evaluate phylogenetic relationships within the genus *Bolboschoenus*.

Genome size estimation

Intraspecific differences in genome size in *Sparganium erectum* were determined using propidium iodide flow cytometry following the simplified two-step procedure with Otto buffers described by Doležel et al. (2007) and an internal reference standard (*Glycine max* cv. Polanka, 2C = 2.50 pg). The relationship between genome size and predefined group (subspecies) was tested by a one-way ANOVA, Kruskal-Wallis test (Kruskal & Wallis 1952) and Tukey's HSD multiple comparison test with the R package multcomp (Hothorn et al. 2008). The genome size estimation of *Bolboschoenus* species is not included in this thesis because no significant difference in genome size was detected in a pilot study.

Morphometric analyses

For each studied complex, characters described in previous works or identification keys were used for morphometric analyses: *Sparganium erectum* – achene size and shape and other distinctive characters (Paper I, Table 1, Fig. 1); *Bolboschoenus* – achene shape and anatomy; inflorescence structure, including lengths of spikelets and peduncles (Paper II and III, Table 1, Fig. 1).

Morphological variation was analyzed using multivariate analyses such as principal component analysis (PCA). Discriminant analyses were performed to identify the most important characters for taxa differentiation. Furthermore, classificatory analyses were used to identify admixed individuals. All analyses were performed with the software Canoco 5 (Ter Braak & Šmilauer 2012) or the Morphotools suite of R scripts (Koutecký 2015).

Results and discussion

Genetic structure

A total of 276 leaf samples of *Sparganium erectum* from 64 natural populations were analysed by AFLP molecular marker and flow cytometry. The results of a STRUCTURE analysis based on 125 AFLP loci identified four separate genetic groups corresponding to *Sparganium erectum* subspecies and a couple of admixed individuals among them (Paper I, Fig. 2A). The group of subsp. *erectum* was well differentiated from all of the other groups by individual genome size, which ranged from 2C = 1.12 to 2C = 1.20 pg (Paper I, Table 3, Fig. 4A). The second group, putative hybrid subsp. *oocarpum*, had an approximately 50:50 proportion of admixture from its parental taxa (subsp. *erectum* and subsp. *microcarpum*) for the solution with K=3 in the STRUCTURE analysis; however, according to the solution K=4, it can alternatively be considered an independent stable hybrid. Similar intermediacy was observed in its genome size (2C = 1.05–1.12 pg, Paper I, Fig. 3). The group with the highest number of individuals was subsp. *microcarpum* (2C= 0.95–1.07 pg), whereas only four non-admixed populations of subsp. *neglectum* were detected, with genome sizes ranging from 2C = 0.97 to 2C = 1.01 pg. Although the genome sizes of these two subspecies partially overlapped, the differences between them were statistically significant. However, genome size alone was not sufficient for accurate determination. Hybridization of subsp. *microcarpum* with other subspecies appears to be frequent as two hybrid groups of individuals (subsp. *erectum* × subsp. *microcarpum* and subsp. *microcarpum* × subsp. *neglectum*) with varying degrees of admixture were found.

For the examination of *Bolboschoenus* species, 279 individuals from 36 populations in central Europe and 90 samples from 87 populations all over the world were analysed. In studying the central European species of *Bolboschoenus* (Paper II), four genetic groups corresponding to the species (*B*. *yagara*, *B*. *laticarpus*, *B*. *planiculmis* and *B*. *maritimus*, Paper II, Fig. 2A) were detected by the STRUCTURE analysis along with admixed groups among them. Individuals of *B*. *laticarpus* were either assigned to an independent group or had a 50:50 proportion of admixture from the species *B*. *yagara* and *B*. *planiculmis*. Similar admixture was later found in Asian *B*. *laticarpus* and Australian *B*. *medianus* (Paper III, Fig. 3). In studying *Bolboschoenus* species from all over the world, the following species formed independent groups:

Mediterranean *B*. *glaucus*, African *B*. *glaucus*, Asian *B*. *affinis* and partly Australian *B*. *caldwellii* (Paper III, Fig. 3–5). African *B*. *grandispicus* was found to share most of its genetic information with African *B*. *glaucus*. Also well differentiated were the North American representatives *B*. *fluviatilis*, *B*. *paludosus* and *B*. *robustus*, whereas *B*. *novae-angliae* was admixed. A more complex admixture was subsequently observed in *B*. *schmidii*, which appears to share genetic information from several species. In addition, subgroups within *B*. *planiculmis* from Asia were observed that corresponded to the geographic distributions of these plants. One of these subgroups was genetically similar to Australian *B*. *caldwellii*.

A similar study of closely related sympatric species and their putative hybrids with intermediate morphology was performed on Chilean *Puya* Molina species. Plastid and nuclear DNA sequence data only partly resolved the relationships in this group. In accordance with the results in this thesis, a STRUCTURE analysis based on AFLP data revealed three genetic groups, recent hybrids with genetic admixture between the parental species and one ancient hybrid with its own genetic group (Schulte et al. 2010).

Admixture of more than two species in hybrids has frequently been reported in the genus *Quercus* L., in which multispecies hybrid zones are known from sympatric populations of five species (*Q*. *castanea* Née, *Q*. *crassifolia* Bonpl., *Q*. *crassipes* Bonpl., *Q*. *laurina* Bonpl. and *Q*. *mexicana* Bonpl.). Whereas allopatric populations formed distinct genetic groups, sympatric populations showed evidence of hybridization and introgression among *Q*. *castanea* and other species (Valencia-Cuaves et al. 2015).

Morphological variation

The morphological characters of achenes considered in this thesis were chosen based on preliminary studies (Cook 1962, Cook & Nicholls 1987, Browning & Gordon-Gray 2000, Kaplan 2002, Hroudová et al. 2007a). However, no comparison of these characters with genetic variation has yet been performed to estimate the importance and suitability of these characters for taxon differentiation and to verify current the classifications of *Sparganium erectum* and members of the genus *Bolboschoenus*. Moreover, in the genus *Bolboschoenus*, unique patterns of achene anatomy and morphology have been observed across its cosmopolitan distribution. Three to four basic morphotypes have been reported in Eurasia, Australia, Africa and North America and

independently described on each continent (Browning & Gordon-Gray 1993, Browning et al. 1995, 1997, 1998, Hroudová et al. 2007a). All available species except the highly different *B*. *nobilis* from Africa were included (Paper III, Fig. 1).

Altogether, 12 morphological characters of *Sparganium erectum* and 8 inflorescence and 8 achene characters of *Bolboschoenus* were analysed to examine morphological variation, detect the most important characters for taxa delimitation and determine admixed individuals.

Principal component analysis (PCA) showed four partially separated groups of *Sparganium* subspecies, which was supported by the canonical discriminant analysis (CDA), which only slightly improved the differentiation (Paper I, Fig. 4B–D).

The most important characters varied among the subspecies (described in detail in discussion of Paper I); however, in general, achene width and length, style length, length of the upper part of the achene and constriction in the middle part of the achene were the characters most correlated with genetic group (subspecies; Paper I, Table 4). In accordance with the STRUCTURE analysis, individuals of putative hybrid *S*. *e*. subsp. *oocarpum* were distributed in an intermediate position between the putative parental taxa, subsp. *erectum* and *microcarpum*. In addition, admixed individuals between genetic groups either morphologically resembled one of the parents or were morphologically intermediate.

In the subsequent investigations, inflorescence characters were found to be insufficient for the differentiation of *Bolboschoenus* species, whereas achene characters distinguished all four morphotypes as well as species with specific types of achenes (*B*. *schmidii*, *B. glaucus*; Paper III, Fig. 8, 9, 10A-G). Putative hybrids of the second morphotype had intermediate characters and were placed between the parental species in scatterplots. Moreover, species were distinguished within morphotypes in separate analyses.

Intermediate morphology in hybrids is quite common and has been reported in other genera of aquatic or wetland plants, such as *Nuphar* (Shiga & Kadono 2007) and *Typha* (Snow et al. 2010). On the other hand, interspecific hybrids may resemble one of the parental species rather than show intermediate morphology (*Carex*, Pedersen et al. 2016).

Migration vs. convergent evolution

Remarkable similarities of *Bolboschoenus* species in achene anatomy and morphology have led to questions about their origin. Although migration of plants between Eurasia and Africa or Australia seems likely, the origin and evolution of North American species remains unknown (Browning & Gordon-Gray 2000). The combined phylogenetic analyses of nuclear and chloroplast regions distinguished neither Eurasian nor Australian species except a *B. yagara* clade consisting of plants from Europe and Asia, *B. schmidii* and *B. glaucus* (Paper III, Fig. 7). In addition, European and African plants of *B. glaucus* were differentiated in the plastid tree. In contrast, North American species were well separated, each represented by a single clade. Furthermore, the plastid tree indicated a common ancestry of *B. yagara* (Eurasia) and *B. fluviatilis* (North America). In addition, it seems that a relationship exists between *B. maritimus* and *B. robustus* (Paper III, Fig. 3–5). Although the third North American species, *B. paludosus*, was clearly separated from the others in the AFLP analyses, its clade had a low support in the phylogenetic analyses. Therefore, earlier transoceanic dispersal or past migration via land connections between continents in the past followed by the divergence of species of each morphotype appears to be a more likely scenario than is convergent evolution for the North American species. A similar scenario was described in a phylogenetic study of the genus *Sparganium*. Genetically and morphologically closely related species were observed among North American and Eurasian species: *S. angustifolium* and *S. emersum* (Ito et al. 2016), *S. fluctuans* B.L.Rob and *S. gramineum* Georgi, *S. eurycarpum* Engelm. and *S. erectum* (Sulman et al. 2013). It was suggested that the these sister groups arose following migration via a land connection between western North America and eastern Eurasia in the Tertiary or via long-distance dispersal and repeated migration of boreal species during the Pliocene and Pleistocene. Migration between continents over land bridges and transoceanic dispersal rather than vicariance was also detected in the tribe Ranunculeae (Ranunculaceae, Emadzade & Hörandl 2011), and frequent transoceanic dispersal was reported in the tribe Schoeneae (Cyperaceae, Viljoen et al. 2013). Although continental drift might have influenced the diversification of some ancient plant species (e.g., of *Podostemaceae*, *Myriophyllum* L. and *Lagarosiphon* Harv.), various aquatic angiosperms dispersed more recently. For example, *Wolffia* Schleid. and *Wolfiella* Hegelm. dispersed across the Atlantic Ocean, and *Lemna* L. dispersed

through Southeast Asia into the North Atlantic Bering sea area (Les et al. 2003).

Hybrid speciation vs. recent hybridization

Several hybrids have been described in the genus *Bolboschoenus* and in the *Sparganium erectum* complex. Convergent morphology of *Bolboschoenus* hybrids has been observed among Eurasia, Australia and North America (Browning et al. 1995, 1997, Marhold et al. 2004). *Sparganium erectum* subsp. *oocarpum* is commonly suggested as a putative hybrid in the *S. erectum* complex. Based on achene morphology, Cook (1961, 1962) hypothesized that subsp. *erectum* and *neglectum* were its parental taxa. This hypothesis was confirmed by the intermediate position of subsp. *oocarpum* individuals in all of the analyses in Paper I. Although Cook (1961) did not detect any ecological differences among subspecies, he observed that subsp. *microcarpum* occurred throughout the British Isles, whereas both parental taxa and their hybrid had similar distributions southward of the bay The Wash (England). Similarly, in the Czech Republic, subsp. *erectum* and hybrid subsp. *oocarpum* often occur in sympatry along rivers, whereas the second parent of the hybrid, subsp. *neglectum*, is much less common (Kaplan et al. 2015). The hybrid might become established following geographical isolation from subsp. *neglectum* due to reduced gene flow. Backcrossing with either parental taxon seems to be rare in subsp. *oocarpum*, and based on its classification as an independent group in the STRUCTURE analysis, its intermediate genome size and its unique morphology, it may be considered as a stable hybrid taxon.

An intermediate genome size between the genome sizes of the parental taxa was also reported in *Narcissus* hybrids *N.* × *alentejanus* (*N. cavanillessi* × *N. serotinus*) and *N.* × *perezlarae* (*N. cavanillessi* × *N. miniatus*) despite high intra-population variance in genome size (Marques et al. 2012). Similarly, intermediacy in genome size was detected in hybrids of central-European *Cirsium* Mill. species, but the genome sizes of diploid hybrids were smaller than the mean value of the parents (Bureš et al. 2004). In contrast, an unusual increase in genome size (of approximately 50% that of the parental taxa) was observed in three *Helianthus* hybrids (*H. anomalus*, *H. deserticola* and *H. paradoxus*, Baack et al. 2005). Although these hybrids have multiple origins, their genome sizes were consistent across populations with the exception of *H. deserticola*, which showed interpopulation variability. Surprisingly, an increase

in genome size has not been observed in any synthetic hybrids or natural hybrids from hybrid zones. Several mechanisms might cause an increase in genome size in hybrids: the existence of rare, large-genome parental plants; the transition of hybrids to new, extreme habitats; or selection for hybrids with larger genomes (Baack et al. 2005).

In the central European *Bolboschoenus* species, the hybrid origin of *B. laticarpus* was confirmed in the second study based on its intermediate genetic information and morphology. However, Asian plants of this hybrid appear to be of more recent origin, and it is possible that they originated from Asian representatives of *B. yagara* and *B. planiculmis* (Paper III, Fig. 3, 4B). As described in the introduction of this thesis, *B. laticarpus* occupies novel habitats (banks of rivers) as well as wet depressions on arable land (Hroudová et al. 1999). Similar ecologies of Australian *B. medianus* and *B. novae-angliae* from North America have been described (Browning et al. 1995, Wilcox 2001). These hybrid taxa occur mainly in river estuaries with brackish water as one of their parent species occupies saline habitats and the other occupies freshwater habitats; in contrast, neither parent species of *B. laticarpus* occurs in saline habitats (Hroudová et al. 1999). Thus, ecological divergence might facilitate the establishment of these hybrids. The parentage of *B. novae-angliae* was confirmed in the molecular analyses by admixture of its parental species, *B. fluviatilis* and *B. robustus*. The origin of second hybrid, *B. medianus*, was not clarified due to the unavailability of one of its likely parents (*B. fluviatilis* resp. *B. yagara*) directly from Australia, but it appears that it raised either from Asian plants of *B. yagara* and *B. planiculmis* or from their divergent counterparts in Australia, *B. caldwellii* and *B. fluviatilis* resp. *B. yagara*.

Divergent habitat is crucial for the establishment of homoploid hybrids and has been reported in several studies (Gross & Rieseberg 2005). For example, all three hybrids of North American sunflowers occur in different and extreme habitats: *H. anomalus* has adapted to desert dunes, *H. deserticola* occurs in high-desert habitats, and *H. paradoxus* inhabits high-salinity areas (Heiser et al. 1969, Rosenthal et al. 2002). Hybridization may further enhance stress tolerance in hybrids. Faster seedling growth rate and more efficient water use allowed the drought-tolerant hybrid *Pinus densata* to inhabit extreme alpine habitats beyond the altitudinal range of both parental species (*P. tabuliformis* and *P. yunnanensis*, Ma et al. 2010). A different habitat from the parental habitats was also observed in *Iris nelsonii*, which exhibits a combination of parental features and occurs in the shady, deep waters of swamps (Arnold

1993). In some cases, hybridization may be followed by the evolution of invasiveness, as observed in *Typha* × *glauca* and *Senecio squalidus* (Schierenbeck & Ellstrand 2009).

Convergent morphological traits of geographically isolated hybrids have been examined in studies of Neotropical *Heliconius* butterflies. A similar wing pattern among divergent *H. erato* races evolved rapidly and independently within the species (Brower 1994, Nadeau et al. 2014). Similar phenotypic traits and ecological niches were described in the algal genus *Fucus* L. along North Atlantic and northeast Pacific shores in marine and estuarine habitats (Neiva et al. 2012). In *F. cottonii* M. J. Wynne & Magne, several convergent salt-marsh morphotypes (dwarf forms) were observed. This study showed that this species consists of an independent population of pure, hybrid or polyploid origin.

In addition to stable hybrids, recent hybridization was detected in both complexes investigated in this thesis. In *Sparganium erectum*, two hybrids with genome sizes and levels of genetic and morphological variation spanning the ranges of the parents were detected. In one case, admixed individuals between subsp. *erectum* and subsp. *microcarpum* were involved (Paper I, Fig. 2A, group B). This hybrid combination was formerly described as *S. microcarpum* × *ramosum* by Ostenfeld-Hansen (1897) and is usually highly sterile (Cook & Nicholls 1987). However, during this study, some fully fertile plants were observed (Introduction, Fig. 2). The second hybrid combination, *S. erectum* subsp. *microcarpum* × subsp. *neglectum*, was morphological variable; its achenes resemble those of both parental taxa and to some extent those of subsp. *oocarpum*, with partial sterility and the allocation of resources to a few wide achenes. Although individuals of this hybrid combination were genetically intermediate between subsp. *microcarpum* and subsp. *neglectum* (Paper I, Fig. 2A, group C), their genome size varied from that of one parent to that of the other. Such morphotypes were formerly described as sterile varieties or forms rather than hybrids (Čelakovský 1896, Neuman 1897, Graebner 1900). In consideration of the different ratios of genetic contributions from the parental subspecies to these two hybrid combinations and their heterogeneous morphologies and genome sizes, recent hybridization seems likely.

Continuous morphological and genome size variation caused by recent introgression has also been observed in central European *Diphasiastrum* Holub. hybrids. Hybrid swarms between species occur in human-disturbed habitats that have likely allowed the unnatural co-occurrence of species and their hybridization (Hanušová et al. 2014).

Similar findings have been reported for the genus *Bolboschoenus*. Recent hybridization has occurred between *B. maritimus* and *B. planiculmis*, *B.*

glaucus or *B. robustus*. In the Czech Republic, the hybrid *B. maritimus* × *B. planiculmis* occurs in field depressions, especially in South Moravia and Slovakia, where it forms hybrid populations of plants with variable morphology ranging from that of *B. planiculmis* to that of *B. maritimus*. It appears that genetic introgression might allow hybrids to occupy less saline habitats, as non-admixed populations of *B. maritimus* were only found in coastal areas or high-salinity inland wetlands (Paper II, Fig. 2A, Appendix 1).

Adaptive introgression has increased abiotic tolerance in *Helianthus annus* (Whitney et al. 2010b), flood tolerance in *Iris* hybrids (Martin et al. 2006) and cold- and drought tolerance in *Populus* introgressed individuals (Suarez-Gonzalez et al. 2016).

The hybrid *B. glaucus* × *B. maritimus* in California was reported to grow in highly disturbed habitats such as rice fields or ditches (Browning et al. 1995). In the third study, plants of this hybrid originated from Azambuja ditch (Portugal) and the shores of Lake Vrana (Croatia, Paper III, Appendix 1). The hybrid combination *B. maritimus* × *B. robustus* is from coastal areas (Nova Scotia, Canada), in accordance with the saline habitat preference of both its parental species.

Habitat disturbance seems to be a common cause of hybridization events, contributing to the formation of hybrid zones between otherwise ecologically divergent and allopatric taxa. The hybrid populations in these zones may consist mainly of F_1 hybrids (*Quercus*, Milne et al. 2003), backcrossed plants (*Populus*, Lexer et al. 2005); *Silene*, Minder et al. 2007) or later-generation hybrids (*Iris*, Arnold 1993) depending on the strengths of the reproductive barriers between the parental species.

Conclusions and future perspectives

A combination of molecular, genome size and morphometric analyses allowed parental and hybrid taxa to be distinguished in *Sparganium erectum* and in the genus *Bolboschoenus* and the suitability of morphological characters that are currently used for taxa delimitation to be verified. Hybrid origins of *S. e.* subsp. *oocarpum*, *B. laticarpus*, *B. medianus* and *B. novae-angliae* were confirmed in the thesis, but more extensive sampling is required for the latter two hybrids. All these hybrids seem to be stable hybrid taxa with intermediate morphology. Our data suggest that the polytopic origin of *Bolboschoenus* hybrids is possible. Further studies should be conducted to examine the origin of Asian hybrids. The divergent ecologies and, to some extent, distributions of hybrids from those of their parental taxa might facilitate their establishment. Moreover, several younger hybrids indicative of recent hybridization were found. Ongoing introgression was detected between *S. e.* subsp. *neglectum* and subsp. *microcarpum* or *erectum*. In hybrid zones between *B. planiculmis* and *B. maritimus*, introgression might allow hybrids to occupy different types of more or less saline habitats. These hybrid zones present great opportunities to investigate possible adaptive introgression. Both old and recent hybrids generally originated and tend to occur in disturbed habitats that provided different habitats from those of their parents, facilitating their successful establishment.

The diversification of the studied cosmopolitan taxa has been influenced by geographical barriers in the form of distant continents. In the genus *Bolboschoenus*, convergence of morphological characters is likely the result of recent migration between continents or long-distance dispersal rather than vicariance; however, molecular dating is required to estimate the times of diversification events in this genus.

Although the methods applied in this thesis were sufficient for taxon differentiation and the investigation of hybridization, other methods such as niche modelling would be useful for examining the divergent habitats of hybrids. In addition, ancestral state reconstruction could be used to detect the origin of morphological convergence. Furthermore, next-generation sequencing, such as RAD-Seq or Hyb-Seq, would be very useful for phylogenetic studies of closely related species with ongoing hybridization; such methods provide high-resolution genetic data that can be used to detect reticulate evolution.

References

Abbott R., Albach D., Ansell S., Arntzen J. W., Baird S. J. E., Bierne N., Boughman J., Brelsford A., Buerkle C. A., Buggs R. & et al. (2013): Hybridization and speciation. – J. Evol. Biol. 26: 229–246.

Abbott R. J., Hegarty M. J., Hiscock S. J. & Brennan A. C. (2010): Homoploid hybrid speciation in action. – Taxon 59: 1375–1386.

Abbott R. J. & Rieseberg L. H. (2012): Hybrid speciation. – John Wiley & Sons, Ltd, Chichester, UK.

Amini Rad M. & Hroudová Z. (2007): Two new records of *Bolboschoenus* (Cyperaceae) from Iran. – Iran. Journ. Bot. 13: 57–62.

Amini Rad M., Hroudová Z. & Marhold K. (2010): The genus *Bolboschoenus* in Iran: taxonomy and distribution. – Nord. J. Bot. 28: 588–602.

Anderson E. (1948): Hybridization of the habitat. – Evolution 2: 1–9.

Anderson E. (1953): Introgressive hybridization. – Biol. Rev. 28: 280–307.

Arnold M. L. (1993): *Iris nelsonii* (Iridaceae): origin and genetic composition of a homoploid hybrid species. – Am. J. Bot. 80: 577.

Arnold M. L. (1997): Natural hybridization and evolution. – Oxford University Press, New York.

Arnold M. L. (2004): Transfer and origin of adaptations through natural hybridization: were Anderson and Stebbins right? – Plant Cell 16: 562–570.

Arnold M. L. & Hodges S. A. (1995): Are natural hybrids fit or unfit relative to their parents? – Trends Ecol. Evol. 10: 67–71.

Ascherson P. F. A. & Graebner P. (1897): Synopsis der mitteleuropäischen Flora, Vol. 1. – Wilhelm Engelman, Leipzig.

Baack E., Melo M. C., Rieseberg L. H. & Ortiz-Barrientos D. (2015): The origins of reproductive isolation in plants. – New Phytol. 207: 968–984.

Baack E. J., Whitney K. D. & Rieseberg L. H. (2005): Hybridization and genome size evolution: timing and magnitude of nuclear DNA content increases in *Helianthus* homoploid hybrid species. – New Phytol. 167: 623–630.

Barker M. S., Arrigo N., Baniaga A. E., Li Z. & Levin D. A. (2016): On the relative abundance of autopolyploids and allopolyploids. – New Phytol. 210: 391–398.

Barton N. H. & Hewitt G. M. (1985): Analysis of hybrid zones. – Annu. Rev. Ecol. Evol. Syst.

Belavskaja A. P. (1984): A contribution to the morphology of fruits of the genus *Sparganium* (Typhaceae) in the flora of the USSR. – Botaničeskij žurnal Akad. Nauk., SSSR 69: 1662–1668.

Brennan A. C., Bridle J. R., Wang A.-L., Hiscock S. J. & Abbott R. J. (2009): Adaptation and selection in the *Senecio* (Asteraceae) hybrid zone on Mount Etna, Sicily. – New Phytol. 183: 702–717.

Brower A. V. (1994): Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. – Proc. Natl. Acad. Sci. U.S.A. 91: 6491–6495.

Browning J. & Gordon-Gray K. D. (1993): Studies in Cyperaceae in southern Africa. 21: The taxonomic significance of the achene and its embryo in *Bolboschoenus*. – S. Afr. J. Bot. 59: 311–318.

Browning J. & Gordon-Gray K. D. (2000): Patterns of fruit morphology in *Bolboschoenus* (Cyperaceae) and their global distribution. – S. Afr. J. Bot. 66: 63–71.

Browning J., Gordon-Gray K. D. & Smith S. G. (1995): Achene structure and taxonomy of North American *Bolboschoenus* (Cyperaceae). – Brittonia 47: 433.

Browning J., Gordon-Gray K. D. & Smith S. G. (1997): Achene morphology and pericarp anatomy of the type specimens of the Australian and New Zealand species of *Bolboschoenus* (Cyperaceae). – Aust. Syst. Bot. 10: 49–58.
Browning J., Gordon-Gray K. D., Smith S. G. & Staden J. (1998): *Bolboschoenus glaucus* (Cyperaceae), with emphasis upon Africa. – Nord. J. Bot. 18: 475–482.
Browning J., Gordon-Gray K. D., Smith S. G. & Staden J. van. (1996): *Bolboschoenus yagara* (Cyperaceae) newly reported for Europe. – Ann. Bot. Fenn. 129–136.
Buerkle C. A., Morris R. J., Asmussen M. A. & Rieseberg L. H. (2000): The likelihood of homoploid hybrid speciation. – Heredity 84: 441–451.
Bureš P., Wang Y.-F., Horová L. & Suda J. (2004): Genome size variation in Central European species of *Cirsium* (Compositae) and their natural hybrids. – Ann. Bot. 94: 353–363.
Burke J. M., Bulger M. R., Wesselingh R. A. & Arnold M. L. (2000): Frequency and spatial pattering of clonal reproduction in Lousiana Iris hybrid populations. – Evolution 54: 137–144.
Casper S. J. & Krausch H.-D. (1980): Pteridophyta und Anthophyta: 1. Teil. Lycopodiaceae bis Orchidaceae. In Ettl H., Gerloff J. & Heynig H. (eds.), Süßwasserflora von Mitteleuropa 23. – Gustav Fischer Verlag, Stuttgart & New York.
Čelakovský L. J. (1896): Ueber die ramosen Sparganien Böhmens. – Oesterr. Bot. Z. 46: 421–433.
Čelakovský L. (1899): Anatomické rozdíly v listech ramósních Sparganií. – Věstník Král. Čes. Spol. Nauk, Cl. Math.-Natur. 1–11.
Ciotir C., Szabo J. & Freeland J. (2017): Genetic characterization of cattail species and hybrids (*Typha* spp.) in Europe. – Aquat. Bot. 141: 51–59.
Cook C. D. K. (1961): *Sparganium* in Britain. – Watsonia.
Cook C. D. K. (1962): *Sparganium erectum* L. (*S. ramosum* Hudson, nom. illeg.). – J. Ecol. 50: 247.
Cook C. D. K. & Nicholls M. S. (1986): A monographic study of the genus *Sparganium* (Sparganiaceae). Part 1, Subgenus *Xanthosparganium* Holmberg. – Bot. Helv. 213–267.
Cook C. D. K. & Nicholls M. S. (1987): A monographic study of the genus *Sparganium* (Sparganiaceae). Part 2, Subgenus *Sparganium*. – Bot. Helv. 1–44.
Darlington C. D. (1937): Recent advences in cytology. – J. and A. Churchill, London.
Dobeš C. H., Mitchell-Olds T. & Koch M. A. (2004): Extensive chloroplast haplotype variation indicates Pleistocene hybridization and radiation of North American *Arabis drummondii*, *A.* × *divaricarpa*, and *A. holboellii* (Brassicaceae). – Mol. Ecol. 13: 349–370.
Doležel J., Greilhuber J. & Suda J. (2007): Flow cytometry with plant cells: analysis of genes, chromosomes and genomes. – John Wiley & Sons, Weinheim.
Donovan L. A., Rosenthal D. R., Sanchez-Velenosi M., Rieseberg L. H. & Ludwig F. (2010): Are hybrid species more fit than ancestral parent species in the current hybrid species habitats? – J. Evol. Biol. 23: 805–816.
Egorova T. V. (1967): Rastenija Centralnoj Azii [The plants of Central Asia], T. 3: 19–22. – Leningrad, Nauka.
Ellstrand N. C. (2003): Dangerous liaisons?: when cultivated plants mate with their wild relatives. – JHU Press.
Ellstrand N. C. & Schierenbeck K. A. (2000): Hybridization as a Stimulus for the Evolution of Invasiveness in Plants? – Proc. Natl. Acad. Sci. U.S.A. 7043–7050.
Ellstrand N. C., Whitkus R. & Rieseberg L. H. (1996): Distribution of spontaneous plant hybrids. – Proc. Natl. Acad. Sci. U.S.A. 93: 5090–5093.
Emadzade K. & Hörandl E. (2011): Northern Hemisphere origin, transoceanic dispersal, and diversification of Ranunculeae DC. (Ranunculaceae) in the Cenozoic. – J. Biogeogr. 38: 517–530.

Gobert V., Moja S., Colson M. & Taberlet P. (2002): Hybridization in the section *Mentha* (Lamiaceae) inferred from AFLP markers. – Am. J. Bot. 89: 2017–2023.
Graebner P. (1900): Sparganiaceae. – In: Das Pflanzenreich IV/8, pp. 1–24. Leipzig.
Grant V. (1981): Plant speciation. – Columbia University Press, New York.
Gross B. L. & Rieseberg L. H. (2005): The ecological genetics of homoploid hybrid speciation. – J. Hered. 96: 241–252.
Guo Y.-P., Vogl C., Van Loo M. & Ehrendorfer F. (2005): Hybrid origin and differentiation of two tetraploid *Achillea* species in East Asia: molecular, morphological and ecogeographical evidence: Alloteraploid speciation in *Achillea*. – Mol. Ecol. 15: 133–144.
Hanušová K., Ekrt L., Vít P., Kolář F. & Urfus T. (2014): Continuous morphological variation correlated with genome size indicates frequent introgressive hybridization among *Diphasiastrum* species (Lycopodiaceae) in Central Europe (P. Hohenlohe, Ed.). – PLoS ONE 9: e99552.
Harrison R. G. (1990): Hybrid zones: windows on evolutionary process. – Oxford Surveys in Evolutionary Biology.
Harrison R. G. (1993): Hybrid zones and the evolutionary process. – Oxford University Press.
Harrison R. G. & Larson E. L. (2014): Hybridization, introgression, and the nature of species boundaries. – J. Hered. 105: 795–809.
Hegi G. (1936): *Sparganium*. In Illustrierte Flora Von Mittel Europa, Ed. 2, Vol. 1, pp. 281–291. – Hansen Verlag, München.
Heiser C. B. (1947): Hybridization between the sunflower species *Helianthus annuus* and *H. petiolaris*. – Evolution 1: 249–262.
Heiser C. B., Smith D. M., Clevenger S. B. & Martin W. C. (1969): The North American sunflowers (*Helianthus*). – Mem. Torrey Bot. Club 22: 1–218.
Hothorn T., Bretz F. & Westfall P. (2008): Simultaneous inference in general parametric models. – Biom. J. 50: 346–363.
Howard D. J., Britch S. C., Braswell W. E. & Marshall J. L. (2004): Evolution in hybrid zones. – In: Singh R. S. & Uyenoyama M. K. (eds.), The evolution of population biology, pp. 297–314. – Cambridge University Press, Cambridge.
Howarth D. G. & Baum D. A. (2005): Genealogical evidence of homoploid hybrid speciation in an adaptive radiation of *Scaevola* (Goodeniaceae) in the Hawaiian islands. – Evolution 59: 948–961.
Hroudová Z., Frantík T. & Zákravský P. (1998a): The differentiation of subspecies in *Bolboschoenus maritimus* based on the inflorescence structure. – Preslia 70: 135–154.
Hroudová Z., Moravcová L. & Zákravský P. (1998b): Differentation of the Central European *Bolboschoenus* taxa based on fruit shape and anatomy. – Thaiszia 8: 91–109.
Hroudová Z., Zákravský P. & Frantík T. (1999): Ecological differentiation of Central European *Bolboschoenus* taxa and their relationship to plant communities. – Folia Geobot. 34(1): 77-96.
Hroudová Z., Zákravský P., Ducháček M. & Marhold K. (2007a): Taxonomy, distribution and ecology of *Bolboschoenus* in Europe. – Ann. Bot. Fenn. 44: 81–102.
Hroudová Z., Zákravský P. & Flegrová M. (2014): The tolerance to salinity and nutrient supply in four European *Bolboschoenus* species (*B. maritimus*, *B. laticarpus*, *B. planiculmis* and *B. yagara*) affects their vulnerability or expansiveness. – Aquat. Bot. 112: 66–75.
Hroudová Z., Zákravský P. & Jarolímová V. (2007b): Notes on *Bolboschoenus glaucus*, a new species to the flora of Portugal. – Portugaliae Acta Biol. 22: 221–220.
Ito Y., Tanaka N., Kim C., Kaul R. B. & Albach D. C. (2016): Phylogeny of *Sparganium* (Typhaceae) revisited: non-monophyletic nature of *S. emersum* sensu lato and resurrection of *S. acaule*. – Plant Syst. Evol. 302: 129–135.

James J. K. & Abbott R. J. (2005): Recent, allopatric, homoploid hybrid speciation: the origin of *Senecio squalidus* (Asteraceae) in the British Isles from a hybrid zone on Mount Etna, Sicily. – Evolution 59: 2533–2547.

Jarolímová V. & Hroudová Z. (1998): Chromosome numbers within the genus *Bolboschoenus* in Central Europe. – Folia Geobot. 33: 415–428.

Kadereit J. W., Uribe-Convers S., Westberg E. & Comes H. P. (2006): Reciprocal hybridization at different times between *Senecio flavus* and *Senecio glaucus* gave rise to two polyploid species in north Africa and south-west Asia. – New Phytol. 169: 431–441.

Kaplan Z. (2002): *Sparganiaceae* Dum. – In: Kubát K., Hrouda L., Chrtek jun. J., Kaplan Z., Kirschner J. & Štěpánek J. (eds.): Klíč ke květeně České republiky [Key to the Flora of the Czech Republic.], pp. 877–878. – Academia, Praha.

Kaplan Z., Danihelka J., Štěpánková J., Bureš P., Zázvorka J., Hroudová Z., Ducháček M., Grulich V., Řepka R., Dančák M., Prančl J., Šumberová K., Wild J. & Trávníček B. (2015): Distributions of vascular plants in the Czech Republic. Part 1. – Preslia 87: 417–500.

Kim M., Cui M.-L., Cubas P., Gillies A., Lee K., Chapman M. A., Abbott R. J. & Coen E. (2008): Regulatory genes control a key morphological and ecological trait transferred between species. – Science 322: 1116–1119.

Koutecký P. (2015): MorphoTools: a set of R functions for morphometric analysis. – Plant Syst. Evol. 301: 1115–1121.

Koyama T. (1980): The genus *Bolboschoenus* Palla in Japan. – Acta Phytotax. Geobot. 31: 139–148.

Kruskal W. H. & Wallis W. A. (1952): Use of ranks in one-criterion variance analysis. – J. Am. Stat. Assoc. 47: 583.

Lai Z., Nakazato T., Salmaso M., Burke J. M., Tang S., Knapp S. J. & Rieseberg L. H. (2005): Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species. – Genetics 171: 291–303.

Les D. H., Crawford D. J., Kimball R. T., Moody M. L. & Landolt E. (2003): Biogeography of discontinuously distributed hydrophytes: a molecular appraisal of intercontinental disjunctions. – Int. J. Plant Sci. 164: 917–932.

Les D. H. & Philbrick C. T. (1993): Studies of hybridization and chromosome number variation in aquatic angiosperms: evolutionary implications. – Aquat. Bot. 44: 181–228.

Levin D. A. (1975): Minority cytotype exclusion in local plant populations. – Taxon 24: 35.

Levin D. A., Francisco-Ortega J. & Jansen R. K. (1996): Hybridization and the extinction of rare plant species. – Conserv. Biol. 10: 10–16.

Lewontin R. C. & Birch L. C. (1966): Hybridization as a source of variation for adaptation to new environments. – Evolution 20: 315–336.

Lexer C., Fay M. F., Joseph J. A., Nica M.-S. & Heinze B. (2005): Barrier to gene flow between two ecologically divergent *Populus* species, *P. alba* (white poplar) and *P. tremula* (European aspen): the role of ecology and life history in gene introgression. – Mol. Ecol. 14: 1045–1057.

Lexer C., Welch M. E., Raymond O. & Rieseberg L. H. (2003): The origin of ecological divergence in *Helianthus paradoxus* (Asteraceae): selection on transgressive characters in a novel hybrid habitat. – Evolution 57: 1989–2000.

Löve Á. & Löve D. (1948): Chromosome numbers of Northern plant species. – Icel. Univ. Inst. Appl. Sci., Dep. Agric., Reykjavík, Rep. Ser. B 3: 1–131.

Ma F., Zhao C., Milne R., Ji M., Chen L. & Liu J. (2010): Enhanced drought-tolerance in the homoploid hybrid species *Pinus densata*: Implication for its habitat divergence from two progenitors. – New Phytol. 185: 204–216.

Mallet J. (2005): Hybridization as an invasion of the genome. – Trends Ecol. Evol. 20: 229–237.

Mallet J. (2007): Hybrid speciation. – Nature 446: 279–283.
Mallet J., Besansky N. & Hahn M. W. (2016): How reticulated are species? – BioEssays 38: 140–149.
Mao J.-F. & Wang X.-R. (2011): Distinct niche divergence characterizes the homoploid hybrid speciation of *Pinus densata* on the Tibetan Plateau. – Am. Nat. 177: 424–439.
Marhold K., Hroudová Z., Ducháček M. & Zákravský P. (2004): The *Bolboschoenus maritimus* group (Cyperaceae) in Central Europe, including *B. laticarpus*, spec. nova. – Phyton (Horn) 44: 1–21.
Marques I., Feliner G. N., Draper Munt D., Martins-Loução M. A. & Aguilar J. F. (2010): Unraveling cryptic reticulate relationships and the origin of orphan hybrid disjunct population in *Narcissus*. – Evolution.
Marques I., Jürgens A., Aguilar J. F. & Feliner G. N. (2016): Convergent recruitment of new pollinators is triggered by independent hybridization events in *Narcissus*. – New Phytol. 210: 731–742.
Marques I., Nieto Feliner G., Martins-Loução M. A. & Fuertes Aguilar J. (2012): Genome size and base composition variation in natural and experimental *Narcissus* (Amaryllidaceae) hybrids. – Ann. Bot. 109: 257–264.
Martin N. H., Bouck A. C. & Arnold M. L. (2006): Detecting adaptive trait introgression between *Iris fulva* and *I. brevicaulis* in highly selective field conditions. – Genetics 172: 2481–2489.
McCarthy E. M., Asmussen M. A. & Anderson W. W. (1995): A theoretical assessment of recombinational speciation. – Heredity 74: 502–509.
Milne R. I., Terzioglu S. & Abbott R. J. (2003): A hybrid zone dominated by fertile F1s: maintenance of species barriers in *Rhododendron*. – Mol. Ecol. 12: 2719–2729.
Minder A. M., Rothenbuehler C. & Widmer A. (2007): Genetic structure of hybrid zones between *Silene latifolia* and *Silene dioica* (Caryophyllaceae): evidence for introgressive hybridization. – Mol. Ecol. 16: 2504–2516.
Nadeau N. J., Ruiz M., Salazar P., Counterman B., Medina J. A., Ortiz-Zuazaga H., Morrison A., McMillan W. O., Jiggins C. D. & Papa R. (2014): Population genomics of parallel hybrid zones in the mimetic butterflies, *H. melpomene* and *H. erato*. – Genome Res. 24: 1316–1333.
Neiva J., Hansen G. I., Pearson G. A., Vliet M. S. V. D., Maggs C. A. & Serrão E. A. (2012): *Fucus cottonii* (Fucales, Phaeophyceae) is not a single genetic entity but a convergent salt-marsh morphotype with multiple independent origins. – Eur. J. Phycol. 47: 461–468.
Neuman L. M. (1897): Om Nomenklatur och artbegränsning inom slägtet *Sparganium*. – Botan. Not. 3: 113–130.
Nieto Feliner G., Álvarez I., Fuertes-Aguilar J., Heuertz M., Marques I., Moharrek F., Piñeiro R., Riina R., Rosselló J. A., Soltis P. S. & Villa-Machío I. (2017): Is homoploid hybrid speciation that rare? An empiricist's view. – Heredity 118: 513–516.
Ostenfeld-Hansen C. (1897): De i Danmark voxende ramose *Sparganium*-Arter. – Bot. Tidskrift. V–IX.
Paun O., Forest F., Fay M. F. & Chase M. W. (2009): Hybrid speciation in angiosperms: parental divergence drives ploidy. – New Phytol. 182: 507–518.
Pedersen A. T. M., Nowak M. D., Brysting A. K., Elven R. & Bjorå C. S. (2016): Hybrid origins of *Carex rostrata* var. *borealis* and *C. stenolepis*, two problematic taxa in *Carex* Section *Vesicariae* (Cyperaceae). – PloS ONE 11: e0165430.
Pollux B. J. A., Jong M. D. E., Steegh A., Verbruggen E., Van Groenendael J. M. & Ouborg N. J. (2007): Reproductive strategy, clonal structure and genetic diversity in populations of the aquatic macrophyte *Sparganium emersum* in river systems. – Mol. Ecol. 16: 313–325.
Pritchard J. K., Stephens M. & Donnelly P. (2000): Inference of population structure using multilocus genotype data. – Genetics 155: 945–959.

Randolph L. F. (1966): *Iris nelsonii*: a new species of Louisiana iris of hybrid origin. – Baileya 14: 143–169.
Reutemann A. G., Ardissone R. E., López M. G., Muchut S. E., Boldrini I., Vegetti A. C. & Giussani L. M. (2018): Phylogenetic relationships in *Bulbostylis* (Abildgaardieae: Cyperaceae) inferred from nuclear and plastid DNA sequence data. – Syst. Biodivers.
Rieseberg L. H. (1991): Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. – Am. J. Bot. 78: 1218.
Rieseberg L. H. (1995): The role of hybridization in evolution: Old Wine in New Skins. – Am. J. Bot. 82: 944–953.
Rieseberg L. H. (1997): Hybrid origins of plant species. – Annu. Rev. Ecol. Evol. Syst. 28: 359–389.
Rieseberg L. H. (2009): Evolution: Replacing Genes and Traits through Hybridization. – Curr. Biol. 19: R119–R122.
Rieseberg L. H., Archer M. A. & Wayne R. K. (1999a): Transgressive segregation, adaptation and speciation. – Heredity 83: 363–372.
Rieseberg L. H., Kim S.-C., Randell R. A., Whitney K. D., Gross B. L., Lexer C. & Clay K. (2007): Hybridization and the colonization of novel habitats by annual sunflowers. – Genetica 129: 149–165.
Rieseberg L. H., Whitton J. & Gardner K. (1999b): Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. – Genetics 152: 713–727.
Roalson E. H. (2008): A synopsis of chromosome number variation in the Cyperaceae. – Bot. Rev. 74: 209–393.
Rosenthal D. M., Schwarzbach A. E., Donovan L. A., Raymond O. & Rieseberg L. H. (2002): Phenotypic differentiation between three ancient hybrid taxa and their parental species. – Int. J. Pl. Sci. 163: 387–398.
Schmickl R., Marburger S., Bray S. & Yant L. (2017): Hybrids and horizontal transfer: introgression allows adaptive allele discovery. – J. Exp. Bot. 68: 5453–5470.
Schulte K., Silvestro D., Kiehlmann E., Vesely S., Novoa P. & Zizka G. (2010): Detection of recent hybridization between sympatric Chilean *Puya* species (Bromeliaceae) using AFLP markers and reconstruction of complex relationships. – Mol. Phylogenetics Evol. 57:1105–1119.
Shiga T. & Kadono Y. (2007): Natural hybridization of the two *Nuphar* species in northern Japan: Homoploid hybrid speciation in progress? – Aquat. Bot. 86: 123–131.
Smith S. G. (2002): *Bolboschoenus*. In Flora of North America: north of Mexico. Volume 23. Magnoliophyta: Commelinidae (in part): Cyperaceae, pp. 37–44. – Oxford University Press, New York.
Snow A. A., Travis S. E., Wildova R., Fer T., Sweeney P. M., Marburger J. E., Windels S., Kubatova B., Goldberg D. E. & Mutegi E. (2010): Species-specific SSR alleles for studies of hybrid cattails (*Typha latifolia* x *T. angustifolia*; Typhaceae) in North America. – Am. J. Bot. 97: 2061–2067.
Soltis D. E., Buggs R. J. A., Doyle J. J. & Soltis P. S. (2010): What we still don't know about polyploidy. – Taxon 59: 1387–1403.
Soltis P. S. & Soltis D. E. (2009): The role of hybridization in plant speciation. – Annu. Rev. Plant Biol. 60: 561–588.
Soltis D. E., Soltis P. S., Pires J. C., Kovarik A., Tate J. A. & Mavrodiev E. (2004): Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. – Biol. J. Linn. Soc. 82: 485–501.
Stebbins G. L. (1959): The role of hybridization in evolution. – Proc. Am. Philos. Soc. 103: 231–251.
Stebbins G. L. (1971): Chromosomal evolution in higher plants. – Edward Arnold Ltd., London.

Steen S. W., Gielly L., Taberlet P. & Brochmann C. (2000): Same parental species, but different taxa: molecular evidence for hybrid origins of the rare endemics *Saxifraga opdalensis* and *S. svalbardensis* (Saxifragaceae). – Biol. J. Linn. Soc. 132: 153–164.

Suarez-Gonzalez A., Hefer C. A., Christe C., Corea O., Lexer C., Cronk Q. C. B. & Douglas C. J. (2016): Genomic and functional approaches reveal a case of adaptive introgression from *Populus balsamifera* (balsam poplar) in *P . trichocarpa* (black cottonwood). – Molecular Ecology 25: 2427–2442.

Sulman J. D., Drew B. T., Drummond C., Hayasaka E. & Sytsma K. J. (2013): Systematics, biogeography, and character evolution of *Sparganium* (Typhaceae): Diversification of a widespread, aquatic lineage. – Am. J. Bot. 100: 2023–2039.

Tatanov I. V. (2007): Taksonomicheskiy obzor roda *Bolboschoenus* (Aschers.) Palla (Cyperaceae) [Taxonomic survey of the genus *Bolboschoenus* (Cyperaceae)]. – Novosti. Sist. Vyssh. Rast. 46–149.

Templeton A. R. (1981): Mechanisms of speciation-A Population Genetic Approach. – Ann. Rev. Ecol. Syst. 12: 23–48.

Ter Braak C. J. F. & Šmilauer P. (2012): Canoco reference manual and user's guide: software for ordination (Version 5.0). – Microcomputer Power, Ithaca, U.S.A.

Valencia-Cuevas L. Mussali-Galante P., Piñero D., Castillo-Mendoza E., Rangel-Altamirano G. & Tovar-Sánchez E. (2015): – Plant Syst. Evol. 301: 1085–1097.

Viljoen J.-A., Muasya A. M., Barrett R. L., Bruhl J. J., Gibbs A. K., Slingsby J. A., Wilson K. L. & Verboom G. A. (2013): Radiation and repeated transoceanic dispersal of *Schoeneae* (Cyperaceae) through the southern hemisphere. – Am. J. Bot. 100: 2494–2508.

Vos P., Hogers R., Bleeker M., Reijans M., Lee T. van de, Hornes M., Friters A., Pot J., Paleman J., Kuiper M. & Zabeau M. (1995): AFLP: a new technique for DNA fingerprinting. – Nucleic Acids Res. 23: 4407–4414.

Wang X.-R., Szmidt A. E. & Savolainen O. (2001): Genetic composition and diploid hybrid speciation of a high mountain pine, *Pinus densata*, Native to the Tibetan Plateau. – Genetics.

Whitham T. G., Morrow P. A. & Potts B. M. (1991): Conservation of hybrid plants. – Science 254: 779–780.

Whitney K. D., Ahern J. R., Campbell L. G., Albert L. P. & King M. S. (2010a): Patterns of hybridization in plants. – Perspect. Plant. Ecol. Syst. 12: 175–182.

Whitney K. D., Randell R. A. & Rieseberg L. H. (2010b): Adaptive introgression of abiotic tolerance traits in the sunflower *Helianthus annuus*. – New Phytologist 187: 230–239.

Wilcox M. D. (2001): *Bolboschoenus* in Auckland. – Auckland Bot. Soc. J 56: 70–71.

Yakimowski S. B. & Rieseberg L. H. (2014): The role of homoploid hybridization in evolution: A century of studies synthesizing genetics and ecology. – Am. J. Bot. 101: 1247–1258.

PAPER I.

Píšová S. & Fér T. (submitted): Homoploid hybrid speciation in *Sparganium erectum*: molecular, genome size and morphometric analyses.

Homoploid hybrid speciation in *Sparganium erectum*: molecular, genome size and morphometric analyses

Homoploidní hybridní speciace *Sparganium erectum*: molekulární a morfometrické analýzy a stanovení velikosti genomu

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Abstract

Aquatic plants tend to exhibit vast phenotypic plasticity leading to taxonomic difficulties in many groups. In the genus *Sparganium*, which comprises about fourteen species, numerous taxa at different ranks have been described. The current classification is based on generative characters on achenes, which are less influenced by the environment than vegetative characters. Nevertheless, the intraspecific division of *Sparganium erectum* has become problematic, especially due to the existence of several intraspecific taxa along with intermediate individuals. In this study we examined four European subspecies of *Sparganium erectum* (subsp. *erectum*, *oocarpum*, *microcarpum* and *neglectum*) from 64 populations in the Czech Republic.

The combination of multivariate morphometrics, AFLP and genome size estimation allowed us to confirm the current subspecies classification and to investigate putative intraspecific hybridization. Four genetic groups corresponding to separate subspecies with different genome size were found. Morphological characters described in previous studies correlated with these genetic groups and thus confirmed the current classification. The most important characters for subspecies differentiation were achene width and length, style length, length of the upper part of the achene and constriction in the middle part of the achene. In addition, admixed individuals between the genetic groups were observed. Subsp. *oocarpum* was identified as a stable hybrid between subsp. *erectum* and subsp. *neglectum*. Moreover, two other hybrid combinations were detected, suggesting recent hybridization (subsp. *erectum* × subsp. *microcarpum* and subsp. *microcarpum* × subsp. *neglectum*).

K e y w o r d s : AFLP, Central Europe, genome size, homoploid hybrid speciation, model-based clustering, multivariate morphometrics, *Sparganium*

Introduction

Aquatic environments are highly dynamic, variable and heterogeneous on a relatively small scale (Sculthorpe 1967). Wetland and aquatic plants are generally well adapted to changing habitat conditions (fluctuations of the water level, changing water turbidity or temperature fluctuations), which is frequently connected with vast morphological variation. High intraspecific variation as an expression of a high level of phenotypic plasticity may complicate taxonomic differentiation (Kaplan 2002a, Santamaria 2002, Barret 2003). Sizes of vegetative organs, such as the length and width of leaves, may vary depending on habitat conditions that make them unreliable for identifying species. Similarly, some species (and sometimes even genera) cannot be distinguished in juvenile or sterile stages (Cook & Nicholls 1986).

Intraspecific morphological variation has also influenced the different taxonomic conceptions of the genus *Sparganium* L (Typhaceae). Even though different authors mentioned within this genus numerous taxa at varying ranks (Cook 1980, Graebner 1900, Ostenfeld-Hansen 1897), most of those taxa probably represent mere phenotypes of one taxon. This has been caused by the use of unstable vegetative characters for taxonomic differentiation (Čelakovský 1896b, Čelakovský 1899, Graebner 1900). The current classification of the genus *Sparganium* is based on less variable morphological characters on achenes and recognizes about fourteen species (Cook & Nicholls 1986, 1987). The taxonomy of the genus in the Czech Republic was recently investigated by Haasová (1997). Although characters such as the length and width of achenes are less appropriate, their ratios can be used as distinguishing characters. The colour of the upper and lower parts of the achene also appears to be useful for the determination of infrageneric taxa (Cook & Nicholls 1987, Kaplan 2002b).

In the Czech Republic, four species: *Sparganium natans* L., *S. erectum* L., S. *emersum* REHMANN and *S. angustifolium* MICHX, have been found growing, the last of which had been considered extinct until recently. Different authors mention various numbers of subspecific taxa (especially within *Sparganium erectum*) as occurring in Central Europe (e.g. Ascherson & Graebner 1897, Casper & Krausch 1980, Hegi 1936). Because vegetative characters were mostly used for their identification, the intraspecific division of *S. erectum* has become unexpectedly problematic.

The present worldwide classification of *S. erectum* is based on the morphology of achenes and distinguishes five subspecies: *S. erectum* L. subsp. *erectum*, *S. e.* subsp. *oocarpum* (ČELAK.) DOMIN, *S. e.* subsp. *neglectum* (BEEDY) K.RICHT., *S. e.* subsp. *microcarpum* (NEUMANN) DOMIN and *S. e.* subsp. *stoloniferum* (GRAEBN.) H.HARA (Graebner 1900, Cook 1980, Cook & Nicholls 1987). Plant material of the Asian subspecies *stoloniferum* was not available to us, so we included only four European taxa in our study. In addition, intermediate individuals between subspecies are found and mixed populations of individuals of different subspecies have been reported, suggesting possible hybridization between subspecies. *Sparganium erectum* subsp. *oocarpum* is supposed to be of hybrid origin, the putative parental taxa being *S. e*. subsp. *neglectum* and *S. e.* subsp. *erectum*. Moreover, previous study of herbarium specimens and achene morphology found subsp. *microcarpum* to have a high level of morphological variation and revealed that many individuals are intermediate between subsp. *microcarpum* and *neglectum* (Haasová 1997). Therefore, Haasová (l. c.) suggested merging both subspecies into one taxon.

To examine intraspecific variation of *Sparganium erectum* and possibly come up with taxonomic conclusions, we decided to use a combination of classical methodical approaches (morphometric analysis) and biosystematic molecular methods such as Amplified Fragment Length Polymorphisms (AFLPs) and flow cytometry (FCM). Such a combination of methods is a powerful tool for assessing intraspecific variation and unravelling the origins of hybrids (Guo 2006, Gobert 2002, Španiel 2011, Píšová et al. 2017). The AFLP method generates a large number of loci distributed throughout the genome and requires no previous sequence knowledge (Vos 1995). Bayesian clustering in STRUCTURE classifies samples into genetic groups (Pritchard et al. 2000, Evanno et al. 2005) and allows subsequent determination of admixed individuals (putative hybrids; Ciotir et al. 2017, Paszko & Nobis 2010, Píšová et al. 2017). The ascertainment of genome size by flow cytometry is a simple and rapid method that has been applied in a wide range of biosystematic studies (Doležel et al. 2007, Loureiro et al. 2010, Slovák et al. 2009, Kolář et al. 2013, Linder et al. 2017) and facilitates the determination of hybrid species (Vít et al. 2014, Bureš et al. 2004). However, these methods were previously never used for the study of genetic relationships and hybridization within the genus *Sparganium*. On the other hand, we did not investigate chromosome numbers because they have been reported to be uniform across the whole genus *Sparganium* (2n= 30, Löve & Löve 1948, 1956; Cook & Nicholls 1986). The

origin of new taxa via hybridization not accompanied by whole-genome duplication and an increase of ploidy level is described as homoploid hybridization speciation (Rieseberg 1997). The establishment of a new homoploid hybrid taxon requires hybrid segregants to be isolated by a sterility barrier or some external isolation mechanism (e.g. by geographic or ecological isolation, Buerkle et al. 2000, Gross & Rieseberg 2005). To investigate homoploid hybridization in *Sparganium erectum* and reassess its current classification, we addressed the following questions: (i) Do the genetic pattern or variation in genome size within *Sparganium erectum* allow the delimitation of clearly separated groups that could be used as a basis for intraspecific taxonomical classification? (ii) Are genetic variation and variation in genome size correlated with morphological variation? (iii) What morphological characters are correlated the most and can they be used for the differentiation of intraspecific taxa? (iv) Is *Sparganium erectum* subsp. *oocarpum* of hybrid origin? Which species are its putative parents? (v) Should the subspecies *neglectum* and *microcarpum* be merged into a single taxon or distinguished as two distinct taxa and which characters can be used for their identification?

Materials and methods

Plant material

Samples of four subspecies of *Sparganium erectum* (subsp. *erectum*, *oocarpum*, *neglectum* and *microcarpum*) from 64 natural populations in the Czech Republic were collected during 2007–2008 (Appendix 1). Typical habitats of *S. erectum* in the Czech Republic occur along river banks and on river floodplains, which corresponds to the distribution of sampling sites predominantly along the rivers Labe (Elbe), Vltava (Moldau), Lužnice and their tributaries. Most plants were sampled in Central and South Bohemia, and one population was sampled in South Moravia (Electronic Appendix 2A, 2B). Material for AFLP analyses (altogether 276 leaf samples), morphological analyses (1770 achenes) and flow cytometry (276 fresh leaves) was collected randomly, but from same individuals, using the following procedures. For AFLP analyses, part of a young leaf was dried immediately in silica gel and another part was used for the estimation of genome size by flow cytometry (up to 5 individuals per population, depending on population size). For morphometric measurements, five ripe achenes were randomly chosen from a mixture of all achenes from each individual (altogether 25 achenes per

population). When putatively mixed or intermediate populations were found, the number of individuals sampled for AFLPs, FCM and morphometric analyses was increased to 15 per population to ensure more thorough examination. Because *S. erectum* is a rhizomatous clonal plant, only individuals located 10 m apart were collected to minimize sampling the same clone several times. Voucher specimens of plants are deposited in the herbarium collection at the Faculty of Sciences, Charles University in Prague (PRC).

AFLP analysis

Total genomic DNA was extracted from silica-dried leaf material using the CTAB isolation buffer following the protocol of Doyle & Doyle (1987). Final DNA pellets were dissolved in 100 µL of 1×TE buffer. The DNA concentration was measured using a Nanodrop 1000 spectrophotometer (Thermo Scientific) and the DNA was diluted to 100 ng μL^{-1}.

AFLP analysis (Vos et al. 1995) was achieved using the AFLP Core Reagent Kit I (Invitrogen) and the AFLP Pre-Amp Primer Mix I (Invitrogen) following the manufacturer's instructions as modified by Záveská et al. (2011) and further modified to yield the procedure described below. Total genomic DNA (~100 ng) was double-digested for 3h at 37°C with 0.5 U of each *Eco*RI and *Mse*I restriction enzymes (Invitrogen) and 1 µl of a 5× reaction buffer (Invitrogen) in a total volume of 5 µl. Subsequently, adaptors were ligated for 3h at 37°C by adding 4.8 µl of an adaptor/ligation solution (Invitrogen) and 0.2 U of T4 DNA ligase (Invitrogen) to the digested DNA (total volume 10 µl). The pre-amplification mixture (total volume 5 µL) contained 0.5 µL of restricted/ligated DNA, 4.0 µL of Pre-Amp Primer Mix I, 0.5 µL of 10× buffer for RedTaq JumpStart (Sigma) and 0.1 U of RedTaq JumpStart DNA polymerase (Sigma). After preamplification, the DNA was 10× diluted with ddH_2O. Three primer combinations were used for selective amplification: *Eco*RI-ACT-(6-FAM)/*Mse*I-CAT, *Eco*RI-AAG-(HEX)/*Mse*I-CTC and *Eco*RI-ACC-(NED)/*Mse*I-CAT (Fér & Pfosser submitted). The reaction mixture for selective amplification contained 2.3 µL of the diluted preamplification mixture, 1 µL of a 10× buffer for RedTaq, 0.2 µM dNTP, 0.5 pmol of an *Eco*RI-selective fluorescence-labelled primer, 2.5 pmol of an *Mse*I-selective primer and 0.2 U of RedTaq JumpStart DNA polymerase (Applied Biosystems) in a total volume of 10 µL. Products of preselective and selective amplifications were checked on an agarose gel. The selective amplification products were electrophoresed on the ABI 3100 Genetic Analyzer (Applied Biosystems) with

GeneScan ROX 500 (Applied Biosystems) as a size standard in the DNA Sequencing Laboratory, Faculty of Science, Charles University in Prague. In total, 276 samples from 64 populations were analysed. The whole AFLP procedure was repeated for 8% (22) of the samples and the error rate was estimated by comparing identical samples (Bonin et al. 2004).

Molecular data analyses

GeneMarker v1.8 (SoftGenetics LLC, PA, USA) was used for analysing AFLP data and transferring them into a binary data matrix. Only unambiguous bands were used for subsequent analyses; faint bands were excluded. Three *Eco*RI/*Mse*I AFLP primer combinations generated 125 AFLP markers, ranging in size from 100 to 500 base pairs.

Bayesian non-hierarchical clustering was employed in STRUCTURE 2.3.2.1 (Pritchard et al. 2000) using a Markov chain Monte Carlo (MCMC) algorithm and an admixture model with correlated allele frequencies to define AFLP groups, to examine the hybrid origin of subsp. *oocarpum* and to assess the degree of admixture among subspecies. Ten replicates for each K=1–10 were done to determine the stability of the results. The burn-in length of 100,000 generations and an additional 1,000,000 generations of MCMC chains after burn-in were run on the Metacentrum VO infrastructure (https://metavo.metacentrum.cz, Falush et al. 2007). The output files were summarized in Structure harvester (Earl & vonHoldt 2012) to determine the optimal number of clusters (K) according to the similarity coefficients between the runs (ΔK), (Evanno et al. 2005, Nordborg et al. 2005). Graphical outputs for selected Ks were generated using CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and Distruct (Rosenberg 2004). Samples with low admixture (up to 15%) were considered members of one of the K AFLP groups and served as predefined groups in morphometric analyses whereas highly admixed individuals (more than 15%, i.e., genetically intermediate between the AFLP groups), were passively projected onto ordination diagrams in subsequent analyses (i.e., PCoA, PCA, CDA; Píšová et al. 2017).

To explore the distribution of genetic variation within and among species and populations, two analyses of molecular variance (AMOVAs, Excoffier et al. 1992; implemented in FAMD 1.3, Schlüter & Harris 2006) were performed: (1) three-level analysis (among species, among populations within species and within populations) and (2) two-level analysis for each subspecies

(among populations and within populations). Only predefined AFLP groups with non-admixed individuals were used for AMOVA calculations.

Principal coordinate analysis (PCoA) implemented in Canoco 5 (ter Braak & Šmilauer 2012) was performed using Jaccard's similarity coefficients (Jaccard 1908) for the calculation of the distance matrix. A neighbour-network was constructed in SPLITSTREE v.4.11.3 (Huson & Bryant 2006).

In all graphical outputs of molecular analyses, AFLP groups are colour-coded in accordance with the presentation of our STRUCTURE results. Highly admixed individuals are marked by grey symbols. In addition, the genetic variability of each population was estimated by calculating the number of genotypes (N_g), Nei's gene diversity (D_{Nei}) and the percentage of polymorphic markers (%poly) using the R script AFLPdat (Ehrich 2006).

Genome size estimation

Estimations of genome sizes were performed using propidium iodide flow cytometry following the simplified two-step procedure using Otto buffers described by Doležel et al. (2007). Intact leaf tissue (1 cm^2) of *Sparganium erectum* together with an appropriate volume of the internal reference standard (*Glycine max* cv. Polanka, 2C = 2.50 pg) was chopped using a sharp razor blade in a Petri dish containing 0.5 ml of the Otto I buffer (water solution of 0.1M citric acid monohydrate and 0.5% Tween 20). The crude suspension was filtered through a nylon mesh (42-µm pore size) and incubated for 15 min at room temperature. After incubation, 1 ml of staining solution containing Otto II buffer (0.4 M $Na_2HPO_4 \times 12\ H_2O$) and propidium iodide (final concentration 50 µg/ml), RNase IIA (50 µg/ml) and β-mercaptoethanol (2 µl/ml) was added. A Partec CyFlow SL (Partec GmbH, Münster, Germany) flow cytometer equipped with a green diode-pumped solid-state laser (100mW, 532-nm, Cobolt Samba, Cobolt, Sweden) as the excitation light source was used for recording the fluorescence intensity of isolated nuclei. Final histograms were evaluated using FloMax software (version 2.4d, Partec GmbH, Münster, Germany) and only analyses with coefficients of variance (CV) of the sample G1 peak below 3% were considered. DNA contents of samples were calculated based on means of peaks (Doležel et al. 2003) using the following formula: Sample 2C DNA content (pg) = (sample G_1 peak mean / standard G_1 peak mean) × standard 2C DNA amount (pg). A one-way ANOVA procedure, a Kruskal-Wallis test (Kruskal & Wallis 1952) and Tukey's HSD multiple comparison tests (as

implemented in the R package multcomp; Hothorn et al. 2008) were performed to determine statistical differences in genome size between seven genetic groups defined using AFLP and visualized using box plots.

Morphometric analyses

Considering the high phenotypic plasticity in vegetative parts of the plant body, we decided to analyse characters only on generative parts (namely achenes) in our morphometric analyses because they appear to be more stable according to the current classification (Cook & Nicholls 1986, Haasová 1997, Kaplan 2002b). The species' achenes are known to change their size and colour during maturation, so only ripe and fully developed achenes were collected. Altogether, 14 morphological characters were used in the primary analysis (Table 1, Fig. 1). However, the characters 'sterility of fruiting heads' and 'shoulder between upper and lower part' were invariable within groups and could not be used for multivariate analyses. The remaining 12 characters were tested for normality by the Shapiro-Wilk statistic, and a non-parametric Spearman correlation coefficient was computed to determine the correlations of morphological characters (both using R version 3.4.0, R Foundation for Statistical Computing, Vienna, Austria). Five achenes from each of 264 individuals were measured and the mean for each individual was used in subsequent analyses. To gain a preliminary insight into the overall morphological variation, principal component analysis (PCA) was performed using the Morphotools suite of R scripts (Koutecký 2015). The AFLP groups were visualized using colours according to the STRUCTURE analysis and highly admixed individuals were presented by grey symbols. Subsequently, a canonical discriminant analysis (CDA) with groups predefined by AFLP (without admixed individuals) was applied to determine variation among these groups and find the most important characters for their differentiation. Highly admixed individuals were passively projected onto the ordination diagram afterwards. Another CDA analysis was employed to differentiate between two morphologically overlapping groups, subsp. *microcarpum* and *neglectum*. Finally, classificatory discriminant analysis was used to verify the accuracy of the classification of individuals into the predefined groups. Determination of highly admixed individuals was performed using predefined groups as a training dataset.

Table 1. – List of morphological characters used for morphometric analyses of achenes from four subspecies of *Sparganium erectum* (See Fig. 1 for graphical explanation). * Invariable characters that were excluded from the total analyses.

Character	Transformation
Infructescence character	
* Sterility of fruit heads	
Achene characters	
Peduncle	Untransformed
AcheneLength – length of achene (without style) (mm)	x^2
AcheneWidth – width of achene (mm)	Untransformed
LengthLower – length of the lower part of achene (mm)	x^0.15
LengthUpper – length of the upper part of achene (mm)	Untransformed
StyleLength –	log10 (x)
* Shoulder – the shoulder between the upper and lower part of achene.	
Number of angles	-1*(x^-2.5)
Constriction of achene below the shoulder: 0 - absent, 1 – present	Untransformed
AcheneLength/ AcheneWidth	log10 (x)
LengthUpper/ LengthLower	log10 (x+1)
StyleLength/ AcheneLength	Untransformed
StyleLength/ AcheneWidth	log10 (x)

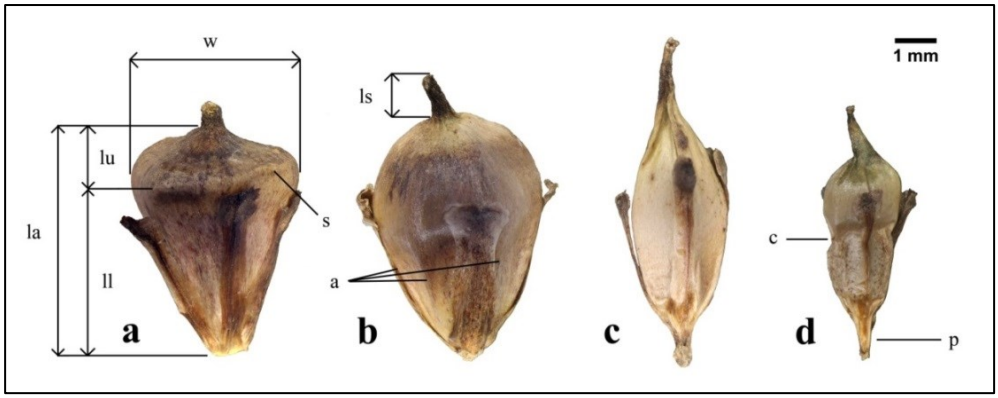

Fig. 1. – Morphological characters of achenes: 1, *Sparganium erectum* subsp. *erectum*; 2, *S. e. oocarpum*; 3, *S. e. neglectum*; 4, *S. e. microcarpum*. Scale bars: 1 mm. a, number of angles; c, constriction; la, length of achene; ll, length of the lower part of achene; lu, length of upper part of achene; ls, length of style; p, peduncle; s, shoulder between upper and lower part; w, width of achene. Phot. S. Píšová.

Results

AFLPs

The three selective primer pairs produced 125 unambiguous AFLP markers (loci), ranging in size from 100 to 500 bp, of which 112 (89.6%) were polymorphic. For 22 replicated samples, the error rate was 4.98% and the average number of loci per individual was 79.43.

The STRUCTURE analysis generated consistent results only for K=2 and K=4. The greater K values and K=3 did not converge towards the same outcomes (Electronic Appendix 3A-E, Electronic Appendix 4). The solution with the greatest ΔK, K=2, separated two groups: The first group morphologically corresponds to *S. erectum* subsp. *erectum* and *microcarpum* (147 samples) and the second group to subsp. *oocarpum* and *neglectum* (71 samples). K=3 resulted in two different outcomes differing in the classification of subsp. *oocarpum* (56 samples). These individuals were either not distinguished from subsp. *neglectum* (for outcomes with lower similarity coefficients, see Electronic Appendix 5) or as an approximately 50:50 admixture of groups corresponding to subsp. *erectum* and subsp. *neglectum* (Fig. 2A). The result with a still high ΔK and also a stable solution with K=4 discriminated four genetic groups (subspecies): *S. e*. subsp. *erectum* (42 samples), *oocarpum* (56 samples), *microcarpum* (105 samples) and *neglectum* (15 samples). To define individual groups and to deal with admixture, we used the ad hoc setting (up to 0.15 assignment probability of clearly placed samples in one of the four genetic groups). Moreover, part of the samples were found to exhibit higher admixture (i.e. with at least 0.15 assignment probability to more than one group), suggesting hybridization also between these genetic groups (Fig. 2A: group A – 5 samples between subsp. *erectum* and subsp. *oocarpum*, group B – 30 samples between subsp. *erectum* and *microcarpum*, group C – 23 samples between subsp. *microcarpum* and *neglectum*).

Principal coordinate analysis (PCoA) confirmed the results of the STRUCTURE analysis. The four subspecies of *S. erectum* were clearly differentiated in the ordination space with admixed individuals in an intermediate position between their parental taxa (the first and second PCoA axes explained 28.17% and 10.43% of the variability, respectively; Fig. 2B). Comparable results were obtained by the neighbour-net network analysis, which well separated the particular subspecies as well as the hybrid individuals between them (Fig. 2C).

Analysis of molecular variation (AMOVA) comprising only four genetic groups corresponding to subspecies determined that most of the variation (77.57%) was attributed to differences between subspecies. Only 15.37% of the variation occurred within subspecies and the remaining 7.06% of the variation was distributed among individuals within populations. The second AMOVA analysis was performed for each subspecies separately. The greatest difference between variation among populations (87.23%) and within populations (12.77%) was found in subsp. *neglectum* whereas the smallest difference was detected in subsp. *erectum* (58.50% and 41.50% of the variation, Table 3).

Maximum genetic diversity was found in populations Db, Po (D_{Nei} = 0.15, %poly = 15.20%) and R (D_{Nei} = 0.15, %poly = 36.0%) whereas minimum genetic diversity was recorded for populations Tr (D_{Nei} = 0.02, %poly = 3.20%), Y (D_{Nei} = 0.02, %poly = 4.8%) and W (D_{Nei} = 0.02, %poly = 4.0%), see Appendix 1.

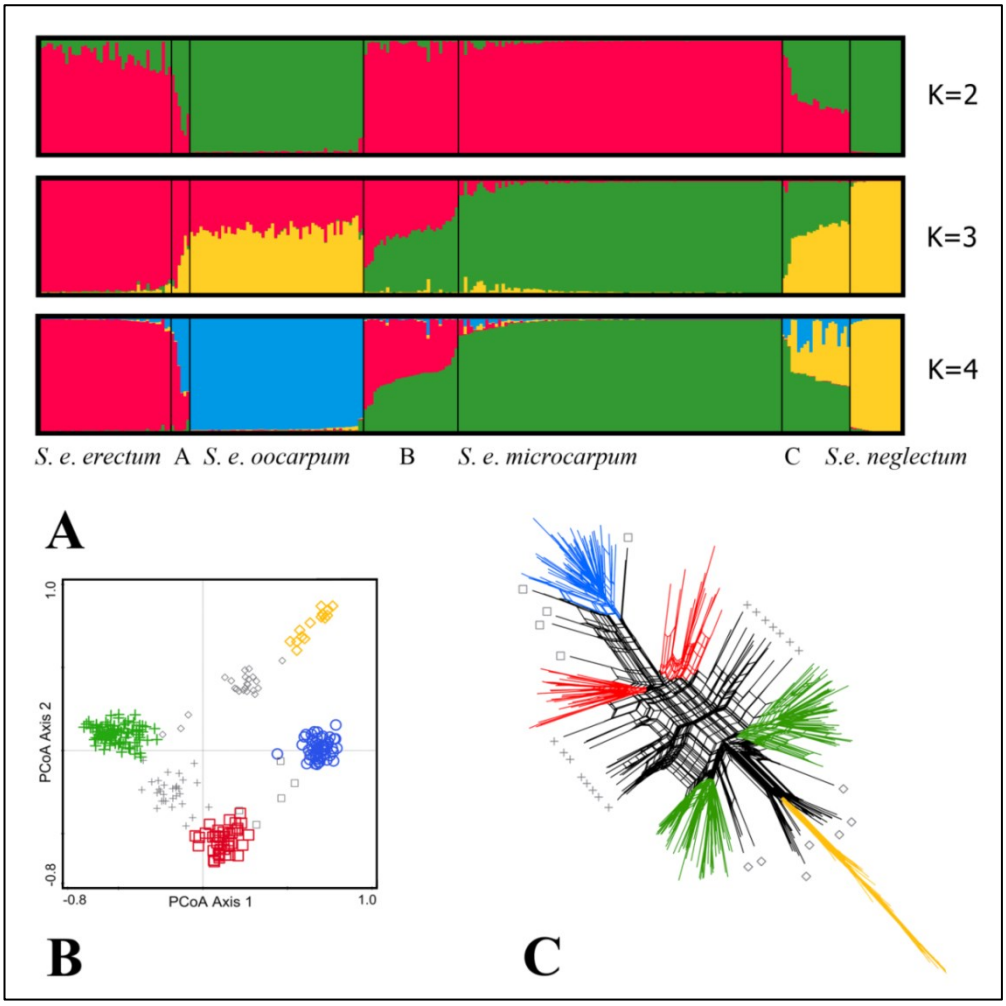

Fig. 2. – Results of molecular analyses of 276 individuals from *Sparganium erectum* based on 125 AFLP loci: (A) Bar plot showing Bayesian assignment probabilities using software STRUCTURE for two, three and four clusters (K=2-4). Four genetic groups (containing individuals with <15% admixture with another group) associated with subspecies were recorded plus intermediate individuals (groups A, B, C) between them. (B) Principal coordinate analysis (PCoA) using Jaccard´s similarity coefficient. The first and second axis explained 28.17% and 10.43% of the variation, respectively. Colours indicate AFLP groups detected by STRUCTURE and passively projected admixed individuals. (C) Neighbour-Network. Colours indicate AFLP groups detected by STRUCTURE. (red lines, *S. e. erectum*; blue lines, *S. e. oocarpum*; green lines, *S. e. microcarpum*; yellow lines, *S. e. neglectum*; black lines, admixed samples: grey square, group A; grey cross, group B; grey diamond, group C).

Table 2. – Analysis of molecular variance (AMOVA) for the total dataset of 218 individuals of *Sparganium erectum* (four subspecies) and separate AMOVA analysis for each subspecies.

Grouping	Source of variation	d.f.	Sum of squares	Variance components	% of total variance
4 subspecies	Among subspecies	3	12.99	0.09	77.57
	Among populations/ within	52	3.91	0.02	15.37
	Within populations	217	1.31	0.01	7.06
S. erectum	Among populations	11	0.86	0.02	58.50
subsp. *erectum*	Within populations	30	0.39	0.01	41.50
S. erectum	Among populations	14	0.49	0.01	61.74
subsp. *oocarpum*	Within populations	41	0.21	0.01	38.26
S. erectum	Among populations	24	2.26	0.02	71.92
subsp. *microcarpum*	Within populations	80	0.66	0.01	28.03
S. erectum	Among populations	3	0.34	0.03	87.23
subsp. *neglectum*	Within populations	11	0.05	0.01	12.77

Intraspecific variation in genome size

Flow cytometry analyses resulted in high-resolution histograms with mean CVs of G_1 peaks of *S. erectum* samples and the internal reference standards of 2.54% (range 1.37–3.24) and 1.99% (range 0.86–3.21), respectively. Intraspecific variation in genome size differed significantly between the subspecies ($p < 0.001$) and ranged from 2C = 0.95 pg to 2C = 1.20 pg (Table 3; Appendix 1). The first AFLP group, corresponding to subsp. *erectum*, had the largest genome size of 2C = 1.16 ± 0.02 pg. The second group (subsp. *oocarpum*) had an intermediate genome size between its parental subspecies *erectum* and subsp. *neglectum* (2C = 1.08 ± 0.02 pg, Fig. 3). The groups of subsp. *microcarpum* and subsp. *neglectum* had similar 2C genome size, albeit still statistically distinguishable; that of subsp. *microcarpum* was 1.02 ± 0.03 pg and that of subsp. *neglectum* was 0.99 ± 0.01 pg (Fig. 4A). The genome sizes of admixed individuals were either similar as the genome size of non-admixed individuals or individuals intermediate between parental subspecies. Admixed individuals from populations Ce, E and R; however, were not assigned by the STRUCTURE analysis to subsp. *oocarpum* (AFLP group A) yet had the same genome size of 2C= 1.07 pg. Similarly, populations Km and Vb (putative subsp. *erectum* × *microcarpum*, group B) possessed the same genome size as subsp. *erectum*. On the other hand, the genome sizes of

other populations were either intermediate between their parental taxa, such as 2C= 1.07 pg (population Za), or the same as subsp. *erectum* 2C= 1.13 pg (population Vp and Vy). Moreover, populations Dk, Ds, Ne, Ps and Tr (subsp. *microcarpum* × *neglectum*, group C) had a genome size with subsp. *microcarpum* and population I with subsp. *neglectum*.

Table 3. – The absolute genome sizes of *Sparganium erectum* subspecies measured using flow cytometry (N=210 individuals). N, number of individuals; Mean (pg), mean value of genome size for given subspecies stated in picogrames; Std., standard deviation; Min., minimum value of genome size; Max., maximum value of genome size.

Subsp.	N	Mean (pg)	Std.	Min.	Max.
erectum	42	1.16	0.02	1.12	1.20
oocarpum	56	1.08	0.02	1.05	1.12
microcarpum	99	1.02	0.03	0.95	1.07
neglectum	13	0.99	0.01	0.97	1.01

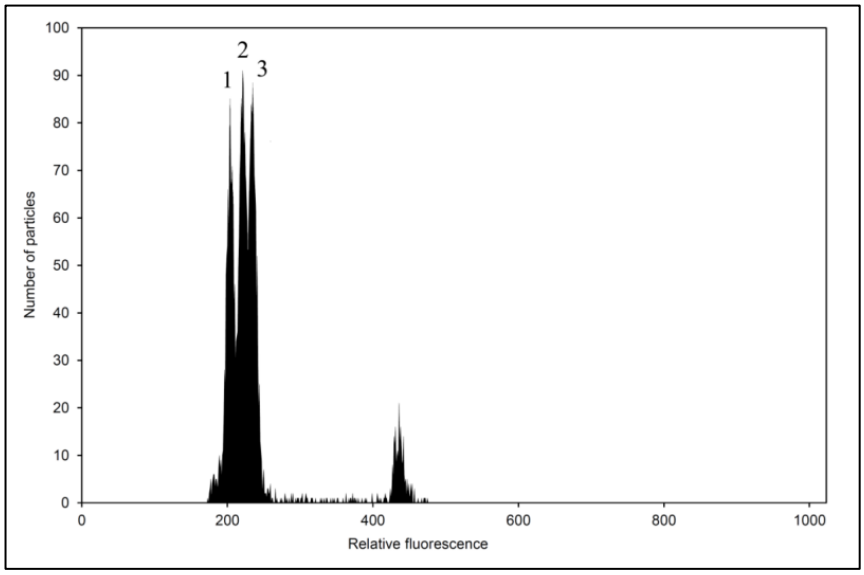

Fig. 3. – Flow cytometry histogram of propidiumiodide-stained nuclei from simultaneously measured leaves of *Sparganium erectum*. Hybrid subsp. *oocarpum* (2) has intermediate genome size (2C nuclear DNA content) between its parental taxa, subsp. *neglectum* (1) and subsp. *erectum* (3).

Morphometric analyses

Most of the measured characters showed departures from a normal distribution, so a non-parametric correlation coefficient (Spearman's) was used and character values were transformed prior to subsequent analyses (Table 1). The correlation coefficients did not exceed 0.9 for any of the character pairs (Electronic Appendix 6). The strongest correlations (reaching the range of 0.8–0.9) were found between four pairs of characters: peduncle and constriction, lengthupper/lengthlower and lengthlower, achenelength /achenewidth and achenewidth, stylelength/achenewidth and stylelength. Two invariable characters (sterility of fruit heads and shoulder) were excluded from further analyses.

Principal component analysis (PCA) based on mean values of achene characters of individuals (264 individuals × 10 characters) showed that four groups of individuals defined by AFLP (representing separate subspecies, Fig. 4B) are closely related and not well separated. Individuals of *S. erectum* subsp. *erectum* and subsp. *oocarpum* were separated along the first axis explaining 45.19% (due to lenghtlower, lenghtupper/lenghtlower and stylelength/achenewidth) whereas individuals of *S. erectum* subsp. *neglectum* along the second axis explaining 20.54% (number of angles and achenelength/achenewidth; PCA eigenvalues are presented in Table 4). However, subsp. *microcarpum* having the most variable achenes overlapped with other subspecies. Additionally, passively projected admixed individuals were situated either in intermediate positions between their parental subspecies or within them.

The CDA analysis performed with four groups predefined by AFLP analysis resulted in better separation of subspecies. Individuals of *S. erectum* subsp. *erectum* and subsp. *microcarpum* as well as subsp. *oocarpum* and subsp. *neglectum* were nearly completely separated by the combination of the first and second canonical axis. However, subsp. *microcarpum* and *neglectum* were partially overlapping. Similarly to the results of PCA, individuals of the hybrid subsp. *oocarpum* were placed between its parental subspecies (Fig. 4D). Admixed individuals of populations Za and Vp were, in accordance with AFLP results (approximately 50:50 admixture of both subsp. *erectum* and *microcarpum* groups), situated in intermediate positions whereas populations Km, Vb, and Vy (with lower admixture of subsp. *microcarpum*, 20–40%) were placed within subsp. *erectum*. Populations I, Ps, Tr (subsp. *microcarpum* ×

neglectum, 50:50) were found in intermediate positions, populations Ds, Ne within subsp. *neglectum* and individuals from population Dk were variously distributed. The characters most highly correlated with the canonical axes were: achenewidth, stylelength/ achenewidth and achenelength/ achenewidth. The first three components explained 66.76% of the total variance (28.24%, 22.71% and 15.81%, respectively). However, the third axis did not provide a better differentiation.

Another CDA was performed to gain better insight into the similarity between the two predefined groups (subsp. *microcarpum* and *neglectum*, Fig. 4C). A canonical scatter-plot showed a partial overlap between the subspecies. The most correlated characters with the canonical axis were 'lengthupper', 'stylelength' and 'constriction' (Table 4).

The classificatory DA correctly assigned 88.43% of individuals to the predefined groups and the remaining 11.57% of individuals were misclassified to other groups (see Appendix 1, CDA). The lowest percentage of individuals was correctly assigned to subsp. *neglectum* (55.56%). These individuals were mostly misclassified to subsp. *microcarpum* and partly to subsp. *oocarpum*. To determine admixed individuals, an additional analysis was performed with four predefined AFLP groups as a training set. The two individuals from population E (group A) were assigned to the subsp. *oocarpum* group. Hybrids of subsp. *erectum* × *microcarpum* (group B), populations Km, Vb, Vy and Za, were assigned to subsp. *erectum* whereas individuals from population Vp were assigned either to subsp. *erectum* or to *microcarpum*. Populations Ds, Dk, Ne, Tr (group C) were assigned to subsp. *oocarpum* and population Ps to subsp. *microcarpum*; population I was partly assigned to subsp. *oocarpum* and subsp. *neglectum*.

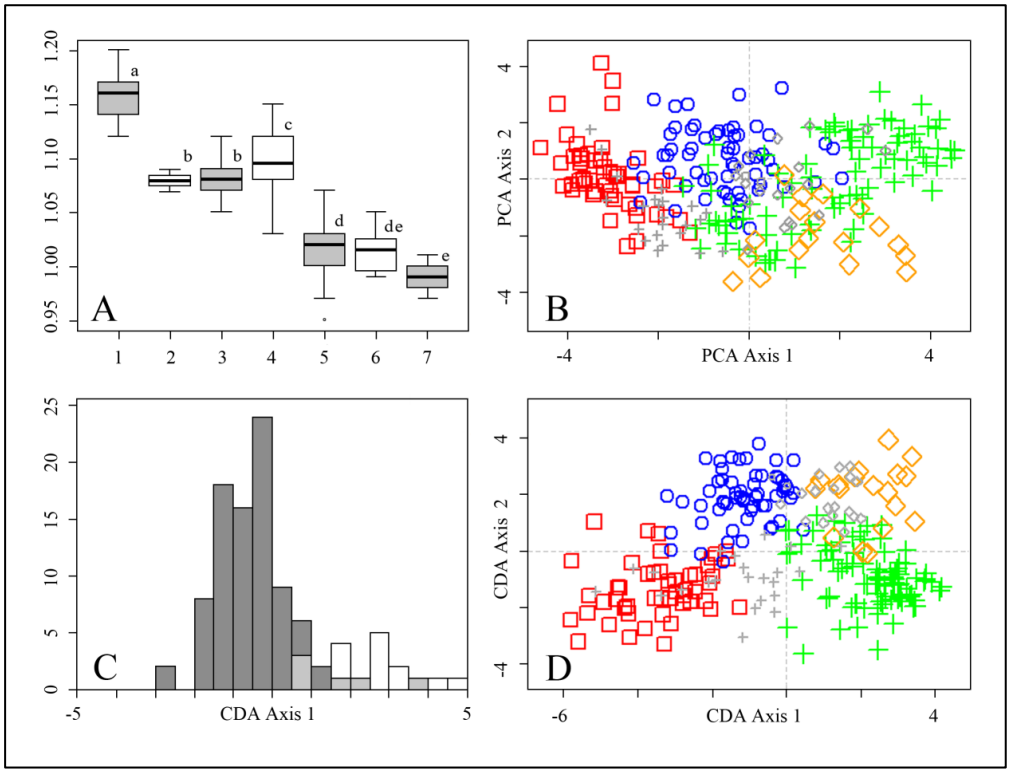

Fig. 4. – (A) Box plot showing the intraspecific distribution of genome size (N=276). Different letters above boxes indicate statistical differences between seven AFLP groups based on a Tukey HSD multiple comparison test ($P < 0.001$). The admixed groups are displayed by white boxes. 1, *Sparganium erectum* subsp. *erectum*; 2, *S. e. erectum* × *S. e. oocarpum*; 3, *S. e. oocarpum*; 4, *S. e. erectum* × *S. e. microcarpum*; 5, *S. e. microcarpum*; 6, *S. e. microcarpum* × *S. e. neglectum*; 7, *S. e. neglectum*.

(B) Principal component analysis (PCA) based on 12 morphological characters of the achenes of 264 individuals from *Sparganium erectum*. The first and second axis explained 45.19% and 20.54% of the variation, respectively. (C) Canonical discriminant analyses (CDA) 91 individuals of subsp. *microcarpum* (dark grey columns) and 18 individuals of subsp. *neglectum* (white columns) with an overlap of both (light grey columns).

(D) Canonical discriminant analyses (CDA) of 264 individuals of *Sparganium erectum* based on 12 morphological characters of achenes. The first and second components explained 28.24% and 22.71% of the variation, respectively.

Table 4. – Results of morphometric analyses based on 12 characters of achenes of *Sparganium erectum*. The three highest PCA eigenvector and total canonical structure values are presented in bold.

Character	PCA1	PCA2	PCA3	CDA1	CDA2	CDA3	CDA4
Peduncle	0.755	0.116	0.507	0.396	-0.271	0.016	-0.196
Achenelength	-0.633	-0.630	0.165	-0.179	-0.187	0.429	0.244
Lengthlower	**-0.803**	-0.484	0.256	-0.311	-0.316	0.343	0.038
Lengthupper	0.514	-0.324	-0.463	0.284	0.387	0.141	**0.623**
Stylelength	0.428	-0.640	-0.509	0.189	0.252	0.196	**0.507**
Achenewidth	-0.789	0.257	-0.442	**-0.677**	0.357	-0.133	0.237
Number of angles	0.403	0.677	0.166	0.133	-0.059	-0.399	-0.381
Constriction	0.697	0.203	0.582	0.401	-0.432	-0.375	**-0.547**
Achenelength/ achenewidth	0.443	-0.662	0.563	0.448	**-0.481**	0.470	-0.002
Lengthupper/ lengthlower	**0.800**	0.168	-0.431	0.364	0.445	-0.121	0.317
Stylelength/ achenelength	0.757	-0.083	-0.493	0.263	0.295	-0.069	0.209
Stylelength/ achenewidth	**0.825**	-0.515	0.037	**0.564**	-0.036	0.216	0.180

Discussion

High levels of phenotypic plasticity known to be common in aquatic plants have resulted in numerous conflicting taxonomical conceptions in various plant groups (Santamaria 2002, Kaplan 2002a). Morphological variation has also complicated the taxonomic treatment of the genus *Sparganium*. In previous studies the inclusion of vegetative parts when describing new taxa led to the description of varying numbers of *Sparganium* taxa, especially at ranks below the species level (Čelakovský 1896, Čelakovský 1899, Belavskaja 1984). The current classification is based on more reliable generative characters. Morphological characters on achenes seem to be stable and allow the differentiation of separate subspecies (Cook 1961). In the present study we assessed the correlation between morphological and genetic variation of *Sparganium erectum* using a combination of molecular, morphometric and flow-cytometric data. Our results provide the first molecular information about intraspecific speciation and hybridization in the genus under study. Our AFLP data differentiated four groups with distinct genome sizes largely corresponding to morphological subspecies and thus confirming the reasonable taxonomic classification of *Sparganium erectum*. Admixed individuals among these groups were also found, indicating recent hybridization. Moreover, the origin of the putative hybrid subspecies *oocarpum* was examined.

Genome size and genetic variation

Many recent studies have employed genetic analyses and the estimation of genome size (Dušková et al. 2010, Chrtek et al. 2009, Chumová et al. 2015, 2017). Nevertheless, so far only a few studies have dealt with the phylogeny of the genus *Sparganium* (Ito et al. 2016, Sulman et al. 2013) or the genetic variation of *Sparganium erectum* (Ishii et al. 2004, Piquot et al. 1996). As intraspecific variation of *Sparganium erectum* had not been investigated, we focused in this study on the evaluation of genetic variability and hybridization and the detection of potential differences in genome size. The STRUCTURE analysis based on AFLP data identified four separate groups and a couple of admixed individuals among them. The first group included only individuals of subsp. *erectum* with low admixture from the other subspecies (up to 15%, as in other groups, Píšová et al. 2017). Populations of this subspecies had the highest intrapopulation genetic variability (41.50%) and the lowest interpopulation variability (58.50%). In accordance with the STRUCTURE analysis, all such individuals were well differentiated by their genome size (2C = 1.12–1.20 pg) from all the other groups. The second group, corresponding to hybrid subsp. *oocarpum*, was found to have an approximately 50:50 proportion of admixture from its parental taxa (subsp. *erectum* and subsp. *microcarpum*) for the solution with K=3 in the STRUCTURE analysis. The genome size of these individuals was also intermediate (2C = 1.05–1.12 pg). Moreover, the solution with K=4 indicated it as a separate, independent taxon (see below). The group with the most individuals was subsp. *microcarpum*, whose genome size ranged from 2C= 0.95 up to 1.07 pg. Hybridization of this subspecies with others seems to be frequent and we found two hybrid groups of individuals with varying degrees of admixture (see below). The last group of subsp. *neglectum* had the highest interpopulation genetic variability (87.23%) while its intrapopulation variability was low (12.77%). Its genome size ranged from 2C = 0.97 to 1.01 pg and even though it partially overlapped with that of subsp. *microcarpum* the difference was statistically significant. Nevertheless, individuals of both subspecies cannot be distinguished solely based on their genome size.

Morphological variation

Morphological characters on achenes have been described as suitable discriminant criteria for subspecies determination in several studies (Cook 1962, Cook & Nicholls 1987, Kaplan 2002b). We compared genetic and morphological variation to estimate the correlations of morphological characters with individual AFLP groups (subspecies). The first group, corresponding to *S. erectum* subsp. *erectum*, was differentiated from other subspecies mainly by the width and length of its achenes, in accordance with other studies that described them as the largest and widest. Additional characters were the ratio between the length of the upper and the lower part of the achene and the ratio between the length of the style and the width of the achene. Even though many authors have regarded a distinct shoulder between the lower and upper part of the achene as an important character, we could not use it in morphological analyses for its invariability within *S. e*. subsp. *erectum*. Morphological analyses placed individuals of the hybrid subsp. *oocarpum* in intermediate position between its parental taxa subsp. *erectum* and subsp. *neglectum*. The ratio between the length of the upper and lower part of the achene separated it from subsp. *erectum* and achene width separated it from subsp. *neglectum*.

The third group, composed of the most variable individuals of subsp. *microcarpum*, was differentiated by the characters stylelength/achenewidth, constriction and achenelength/achenewidth from subsp. *erectum* and subsp. *oocarpum*. An additional discriminant analysis was performed to find the most important characters distinguishing subsp. *microcarpum* and *neglectum*. The characters constriction and number of angles delimited subsp. *microcarpum* and subsp. *neglectum* was separated by the characters length of the upper part of the achene and style length. These results are in accordance with other studies mentioning style length as an important character for identification of subsp. *neglectum* and the characters constriction and visible angles for the determination of subsp. *microcarpum* (Cook 1962, Kaplan 2002).

Hybrid origin of S. erectum subsp. *oocarpum*

Speciation as a result of polyploidization is a topic of many studies (Soltis & Soltis 2009, Soltis et al. 2014, Vít et al. 2017) whereas studies dealing with homoploid speciation are rare (Feliner et al. 2017, Lai et al. 2005, Masuelli et al. 2009). Homoploid hybridization more often results in the formation of hybrid zones by introgression and the establishment of a new hybrid taxon can be promoted ecological selection or geographic isolation (Abbott & Riseberg 2012, Abbott et al. 2013, Buerkle et. al 2000, Yakimowski & Rieseberg 2014). All homoploid hybrid species appear to be ecologically divergent from their parental taxa (Gross & Rieseberg 2005). However, the ecology of recognized subspecies of *Sparganium erectum* is not well known, and comparative studies (Cook 1962, Cook & Nicholls 1987) describe no ecological differences between the subspecies. The distribution of the four subspecies of *Sparganium erectum* in the Czech Republic was investigated and summarized in detail by Kaplan (2015). Subsp. *erectum* and the hybrid subsp. *oocarpum* have quite similar distribution ranges and ecology, in contrast to its second parent subsp. *neglectum*, which is much less common. Reduced gene flow from subsp. *neglectum* (which is the rarest subspecies) compared to subsp. *erectum* might have facilitated the establishment of subsp. *oocarpum* as a descendant of subsp. *neglectum* than of subsp. *microcarpum* (which is the most common subspecies). Both parental taxa and their hybrid have similar distribution in the British Isles and tend to occur south of the Wash whereas subsp. *microcarpum* occurs throughout the British Isles (Cook 1961). The parentage of the hybrid subsp. *oocarpum* was proposed by Cook (1961, 1962) on the basis of achene morphology. This contradicts Čelakovský (1896b), who was convinced that subsp. *oocarpum* was only a variety of subsp. *neglectum*. The intermediate position of subsp. *oocarpum* in genome size (2C= 1.08 pg), genetic and morphometric analyses in this paper affirms its origin as a hybrid of subsp. *erectum* and *neglectum*.

Recent hybridization among subspecies

In addition to the stabilized hybrid subsp. *oocarpum*, we identified two other cases of hybridization have been identified. The first case is *S. erectum* subsp. *erectum* × subsp. *microcarpum* (group B, Fig. 2A), formerly described as *S. microcarpum* × *ramosum* (Ostenfel-Hansen 1897), which usually has highly sterile heads, as also mentioned by Cook & Nicholls (1987). Individuals of

populations Km, Vb, Vp, Vy and Za were admixed with different ratios of genetic contributions from their parents. Their achenes resembled rather subsp. *erectum*; however, population Vp, which was morphologically intermediate between subsp. *erectum* and subsp. *microcarpum*. The genome size of these admixed individuals corresponded with the ratio of genetic makeup inherited from their parents. Individuals with a higher percentage of genetic makeup from subsp. *erectum* (64–77%, populations Km and Vb) had the same genome size as subsp. *erectum* (2C = 1.14 pg) whereas among genetically intermediate individuals (40–57% percentage of genetic information from subsp. *erectum*) genome size varied from intermediate (2C = 1.09 pg, population Za) to similar to subsp. *erectum* (populations Vp and Vy).

The second case was detected between subsp. *microcarpum* and *neglectum* (group C, Fig. 2A). However, such individuals are difficult to determine. Their achenes resemble those of subsp. *microcarpum*, but they are elongated such as those of subsp. *neglectum* and larger such as those of subsp. *oocarpum*, which had a partly sterile, in accordance with the observations of Cook & Nicholls (1987). Individuals of this hybrid were genetically intermediate between subsp. *microcarpum* and subsp. *neglectum*. Populations Dk, Ds, Ne and Tr morphologically resembled subsp. *oocarpum*, though their genome size corresponded to subsp. *microcarpum* (2C = 1.02 pg). By contrast, population Ps had achenes that were more similar to subsp. *microcarpum*. The same genome size as for subsp. *neglectum* (2C = 0.99 pg) was found in population I, which was morphologically intermediate between subsp. *oocarpum* and subsp. *neglectum*. These hybrid individuals may be the plants that Čelakovský (1896b) described as *S. neglectum* var. *oocarpum*, Neuman (1897) as *S. ramosum* f. *substerile* and Graebner (1900) as *S. ramosum* subsp. *polyedrum* var. ζ *substerile*. Different ratios of genetic contributions from the parental subspecies were detected for the two hybrid combinations, indicating recent hybridization. These individuals either had a similar genome size or as their parental subspecies or were similar in morphology. On the opposite, we consider subsp. *oocarpum* a stable hybrid taxon that differs in genome size, morphology and an intermediate genetic pattern.

Several studies have investigated speciation through homoploid hybridization and demonstrated different degrees of the ‘intermediateness’ of hybrids. For example, natural co-occurrence of diploid *Helianthus annuus* and *H. petiolaris* has led to the formation of the reproductively isolated hybrid species *H. anomalus*, *H. deserticola* and *H. paradoxus*, whose genome sizes

and habitat preferences differ both from each other as well as from their parental taxa (Baack et al. 2005, Rieseberg et al. 2003, Lexer et al. 2003). The distinct ecology and distribution pattern also facilitated the establishment of the hybrid species *Bolboschoenus laticarpus* (Hroudová et al. 2007, Píšová et al. 2017). On the other hand, a new hybrid species may be formed without strong reproductive isolation, as documented, for example by the case of *Senecio squalidus*, which got eco-geographically isolated from its parental species after its human-mediated introduction to the British Isles from its hybrid zone on Mt Etna (James & Abbott 2005, Abbott et al. 2010). In *Carex* sect. Vesicariae, the origin of two hybrid taxa was examined by Pedersen et al. (2016): *Carex rostata* var. *borealis* (*Carex rostrata* × *C. rotundata*) and *C. stenolepis* (*C. vesicaria* × *C. saxatilis*). Both hybrids had an intermediate AFLP pattern, but both were closer to one of the parental species. An increasing number of studies show that homoploid hybridization is more common than has been assumed.

Conclusions

In the present study, the combination of AFLPs, flow cytometry and morphometric analyses allowed us to investigate genetic and morphological variation of *Sparganium erectum*. The results confirm the current intraspecific classification. Four AFLP groups were identified, representing the four European subspecies and three more or less numerous hybrid groups between them. Genome size values corresponded to the delimitation of these AFLP groups. Morphological analyses identified well separated groups with partial overlaps. Both genetic and morphometric analyses confirmed the hybrid origin of subsp. *oocarpum* by placing it in intermediate position between its parental taxa subsp. *erectum* and *neglectum.* Recent hybridization was detected, between subsp. *microcarpum* and either *erectum* or *neglectum*. Important distinguishing characters were achene width and length (for differentiating subsp. *erectum*), ratio between the lengths of the upper and lower part of the achene together with achene width (for subsp. *oocarpum*), ratio between style length and achene width together with the ratio of achene length to achene width (for subsp. *microcarpum*), and length of the upper part of the achene and style length (for subsp. *neglectum*).

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Souhrn

Značná fenotypová plasticita, typická pro vodní a mokřadní rostliny, způsobila problémy v taxonomickém pojetí i v rámci rodu *Sparganium*. V minulosti bylo popisováno množství variet a forem zvláště u druhu *Sparganium erectum*. Použití plastických znaků na listech se neuplatnilo a současné členění rodu je založeno hlavně na tvaru a barvě nažek, která je stabilní a specifická. Celkem je odlišováno pět poddruhů *Sparganium erectum* a to: poddruh *erectum*, předpokládaný kříženec *oocarpum*, *microcarpum*, *neglectum* a *stoloniferum*. Kromě posledně jmenovaného se ostatní čtyři vyskytují v Evropě a také v České republice. Mezi poddruhy byly zaznamenány i přechodné populace naznačující možnou hybridizaci. Pro ověření oprávněnosti současného vnitrodruhového členění tohoto druhu a zjištění míry hybridizace bylo navštíveno 64 lokalit v České Republice a odebrán materiál pro morfometrické a molekulární analýzy. Pro porovnání genetické a morfologické variability byla použita kombinace AFLP (Amplified Fragment Length Polymorphism) metody jako molekulárního markeru, průtokové cytometrie pro stanovení velikosti genomu a mnohorozměrné morfometrické analýzy. Výsledky přinesly dobré odlišení všech čtyř poddruhů s přechodnými jedinci mezi nimi. Současné členění odpovídá nalezeným genetickým rozdílům a rovněž rozdílům ve velikosti genomu. Předpokládaný hybridní původ poddruhu *oocarpum* byl potvrzen na základě pozice mezi jeho rodičovskými poddruhy *erectum* a *neglectum* v genetických a morfometrických analýzách a také ve velikosti genomu. Vzhledem k vzácnému výskytu zpětného křížení a samostatnému odlišení v analýzách ho považujeme za stabilní hybridogenní poddruh. Zároveň byly zjištěny dvě skupiny kříženců s přechodnými znaky: *S. e. erectum* × *microcarpum* a *S. e. microcarpum* × *neglectum* naznačující současnou hybridizaci.

References

Abbott R., Hegarty M. J., Hiscock S. J. & Brennan A. C. (2010): Homoploid hybrid speciation in action. – Taxon 59 (5): 1375–1386.

Abbott R. & Rieseberg L. H. (2012): Hybrid speciation. – eLS. John Wiley & Sons, Chichester.

Abbott R., Albach D., Ansell S., Arntzen J. W., Baird S. J., Bierne N., Boughman J., Brelsford A., Buerkle C. A., Buggs R., Butlin R. K., Dieckmann U., Eroukhmanoff F., Grill A., Cahan S. H., Hermansen J. S., Hewitt G., Hudson A. G., Jiggins C., Jones J., Keller B., Marczewski T., Mallet J., Martinez-Rodriguez P., Möst M., Mullen S., Nichols R., Nolte A. W., Parisod C., Pfennig K., Rice A. M., Ritchie M. G., Seifert B., Smadja C. M., Stelkens R., Szymura J. M., Väinölä R., Wolf J. B. W. & Zinner D. (2013): Hybridization and speciation. – J. Evol. Biol. 26 (2): 229–246.

Ascherson P. & Graebner P. (1897): Synopsis der mitteleuropäischen Flora 1. Wilhelm Engelman, Leipzig.

Baack E. J., Whitney K. D. & Riesenberg L. H. (2005): Hybridization and genome size evolution: timing and magnitude of nuclear DNA content increases in *Helianthus* homoploid hybrid species. – New Phytol. 167 (2): 623–630.

Barrett S. C. H., Echert C. G. & Husband B. C. (1993): Evolutionary processes in aquatic plant populations. – Aquat. Bot. 44: 105–145.

Belavskaja A. P. (1984): A contribution to the morphology of fruits of the genus *Sparganium* (Typhaceae) in the flora of the USSR. – Botaničeskij žurnal Akad. Nauk. SSSR 69 (12): 1662–1668.

Benham J. J. (2001): Genographer. Montana State University. Available from http://hordeum.oscs.montana.edu/genographer/.

Bonin A., Bellemain E., Eidesen P. B., Pompanon F., Brochmann C. & Taberlet P. (2004): How to track and assess genotyping errors in population genetics studies. – Mol. Ecol. 13: 3261–3273.

Buerkle C. A., Morris R. J., Asmussen M. A. & Rieseberg L. H. (2000): The likelihood of homoploid hybrid speciation. – Heredity 84 (4): 441–451.

Bureš P., Wang Y. F., Horová L. & Suda J. (2004): Genome size variation in Central European species of *Cirsium* (Compositae) and their natural hybrids. – Ann. Bot. 94 (3): 353–363.

Casper S. J. & Krausch H. D. (1980): *Pteridophyta* und *Anthophyta*. 1. Teil: Lycopodiaceae bis Orchidaceae. In: Ettl H., Gerloff J. & Heynig H. (eds.), Süßwasserflora von Mitteleuropa 23, Gustav Fischer Verlag, Stuttgart & New York.

Ciotir C., Szabo J. & Freeland J. (2017): Genetic characterization of cattail species and hybrids (*Typha* spp.) in Europe. – Aquat. Bot. 141: 51–59.

Cook C. D. K. (1961): *Sparganium* L. in Britain. – Watsonia 5 (1): 1–10.

Cook C. D. K. (1962): *Sparganium erectum* L. (*S. ramosum* Hudson, nom. illeg.) – J. Ecol. 50 (1): 247–255.

Cook C. D. K. (1980): Sparganiaceae. – In: Tutin T. G., Heywood V. H., Burges N.A., Moore D. M., Valentine D. H., Walters S. M. & Webb D.A. (eds.), Flora Europaea 5: 274–275, Cambridge Univ. Press, Cambridge.

Cook C. D. K. & Nicholls M. S. (1986): A monographic study of the genus *Sparganium* (Sparganiaceae). Part 1. Subgenus *Xanthosparganium* Holmberg. – Bot. Helv. 96: 213–267.

Cook C. D. K. & Nicholls M. S. (1987): A monographic study of the genus *Sparganium* (Sparganiaceae). Part 2. Subgenus *Sparganium*. – Bot. Helv. 97: 1–44.

Čelakovský L. J. (1896a): Analytická květena Čech, Moravy a Rak. Slezka. Praha.

Čelakovský L. J. (1896b): Ueber die ramosen Sparganien Böhmens. – Oesterr. Bot. Z. 46: 421–433.

Čelakovský L. (1899): Anatomické rozdíly v listech ramósních Sparganií. – Věstník Král. Čes. Spol. Nauk, Cl. Math.-Natur. 5:1–11.

Doležel J., Greilhuber J. & Suda J. (eds) (2007): Flow cytometry with plant cells. Wiley-VCH, Weinheim.

Doležel J., Bartoš J., Voglmayr H. & Greilhuber J. (2003): Nuclear DNA content and genome size of trout and human. – Cytometry 51: 127–128.

Dušková E, Kolář F., Sklenář P., Rauchová J., Kubešová M., Fér T., Suda J. & Marhold K. (2010): Genome size correlates with growth form, habitat and phylogeny in Andean genus *Lasiocephalus* (Asteraceae). – Preslia 82: 127–148.

Doyle J. J. & Doyle J. L. (1987): A rapid DNA isolation procedure for small amounts of fresh leaf tissue. – Phytochem. Bull. 19: 11–15.

Earl D. A. & vonHoldt B. M. (2012): STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. – Conserv. Genet. Resour. 4: 359–361.

Ehrich D. (2006): AFLPDAT: a collection of R functions for convenient handling of AFLP data. – Mol. Ecol. Notes 6: 603–604.

Evanno G., Regnaut S. & Goudet J. (2005): Detecting the number of clusters of individuals using the software structure: a simulation study. – Mol. Ecol. 14: 2611–2620.

Excoffier L., Smouse P. & Quattro J. (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. – Genetics 131: 479–491.

Falush D., Stephens M. & Pritchard J. K. (2007): Inference of population structure using multilocus genotype data: dominant markers and null alleles. – Mol. Ecol. Notes 7: 574–578.

Feliner G. N., Álvarez I., Fuertes-Aguilar J., Heuertz M., Marques I., Moharrek F., Piñeiro R., Riina R., Rosselló J.A., Soltis P.S. & Villa-Machío I. (2017): Is homoploid hybrid speciation that rare? An empiricist's view. – Heredity 118 (6): 513–516.

Fér T. & Pfosser M.: Tracing plant dispersal in river systems: AFLP analysis of *Sparganium erectum* L. (submitted)

Graebner P. (1900): Sparganiaceae. – In: Engler A., Das Pflanzenreich IV/8. 10: 1–24. Leipzig.

Gobert V., Moja S., Colson M. & Taberlet P. (2002): Hybridization in the section *Mentha* (Lamiaceae) inferred from AFLP markers. – Am. J. Bot. 89: 2017–2023.

Gross B. L. & Rieseberg L. H. (2005): The ecological genetics of homoploid hybrid speciation. – J. Hederity 96: 241–252.

Guo Y. P., Vogl C., van Loo M. & Ehrendorfer F. (2006): Hybrid origin and differentiation of two tetraploid *Achillea* species in East Asia: molecular, morphological and ecogeographical evidence. – Mol. Ecol. 15: 133–144.

Haasová M. (1997): Variabilita druhu *Sparganium erectum* L. v České republice. [Variability of *Sparganium erectum* in the Czech Republic]. – Ms. Thesis; depon. in: Bot. Library, Fac. of Sci., Charles Univ., Prague.

Hegi G. (1936): *Sparganium*. Illustrierte Flora von Mittel-Europa Ed.2, Vol.1. 281–291, Carl Hanser Verlag, München.

Hothorn T., Bretz F. & Westfall P. (2008): Simultaneous inference in general parametric models. – Biometr. J. 50: 346–363.

Hroudová Z., Zákravský P., Ducháček M. & Marhold K. (2007): Taxonomy, distribution and ecology of *Bolboschoenus* in Europe. – Ann. Bot. Fenn. 44: 81–102.

Huson D. H. & Bryant D. (2006): Application of phylogenetic networks in evolutionary studies. – Mol. Biol. Evol. 23: 254–267.

Chrtek J., Zahradníček J., Krak K. & Fehrer J. (2009): Genome size in *Hieracium* subgenus *Hieracium* (Asteraceae) is strongly correlated with major phylogenetic groups. – Ann. Bot. 104 (1): 161–178.

Ishii T., Nakayama Y., Kobayashi M. & Yamaguchi H. (2004): A note on the genetic diversity of the vulnerable aquatic macrophyte *Sparganium erectum* and its congeners (Sparganiaceae) in rural wetland in Japan. Weed Biol. Manag. 4: 230–234.

Ito Y., Tanaka N., Kim C., Kaul R. B. & Albach D. C. (2016): Phylogeny of *Sparganium* (Typhaceae) revisited: non-monophyletic nature of *S. emersum* sensu lato and resurrection of *S. acaule*. – Plant Syst. Evol. 302: 129–135.

Jaccard P. (1908): Nouvelles recherches sur la ristribution florale. – Bull. Soc. Vaud. Sci. Nat. 44: 223–270.

Jakobsson M. & Rosenberg N. A. (2007): CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. – Bioinformatics 23: 1801–1806.

James J.K. & Abbott R.J. (2005): Recent, allopatric, homoploid hybrid speciation: the origin of *Senecio squalidus* (Asteraceae) in the British Isles from a hybrid zone on Mount Etna, Sicily. – Evolution 59 (12): 2533–2547.

Kaplan Z. (2002a): Phenotypic plasticity in *Potamogeton* (Potamogetonaceae). – Folia Geobot. 37: 141–170.

Kaplan Z. (2002b): *Sparganiaceae* Dum. – In: Kubát K., Hrouda L., Chrtek jun. J., Kaplan Z., Kirschner J. & Štěpánek J. (eds.): Klíč ke květeně České republiky [Key to the Flora of the Czech Republic.], 877–878. Academia, Praha.

Kaplan Z., Danihelka J., Štěpánková J., Bureš P., Zázvorka J., Hroudová Z., Ducháček M., Grulich V., Řepka R., Dančák M., Prančl J., Šumberová K., Wild J. & Trávníček B. (2015): Distributions of vascular plants in the Czech Republic. Part 1. – Preslia 87: 417–500.

Kolář F., Lučanová M., Vít P., Urfus T., Chrtek J., Fér T., Ehrendorfer F. & Suda J. (2013): Diversity and endemism in deglaciated areas: ploidy, relative genome size and niche differentiation in the *Galium pusillum* complex (Rubiaceae) in Northern and Central Europe. – Ann. Bot. 111: 1095–1108.

Koutecký P. (2015): MorphoTools: a set of R functions for morphometric analysis. – Plant Syst. Evol. 301: 1115–1121.

Lai Z., Nakazato T., Salmaso M., Burke J. M., Tang S., Knapp S. J. & Rieseberg L. H. (2005): Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species. – Genetics 171 (1): 291–303.

Lexer C., Welch M. E., Raymond O. & Rieseberg L. H. (2003): The origin of ecological divergence in *Helianthus paradoxus* (Asteraceae): selection on transgressive characters in a novel hybrid habitat. – Evolution 57 (9): 1989–2000.

Linder P. H., Suda J., Weiss-Schneeweiss H., Trávníček P. & Bouchenak-Khelladi Y. (2017): Patterns, causes and consequences of genome size variation in Restionaceae of Cape flora. – Bot. J. Linn. Soc. 183(4): 515–531.

Loureiro J., Trávníček P., Rauchová J., Štech M. & Suda J. (2010): The impact of flow cytometry on biosystematics, ecology and population biology of homoploid plant groups. – Preslia 82: 3–21.

Löve Á. & Löve D. (1948): Chromosome numbers of Northern plant species. – Icel. Univ. Inst. Appl. Sci., Dep. Agric. Reykjavík, Rep. Ser. B. 3: 1–131.

Mandák B., Vít P., Krak K., Trávníček P., Havrdová A., Hadincová V., Zákravský P., Jarolímová V., Bacles C. F. & Douda J. (2016): Flow cytometry, microsatellites and niche models reveal the origins and geographical structure of *Alnus glutinosa* populations in Europe. – Ann. Bot. 117 (1): 107–120.

Masuelli R. W., Camadro E. L., Erazzú L. E., Bedogni M. C. & Marfil C. F. (2009): Homoploid hybridization in the origin and evolution of wild diploid potato species. – Plant Syst. Evol. 277(3-4): 143–151.

Neuman L. M. (1897): Om nomenklatur och artbegränsning inom slägtet *Sparganium*. I. – Botan. Notiser. 113-130

Nordborg M., Hu T. T., Ishino Y., Jhaveri J., Toomajian C., Zheng H. G., Bakker E., Calabrese P., Gladstone J., Goyal R., Jakobsson M., Kim S., Morozov Y., Padhukasahasram B., Plagnol V., Rosenberg N. A., Shah C., Wall J. D., Wang J., Zhao K. Y., Kalbfleisch T., Schulz V., Kreitman M. & Bergelson J. (2005): The pattern of polymorphism in *Arabidopsis thaliana*. – PLoS Biol. 3: 1289–1299.

Ostenfeld – Hansen C. (1897): De i Danmark voxende ramose Sparganium-Arter – Bot. Tidskrift. 21 (1): V-IX.

Paszko B. & Nobis M. (2010): The hybrid origin of *Calamagrostis* × *gracilescens* (Poaceae). – Acta Soc. Bot. Pol. 79 (1): 51–61.

Pedersen A. T. M., Nowak M. D., Brysting A. K., Elven R. & Bjorå, C. S. (2016): Hybrid origins of *Carex rostrata* var. *borealis* and *C. stenolepis*, two problematic taxa in *Carex* section *Vesicariae* (Cyperaceae). – PloS ONE 11 (10): 1–18.

Píšová S. (2009): Zhodnocení vnitrodruhové variability *Sparganium erectum* s využitím morfometrie, AFLP a průtokové cytometrie [Evaluation of variability within *Sparganium erectum* using morphometrics, AFLP and flow cytometry.]. – Ms. Thesis; depon. in: Bot. Library, Fac. of Sci., Charles Univ., Prague.

Píšová S., Hroudová Z., Chumová Z. & Fér T. (2017): Ecological hybrid speciation in central-European species of *Bolboschoenus*: genetic and morphological evaluation. – Preslia 89: 17–39.

Piquot Y., Saumitou-Laprade P., Petit D., Vernet P. & Epplen J. T. (1996): Genotypic diversity revealed by allozymes and oligonucleotide DNA fingerprinting in French populations of the aquatic macrophyte *Sparganium erectum*. – Mol. Ecol. 5 (2): 251–258.

Pritchard J. K., Stephens M. & Donnelly P. (2000): Inference of population structure using multilocus genotype data. – Genetics 155: 945–959.

R Development Core Team (2008): R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org.

Rieseberg L. H. (1997): Hybrid origins of plant species. – Annu. Rev. Ecol. Syst. 28 (1): 359–389. Rieseberg L. H., Raymond O., Rosenthal D. M., Lai Z., Livingstone K., Nakazato T., Durphy J. L., Schwarzbach A. E., Donovan L. A. & Lexer C. (2003): Major ecological transitions in wild sunflowers facilitated by hybridization. – Science 301: 1211–1216.

Rosenberg N. A. (2004): DISTRUCT: a program for the graphical display of population structure. – Mol. Ecol. Notes 4: 137–138.

Santamaria L. (2002): Why most aquatic plants are broadly distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. – Acta Oecol. 23: 137–154.

Schlüter P. M. & Harris S. A. (2006): Analysis of multilocus fingerprinting data sets containing missing data. – Mol. Ecol. Notes 6: 569–572.

Sculthorpe C. D. (1967): The biology of aquatic vascular plants. Edward Arnold, London. 610 pp.

Slovák M., Vít P., Urfus T. & Suda J. (2009): Complex pattern of genome size variation in the polymorphic species *Picris hieracioides* (Compositae). – Plant Syst. Evol. 278: 187–201.

Soltis D. E., Visqer C.J. & Soltis P. S. (2014): The polyploidy revolution then…and now: Stebbins revisited.

Soltis P. S. & Soltis D. E. (2009): The role of hybridization in plant speciation. – Annu. Rev. Plant. Biol. 60: 561–588.

Sulman J. D., Drew B. T., Drummond C., Hayasaka E. & Systma K. J. (2013): Systematics, biogeography, and character evolution of *Sparganium* (Typhaceae): diversification of a widespread aquatic lineage. – Amer. J. Bot. 100: 2023–2039.

Španiel S., Marhold K., Filová B. & Zozomová-Lihová J. (2011): Genetic and morphological variation in the diploid–polyploid *Alyssum montanum* in Central Europe: taxonomic and evolutionary considerations. – Plant Syst. Evol. 294: 1–25.

ter Braak C. J. F. & Šmilauer P. (2012): Canoco reference manual and user's guide: software for ordination, version 5.0. – Microcomputer Power, Ithaca, USA.

Vít P., Wolfová K., Urfus T., Tájek P. & Suda J. (2014): Interspecific hybridization between rare and common plant congeners inferred from genome size data: assessing the threat to the Czech serpentine endemic *Cerastium alsinifolium* (Caryophyllaceae). – Preslia 86: 95–117.

Vít P., Douda J., Krak K., Havrdová A. & Mandák B. (2017): Two new polyploid species closely related to *Alnus glutinosa* in Europe and North Africa – An analysis based on morphometry, karyology, flow cytometry and microsatellites. – Taxon 66 (3): 567–583.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. & Zabeau M. (1995): AFLP: a new technique for DNA fingerprinting. – Nucl. Acids Res. 23: 4407–4414.

Yakimowski S. B. & Rieseberg L. H. (2014): The role of homoploid hybridization in evolution: a century of studies synthesizing genetics and ecology. – Amer. J. Bot. 101 (8): 1247–1258.

Záveská E., Fér T., Šída O., Leong-Škorničková J., Sabu M. & Marhold K. (2011): Genetic diversity patterns in *Curcuma* reflect differences in genome size. – Bot. J. Linn. Soc. 165: 388–401.

Appendix 1. – List of the populations of the subspecies of *Sparganium erectum* studied and their characteristics. AFLP group – determination of each population based on AFLP data (STRUCTURE 85%): E – *S. erectum* subsp. *erectum*, A – *S. e. erectum* × *S. e. oocarpum*, O – *S. e. oocarpum*, B – *S. e. erectum* × *S. e. microcarpum*, M – *S. e. microcarpum*, C – *S. e. microcarpum* × *S. e.* subsp. *neglectum*, N – *S. e. neglectum*. Nind – number of individuals analyzed for AFLPs; Ng – number of genotypes; DNei – Nei´s gene diversity; %poly – percentage of AFLP markers demonstrating intra-population polymorphism. FCM – The average genome size of the populations of the subspecies of *Sparganium erectum* (2C value, picograms, pg); subsp. – subspecies determination of each population based on its genome size. CDA – subspecies determination of each population based on classificatory discriminant analyses of (E – *S. e. erectum*, O – *S. e. oocarpum*, M – *S. e. microcarpum*, N – *S. e.* subsp. *neglectum*).

		AFLP group											FCM		CDA
Acronym	**Locality and date of collection**	**E**	**A**	**O**	**B**	**M**	**C**	**N**	N_i	N_g	D_{Nei}	**%poly**	**2C (pg)**	**subsp.**	**subsp.**
Sparganium erectum* subsp. *erectum															
D	CZ-CB, The Cidlina river near the railway bridge, 1.13 km NW from the village Sány, 50°7.707'N, 15°13.945'E, 192 m, 29.8.2007	3							3	2	0.10	14.4	1.14	E	E
Do	CZ-CB, The Cidlina river near the road bridge, 400m N from the village Dobšice nad Cidlinou, 50°8.24'N, 15°16.00'E, 200 m, 29.8.2007	5							5	4	0.07	15.2	1.15	E	E
Ha	CZ-SB, The Přední Sax fishpond, The village of Hamr, 1km N from the village Hamr, 49°9.77'N, 14°45.60'E, 410 m; 29.9.2008	2							2	1	0.00	0.0	1.18	E	E, O
Ch	CZ-CB, The Krčský fishpond 2.5km NW from the town Městec Králové, 50°13.23'N, 15°16.17'E, 210 m, 9.7.2008	4							4	4	0.09	16.8	1.17	E	E, O
Km	CZ-CB, The Elbe river, 3.10 km SW from the town Nymburk, 50°10.37'N, 15°0.33'E, 180 m, 24.9.2008				3				3	3	0.10	15.2	1.14	E	E
L	CZ-CB, The fire reservoir in the town Libice nad Cidlinou, 50°7.73'N, 15°10.73'E, 190 m, 25.8.2007	5							5	1	0.04	7.2	1.15	E	E
O	CZ-CB, The Kupecká ditch, 760m from the village Oseček, 50°6.39'N, 15°8.533'E, 180 m, 14.9.2007	4							4	4	0.12	24.0	1.14	E	E
Po	CZ-CB, Prague city, distr. Dolní Počernice, The Počernický fishpond, 50°5.08'N, 14°35.45'E, 230 m, 14.9.2008	2							2	2	0.15	15.2	1.19	E	E
Ro	CZ-CB, The Třebonický fishpond, 1.28km from the town Rožďalovice, 50°17.85'N, 15°11.01'E, 200 m, 31.8.2008	4							4	4	0.11	20.0	1.13	E	E, O
Sa	CZ-CB, Prague city, dist. Hrnčíře, The Hrnčířský fishpond, 50°0.29'N, 14°30.48'E, 290 m, 10.9.2008	2							2	2	0.11	16.8	1.17	E	E, O

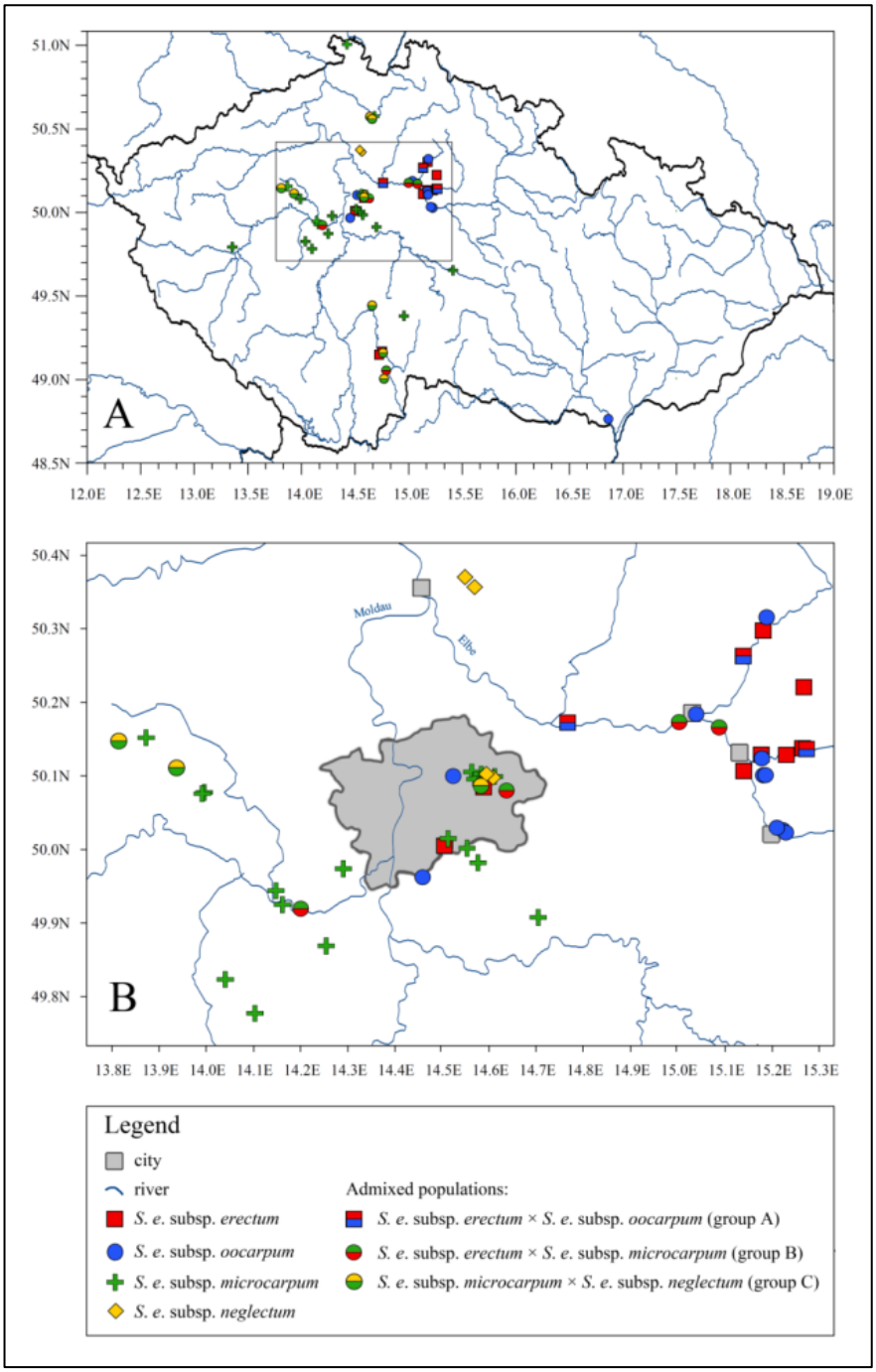
Acronym	Locality and date of collection	AFLP group							N_i	N_g	D_{Nei}	%poly	FCM 2C (pg)	FCM subsp.	CDA subsp.
		E	A	O	B	M	C	N							
Vl	CZ-SB, The Vlkovský fishpond, 800m from the village Vlkov nad Lužnicí, 49°8.72'N, 14°43.94'E, 390 m, 29.9.2008	4							4	4	0.12	22.4	1.16	E	E
Vy	CZ-SB, The Dolní u Smítky fishpond behind the Rožmberk fishpond, 3.37km SE from the village Lužnice, 49°3.07 E 14°47.91'E, 430 m, 29.9.2008				3				3	2	0.08	12.0	1.10	E	E, O
Vb, Vz	CZ-CB, The Elbe river by the road bridge, 1 km from the village Velké Zboží, 50°9.951'N, 15°5.42'E, 184 m, 5.9.2008	1		4	3				8	8	0.14 0.08	25.6 14.4	1.13 1.08	E O	E O
Sparganium erectum* subsp. *oocarpum															
C	CZ-CB, The Cidlina river near the road bridge, Libice nad Cidlinou, 50°7.42'N, 15°10.84'E, 186 m, 25.8.2007			5					5	5	0.10	20.8	1.07	O	O
Ce	CZ-CB, The Elbe river by the railway bridge, 1.70 km NE from the town Čelákovice, 50°10.33'N, 14°46.16'E, 170 m, 14.9.2007		2	3					5	5	0.12	26.4	1.08	O	-
E	CZ-CB, The Steklá ditch by the Žehuňský fishpond, 1.26 km W from the village Žehuň, 50°8.18'N, 15°16.49'E, 200 m, 29.8.2007		2	2					4	4	0.13	23.2	1.07	O	O
G	CZ-CB, The Pazderák fishpond in the village Dolní Břežany, 49°57.744'N, 14°27.73'E, 360 m, 4.9.2007			4						2	0.05	10.4	1.07	O	O
H	CZ-SM, The Bruksa pool near the Stará Dyje river, 1.28 km W from the town Břeclav, 48°45.68'N, 16°52.10'E, 157 m, 7.9.2007			4					4	4	0.09	16.8	1.07	O	O
Ho	CZ-CB, Prague city, distr. Vysočany, The Hořejší fishpond, 50°5.975'N, 14°31.607'E, 200 m, 17.9.2008			3					3	3	0.11	16.8	1.09	O	O
K1	CZ-CB, The Staré Labe blind stream branch of the Elbe river, 1.74 km E from the Kolín. 50°1.56'N, 15°13.52'E, 195 m, 31.8.2008			3					3	3	0.07	10.4	1.09	O	O
K2	CZ-CB, The blind stream branch of the Elbe river, 2.20 km from the town Kolín, 50°1.37'N, 15°13.885'E, 190 m, 31.8.2008			4					4	4	0.10	18.4	1.09	O	O
Ko	CZ-CB, The Elbe river by the railway bridge in the town Kolín, 50°1.76'N, 15°12.75'E, 200 m; 31.8.2008			4					4	2	0.06	12.0	1.12	O	O

Acronym	Locality and date of collection	AFLP group							N_i	N_g	D_{Nei}	%poly	FCM 2C (pg)	FCM subsp.	CDA subsp.
		E	A	O	B	M	C	N							
Ny	CZ-CB, The Elbe river by the Kamenný most bridge in the town Nymburk, 50°11.02'N, 15°2.49'E, 180 m, 31.8.2008			5					5	4	0.08	16.8	1.09	O	E, O
Rz	CZ-CB, The Bučický fishpond, 1.97 km NE from the town Rožďalovice, 50°18.938'N. 15°11.447'E. 200m. 16.7.2008			4					4	1	0.03	6.4	1.11	O	O
V	CZ-CB, The Máčidlo fishpond by Libický luh nature reserve, in the town Velký Osek, 50°6.05'N, 15°11.01'E, 180 m, 2.8.2008			5					5	2	0.05	10.4	1.07	O	O
Vo	CZ-CB, The Bačovka stream by the main road in the town Velký Osek; 50°6.06'N, 15°11.30'E, 180 m, 2.8.2008			4					4	2	0.07	11.2	1.07	O	O
R	CZ-CB, The Mrlina river by the railway bridge in the village Křinec, 50°15.76'N, 15°8.47'E, 210 m, 13.9.2007	6	1	2					9	9	0.15	36.0	1.16 1.07	E O	E O
Sparganium erectum* subsp. *microcarpum															
Al	CZ-CB, Prague city, distr. Dolní Počernice, The Aloisov fishpond, 50°6.30'N, 14°33.95'E, 230 m,14.9.2008					3			3	2	0.07	10.4	1.01		M
F	CZ-NB, The small wetland under a farm in the village Císařský, 1.14 km W from the town Šluknov, 51°0.06'N, 14°25.95'E, 400 m, 2.9.2007					4			4	3	0.06	12.8	1.02		M
M	CZ-CB, The Bojovský stream by Zámecký fishpond in the town Mníšek pod Brdy, 49°52.124'N, 14°15.454'E, 390 m, 17.8.2007					5			5	3	0.08	16.0	1.02		M
Ma	CZ-CB, Prague city, distr. Dolní Počernice,The Svépravický stream by Vidlák fishpond, 50°5.72'N, 14°34.33'E, 220 m, 14.9.2008					2			2	2	0.06	5.6	1.03		M
N	CZ-EB, The Závidkovický stream, 1.13 km from the village Nová Ves u Světlé nad Sázavou, 49°38.92'N, 15°25.27'E, 430 m, 19.8.2007					4			4	4	0.10	18.4	1.02		M, N
Pl	CZ-EB, The forest wetland of the Bolevecký stream by Strženka fishpond, 5.20 km from the Plzeň city, 49°47.28'N, 13°21.82'E, 350 m, 16.9.2007					4			4	3	0.07	12.0	0.99		M
Q	CZ-CB, The Lomnický stream entry to the Štičí fishpond in the village Mirošovice, 49°54.44'N, 14°42.41'E, 341 m, 15.9.2007					5			5	5	0.12	24.0	1.03		M
S	CZ-CB, The streamlet 320 m to the right from the road to the Hřebeny mountains, 1.78 km NW from the village Sychrov u Dobříše, 49°46.64'N, 14°6.41'E, 440 m, 22.8.2007					5			5	1	0.03	6.4	1.00		M

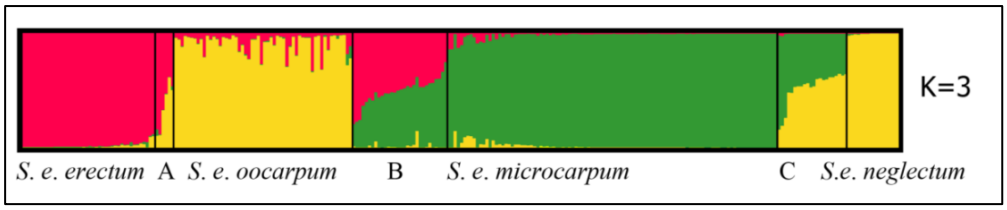
Acronym	Locality and date of collection	AFLP group							N_i	N_g	D_{Nei}	%poly	FCM 2C (pg)	CDA subsp.
		E	A	O	B	M	C	N						
X	CZ-CB, Prague city, distr. Černý most, The Chvalka stream by the slip-road, 50°6.144'N, 14°35.171'E, 230 m, 15.9.2007					4			4	2	0.04	8.0	0.97	M
Y	CZ-CB, Prague city, distr. Černý most, The Svépravický stream among the slip-roads, 50°5.938'N, 14°35.628'E, 230 m, 15.9.2007					5			5	1	0.02	4.8	1.00	M
Ba	CZ-CB, The Pomezní rybník, 1.5 km NW from the village Bratronice, 50°4.64'N, 13°59.96'E, 400 m, 4.10.2008					2			2	2	0.05	4.8	1.02	O, M
Br	CZ-CB, The U paseky fishpond, 1.67 km NW from the village Bratronice, 50°4.54'N, 13°59.69'E, 400 m, 4.10.2008					3			3	3	0.11	16.8	0.99	M, N
Db	CZ-CB, Prague city, distr. Dobřejovice, The Dobřejovický stream by the bus stop Na Návsi, 49°58.902'N, 14°34.752'E, 330 m, 11.9.2008					2			2	2	0.15	15.2	0.99	M
Dk	CZ-NB, The Robečský stream by the railway bridge near the Máchovo jezero lake in the town Doksy, 50°34.201N, 14°39.37'E, 270 m, 12.9.2008						5		5	5	0.13	28.0	1.02	O, M
Ds	CZ-NB, The Robečský stream below the Čepelský fishpond in the town Doksy, 50°33.669'N, 14°39.503'E, 280 m, 12.9.2008						2		2	2	0.08	8.0	1.02	O
Hp1	CZ-CB, Prague city, distr. Horní Počernice (Svépravice), The wetland above the Eliška fishpond, 50°5.95'N, 14°36.87'E, 230 m, 7.9.2008					4			4	4	0.09	17.6	1.02	-
Hs	CZ-CB, The Chumava stream in the village Hostomice, 49°49.397'N, 14°2.619'E, 340 m, 12.9.2008					2			2	1	0.03	3.2	1.01	M
I	CZ-SB, The Malý Jordán fishpond, 3 km N from the city Tábor, 49°26.14'N, 14°40.08'E, 430 m, 9.9.2007						5		5	2	0.08	16.0	0.99	O, N
J	CZ-SB, The Loucký fishpond in the town Černovice, 49°22.71'N, 14°57.74'E, 630 m, 12.9.2007					3			3	3	0.07	10.4	1.01	M
Jo	CZ-NB, The Jordán stream by the road from the town Doksy to the village Břehyně, 50°34.27'N, 14°40.81'E, 260 m, 12.9.2008					3			3	3	0.10	15.2	1.04	O, M
K	CZ-CB, The small pond by the road from the town Karlštejn to the village Krupná, 49°55.491'N,14°9.902'E, 300 m, 29.7.2008					5			5	4	0.07	13.6	1.00	M

Acronym	Locality and date of collection	AFLP group							N_i	N_g	D_{Nei}	%poly	FCM 2C (pg)	FCM subsp.	CDA subsp.
		E	A	O	B	M	C	N							
La	CZ-CB, The fishpond on the right side of the Pánovka fishpond by Pánova louka grassfield, 1.83 km S from the village Lány, 50°6.50'N, 13°56.67'E, 400 m, 30.9.2008					1	1		2	2	0.14	13.6	1.00		M
Ne	CZ-SB, The Nežárka river below the weir in the village Hamr, 49°9.45'N, 14°45.995'E, 420 m, 29.9.2008						2		2	2	0.07	7.2	1.02		O
P	CZ-CB, The Botič stream below the road and Průhonice Castle in the village Průhonice, 50°0.10'N, 14°33.36'E, 290 m, 24.8.2007					5			5	5	0.09	17.6	1.01		M
Ps	CZ-CB, Prague city, distr. Dolní Počernice, The stream along the Počernický fishpond, 50°5.25'N, 14°35.09'E, 230 m, 14.9.2008						3		3	2	0.09	12.8	1.01		M
Re	CZ-CB, The small forest fishpond in the Prameny Klíčavy nature reserve, 5.85 km W from the town Nové Strašecí, 50°8.892'N, 13°49.102'E, 420 m, 13.9.2008					1	1		2	2	0.10	10.4	1.01		M
Sr	CZ-CB, The small fishpond on the Bubovický stream, 1.34 km NE from the village Srbsko, 49°56.64'N, 14°9.07'E, 400 m, 6.10.2008					4			4	3	0.06	12.0	1.05		-
St	CZ-CB, The Novostrašecký fishpond, 1.70 km W from the town Nové Strašecí,; 50°9.120'N, 13°52.58'E, 410 m; 13.9.2008					2			2	1	0.00	0.0	1.07		M
T	CZ-CB, The Louže pond in the village Třebotov, 49°58.437'N, 14°17.62'E, 360 m, 8.8.2007					15			15	6	0.07	24.0	1.02		M
Tr	CZ-SB, The Prostřední stoka canal near the railway station, Třeboň, 49°0.39'N, 14°46.62'E, 430 m, 28.9.2008						3		3	3	0.02	3.2	1.02		O, M
U	CZ-CB, Prague city, distr. Újezd nad Lesy, The Blatovský fishpond in the village Blatov, 50°4.79'N, 14°38.38'E, 245 m, 25.7.2007				2	12			14	8	0.10	34.4	1.03		E, M
Vp	CZ-CB, Prague city, distr. Šeberov, The Kovářský fishpond, 50°0.88'N, 14°30.96'E, 290 m, 8.9.2008				14				14	12	0.10	28.0	1.09		E, O, M
Za	CZ-CB, The Berounka river by the railway station Zadní Třebáň, 49°55.16'N, 14°12.22'E, 200 m, 6.10.2008				5				5	4	0.09	17.6	1.08		E

Acronym	Locality and date of collection	AFLP group											FCM	CDA
		E	A	O	B	M	C	N	N_i	N_g	D_{Nei}	%poly	2C (pg)	subsp.
Sparganium erectum* subsp. *neglectum														
Hp2	CZ-CB, Prague city, distr. Horní Počernice (Svépravice), The Eliška fishpond, r. 50°5.87'N, 14°36.67'E, 230 m, 7.9.2008							3	3	3	0.05	8.0	1.00	O, M, N
Hp3	CZ-CB, Prague city, distr. Horní Počernice (Svépravice), The Xaverovský fishpond II, 50°6.15'N, 14°35.83'E, 230 m, 7.9.2008						1	4	5	4	0.09	20.0	0.99	O, N
W	CZ-CB, The wetland of the Pšovka river, 1km SE from the village Hleďsebe; 50°21.40'N, 14°34.32'E, 200 m, 17.9.2007							5	5	1	0.02	4.0	1.00	M, N
Z	CZ-CB, The Pšovka river, Lhotka u Mělníka, 50°22.20'N, 14°33.10'E, 210 m, 17.9.2007							3	3	1	0.03	4.8	0.99	M, N



Electronic Appendix 2. – Map of localities of *Sparganium erectum* in the Czech Republic (A) and in Central Bohemia and the Elbe river basin (B, for list of localities see Appendix 1).



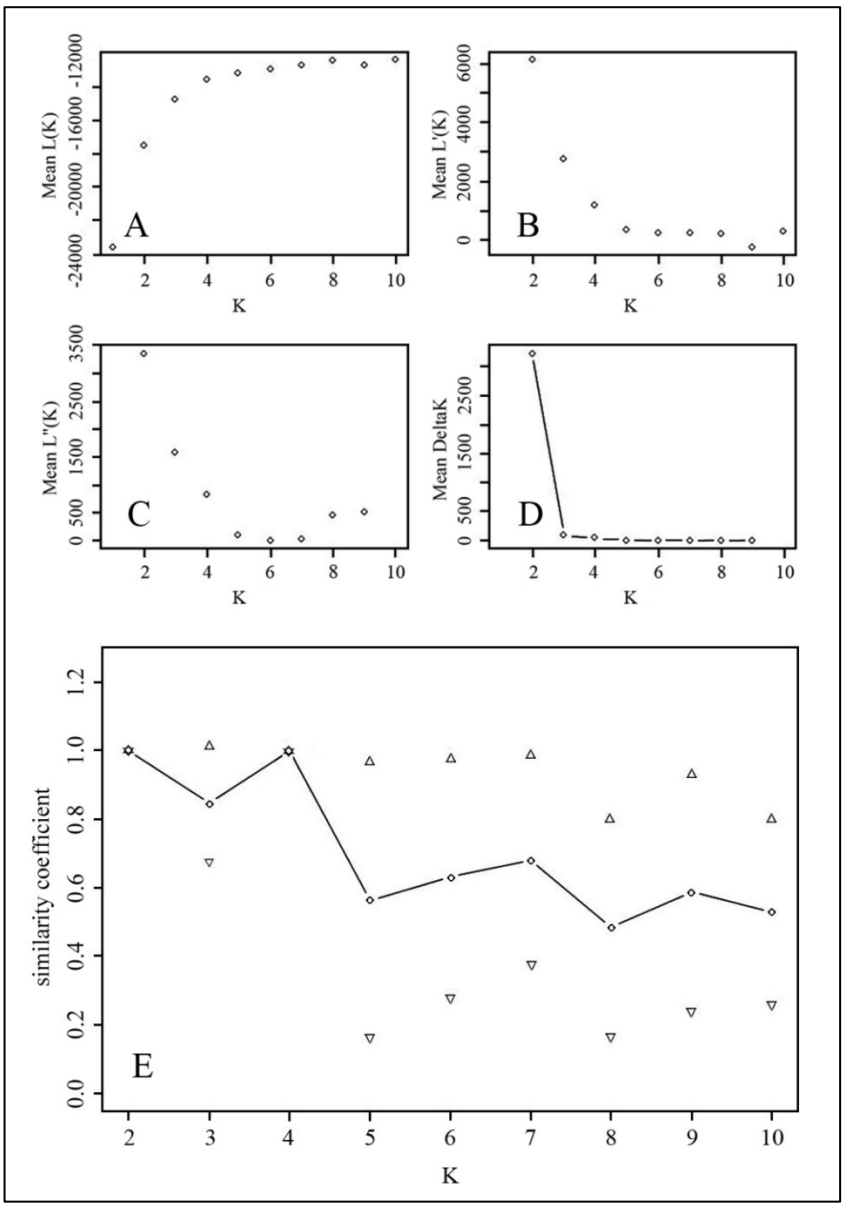
Electronic Appendix 3. – STRUCTURE analysis of 276 individuals of *Sparganium erectum* based on 125 AFLP loci. Graphical representation of estimated: (A) mean L(K), (B) meanL'(K), (C) meanL"(K), (D) mean DeltaK and (E) similarity coefficient computed using Structure harvester.

Electronic Appendix 4. – Results of STRUCTURE simulations for K 1-10 based on 276 individuals of *Sparganium erectum* and 125 AFLP loci. Mean similarity coefficient, standard deviation and ΔK was computed using Structure harvester.

Cluster (K)	Number of runs	Mean similarity coeff.	standard dev.	ΔK
1	10	-23594.84	11.51	-
2	10	-17467.43	1.04	3226.28
3	10	-14706.81	18.68	84.56
4	10	-13526.10	22.45	36.86
5	10	-13172.95	49.70	1.87
6	10	-12912.64	39.30	0.05
7	10	-12650.46	41.52	0.71
8	10	-12417.95	145.06	3.11
9	10	-12636.97	1772.39	0.28
10	10	-12355.49	487.30	-

Electronic Appendix 6. – Spearman correlation coefficient of 12 morphological characters of achenes of *Sparganium erectum*.

Character	Ped.	Al	Ll	Lu	Sl	Aw	Na	Con.	Al/aw	Lu/Ll	Sl/Al	Sl/Aw
Peduncle (Ped.)	1.00	-0.38	-0.51	0.21	-0.01	-0.69	0.44	0.90	0.52	0.48	0.27	0.54
Achenelength (Al)	-0.38	1.00	0.88	-0.01	0.10	0.35	-0.52	-0.40	0.20	-0.61	-0.57	-0.24
Lengthlower (Ll)	-0.51	0.88	1.00	-0.34	-0.08	0.43	-0.53	-0.47	0.03	-0.88	-0.65	-0.40
Lengthupper (Lu)	0.21	-0.01	-0.34	1.00	0.57	-0.21	-0.03	0.12	0.25	0.71	0.39	0.46
Stylelength (Sl)	-0.01	0.10	-0.08	0.57	1.00	-0.18	-0.24	-0.10	0.26	0.31	0.70	0.66
Achenewidth (Aw)	-0.69	0.35	0.43	-0.21	-0.18	1.00	-0.13	-0.66	-0.83	-0.41	-0.41	-0.84
Number of angles (Na)	0.44	-0.52	-0.53	-0.03	-0.24	-0.13	1.00	0.52	-0.14	0.35	0.17	0.02
Constriction (Con.)	0.90	-0.40	-0.47	0.12	-0.10	-0.66	0.52	1.00	0.48	0.39	0.21	0.47
Al/Aw	0.52	0.20	0.03	0.25	0.26	-0.83	-0.14	0.48	1.00	0.10	0.11	0.75
Lu/Ll	0.48	-0.61	-0.88	0.71	0.31	-0.41	0.35	0.39	0.10	1.00	0.66	0.51
Sl/Al	0.27	-0.57	-0.65	0.39	0.70	-0.41	0.17	0.21	0.11	0.66	1.00	0.71
Sl/Aw	0.54	-0.24	-0.40	0.46	0.66	-0.84	0.02	0.47	0.75	0.51	0.71	1.00



Electronic Appendix 5. – Results of molecular analyses of 276 individuals from *Sparganium erectum* based on 125 AFLP loci. Bar plot showing Bayesian assignment probabilities using software STRUCTURE for three clusters (K=3, second solution). Four genetic groups (containing individuals with <15% admixture with another group) associated with subspecies were recorded plus intermediate individuals (groups A, B, C) between them.

PAPER II.

Píšová S., Hroudová Z., Chumová Z. & Fér T. (2017): Ecological hybrid speciation in central-European species of *Bolboschoenus*: genetic and morphological evaluation. – Preslia. 89: 17–39.

Ecological hybrid speciation in central-European species of *Bolboschoenus*: genetic and morphological evaluation

Ekologická hybridní speciace středoevropských kamyšníků (*Bolboschoenus*) – genetické a morfologické zhodnocení

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Abstract

Divergent natural selection is known to facilitate speciation in many taxa. The genus *Bolboschoenus* (Cyperaceae) is a model group for investigating ecological and homoploid hybrid speciation. Four taxa of *Bolboschoenus* occur in central Europe: the halophyte *B*. *maritimus* and glycophytes *B*. *laticarpus*, *B*. *planiculmis* and *B*. *yagara*. These species differ in their ecological niches. Such ecological and/or geographical isolation is critical for homoploid hybrid speciation. The determination of species of *Bolboschoenus* is based on morphological characters of the inflorescence and on achene shape and anatomy. On the basis of its intermediate morphology, chromosome number and ecological amplitude *B*. *laticarpus* is thought to be a hybrid. In order to determine the validity of morphological species and the possible hybrid origin of *B*. *laticarpus* we used amplified fragment length polymorphisms (AFLPs) as molecular markers and compared different genetic groups defined using STRUCTURE analysis with morphological data. The morphological classification of central-European species of *Bolboschoenus* was confirmed. Plants of heterogeneous genotypes were also found to be intermediate individuals resulting from spontaneous hybridization. Hybrid origin of *B*. *laticarpus*, which is genetically and morphologically intermediate between *B*. *yagara* and *B*. *planiculmis*, was elucidated. Inflorescence characters were less important for determining species than anatomical characters of achenes (widths of the exocarp and mesocarp).

K e y w o r d s: AFLP, *Bolboschoenus*, central Europe, hybridization, model-based clustering, morphometrics, speciation

Introduction

The evolutionary histories of groups of closely related species of plants have been the focus of interest of much research in recent years, especially the process of speciation (Kaplan et al. 2013, Kolář et al. 2014, 2015). Moreover, questions concerning reproductive isolating mechanisms and the genetics and genomics of speciation have been highlighted by a major European initiative as the main subjects for further research (Butlin et al. 2012). Ecological speciation is one of the main modes of speciation (Schluter 2001). It occurs as a result of reproductive isolation due to divergent selection of organisms in different environments and can arise even where species occur sympatrically (Sobel et al.2009). Studying microevolution within groups of closely related species differing in ecology may thus reveal the role of such environmental differences in their origin. Hybridization can result in both gene flow among plant taxa and generation of new species. Some families and genera have relatively high numbers of hybrids, with the family Cyperaceae being one of them (Ellstrand et al. 1996).

The European species of *Bolboschoenus* (Asch.) Palla (Cyperaceae) provide an example of a putative hybrid complex for which ecological selection, geographic isolation and hybridization are thought to have been important in their speciation, with the study of their genetic variation likely to shed light on their speciation processes. Of the ~14 species of *Bolboschoenus* that are distinguished worldwide (Browning & Gordon-Gray 2000, Tatanov 2007), four are native to Europe (Hroudová et al. 2007): *Bolboschoenus maritimus* (L.) Palla, *B. laticarpus* Marhold, Hroudová, Ducháček et Zákravský, *B. planiculmis* (Schmidt) T. V. Egorova, and *B. yagara* (Ohwi) Y. C. Yang et M. Zhan. Their native ranges include at least some part of Europe, with *Bolboschoenus maritimus* and *B. laticarpus* occurring mainly in Europe, whereas *B. planiculmis* and *B. yagara* reach the western borders of their ranges in central Europe and are continuously distributed through Eurasia to the Far East (Egorova & Tatanov 2003, Tatanov 2003, Hroudová et al. 2007). Central Europe is thus the area where these four taxa co-occur. Indeed, in some regions of central Europe, individuals of different species form mixed populations. In such cases, plants with intermediate morphology occur, indicating possible spontaneous hybridization. Such morphotypes are recorded frequently, especially those intermediate between *B. maritimus* and *B. planiculmis* (Ducháček 2002, Hroudová et al. 2006). Nevertheless, it is difficult to

determine whether such intermediate plants have resulted from present-day spontaneous hybridization or simply represent overlapping variation in the two species' morphological characters.

The species differ in their ecological niches (Hroudová et al. 1999, 2007, Kaplan et al. 2015): *Bolboschoenus maritimus* inhabits saline habitats (mainly remnants of natural halophyte vegetation), *B. planiculmis* inhabits secondary habitats (temporarily flooded field depressions, wet meadows, arable land, inland shores of fishponds and other reservoirs, ruderal and other anthropogenic habitats), *B. yagara* occurs predominantly in the littoral of fishponds, mainly in several fishpond basins, and *B. laticarpus* has a wide habitat range, including along streams and many secondary habitats (flooded depressions in fields, wet ditches and channels, and as a weed in arable land). This obvious ecological differentiation might indicate a crucial role of environmentally-driven selection in the evolutionary process leading to speciation within the genus *Bolboschoenus*.

Current classification is based on morphological and anatomical characters of achenes, which are more reliable than inflorescence characters (Oteng-Yeboah 1974, Browning & Gordon-Grey 1993, Browning et al. 1997b, Browning & Gordon-Grey 2000). Individuals without ripe achenes cannot be determined unambiguously. *Bolboschoenus yagara* is distinguished from other species by narrowly obovate achenes, triangular in transverse section and a particularly thin exocarp. *Bolboschoenus planiculmis* has obovate achenes, concave on the abaxial side and exocarp approximately as thick as the sclerenchymatic mesocarp. *Bolboschoenus maritimus* is distinguished by lenticular achenes convex on the abaxial side, with exocarp thicker than mesocarp. *Bolboschoenus laticarpus* has trigonous achenes with thicker mesocarp than exocarp. Its achene shape varies, ranging from trigonous achenes with the edge sharp through flattened-trigonous to slightly convex on the abaxial side. In summary, the most important characters for species determination are the shape of the achene in transverse section and the widths of the exocarp and mesocarp (Hroudová et al. 2007).

Bolboschoenus laticarpus (n = 54, 55) is a taxon that is morphologically and anatomically intermediate between *B. yagara* (n = 55) and *B. maritimus* (n = mostly 55) or *B. planiculmis* (n = mostly 54; meiotic chromosomes counted by Jarolímová & Hroudová 1998). Browning et al. (1996) hypothesize that this taxon is of hybrid origin and call it *B. maritimus* × *B. yagara*. Marhold et al. (2004) consider it to be a stable taxon of hybrid origin with possible parentage

B. *yagara* × *B*. *planiculmis*, based on the numbers of style branches and chromosomes (Jarolímová & Hroudová 1998). Finally, hybridization in nature is most likely to occur between *B*. *yagara* and *B*. *planiculmis,* with overlapping distributions across Eurasia, than between *B*. *yagara* and *B*. *maritimus*, with distinct distributions and quite different habitats. There are no molecular studies of *Bolboschoenus* and thus study of its genetic variation provides a suitable way of elucidating the processes leading to the differences in the distributions and ecologies of the species within this genus. Moreover, molecular analyses would reveal whether *B*. *laticarpus* is of hybrid origin, and, if so, its likely parentage.

The AFLP method is a suitable molecular marker to generate a large number of variable loci distributed throughout the genome, and requires no previous sequence knowledge (Vos et al. 1995). This method offers powerful tools for the assessment of intraspecific variation and detection of hybrid origin (Gobert et al. 2002, Guo et al. 2006, Španiel et al. 2011, Záveská et al. 2011). We analysed populations of *B*. *yagara*, *B*. *laticarpus*, *B*. *maritimus* and *B*. *planiculmis* using AFLPs, with the objective of determining the mechanisms that resulted in inter-specific differentiation during their evolution. In particular, whether genetic variation among the species corresponds to their morphological variation, indicating gene flow during evolution, or whether species differentiation resulted predominantly from ecological (ecophysiological) speciation due to selection. Both the role of hybridization in speciation, and possible recent spontaneous hybridization between some species were also studied.

We aimed to answer the following questions: (i) Does morphological variation of the species studied correspond to the genetic variation? (ii) What morphological and anatomical characters can be used to differentiate among individual species? (iii) Does spontaneous hybridization occur in natural populations of the species, and to what extent is this reflected by morphology? (iv) Is *B*. *laticarpus* of hybrid origin, and if so, what are its parental species?

Materials and methods

Plant material

In order to include all the variation in European taxa of *Bolboschoenus*, samples of four species of *Bolboschoenus* (*B*. *maritimus*, *B*. *laticarpus*, *B*. *planiculmis* and *B*. *yagara*) from 36 natural populations in the Czech Republic, Slovakia, Austria and Hungary were collected during 2010–2011 (Appendix 1).

Species were pre-identified in the field using identification keys and the nomenclature follows Hroudová et al. (2007). When mixed populations were found (rarely the case) only individuals morphologically resembling a particular taxon were collected. The number of individuals sampled for AFLPs (as well as inflorescences and achenes) was increased in order to collect all species in the same way. *Bolboschoenus maritimus* material from natural populations was complemented with material from plants cultivated from seeds (collected in the wild in Germany, France and Iran) in an experimental garden of the Institute of Botany ASCR in Průhonice (49°59.69'N, 14°34.00'E). For this cultivation, for each population, descendants of particular individuals were used in order to reflect intrapopulation genetic and morphological variation. The results of earlier work (Hroudová et al. 1998a) confirmed that the morphological characteristics recorded in natural populations of *Bolboschoenus* persisted after transfer into cultivation, i.e. plants from cultivation corresponded to plants from natural habitats.

Material for AFLP analyses (altogether 279 leaf samples), morphological analyses (855 inflorescences) and anatomical measurements (875 achenes) was collected using the following procedures. For AFLP analyses, undamaged part of fresh leaves usually from 10 individuals per population (whenever possible, depending on population size) were sampled randomly and immediately dried in silica gel. Due to the extensive damage to leaves by rusts that occurs when the inflorescences are fully developed and prevent the isolation of DNA suitable for AFLP analyses, we collected fresh young leaves from different individuals (but in the same populations) than those used for morphometrics. In addition, the breaking up of inflorescences and spikelets at maturity made it impossible to obtain both inflorescences and ripe achenes from the same individuals. Whole welldeveloped inflorescences of 25 individuals in each population were collected and 25 ripe achenes randomly chosen for the morphological analyses. Samples were always collected at least 10 m apart in order to minimize collection from within the same clone. Voucher specimens are deposited in the Charles University Herbarium (PRC).

AFLP analysis

Total genomic DNA was extracted using the Invisorb Spin Plant Mini Kit (Invitek). DNA pellets were dissolved in 40 µl of Elution Buffer D. DNA concentration was then measured using a Nanodrop 1000 spectrophotometer (Thermo Scientific) and DNA was diluted to 50 $ng \cdot \mu l^{-1}$.

AFLP analysis (Vos et al. 1995) was carried out using the AFLP Core Reagent Kit I (Invitrogen) and the AFLP Pre-Amp Primer Mix I (Invitrogen), following the manufacturer's instructions as modified by Záveská et al. (2011) and then further modified to yield the procedure described below. Total genomic DNA of about 50 ng was restricted for 12 h at 37° C with 0.5 U each of *Eco*RI and *Mse*I restriction enzymes (Invitrogen) and 1 µl 5 × reaction buffer (Invitrogen) in a total volume of 5 µl. Adaptors were ligated for 12 h at 37° C by adding 4.8 µl adaptor/ligation solution (Invitrogen) and 0.2 U T4 DNA ligase (Invitrogen) to the digested DNA (total volume 10 µl). Preamplification reactions (total volume 5 µl) contained 0.5 µl of restricted/ligated DNA, 4.0 µl Pre-Amp Primer Mix I, 0.5 µl 10× buffer for RedTaq JumpStart (Sigma) and 0.1 U RedTaq JumpStart DNA polymerase (Sigma). After preamplification, DNA was 10× diluted with water. Four primer combinations (selected after an initial screening of 72 primer combinations) were used for selective amplification: *Eco*RI-ATC-(6-FAM)/*Mse*I-CAA, *Eco*RI-AAG(VIC)/*Mse*I-CTC, *Eco*RI-AAC-(NED)/*Mse*I-CAG, *Eco*RI-ACA-(PET)/*Mse*I-CAT. Selective amplification was done using 2.3 µl of the diluted preamplification mixture, 1 µl 10× buffer for RedTaq, 0.2 µM dNTP, 0.5 pmol EcoRI-selective fluorescence-labelled primer, 2.5 pmol Mse I-selective primer and 0.2 U RedTaq JumpStart DNA polymerase (Applied Biosystems) in a total volume of 10 µl. Selective amplification products were mixed with GeneScan LIZ 600 (Applied Biosystems) size standard and electrophoresed on an ABI 3130xl Avant Genetic Analyzer (Applied Biosystems) in the DNA Sequencing Laboratory, Faculty of Science, Charles University in Prague). Altogether, 279 samples from 36 populations were analysed. The whole AFLP procedure was repeated for 10% (29) of the samples and the error rate assessed by comparisons of identical samples (Bonin et al. 2004).

Molecular data analyses

AFLP data were analysed using GeneMarker software v1.8 (SoftGenetics LLC, PA, USA) and transferred into a binary data matrix. Only well-scorable, unambiguous fragments were recorded. Bayesian non-hierarchical clustering was performed in STRUCTURE 2.3.2.1 (Pritchard et al. 2000) to explore the genetic structure of the whole dataset, to define AFLP groups and assess the degree of admixture among species. The admixture model was used and independent allele frequencies were assumed. As AFLPs are dominant markers, a recessive allele model was used. The number of clusters (K) ranged from 1 to 10. For each K, ten runs were done to determine the stability of the results. The length of the burn-in period was set to 100,000 and the MCMC chains after burn-in were run through an additional 1,000,000 replicates (Falush et al. 2007). All computations were done on the freely available Bioportal computer cluster (University of Oslo, http://www.bioportal.uio.no). The R-script (R Development Core Team 2008) Structuresum-2009 (Ehrich et al. 2007) was used to summarize the output files and to calculate similarity coefficients between the replicate runs (Nordborg et al. 2005) and K (Evanno et al. 2005). The optimal number of groups (K) was the one with consistent results over ten repeats, high similarity coefficient and highest K. The software CLUMPP 1.1.1 (Jakobsson & Rosenberg 2007) and Distruct (Rosenberg 2004) were used to create graphical outputs for selected Ks. Samples with low admixture (up to 15%; Rossi et al. 2009, Roulier et al. 2013) were classified as members of one of the K AFLP groups and populations were treated as "pure" when all individuals were clearly assigned to the same group. Such populations were treated as "pure" also for analyses based on inflorescences and measurements of achenes (see below). Highly admixed individuals (more than 15%), i.e. genetically intermediate between the AFLP groups were passively projected onto ordination diagrams in successive analyses (i.e. PCoA, PCA, CDA; Koutecký 2015; see below). A matrix of pair-wise Jaccard's similarity coefficients (Jaccard 1908) was used for the calculation of the principal coordinate analysis (PCoA) implemented in Canoco 5 (ter Braak & Šmilauer 2012) and for construction of neighbour-network in SPLITSTREE v.4.11.3 (Huson & Bryant 2006). In the graphical outputs of these two analyses, AFLP groups were coloured according to STRUCTURE results, with highly admixed individuals remaining in black or represented by grey symbols. To explore partitioning of genetic variation within and among species and populations, two analyses of molecular variance (AMOVAs,

Excoffier et al. 1992; implemented in FAMD 1.3, Schlüter & Harris 2006) were performed: (i) three-level analysis (among species, among populations within species, within populations), (ii) two-level analysis for each species separately (among populations, within populations). Only populations with all samples classified to AFLP groups were used for AMOVA calculations.

Even though our sampling design was not intended to determine clonal structure within populations, we assessed genetic variability of each population by calculating the number of genotypes (Ng), Nei's gene diversity (DNei) and percentage of polymorphic markers (%poly) using the R script AFLPdat (Ehrich 2006).

Morphometric analyses

The structure of inflorescences and the morphology and pericarp anatomy of achenes are taxonomically important characters for determining species of *Bolboschoenus*. The selection of characters for species differentiation was based on the literature (Hroudová et al. 1997, 1998b, 2002, 2007, Ducháček 2002). Morphological and anatomical characters are given in Table 1 and Fig. 1. Morphometric analyses were done in order to evaluate the fit of morphological characters to genetically well-defined AFLP groups and reveal correspondence of taxonomic concept with genetic analysis. In order to obtain suitable sections of achenes for repeated measurements of pericarp anatomy, particular achenes were destroyed so the same achenes could not be used for further measurements. Therefore, it was necessary to analyse the four data sets separately. The first data set contained information on seven morphological characters (e.g. numbers and lengths of peduncles and spikelets) of 855 inflorescences. The second data set consisted of information on two morphological characters (achene length and width) of 875 achenes. The third data set included data on two morphological characters (achene width and thickness in transverse section) of 875 achenes. The fourth data set included information on six pericarp anatomical characters of 875 achenes. Morphological characters of inflorescences were measured using a digital calliper (first data set). Width and length of achenes were measured on whole achenes using a dissecting microscope with a digital camera to record images (second data set). Subsequently, width and thickness were obtained by cutting achenes with a razor in the widest part (third data set). Finally, we prepared the achenes so that anatomical characters of the pericarp (fourth data set, width of exo-, meso- and endocarp in the narrowest and in the widest parts) could be recorded.

Table 1. – List of morphological characters used in the study. * Highly correlated character that was excluded from the total analyses.

Inflorescence characters	**Achene characters**
Number of peduncles	Length/width of achenes (L/W)
Number of spikelets	Width/thickness of achenes (W/T)
Length of peduncles	**Anatomical characters of pericarp**
Length of spikelets	Width of exocarp at the widest part (exow)
Number of spikelets/ number of	Width of mesocarp at the widest part (mesow)
Length of peduncles/ number of spikelets	Width of endocarp at the widest part (endow)
* Length of peduncles / length of	Width of exocarp at the narrowest part (exon)
	Width of mesocarp at the narrowest part
	Width of endocarp at the narrowest part (endon)

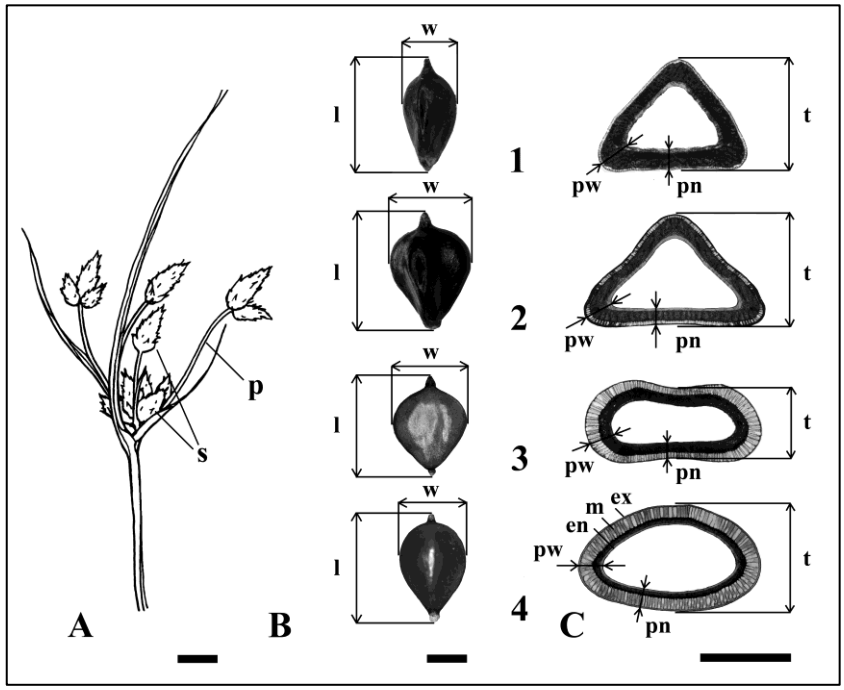

Fig. 1. – Morphological and anatomical characters. (A) Inflorescence. (B) Achenes. (C) Achene anatomical characters in cross sections. 1, *Bolboschoenus yagara*; 2, *B. laticarpus*; 3, *B. planiculmis*; 4, *B. maritimus*. Scale bars: 1 cm (A), 1 mm (B, C). p, peduncle; s, spikelet. l, length of achene; w, width of achene. t, thickness of achene; pw, width of pericarp layers at the widest part; pn, width of pericarp layers at the narrowest part; ex, exocarp; m, mesocarp; en, endocarp. (A) Del. Z. Hroudová. (B), (C) Phot. S. Píšová.

Achenes of *Bolboschoenus* are hard to cut; therefore, we had to cut off the base of achenes and put them in water for one week to soften them.

Anatomical and morphological characters remained unchanged (Hroudová et al. 1997). Cross sections of achenes 20 μm thick were obtained using Shadon Cryotome® microtome (77200226, Shandon Sci., Astmoor Runcorn, UK). Olympus BX50 microscopes with digital camera Olympus E 10 were used to record images. We measured all the achene characters using ImageJ software (National Institute of Health, USA).

The first data set of inflorescence characters and fourth data set of anatomical characters of achenes we subjected to multivariate morphometric analyses. These data sets were tested using the Shapiro-Wilk statistic for normality and non-parametric Spearman correlation coefficients were computed to investigate the correlations between the characters. Characters that were highly correlated with one another (exceeding 0.95 – length of peduncles / length of spikelets) were excluded from further analyses. Principal component analysis (PCA) with both individual plants and population means as operational units was done using Canoco 5 (ter Braak & Šmilauer 2012) for initial insight into the overall morphological variation. Canonical discriminant analysis was used to determine variation among predetermined AFLP groups (species) and the most important characters for their differentiation using R-script Morphotools (Koutecký 2015). In consequence, highly admixed individuals were passively projected. Classificatory discriminant analyses in R-script Morphotools were used to compute the percentage of genetically "pure" (i.e. admixed up to 15%) individuals correctly assigned to predefined AFLP groups. These individuals subsequently served as the training data set for determination of populations with higher admixtures. A one-way ANOVA procedure, Kruskal-Wallis test (Kruskal & Wallis 1952), Tukey HSD multiple comparison test and graphic representation by box plots were used to determine statistical differences among AFLP groups in the second (length/width ratio) and third data sets (width/thickness ratio) in R package multcomp (Hothorn et al. 2008). Admixed individuals were also additionally displayed for demonstration.

Results

AFLP analysis

The four selective primer combinations generated 122 AFLP markers (all polymorphic), ranging in size from 100–500 bp. The technical error rate based on repeated AFLP analysis of 29 samples was 4.47%. STRUCTURE analysis yielded highly consistent results (i.e. similarity coefficient for ten runs) for K = 2–4; higher K values did not converge towards the same outcomes. Results for K = 2 discriminated two morphological groups generally corresponding to *B*. *yagara* and *B*. *planiculmis*. The species *B*. *maritimus* was not distinguished from *B*. *planiculmis*. Samples determined as *B*. *laticarpus* were approximately a 50:50 admixture of both groups (Fig. 2A). K = 3 discriminated AFLP groups corresponding to species: *B*. *yagara*, *B*. *planiculmis* and *B*. *maritimus*. The putative hybrid *B*. *laticarpus* was an even admixture of *B*. *yagara* and *B*. *planiculmis*. Result with highest K, K = 4 discriminated all four species: *B*. *yagara* (81 samples), *B*. *laticarpus* (57 samples), *B*. *planiculmis* (78 samples) and *B*. *maritimus* (32 samples). Most of the individuals of *B*. *laticarpus* were classified in an independent group (species). Nevertheless, some individuals were not differentiated as putative hybrids of *B*. *yagara* and *B*. *planiculmis*. In all analyses, the majority of the samples were clearly (i.e. with an up to 0.15 assignment probability to another group; ad hoc setting for dealing with admixtures) placed in one of the four AFLP groups. However, some of the individuals were highly admixed (i.e. with at least a 0.15 assignment probability) and assumed to be hybrids or results of introgression between AFLP groups. For the better differentiation of these admixed samples they were split into three groups in the graphs (Fig. 2A: group A – 10 samples between *B*. *yagara* and *B*. *laticarpus*, group B – seven samples between *B*. *laticarpus* and *B*. *planiculmis* and group C – 14 samples between *B*. *planiculmis* and *B*. *maritimus*). Moreover, two samples from Dolní Věstonice were an admixture of more than two groups (putative hybrid individuals of *B*. *yagara* × *B*. *planiculmis* with admixture of *B*. *maritimus*).

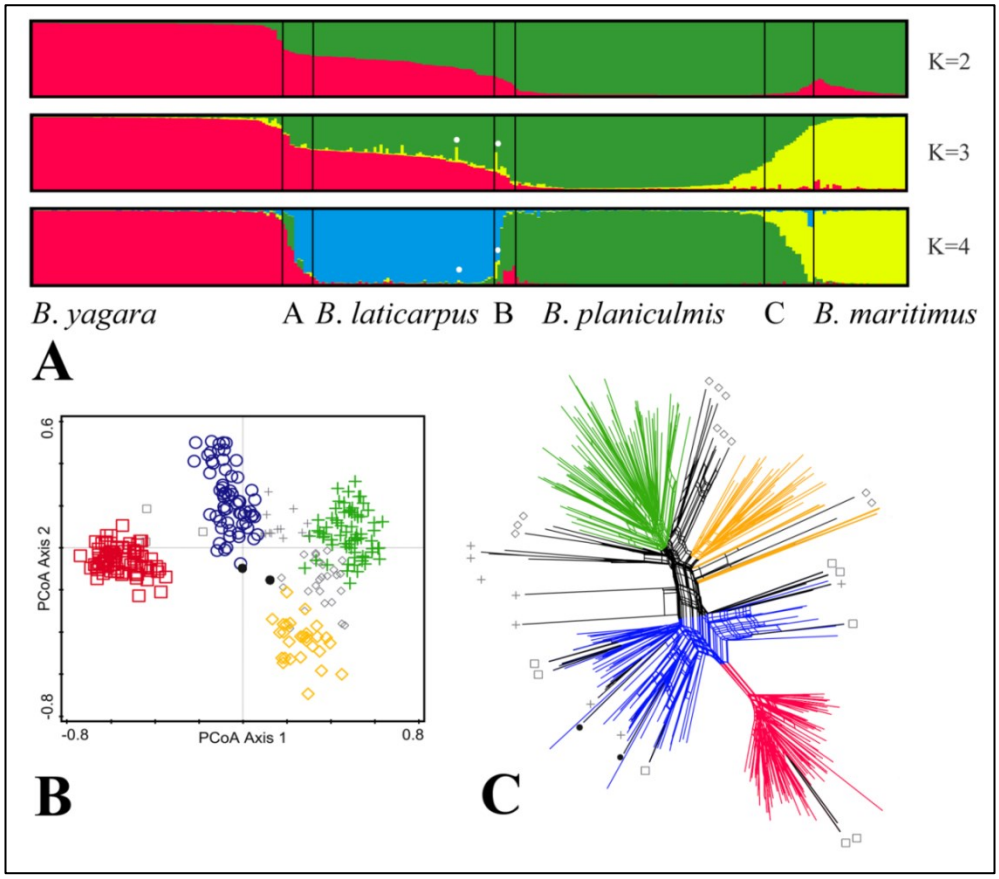

Fig. 2. – (A) Bar plot showing Bayesian assignment probabilities using software STRUCTURE for two, three and four clusters (K = 2–4) based on 122 AFLP loci and 279 individuals of *Bolboschoenus*. Four genetic groups associated with the species were recorded plus intermediate individuals (groups A, B, C; white dots – two samples of *B. laticarpus* with admixture of *B. maritimus*) between them. (B) Principal coordinate analysis (PCoA) using Jaccard's similarity coefficient. The first two axes explain 18.1% and 4.9% of the variation. Colours indicate AFLP groups detected by STRUCTURE and passively projected admixed individuals (red square, *B. yagara*; blue circle, *B. laticarpus*; green cross, *B. planiculmis*; yellow diamond, *B. maritimus*; grey square, group A; grey cross, group B; grey diamond, group C; black dots – two samples of *B. laticarpus* with admixture of *B. maritimus*). (C) Neighbour net diagram of 279 individuals of *Bolboschoenus*. Colours indicate AFLP groups detected by STRUCTURE (red lines, *B. yagara*; blue lines, *B. laticarpus*; green lines, *B. planiculmis*; yellow lines, *B. maritimus*; black lines, admixed samples: grey square, group A; grey cross, group B; grey diamond, group C; black dots – two samples of *B. laticarpus* with admixture of *B. maritimus*).

Combination of the first two PCoA axes (explaining 18.1% and 4.9% of the variability; Fig. 2B) and a neighbour-net diagram (Fig. 2C) largely confirmed the STRUCTURE results. In both analyses, *B. laticarpus* was in an intermediate position between the presumed parental species (*B. yagara* and *B. planiculmis*). The majority of admixed individuals were also placed between the AFLP groups.

The AMOVA for separate AFLP groups indicate that AFLP groups (species) are well differentiated, as 49.6% of the total variation was attributed to the differences among groups (Table 2). About 18.5% of the variation was among populations within species and the rest (31.9%) to differences among individuals within populations.

The estimates of number of genotypes, Nei's gene diversity and percentage of polymorphic markers for each population are shown in Appendix 1. Maximum genetic diversity was recorded for the *B. maritimus* populations HU (DNei = 0.29, %poly = 68.03%) and B (DNei = 0.23, %poly = 63.93%). Minimum genetic diversity was recorded for populations NE (DNei = 0.11, %poly = 17.21%) of *B. laticarpus*, and TO (DNei = 0.13, %poly = 19.67%) of *B. yagara*. No species-specific markers were found. The populations sampled were highly diverse and most plants had different genotypes. Investigation of clonal reproduction, however, was not the main goal of this study and further research is needed.

Table 2. – Analysis of molecular variance (AMOVA) of AFLP data (N = 247 individuals).

Grouping	Source of variation	d.f.	Sum of squares	Variance components	% of total variance
4 species	Among species	3	12.98	0.06	49.63
	Among populations/ within groups	32	6.87	0.02	18.46
	Within populations	227	9.51	0.04	31.91
B. yagara	Among populations	9	0.76	0.01	15.64
	Within populations	73	2.43	0.03	84.35
B. laticarpus	Among populations	8	3.49	0.06	65.35
	Within populations	61	1.87	0.03	34.64
B. planiculmis	Among populations	10	2.45	0.03	35.25
	Within populations	71	3.52	0.05	64.75
B. maritimus	Among populations	5	1.14	0.03	32.65
	Within populations	26	1.70	0.07	67.35

Morphometric analyses

A principal component analysis (PCA) based on mean values of inflorescence characters (first data set, 855 individuals × 6 characters) revealed four overlapping groups partially separated along the first axis (number and length of peduncles, Fig. 3A). Individuals of the putative hybrid taxon *B. laticarpus* were placed between its supposed parental taxa, *B. yagara* and *B. planiculmis*. Additionally, passively projected admixed individuals were scattered within and between these groups. PCA eigenvectors are given in Table 3. PCA based on anatomical characters of achenes (fourth data set, 875 individuals × 6 characters) revealed four well-separated AFLP groups along the first axis. This differentiation is attributable to exocarp and mesocarp widths at narrowest part. The *B. laticarpus* was also placed between its parental taxa and the admixed individuals were scattered within and between these groups (Fig. 3C, Table 4). The samples from AFLP group C were the most variable.

The CDA, based on the morphological characters of inflorescences, was done using four predefined AFLP groups and passively projected admixed individuals. It also revealed partial overlaps between groups (first data set, Fig. 3B). The individuals of *B. laticarpus* and *B. planiculmis* were slightly separated along the first canonical axis and those of *B. yagara* and *B. maritimus* along the second axis. Moreover, the individuals of the hybrid taxon *B. laticarpus* were placed between their parental taxa as in the PCA. The third axis (not shown) explained 4.6% of the variability and did not provide a differentiation that differed from that provided by the first and second axes. The characters most highly correlated with the canonical axes were: number of peduncles, number of peduncled spikelets, number of sessile spikelets/number of peduncled spikelets, length of sessile spikelets/length of peduncles of peduncled spikelets, length of peduncled spikelets and number of peduncled spikelets/number of peduncles of peduncled spikelets (Table 3). The CDA based on the anatomical characters of achenes (fourth data set) separated individual AFLP groups (species) even better (with less overlap). Admixed individuals were situated within and between them (Fig. 3D). The third axis (not shown) explained only 2.8% of the variability and did not provide a differentiation that differed from that provided by the first two axes. The most important characters for differentiation of separate taxa were exocarp and mesocarp widths (Table 4).

The classificatory DA based on morphological characters of inflorescences (first data set) correctly assigned 77.4% of the individuals to

predefined AFLP groups (80.3% of *B*. *yagara*, 62.7% *B*. *laticarpus*, 75.4% *B*. *planiculmis*, 85.0% *B*. *maritimus*). The classificatory DA based on anatomical characters of achenes (fourth data set) was more successful and correctly assigned 96.4% individuals (98.2% of *B*. *yagara*, 98.7% *B*. *laticarpus*, 88.8% *B*. *planiculmis*, 100% *B*. *maritimus*). The rest of the samples (3.6%) were misclassified in other groups. A part of population NZ was misclassified in the *B*. *maritimus* group. Populations with a high admixture were analysed for species determination with four predefined AFLP groups as a training set. Population KR (group A) was classified in the *B*. *yagara* group, while populations BO, DP, LI, LD-L, SK4 and AU2, DV_L (group B) were classified in the *B*. *laticarpus* group. In addition, populations DI, JK and DJ-PL, TD (group C) were classified in the *B*. *planiculmis* group. Samples of intermediate populations SK-KU and B were partially classified in the *B*. *planiculmis* and *B*. *maritimus* groups. (Appendix 1, CDA species).

The variation in the length/width ratio (second data set, Fig. 4A) is significantly different among species (Kruskal-Wallis test, $H = 410.18$, $df = 3$, $P < 0.0001$) as well as differences in width/thickness ratio (third data set, Fig. 4B, Kruskal-Wallis test, $H = 440.19$, $df = 3$, $P < 0.0001$). The four AFLP groups were well separated based on Tukey HSD multiple comparison test ($P < 0.001$). Admixed individuals (groups A, B and C) are also displayed between AFLP groups for comparison.

Table 3. – Results of morphometric analyses based on characters of inflorescences. The three highest PCA eigenvector and total canonical structure values are presented in bold.

Character	PCA 1	PCA 2	PCA 3	CDA 1	CDA 2	CDA 3
Number of peduncles	**–0.945**	0.004	0.084	**0.689**	0.193	–0.074
Number of spikelets	–0.706	0.626	0.241	0.237	–0.326	0.035
Length of peduncles	**–0.955**	0.052	0.210	**0.588**	–0.119	0.226
Length of spikelets	0.481	–0.278	0.830	–0.169	0.325	0.022
Number of spikelets / number of peduncles	0.369	**0.858**	0.066	–0.176	**–0.570**	–0.566
Length of peduncles / number of spikelets	–0,797	–0.392	–0.033	0.481	–0.172	0.228

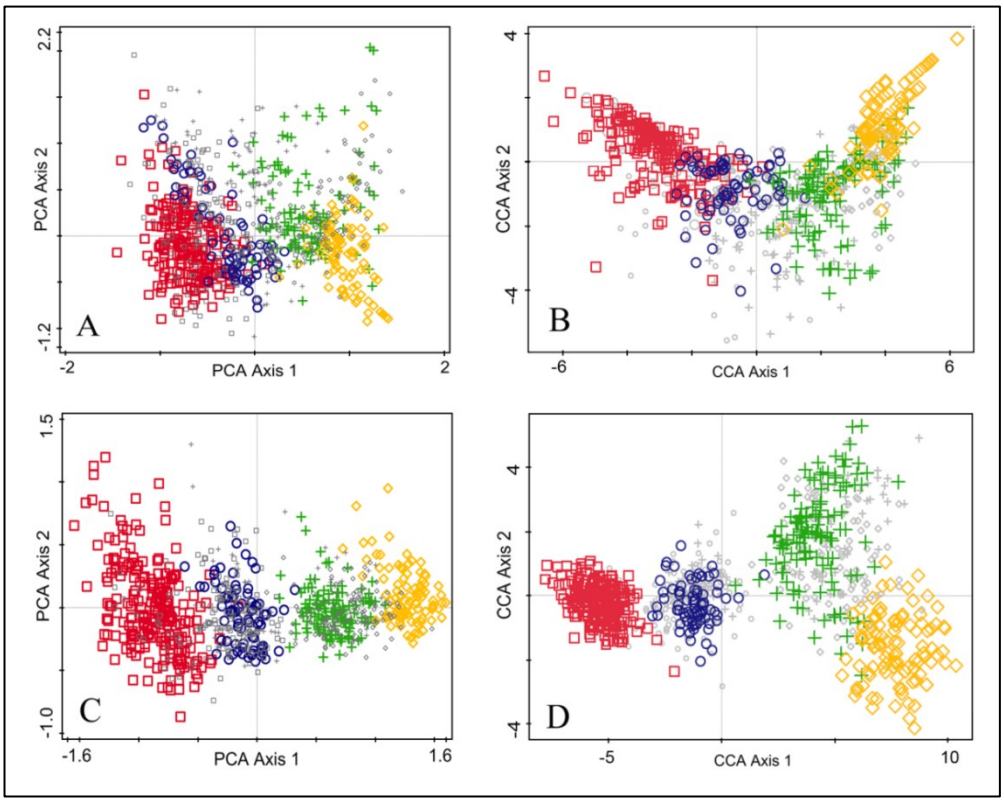

Fig. 3. – (A) Principal component analysis (PCA) based on six morphological characters of the inflorescences of 855 individuals of *Bolboschoenus*. The first two axes explain 55.1% and 22.7% of the variation. (B) Canonical discriminant analyses (CDA) of 855 individuals of *Bolboschoenus* based on six morphological characters of inflorescences. The first two components explain 29.8% and 12.6% of the variation. (C) Principal component analysis (PCA) based on six anatomical characters of 875 achenes. The first two axes explain 78.4% and 8.3% of the variation. (D) Canonical discriminant analyses (CDA) of 875 individuals based on six anatomical characters on achenes. The first two components explain 32.1% and 19.1% of the variation. Colours indicate AFLP groups detected by STRUCTURE and passively projected admixed individuals (red square, *B. yagara*; blue circle, *B. laticarpus*; green cross, *B. planiculmis*; yellow diamond, *B. maritimus*; grey square, group A; grey cross, group B; grey diamond, group C).

Table 4. – Results of morphometric analyses based on anatomical characters of achenes. The three highest PCA eigenvector and total canonical structure values are presented in bold.

Character	PCA 1	PCA 2	PCA 3	CDA1	CDA2	CDA3
Width of exocarp at the widest part	**0.921**	0.119	-0.084	**0.650**	0.606	0.428
Width of mesocarp at the widest part	**-0.910**	-0.165	0.055	-0.358	-0.020	**0.784**
Width of endocarp at the widest part	-0.766	0.620	0.165	-0.181	-0.045	0.297
Width of exocarp at the narrowest part	0.907	0.208	-0.084	0.512	**-0.603**	0.423
Width of mesocarp at the narrowest part	**-0.932**	-0.130	0.099	-0.432	0.277	0.240
Width of endocarp at the narrowest part	-0.866	0.109	-0.488	-0.261	0.037	0.355

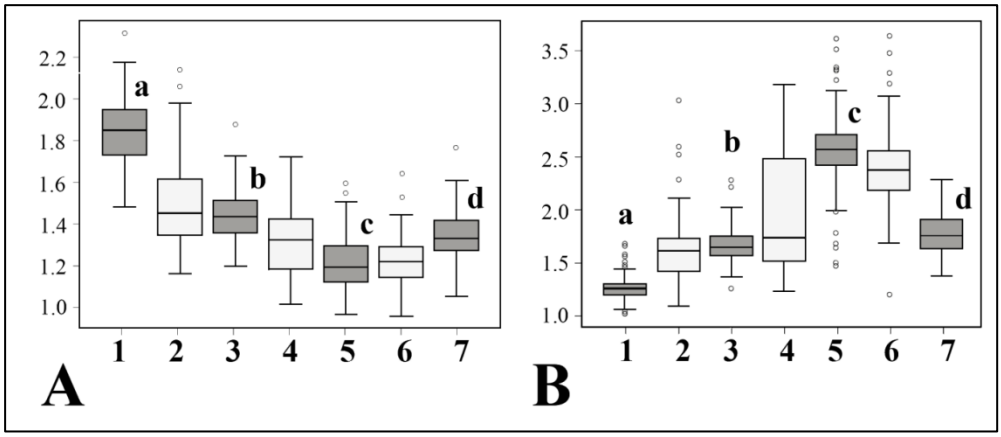

Fig. 4. – (A) Box plot showing the distribution of the ratio length to width of achenes (N = 875). (B) Box plot showing the distribution of the ratio width to thickness of achenes (n = 875). Different letters above boxes indicate statistical differences between four AFLP groups based on a Tukey HSD multiple comparison test ($P < 0.001$). The admixed groups are also displayed. 1, *B. yagara*; 2, group A; 3, *B. laticarpus*; 4, group B; 5, *B. planiculmis*; 6, group C; 7, *B. maritimus*.

Discussion

In this study, we focused on speciation in central-European species of *Bolboschoenus*. Molecular variation and morphological characters were compared and hybrid status of *B. laticarpus* assessed. Although we did not search for morphologically intermediate individuals the specimens of the species studied include both individuals that were morphologically typical and intermediate between species. Intermediate individuals were recorded especially between *B. planiculmis* and *B. maritimus*, and impossible to determine unambiguously using a classificatory function. The STRUCTURE analysis of AFLP data revealed genetic groups that mostly corresponded to morphologically defined species. Similar to other studies (Rossi et al. 2009, Roullier et al. 2013) individuals with posterior assignment probabilities above 0.85 (i.e. with less than 15% admixture) were assigned to a specific AFLP group (corresponding to a particular species), while individuals with higher assignment probabilities were considered to be admixed (i.e. hybrids). We found four AFLP genetic groups corresponding to morphological and anatomical differences in the achenes. The current classification of European species of *Bolboschoenus* was thereby confirmed, and in accordance with previous studies we accept the traditional morphological concept (Marhold et al. 2004, Hroudová et al. 2005, 2006, 2007).

Important morphological and anatomical characters for species determination

The first genetic group, with the least genetic variation corresponded to the species *B. yagara* (= *B. maritimus* subsp. *maritimus* with narrow fruits – Hroudová et al. 1998b or *B. fluviatilis* subsp. *yagara* – Browning et al. 1997a, Hayasaka & Ohashi 2002). The inflorescence characters partially differentiating this species are the higher number of peduncles, higher number of peduncled spikelets and lower ratio of sessile/peduncled spikelets. Ducháček (2002) extensively investigated the morphological variation of inflorescence characters and mentions the same characters for species differentiation. However, he also reports partial overlaps among species. We found that the shape of achenes (recorded here as the length/width and width/thickness ratios) was more important than inflorescence characters for determining species. For example, achenes of *B. yagara* were narrow, elongated and triangular in transverse section. Not even the shape of achenes was sufficient for species differentiation. The most reliable distinguishing characters proved to be anatomical characters:

very thin exocarp and thick mesocarp. This study confirmed their importance, first emphasized by Browning & Gordon-Gray (1993), and subsequently used in determination keys by Hroudová (2002) and Hroudová et al. (2007).

The second genetic group included individuals of the putative hybrid *B. laticarpus* noted as *B. maritimus* subsp. *maritimus* with wide fruits (Hroudová et al. 1998b), *B. maritimus* × *B. yagara* (Browning et al. 1996) and *B. yagara* × *B. koshewnikowii* (Hroudová 2002). Inflorescence characters were intermediate between those of *B. yagara* and *B. planiculmis*, as in Ducháček (2002) and Marhold et al. (2004). *Bolboschoenus laticarpus* differed from *B. yagara* mainly in having a lower number of branches and peduncled spikelets. Achenes were somewhat wider than those of *B. yagara*, elongated and trigonous with a somewhat thicker exocarp (see below for more details about hybrid origin of *B. laticarpus*).

The third genetic group included the most variable species *B. planiculmis* (*B. koshewnikowii* in Kozhevnikov 1988 or *B. maritimus* subsp. *compactus* in Hroudová et al. 1998b). Inflorescences consisted of sessile spikelets, sometimes accompanied by a few peduncled spikelets. This species had very wide and short achenes, concave to flat dorsally. The most reliable character was exocarp approximately as thick as the mesocarp. Ducháček (2002) considers *B. planiculmis* to be a readily distinguishable species. Nevertheless, he, like Hroudová et al. (2006), mentions individuals intermediate between *B. planiculmis* and *B. maritimus* for which determination is impossible.

The fourth group included populations of *B. maritimus* from coastal and inland saline habitats (*B. maritimus* s. str.) with head-like inflorescence consisting of sessile spikelets and a few peduncled spikelets. This species was distinguished by having a thicker exocarp than mesocarp. Achenes were wide, short and convex dorsally. However, achenes may also sometimes be lenticular like those of *B. planiculmis* (Ducháček 2002, Hroudová et al. 2007).

In summary, morphological characters of inflorescences appear to be less reliable than the morphological and anatomical characters of achenes. In particular, we consider exocarp and mesocarp widths and achene shape in transverse section to be the most important characters for species determination.

Hybrid origin of Bolboschoenus laticarpus

Hybridization influences plant evolution, inducing diversification and speciation and affecting genetic variation (Lihová et al. 2007). The frequency of spontaneous hybridization is higher in certain families and genera such as *Bolboschoenus* (Ellstrand et al. 1996). *Bolboschoenus laticarpus*, which is assumed to be of hybrid origin because of its intermediate morphology (Browning et al. 1996, Hroudová 2002, Marhold et al. 2004) is in the second genetic group. All samples of *B. laticarpus* were intermediate between *B. yagara* and *B. planiculmis*, with genetic information from both parental taxa (almost in the ratio 50:50 for division K = 3, Fig. 2A). Backcrossing with the parental species is rare and was recorded only for *B. planiculmis* in population SK4 and SK_Ma. Although *B. laticarpus* is closer to *B. yagara* in inflorescence structure (compound inflorescence with sessile and peduncled spikelets) and in fruit shape and anatomy (trigonous fruits with thicker mesocarp than exocarp), an overall comparison of its morphological and anatomical characters indicates it occupies an intermediate position between *B. yagara* and *B. planiculmis*.

The chromosome number of *B. yagara* is n = 55, *B. planiculmis* n = 54 (only exceptionally n = 55), while in *B. laticarpus* both n = 55 and n = 54 are recorded (Jarolímová & Hroudová 1998). This is in agreement with the parentage of *B. laticarpus* determined in this study, because it is unlikely that *B. maritimus* with n = 55 could be the second parent.

The STRUCTURE result for division K = 4 indicate that *B. laticarpus* is a stable hybridogenous species. It differs from other European species of *Bolboschoenus* by its wide ecological amplitude and in inhabiting a broad range of habitats (Marhold et al. 2004), which is reflected in its role in plant communities. This species appears to occur very frequently in central Europe, spreading along rivers, (e.g. association *PhalaridoBolboschoenetum laticarpi* Passarge 1999 corr. Krumbiegel 2006; Hroudová et al. 1999, 2009) in river floodplains and also as a weed in arable land (Hroudová et al. 2007). Such success contrasts with that of many interspecific hybrids that are less successful than their parental taxa (Yakimowski & Rieseberg 2014). Indeed, interspecific hybridization sometimes even leads to sterility, which was not recorded for *B. laticarpus* (Moravcová et al. 2002). However, in other cases gene combinations can give rise to hybrids that are fitter than their parental taxa, able to inhabit unoccupied niches, and with the origins of some hybrid species connected with habitat disturbance in man-influenced landscapes (Schemske 2000). In the case

of *B. laticarpus*, hybridization led to an increase in fitness, which enabled it to inhabit a wide range of habitats including secondary ones (e.g. arable land) (Hroudová et al. 2014). The life-history characteristics of *B. laticarpus* correspond to the adaptive traits associated with the formation of new species during evolution (Ellstrand et al. 1996): (i) outcrossing, (ii) developmental and ecological flexibility, (iii) perennial habit and vegetative reproduction.

The question regarding the centre of origin of *B. laticarpus* remains. Its current distribution is mainly in central Europe, along some rivers and even reaches the sea coast (Hroudová et al. 2007). This indicates its possible origin in central Europe, where the areas of distribution of *B. yagara* and *B. planiculmis* also overlap and mixed populations occur (e.g. AU2). However, in contrast to *B. laticarpus*, the continuous distributions of both its parental taxa extend eastwards through Russia to the Far East (Egorova & Tatanov 2003, Tatanov 2003, Hroudová et al. 2007), and plants very similar morphologically to European *B. laticarpus* occur in Japan, under the name *Bolboschoenus fluviatilis* subsp. *yagara*, type B (Hayasaka & Ohashi 2002), in Kazakhstan and East Asia (Tatanov 2007). This suggests a possible polytopic origin of this hybrid species.

Recent hybridization among species

The percentage of species that hybridize varies among families, but around 25% of plant species hybridize with at least one other species (Mallet 2007). Hybridization undoubtedly is an important process in *Bolboschoenus* evolution and a means of adapting to habitat conditions. Hybridization occurred not only in the past [leading to the establishment of the stable hybridogenous *B. laticarpus* with a different ecological niche, area of distribution (Hroudová et al. 2007) and special biological traits (Hroudová et al. 2014); see above] but also recently. Distributions of central-European species of *Bolboschoenus* overlap, which provides the opportunity for hybridization. We recorded 31 individuals (11%) that were placed between AFLP groups, reflecting a genetic admixture, presumably a result of spontaneous hybridization. These genetically intermediate groups (A, B, C) differ in their representation. The least numerous is the group between *B. laticarpus* and *B. planiculmis* (2%). Slightly more numerous is the admixture group between *B. yagara* and *B. laticarpus* (4%), and it is not excluded that some backcrossing between them has occurred. Finally, we recorded many admixtures (5%) intermediate between *B.*

planiculmis and *B. maritimus*; in some cases whole populations consisted of admixtures. However, such admixed populations (e.g. population B) were morphologically clearly identified as *B. maritimus* (Ducháček 2002).

The comparison of populations of *B. maritimus* from highly saline areas (e.g. sea coasts) and localities with lower salinity (some inland saline marshes) revealed genetical differences. Samples from habitats with a high salinity were genetically pure, even though the two localities were distantly remote from one another (France, Iran). On the other hand, some inland samples (from areas where the distribution of *B. maritimus* and *B. planiculmis* overlapped) contained admixtures of *B. planiculmis* genotypes. *Bolboschoenus planiculmis* and *B. maritimus* occur most often together in inland freshwaters or slightly saline habitats (e.g. in southern Moravia, southern Slovakia, Lower Austria: Ducháček 2002, Hroudová et al. 2007, 2014), and intermediate morphotypes are frequently found there. We suppose that these intermediate morphotypes are spontaneous hybrids that cannot arise in areas where only *B. maritimus* occurs in habitats with high salinity (sea coast, inland salt lakes). Nevertheless, introgression or backcrossing can only be properly studied using variable codominant microsatellite markers (e.g. Snow et al. 2010).

The finding that the genotypes of some of the plants morphologically similar to *B. maritimus*, had admixtures of *B. planiculmis* genotypes (e.g. in populations B and HU), may account for some irregularities and changes in plant occurrence. The admixture of *B. planiculmis* genotype may influence their biological traits, e.g. survival in a wider range of habitats (not saline or slightly saline). This might account for the occurrence of *B. maritimus* in some unusual habitats, small fishponds, sand pits or depressions in fields.

On the other hand, hybridization between *B. yagara* and *B. laticarpus* is probably less frequent. Although both species are usually fully fertile and sometimes occur together their seedlings rarely become established. Their seeds germinate and seedlings establish only on the water-saturated exposed bottoms of fishponds (Hroudová et al. 1996). We suppose that their limited generative reproduction due to unstable habitat conditions results in prevalence of clonal growth and also in a limited occurrence of recent spontaneous hybrids of these species. Moreover, two individuals appeared to be an admixture of three genetic groups. These plants originated from mixed populations of *B. laticarpus* and *B. planiculmis* (population DV), and thus we interpret their occurrence as resulting from backcrossing between *B. laticarpus* and *B. planiculmis*, with an admixture of *B. maritimus*.

Although the aim of this study was not to determine the clonal structure of populations and our sampling design did not allow us to properly address clonal structure, we found some indications that species might differ in their degree of clonality. Populations were highly diverse and most individuals belonged to separate genotypes. Clones occurred occasionally in populations of *B*. *laticarpus* and *B*. *planiculmis*, but in only one population of *B*. *yagara*.

Conclusions and perspectives for future studies

The present study confirmed the most recent taxonomic classification of European species of *Bolboschoenus*. Based on AFLP data, four genetic groups corresponding to the species were found. Correlations of important determination characters with genetic groups were detected using canonical discriminant analysis, which confirmed the importance of morphological and anatomical characters of achenes. We consider exocarp and mesocarp widths and achene shape in transverse section to be the most reliable characters for species determination. Moreover, admixed individuals were recorded between genetic groups, indicating possible spontaneous hybridization, mostly between *B*. *planiculmis* and *B*. *maritimus*. Populations of *B*. *maritimus* from inland areas contained admixtures of *B*. *planiculmis* genotypes, while populations of *B*. *maritimus* from coastal areas and habitats with a high salinity were genetically pure. Nevertheless, for a further elucidation of this pattern of variation a more detailed study of *B*. *maritimus* throughout its distribution is needed.

Hybrid origin of *B*. *laticarpus* was confirmed, with *B*. *yagara* and *B*. *planiculmis* as its parental taxa. It is genetically and morphologically intermediate and is a stable hybrid. Further study should investigate the possibility of polytopic origins of *B*. *laticarpus* in Japan or China and further verifying its supposed hybrid parentage.

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Souhrn

Rod *Bolboschoenus* (kamyšník, Cyperaceae) představuje vhodnou modelovou skupinu ke studiu ekologické a homoploidní hybridní speciace. Ve střední Evropě se přirozeně vyskytují čtyři druhy tohoto rodu: *B. maritimus* (k. přímořský), *B. laticarpus* (k. širokoplodý), *B. planiculmis* (k. polní) a *B. yagara* (k. vrcholičnatý). V rámci skupiny těchto čtyř taxonů, dříve označované jako široce pojatý druh *B. maritimus*, můžeme pozorovat odlišné ekologické nároky. Právě taková ekologická či zeměpisná izolace je při homoploidní hybridní speciaci rozhodující. Současné taxonomické členění rodu *Bolboschoenus* je založeno na morfologii květenství a tvaru a anatomii nažek. Pro jeho ověření jsme porovnali morfologická data se čtyřmi genetickými skupinami předdefinovanými na základě dat získaných pomocí molekulárního markeru AFLP (Amplified Fragment Length Polymorphism). Navíc nám tato metoda umožnila prozkoumat i předpokládaný hybridogenní původ druhu *B. laticarpus*, který je založený na jeho přechodných morfologických znacích, počtu chromozomů i široké ekologické amplitudě. Výsledky potvrdily současnou klasifikaci středoevropských kamyšníků – morfologická diferenciace z velké části odpovídá genetickým skupinám. Potvrzen byl rovněž hybridní původ druhu *B. laticarpus* jako výsledek křížení mezi druhy *B. yagara* a *B. planiculmis*. V současné době se ovšem v přírodních populacích vyskytuje i určitý podíl rostlin s heterogenním genotypem, představujících jedince vzniklé spontánní hybridizací (zejména mezi *B. maritimus* a *B. planiculmis* v oblastech, kde jsou rozšířeny oba druhy).

References

Bonin A., Bellemain E., Eidesen P. B., Pompanon F., Brochmann C. & Taberlet P. (2004): How to track and assess genotyping errors in population genetics studies. – Mol. Ecol. 13: 3261–3273.

Browning J. & Gordon-Gray K. D. (1993): Studies in Cyperaceae in southern Africa. 21: The taxonomic significance of the achene and its embryo in *Bolboschoenus*. – S. Afr. J. Bot. 59: 311–318.

Browning J. & Gordon-Gray K. D. (2000): Patterns of fruit morphology in *Bolboschoenus* (Cyperaceae) and their global distribution. – S. Afr. J. Bot. 66: 63–71.

Browning J., Gordon-Gray K. D. & Smith S. G. (1997a): Achene morphology and pericarp anatomy of the type specimens of the Australian and New Zealand *Bolboschoenus* species (Cyperaceae). – Austral. Syst. Bot. 10: 49–58.

Browning J., Gordon-Gray K. D., Smith S. G. & van Staden J. (1996): *Bolboschoenus yagara* (Cyperaceae) newly reported for Europe. – Ann. Bot. Fenn. 33: 129–136.

Browning J., Gordon-Gray K. D., Smith S. G. & van Staden J. (1997b): *Bolboschoenus maritimus* s.l. in the Netherlands: a study of pericarp anatomy based on the work of Irene Robertus-Koster. – Ann. Bot. Fenn. 34: 115–126.

Butlin R., Debelle A., Kerth C., Snook R. R., Beukeboom L. W., Cajas R. F. C., Diao W., Maan M. E., Paolucci S., Weissing F. J., van de Zande L., Hoikkala A., Geuverink E., Jennings J., Kankare M., Knott K. E., Tyukmaeva V. I., Zoumadakis C., Ritchie M. G., Barker D., Immonen E., Kirkpatrick M., Noor M., Macias Garcia C., Schmitt T. & Schilthuizen M. (2012): What do we need to know about speciation? – Trends Ecol. Evol. 27: 27–39.

Ducháček M. (2002): Variabilita a rozšíření taxonů rodu *Bolboschoenus* (L.) Palla (kamyšník) v ČR [Variability and distribution of *Bolboschoenus* taxa in the Czech Republic]. – Ms. Thesis, Faculty of Science, Charles Univ., Prague.

Egorova T. V. & Tatanov I. V. (2003): O sistematicheskom polozhenii *Bolboschoenus planiculmis* i *Bolboschoenus koshewnikowii* (Cyperaceae) [On systematic position of *Bolboschoenus planiculmis* and *B. koshewnikowii* (Cyperaceae)]. – Bot. Zhurn. 88: 131–142.

Ehrich D. (2006): AFLPDAT: a collection of R functions for convenient handling of AFLP data. – Mol. Ecol. Notes 6: 603–604.

Ehrich D., Gaudeul M., Assefa A., Koch M., Mummenhoff K., Nemomissa S., Consortium I. & Brochmann C. (2007): Genetic consequences of Pleistocene range shifts: contrast between the Arctic, the Alps and the East African mountains. – Mol. Ecol. 16: 2542–2559.

Ellstrand N. C., Whitkus R. & Rieseberg L. H. (1996): Distribution of spontaneous plant hybrids. – Proc. Nat. Acad. Sci. USA 93: 5090–5093.

Evanno G., Regnaut S. & Goudet J. (2005): Detecting the number of clusters of individuals using the software structure: a simulation study. – Mol. Ecol. 14: 2611–2620.

Excoffier L., Smouse P. & Quattro J. (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. – Genetics 131: 479–491.

Falush D., Stephens M. & Pritchard J. K. (2007): Inference of population structure using multilocus genotype data: dominant markers and null alleles. – Mol. Ecol. Notes 7: 574–578.

Gobert V., Moja S., Colson M. & Taberlet P. (2002): Hybridization in the section *Mentha* (Lamiaceae) inferred from AFLP markers. – Am. J. Bot. 89: 2017–2023.

Guo Y. P., Vogl C., van Loo M. & Ehrendorfer F. (2006): Hybrid origin and differentiation of two tetraploid *Achillea* species in East Asia: molecular, morphological and ecogeographical evidence. – Mol. Ecol. 15: 133–144.

Hayasaka E. & Ohashi H. (2002): Achene gross morphology and pericarp anatomy of Japanese *Bolboschoenus* (Cyperaceae). – J. Jap. Bot. 77: 9–23.

Hothorn T., Bretz F. & Westfall P. (2008): Simultaneous inference in general parametric models. – Biometr. J. 50: 346–363.

Hroudová Z. (2002): *Bolboschoenus* Palla – kamyšník. – In: Kubát K., Hrouda L., Chrtek J. jun., Kaplan Z., Kirschner J. & Štepánek J. (eds), Klíč ke květeně České republiky [Key to the flora of the Czech Republic], p. 794–795, Academia, Praha.

Hroudová Z., Frantík T. & Zákravský P. (1998a): The differentiation of subspecies in *Bolboschoenus maritimus* based on the inflorescence structure. – Preslia 70: 135–154.

Hroudová Z., Hrivnák R. & Chytrý M. (2009): Classification of inland *Bolboschoenus*-dominated vegetation in Central Europe. – Phytocoenologia 39: 205–215.

Hroudová Z., Marhold K. & Jarolímová V. (2006): Notes on the *Bolboschoenus* species in Austria. – Neilreichia 4: 51–73.

Hroudová Z., Moravcová L. & Zákravský P. (1996): Poznámky k semennému rozmnožování *Bolboschoenus maritimus* [Notes on seed reproduction of *Bolboschoenus maritimus*]. – Zprávy Čes. Bot. Společ. 31: 71–74.

Hroudová Z., Moravcová L. & Zákravský P. (1997): Effect of anatomical structure on the buoyancy of achenes of two subspecies of *Bolboschoenus maritimus*. – Folia Geobot. Phytotax. 32: 377–390.

Hroudová Z., Moravcová L. & Zákravský P. (1998b): Differentiation of the Central European *Bolboschoenus* taxa based on fruit shape and anatomy. – Thaiszia 8: 91–109.

Hroudová Z., Zákravský P., Ducháček M. & Marhold K. (2007): Taxonomy, distribution and ecology of *Bolboschoenus* in Europe. – Ann. Bot. Fenn. 44: 81–102.

Hroudová Z., Zákravský P. & Flegrová M. (2014): The tolerance to salinity and nutrient supply in four European *Bolboschoenus* species (*B. maritimus*, *B. laticarpus*, *B. planiculmis* and *B. yagara*) affects their vulnerability or expansiveness. – Aquat. Bot. 112: 66–75.

Hroudová Z., Zákravský P. & Frantík T. (1999): Ecological differentiation of Central European *Bolboschoenus* taxa and their relationship to plant communities. – Folia Geobot. 34: 77–96.

Hroudová Z., Zákravský P., Wójcicki J. J., Marhold K. & Jarolímová V. (2005): The genus *Bolboschoenus* (Cyperaceae) in Poland. – Polish Bot. J. 50: 117–137.

Huson D. H. & Bryant D. (2006): Application of phylogenetic networks in evolutionary studies. – Mol. Biol. Evol. 23: 254–267.

Jaccard P. (1908): Nouvelles recherches sur la distribution florale. – Bull. Soc. Vaud. Sci. Nat. 44: 223–270.

Jakobsson M. & Rosenberg N. A. (2007): CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. – Bioinformatics 23: 1801–1806.

Jarolímová V. & Hroudová Z. (1998): Chromosome numbers within the genus *Bolboschoenus* in Central Europe. – Folia Geobot. 33: 415–428.

Kaplan Z., Danihelka J., Štěpánková J., Bureš P., Zázvorka J., Hroudová Z., Ducháček M., Grulich V., Řepka R., Dančák M., Prančl J., Šumberová K., Wild J. & Trávníček B. (2015): Distributions of vascular plants in the Czech Republic. Part 1. – Preslia 87: 417–500.

Kaplan Z., Jarolímová V. & Fehrer J. (2013): Revision of chromosome numbers of Potamogetonaceae: a new basis for taxonomic and evolutionary implications. – Preslia 85: 421–482.

Kolář F., Kaplan Z., Suda J. & Štech M. (2015): Populations of *Knautia* in ecologically distinct refugia on the Hercynian massif belong to two endemic species. – Preslia 87: 363–386.

Kolář F., Lučanová M., Koutecký P., Dortová M., Knotek A. & Suda J. (2014): Spatio-ecological segregation of diploid and tetraploid cytotypes of *Galium valdepilosum* in central Europe. – Preslia 86: 155–178.

Koutecký P. (2015): MorphoTools: a set of R functions for morphometric analysis. – Plant Syst. Evol. 301: 1115–1121.

Kozhevnikov A. E. (1988): Klubnekamysh – *Bolboschoenus* (Aschers.) Palla. – In: Charkevich S. S. (ed.), Sosudistye rasteniya sovetskogo Dal'nego Vostoka [Vascular plants of the Russian Far East] 3: 187–190, Leningrad.

Kruskal W. H. & Wallis W. A. (1952): Use of ranks in one-criterion variance analysis. – J. Am. Stat. Assoc. 47: 583–621.

Lihová J., Kučera J., Perný M. & Marhold K. (2007): Hybridization between two polyploid *Cardamine* (Brassicaceae) species in northwestern Spain: discordance between morphological and genetic variation patterns. – Ann. Bot. 99: 1083–1096.

Mallet J. (2007): Hybrid speciation. – Nature 446: 279–283.

Marhold K., Hroudová Z., Ducháček M. & Zákravský P. (2004): The *Bolboschoenus maritimus* group (Cyperaceae), in Central Europe, including *B. laticarpus*, spec. nova. – Phyton Ann. Rei Bot. 44: 1–21.

Moravcová L., Zákravský P. & Hroudová Z. (2002): Germination response to temperature and flooding of four Central European species of *Bolboschoenus*. – Preslia 74: 333–344.

Nordborg M., Hu T. T., Ishino Y., Jhaveri J., Toomajian C., Zheng H. G., Bakker E., Calabrese P., Gladstone J., Goyal R., Jakobsson M., Kim S., Morozov Y., Padhukasahasram B., Plagnol V., Rosenberg N. A., Shah C., Wall J. D., Wang J., Zhao K. Y., Kalbfleisch T., Schulz V., Kreitman M. & Bergelson J. (2005): The pattern of polymorphism in *Arabidopsis thaliana*. – PLoS Biol. 3: 1289–1299.

Oteng-Yeboah A. A. (1974): Taxonomic studies in Cyperaceae-Cyperoideae. – Notes R. Bot. Gard. Edinburgh 33: 311–316.

Pritchard J. K., Stephens M. & Donnelly P. (2000): Inference of population structure using multilocus genotype data. – Genetics 155: 945–959.

R Development Core Team (2008): R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org.

Rosenberg N. A. (2004): DISTRUCT: a program for the graphical display of population structure. – Mol. Ecol. Notes 4: 137–138.

Rossi M., Bitocchi E., Bullucci E., Nanni L., Rau D., Attene G. & Papa R. (2009): Linkage disequilibrium and population structure in wild and domesticated populations of *Phaseolus vulgaris* L. – Evol. Appl. 2: 504–522.

Roullier C., Benoit L., McKey D. B. & Lebot V. (2013): Historical collections reveal patterns of diffusion of sweet potato in Oceania obscured by modern plant movements and recombination. – Proc. Natl. Acad. Sci. USA 110: 2205–2210.

Schemske D. W. (2000): Understanding the origin of species. – Evolution 54: 1069–1073.

Schluter D. (2001): Ecology and the origin of species. – Trends Ecol. Evol. 16: 372–380.

Schlüter P. M. & Harris S. A. (2006): Analysis of multilocus fingerprinting data sets containing missing data. – Mol. Ecol. Notes 6: 569–572.

Snow A. A., Travis S. E., Wildová R., Fér T., Sweeney P. M., Marburger J. E., Windels S., Kubátová B., Goldberg D. E. & Mutegi E. (2010): Species-specific SSR alleles for studies of hybrid cattails (*Typha latifolia* × *T. angustifolia*, Typhaceae) in North America. – Am. J. Bot. 97: 2061–2067.

Sobel J. M., Chen G. F., Watt L. R. & Schemske D. W. (2009): The biology of speciation. – Evolution 64: 295–315.

Španiel S., Marhold K., Filová B. & Zozomová-Lihová J. (2011): Genetic and morphological variation in the diploid–polyploid *Alyssum montanum* in Central Europe: taxonomic and evolutionary considerations. – Plant Syst. Evol. 294: 1–25.

Tatanov I. V. (2003): Kriticheskie zametki o vidakh *Bolboschoenus desoulavii* (Drob.) A. E. Kozhevnikov i *Bolboschoenus yagara* (Ohwi) Y. C. Yang et M. Zhan (Cyperaceae) [Critical comments on the species *Bolboschoenus desoulavii* (Drob.) A. E. Kozhevnikov and *Bolboschoenus yagara* (Ohwi) Y. C. Yang et M. Zhan (Cyperaceae)]. – Novist. Sist. Vyssh. Rast. 35: 51–62.

Tatanov I. V. (2007): Taksonomicheskiy obzor roda *Bolboschoenus* (Aschers.) Palla (Cyperaceae) [Taxonomic survey of the genus *Bolboschoenus* (Cyperaceae)]. – Novist. Sist. Vyssh. Rast. 39: 46–149.

ter Braak C. J. F. & Šmilauer P. (2012): Canoco reference manual and user's guide: software for ordination, version 5.0. – Microcomputer Power, Ithaca, USA.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. & Zabeau M. (1995): AFLP: a new technique for DNA fingerprinting. – Nucl. Acids Res. 23: 4407–4414.

Yakimowski S. B. & Rieseberg L. H. (2014): The role of homoploid hybridization in evolution: a century of studies synthesizing genetics and ecology. – Am. J. Bot. 101: 1247–1258.

Záveská E., Fér T., Šída O., Leong-Škorničková J., Sabu M. & Marhold K. (2011): Genetic diversity patterns in *Curcuma* reflect differences in genome size. – Bot. J. Linn. Soc. 165: 388–401.

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Appendix 1. – List of the populations of the species of *Bolboschoenus* studied and their characteristics. AFLP group – determination of each population based on AFLP data (STRUCTURE 85%): Y – *B. yagara*, A – *B. yagara* × *B. laticarpus*, L – *B. laticarpus*, B – *B. laticarpus* × *B. planiculmis*, P – *B. planiculmis*, C – *B. planiculmis* × *B. maritimus*, M – *B. maritimus*. Nind – number of individuals analysed for AFLPs; Ng – number of genotypes; DNei – Nei's gene diversity; %poly – percentage of AFLP markers demonstrating intra-population polymorphism. N – number of inflorescences used in the morphometric analysis. CDA – species determination of each population based on classificatory discriminant analyses of anatomical characters of pericarp (Y – *B. yagara*, L – *B. laticarpus*, P – *B. planiculmis*, M – *B. maritimus*.

		AFLP group												CDA
Acronym	**Locality and date of collection**	**Y**	**A**	**L**	**B**	**P**	**C**	**M**	**N_{ind}**	**N_g**	**D_{Nei}**	**%poly**	**N**	**sp.**
Bolboschoenus yagara														
DZA	CZ-SB, the fishpond Zadní ca. 1.5 km S of the village Domanín, 5 km SW of the town of Třeboň, 48°57.583'N, 14°44.983'E, 450 m, 26.7.2011	7							7	7	0.16	36.06	25	Y
HR	CZ-SB, the fishpond Hrachovišťský at western border of the village Hrachoviště, 8 km S of the town of Třeboň, 48°55.733'N, 14°45.85'E, 460 m, 20.9.2010	8							8	8	0.12	30.33	25	Y
KO	CZ-SB, north-eastern shore of the fishpond Koclířov, ca 1 km SW of the town Lomnice n. Lužnicí, 49°4.633′N, 14°42.083′E, 425 m, 16.6.2011	10							10	10	0.14	42.62	25	Y
KR	CZ-SB, the fishpond Králek 3.2 km ESE of the town Kardašova Řečice, 49°10.583′N, 14°53.767′E, 470 m, 28.7.2011	6	2						8	8	0.17	44.26	25	Y
OB	CZ-SB, the fishpond Oběšený near south-western border of the village Mláka, 8 km NE of the town of Třeboň, 49°3.4′N, 14°50.35′E, 445 m, 29.7.2010	9							10	10	0.18	49.18	11	Y
OS	CZ-SB, the fishpond Ostrý ca. 600 m S of the village Kolence, 5 km E of the town Lomnice n. Lužnicí; 49°5.067′N, 14°47.083′E, 420 m, 29.7.2010	10							10	9	0.11	30.33	17	Y
SH	CZ-SB, the Stehlík fishpond 500 m SE of the village Klec, 2.5 km NE of the town Lomnice n. Lužnicí, 49°5.55′N, 14°45.183′E, 420 m, 27.7.2011	9							9	9	0.16	45.08	25	Y

		AFLP group												CDA
Acronym	**Locality and date of collection**	**Y**	**A**	**L**	**B**	**P**	**C**	**M**	**N_{ind}**	**N_g**	**D_{Nei}**	**%poly**	**N**	**sp.**
TI	CZ-SB, the fishpond Velký Tisý , littoral in the bay at western shore of the Lůsy penninsula, ca. 3.3 km S of the town Lomnice n. Lužnicí, 49°4.1′N, 14°42.4′E, 420 m, 25.7.2011	9							9	9	0.13	36.06	25	Y
TO	CZ-SB, the fishpond Tobolky near the village Branná, 4 km S of the town Třeboň, 48°57.65′N, 14°46.333′E, 440 m, 28.7.2010	3							3	3	0.13	19.67	25	Y
VO	CZ-SB,the fishpond Velká Ochoz, ca. 2.2 km S of the town Kardašova Řečice, 49°10.167′N, 14°50.467′E, 430 m, 20.9.2010	10							10	10	0.14	33.61	25	Y
Bolboschoenus laticarpus														
AU2	A, Lower Austria, the fishpond shore near bridge over railway line at eastern border of the town Bernhardsthal, 48°41.567′N, 16°52.633′E, 160 m, 4.8.2011		1	5	2				8	8	0.21	55.74	25	L
BO	CZ-NM, field depression near north-western border of the village Bohuslavice, ca. 6 km N of the town Mohelnice, 49°49.667′N, 16°56.167′E, 260 m, 5.8.2011		1	7					9	7	0.17	42.62	25	L
DJ-L	CZ-EB, depression in field near north-eastern border of the village Dolní Jelení, 11 km NNW of the town Vysoké Mýto, 50°3.133′N, 16°6.583′E, 280 m, 5.8.2011			5					6	6	0.16	38.52	25	L
DP	CZ-CB, Prague city, flooded depression in field at north-eastern border of the Dolní Počernice suburb, near the road to Svépravice, 50°5.45′N, 14°35.25′E, 230 m, 21.7.2010		1	3					4	3	0.14	20.49	25	L
DV	CZ-SM, wet depression in field 0.5 km W of the village Dolní Věstonice, 48°53.183′N, 16°37.817′E, 170 m, 4.8.2011			9	1				10	9	0.18	45.90	25	L
LD-L	CZ-SM, wet depression in field, 1.6 km NE of the town Lanžhot, 48°43.9′N, 16°58.817′E, 150 m, 1.9.2010		1						2	2	0.29	28.69	25	L
LI	CZ-CB, Prague city, wet depression in field near the fishponds in protected area „Litožnice“ ca. 1.5 km SSE of the railway station Běchovice, 50°4.2′N, 14°36.417′E, 230 m, 21.7.2010		3	6					9	8	0.14	36.88	25	L

		AFLP group												CDA
Acronym	**Locality and date of collection**	**Y**	**A**	**L**	**B**	**P**	**C**	**M**	**N_{ind}**	**N_g**	**D_{Nei}**	**%poly**	**N**	**sp.**
NE	CZ-CB, Prague city, wet depression in field near Netluky farm-house, near the road Uhříněves – Koloděje, 50°2.667′N, 14°36.933′E, 270 m, 21.7.2010			6					6	3	0.11	17.21	25	L
SK-MA	SK, Záhorie lowland, wet depression in field N of the road Plavecký Štvrtok – Láb, ca. 800 m NE of the village Láb, 48°22.183′N, 16°58.85′E, 190 m, 3.8.2011			7					7	6	0.09	20.49	25	L
SK4	SK, Záhorie lowland, wet depression in field near the road Vysoká pri Morave – Záhorská ves, 2.4 km NW of the village Vysoká pri Morave, 48°20.833′N, 16°53.517′E, 180 m, 3.8.2011		1	9					10	10	0.15	38.52	25	L
Bolboschoenus planiculmis														
AU1	A, Lower Austria, shore of the small fishpond near the road Poysdorf – Herrnbaumgarten, near southern border of the village Herrnabumgarten, 48°41.317′N, 16°40.333′E, 190 m, 4.8.2011					8			8	7	0.22	51.64	25	P
DI	CZ-SM, wet depression in field near the Haraska brook, S of the Martinice farm-house, ca. 3 km WNW of the town Klobouky u Brna, 49°0.317′N, 16°49.35′E, 230 m, 31.8.2010				1	7			8	7	0.17	45.08	25	P
DJ-PL	CZ-EB, depression in field near north-eastern border of the village Dolní Jelení, 11 km NNW of the town Vysoké Mýto, 50°3.133′N, 16°6.583′E, 280 m, 5.8.2011					7	1		8	8	0.14	43.44	25	P
JK	CZ-SM, wet depression in the meadow near the pool Kutnar, 3 km SW of the village Rakvice, 48°50.2′N, 16°47.617′E, 180 m, 29.6.2011				1	8			9	7	0.23	57.38	25	P
LD-PL	CZ-SM, wet depression in field near the highway to Slovakia, 1.6 km NE of the town Lanžhot, 48°43.9′N, 16°58.817′E, 150 m, 1.9.2010					10			6	5	0.09	20.49	25	P
NO	CZ-SM, salt marsh at the north-eastern border of the village Novosedly, 48°50.35′N, 16°29.833′E, 180 m, 31.8.2010					8			8	8	0.20	46.72	25	P
NZ	CZ-SM, wet depression in field near the southern bay of the Nesyt fishpond, 3.5 km NW of the town Valtice, 48°45.783′N, 16°43.7′E, 170 m, 31.8.2010					10			10	8	0.15	36.88	25	P, M

		AFLP group												CDA
Acronym	**Locality and date of collection**	**Y**	**A**	**L**	**B**	**P**	**C**	**M**	**N_{ind}**	**N_g**	**D_{Nei}**	**%poly**	**N**	**sp.**
SK-KU	SK, Záhorie lowland, depression in field at southern border of the town Kúty, 48°39.083′N, 17°0.717′E, 190 m, 31.8.2010					3	5		8	7	0.13	36.88	25	P, M
TD	CZ-SM, salt marsh near the Trkmanský Dvůr, 3.5 km NE of the village Rakvice, 48°51.85′N, 16°50.717′E, 170 m, 28.6.2011					7	1		8	8	0.24	56.56	25	P
ZA	CZ-SM, restored salt marsh 1.2 km N of the village Terezín, 48°57.933′N, 16°56.35′E, 175 m, 31.8.2010					10			10	10	0.21	54.10	25	P
Bolboschoenus maritimus														
AZ	IR, West Azerbaijan, Makou to Buralan							7	7	7	0.16	36.06	10	M
B	CZ-NB, depression in field near the Bečovský potok brook, l km S of the town Bečov, near the road Bečov – Volevčice, 50°26.45′N, 13°42.567′E, 220 m, 29.8.2010				2	1	5	1	9	9	0.23	63.93	25	P, M
HU	HU, Hortobágy Puszta, salt marsh 8 km E of the town Hortobágy, 47°34.617′N, 21°15.05′E, 100 m, 19.5.2010						2	5	7	7	0.29	68.03	5	M
TE	D, Sachsen-Anhalt, surroundings of saline "slagheap", Salzstelle Teutschenthal, W of the town Halle, 51°27.183′N, 11°48.933′E, 100 m							7	7	7	0.17	39.34	25	M
TU1	F, la Camarque, Rhône Delta, Tour du Valat Wildlife Reserve 1, marsh Emprunt Nord Tamarquiron, 43º30´N, 4º30´E							8	8	8	0.21	50.00	25	M
TU2	F, la Camarque, Rhône Delta, Tour du Valat Wildlife Reserve 2, marsh Relongue Nord, 43º30´N, 4º30´E							4	4	4	0.12	20.95	25	M

PAPER III.

Píšová S., Hroudová Z., Chumová Z., Schmickl R. & Fér T. (manuscript): Convergent evolution, migration and homoploid hybridization in the genus *Bolboschoenus* (Cyperaceae) on a worldwide scale.

Convergent evolution, migration and homoploid hybridization in the genus *Bolboschoenus* (Cyperaceae) at a worldwide scale

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Abstract

Cosmopolitan taxa often include morphologically similar species in different regions of the world. The recent patterns of their distribution may either represent migration of species between continents, be due to convergent evolution or be a combination thereof. An investigation was undertaken to explore which of these processes influenced the present distribution pattern and species differentiation within the wetland plant genus *Bolboschoenus*. Approximately four nearly identical morphotypes occur in different continents, mainly in Europe, Asia, North America and Australia. Analysis of molecular markers (AFLPs, amplified fragment length polymorphisms, ITS region of rDNA, and two chloroplast regions: *trn*H-*psb*A and *trn*C-*psb*MR) and morphometric analyses of plants from different continents were performed using a large collection of cultivated plants complemented by plants from natural populations. We found evidence for migration rather than convergent evolution underlying the nearly identical morphologies of the Eurasian and Australian species. In contrast, the North American species present early separation from the Eurasian taxa.

Homoploid hybrids usually need to overcome an initial phase of selective disadvantage compared to their parents. Evolving phenotypic and ecological differentiation from its parents can greatly facilitate the hybrid's ability to find its own ecological niche and maintain stable populations. We tested whether hybrid origin may be found in three *Bolboschoenus* species, each occurring either in Europe, North America or Australia, based on morphology and molecular markers. There is evidence for all of these species to be homoploid hybrids, possibly formed through convergent origin on these three continents. At least one of them is strongly phenotypically and ecologically differentiated from its parents, which suggests successful hybrid establishment.

Keywords: AFLP, *Bolboschoenus*, convergent evolution, homoploid hybridization, morphometrics, speciation

Introduction

Aquatic vascular plants have a ubiquitous presence depending on wetland habitats that occur on every continent except Antarctica. Some of these plants, with a geographical distribution ranging over several continents, are classified as cosmopolitan (Cronk & Fennessy 2016, Santamaria 2002). Similar forms, shapes, structures and functions can often be found in aquatic plants. These similarities are generally attributed to common ancestry due to migration between continents in the past (Went 1971). In other cases, they may be a result of convergent evolution through adaptation to the general uniformity of aquatic habitats (Williamson & Schneider 1996, Les et al. 1991, Sculthorpe 1967). Many groups of aquatic plants are difficult to classify, either due to convergent morphology, low taxonomic differentiation or extensive phenotypic plasticity (Barret et al. 1993). Convergent morphology and anatomy of achenes also causes difficulties in the intrageneric classification of the wetland monocot genus *Bolboschoenus* (Asch.) Palla. Determination of species is currently based mainly on the ratio of pericarp layers (width of exocarp and mesocarp). Three to four basic achene morphotypes occur and were independently described under different names in each of following continents: Eurasia, Australia, Africa and North America (Fig. 1, 2). Nevertheless, these names might be misleading, and the Eurasian and Australian species of the same morphotypes in particular might be the same species. Such unique worldwide convergence provides a great opportunity for examination of the evolutionary processes that have led to this morphological pattern. Either migration between continents in the past or recent long-distance dispersal rather than continental drift is a possible cause of convergence in cosmopolitan aquatic plants (Les et al. 2003).

The current classification of *Bolboschoenus* distinguishes from 14 (Tatanov 2007a) to 15 species (Browning & Gordon-Grey 2000). Nevertheless, delimitation of some species is still unclear, and this genus is in need of a thorough worldwide revision.

The taxonomy and distribution of *Bolboschoenus* species have been studied in detail since 1990, especially by J. Browning and her collaborators in Africa (Browning & Gordon-Grey 1992, 1993, 1999; Browning et al. 1998), North America (Browning et al. 1995), Australia and New Zealand (Browning et al. 1997a) and Europe (Browning et al. 1996, 1997b). Synopses of the genus and inter- and intrageneric hybrids were described by I. Tatanov

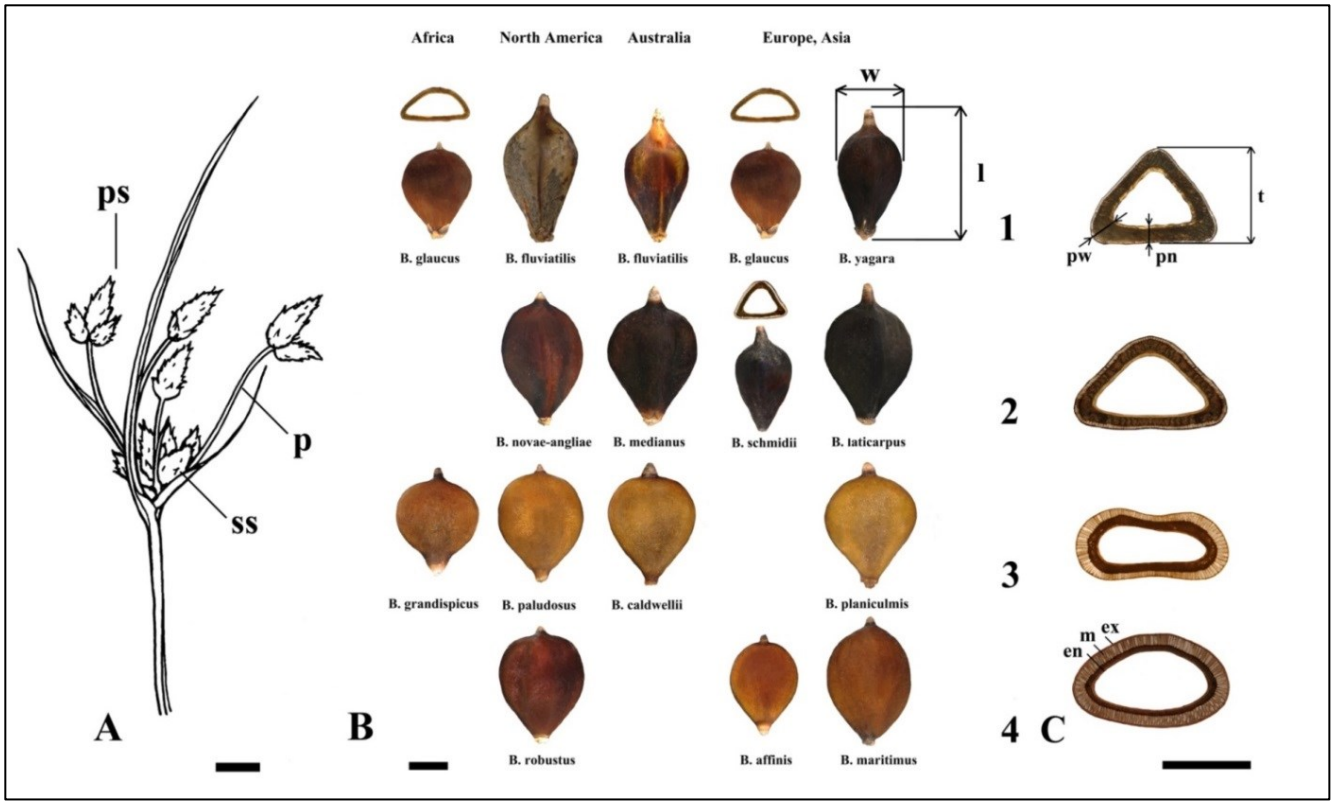

Fig. 1. – Morphological and anatomical characters. (A) Inflorescence. (B) Four achene morphotypes. (C) Achene anatomical characters in cross-section. Scale bars: 1 cm (A), 1 mm (B, C); p, peduncle; s, spikelet; l, length of achene; w, width of achene. t, thickness of achene; pw, width of pericarp layers at the widest part; pn, width of pericarp layers at the narrowest part; ex, exocarp; m, mesocarp; en, endocarp. (A) Del. Z. Hroudová. (B), (C) Phot. S. Píšová.

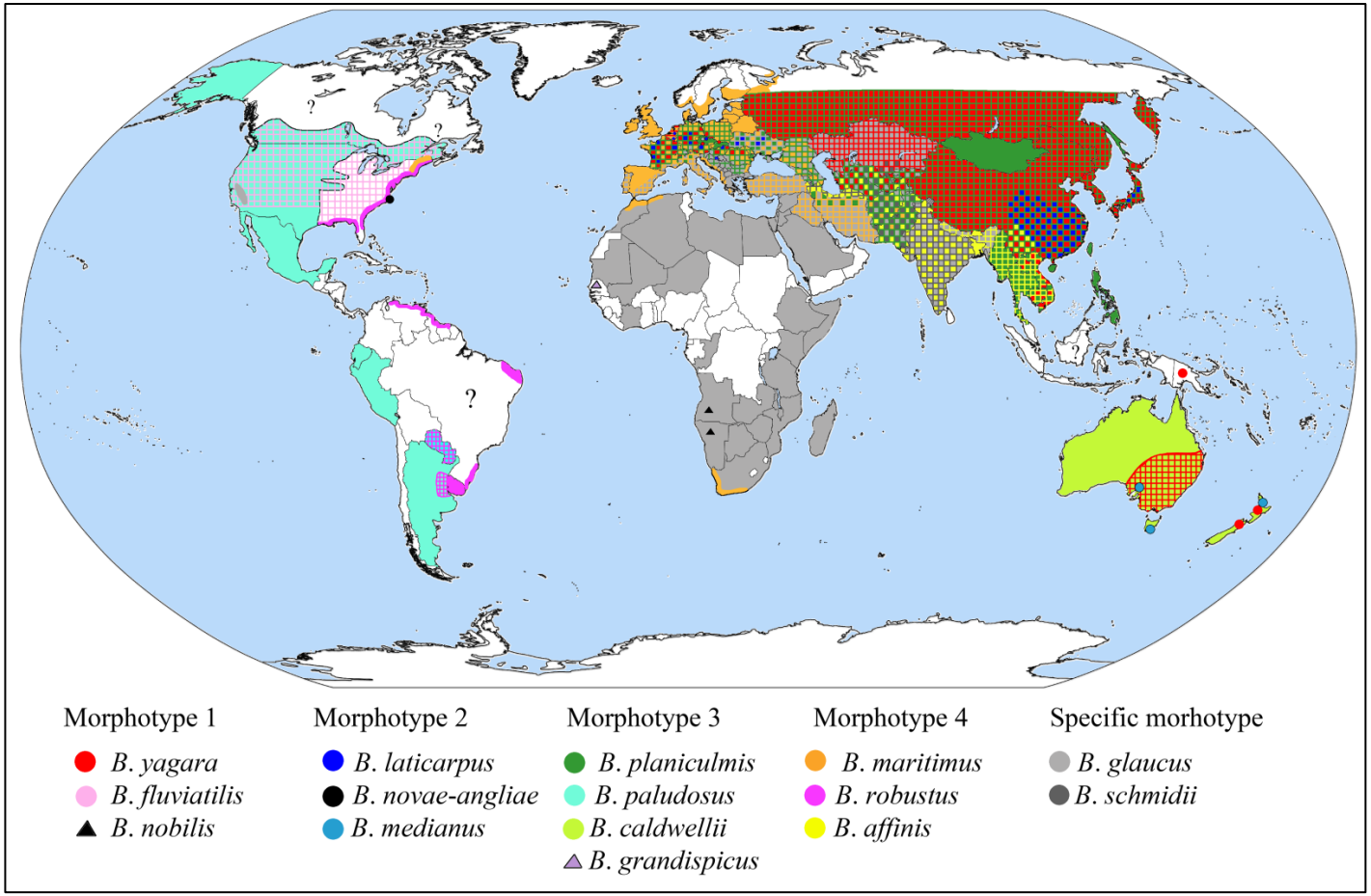

Fig. 2. – World map showing distribution of *Bolboschoenus* species and morphotypes. The map is based on the distribution of species desribed in Browning et al. (2000), Hroudová et al. (2007) and Tatanov (2007a).

(Tatanov 2007a, b) and by other authors in Europe (Marhold et al. 2004a, Hroudová et al. 2007). In Asia, *Bolboschoenus* species were investigated by Koyama (1980), Haysaka & Ohashi (2002) and Egorova & Tatanov (2003). Nevertheless, very little is known about the *Bolboschoenus* species in South America.

In addition to convergence, hybridization has been recognized as the next most important evolutionary force. Approximately 10% of plant species hybridize, but hybridization is unevenly distributed, and some plant groups have a higher propensity for hybridization than others (e.g., Cyperaceae, Ellstrand et al. 1996). Speciation via hybridization and genome doubling (allopolyploidy) is much more common, and many recent studies were devoted to polyploidization (Soltis & Soltis 2009, Alix et al. 2017, Vallejo-Marín et al. 2015, Winterfeld et al. 2014). In contrast, homoploid hybrid speciation seems to be rare, partly due to the lack of reproductive isolation from parents and to reduced fitness in early generations of hybrids and partly because only a few examples have been found (Riley 1938, Rieseberg et al. 2007). Before a new hybrid species can be established, reproductive isolation is achieved by chromosomal rearrangements or a geographical and/or ecological barrier (Stebbins 1959).

Moreover, in the absence of an open habitat for new hybrids, the formation of stable hybrid zones is more likely than adaptive introgression or homoploid hybrid speciation (Buerkle et al. 2000, Kim et al. 2008). The presence of unoccupied ecological niches is thus crucial for the establishment of new hybrid species. Several examples of divergent habitat of hybrid species from their parental taxa have been reported (Donovan et al. 2010, Wang et al. 2001, Hroudová et al. 2014.). However, hybrids are expected to have a range of intermediate phenotypes, from characters that are identical to those of one parent, to those that are intermediate, to novel characters (Soltis & Soltis 2009, Rieseberg 1995). At least three putative hybrid species with intermediate morphology were described in the genus *Bolboschoenus*, each on a different continent: *B. laticarpus* in Europe, *B. medianus* in Australia and *B. novae-angliae* in North America. Whether these species are of hybrid origin and stable or are recent hybrids is still unknown. Similarly, it is unclear whether they are of polytopic origin from the same parental taxa or are the results of convergence between different parental pairs on each continent.

A previous examination of four central-European *Bolboschoenus* species revealed the hybrid origin of *B. laticarpus* and confirmed their current

classification based on achene shape and anatomy (Píšová et al. 2017). In the present study, we focused on the convergent morphology of achenes between continents and the detection of hybrid origins of the remaining two putative hybrid species. Chromosome counts were obtained in a study of European *Bolboschoenus* by Jarolímová & Hroudová (1998); only counts of n=54 and n=55 were observed, and similar records are reported in the literature (Roalson 2008). We suspect that *Bolboschoenus* species are all diploids, as further investigation of species from other continents suggests, and that their hybridization thus results in homoploid hybrids (Jarolímová & Hroudová in prep.).

Molecular markers such as AFLPs (amplified fragment length polymorphisms) that provide a large number of variable loci distributed throughout the genome allow the assessment of genetic structure and detection of hybridization (Vos et al. 1995, Guo et al. 2006, Španiel et al. 2011). This type of molecular marker has also proved to be useful in studies of complexes of closely related taxa (Marhold et al. 2004b, Després et al. 2003, Whittall et al. 2004). Moreover, sequencing of the ITS region of rDNA and of chloroplast regions is usually used to evaluate intrageneric phylogenetic relationships (Baldwin 1992, Shaw et al. 2005, Yano & Hoshino 2005).

The aims of this study are to answer the following: (1) What is the overall pattern of genetic structure among *Bolboschoenus* species of the studied morphotypes worldwide? (2) Does the present distribution pattern reflect separate phylogenetic lineages with convergent morphology or migration between continents in the past? (3) What is the role of hybridization in the speciation process? Are species with an intermediate morphology (*B. laticarpus* from Eurasia, *B. medianus* from Australia and *B. novae-angliae* from North America, second morphotype) of a hybrid origin? Are they stable hybrid species or a result of current hybridization?

Materials and methods

Plant material

Altogether, material from 14 species (87 populations) in the field or cultivated in the experimental garden of the Institute of Botany of the Czech Academy of Sciences in Průhonice (49°59.69′N, 14°34′E) was collected (Appendix 1). The seeds for the cultivation have been provided (during the last few decades) by collaborators in Europe, Iran, China, Japan, Australia and the U.S.A. Nevertheless, we were unable to obtain samples of *B*. *nobilis* (Namibia, Africa), *B*. *fluviatilis* from Australia (old herbarium specimen) and *B*. *popovii* from Asia. Material for genome size estimation and molecular analyses (all leaf samples), morphometric analyses (1390 inflorescences) and anatomical measurements (656 achenes) was collected. Specifically, one fresh leaf per population was sampled and then dried in silica gel for the molecular analyses. Further, up to 25 well-developed inflorescences from each population were collected, and up to 25 ripe achenes were randomly chosen for the morphometric analysis. Voucher specimens are deposited in the PRC (Herbarium of Charles University), PRA (Herbarium of the Institute of Botany of the Academy of Sciences of the Czech Republic) and PR (Herbarium of the National Museum in Prague) herbaria.

AFLP analysis

Genomic DNA was extracted using the Invisorb Spin Plant Mini Kit (Invitek), and its concentration was measured by a Nanodrop 1000 spectrophotometer (Thermo Scientific) and adjusted to 25 $ng.\mu l^{-1}$.

AFLP analysis (Vos et al. 1995) was performed with an AFLP Core Reagent Kit I (Invitrogen) and an AFLP Pre-Amp Primer Mix I (Invitrogen), following the manufacturer's instructions as modified and described in a previous study (Píšová et al. 2017). Four primer combinations were used for selective amplification: *Eco*RI-ATC-(6-FAM)/*Mse*I-CAA, *Eco*RI-AAG-(VIC)/*Mse*I-CTC, *Eco*RI-AAC-(NED)/ *Mse*I-CAG, and *Eco*RI-ACA-(PET)/*Mse*I-CAT. Selective amplification products were mixed with GeneScan LIZ 600 (Applied Biosystems) size standard and electrophoresed on an ABI 3130xl Avant Genetic Analyzer (Applied Biosystems) in the DNA Sequencing Laboratory, Faculty of Science, Charles University, Prague. Altogether, 90

samples from 87 populations were analysed. The entire AFLP procedure was repeated for 17 samples to calculate the AFLP error rate (Bonin et al. 2004).

Molecular data analyses

The AFLP data were scored as absence/presence of bands with GeneMarker software v1.8 (SoftGenetics LLC, PA, U.S.A.). Only unambiguous fragments were recorded. Bayesian non-hierarchical clustering was carried out in STRUCTURE 2.3.2.1 (Pritchard et al. 2000). An admixture model was applied along with a model of independent allele frequencies and a recessive allele model for simulations. Ten replicates were computed for each K number of clusters (K=1 to K=15) with a burn-in period of 100,000 generations and MCMC simulations of 1,000,000 iterations with the Metacentrum VO infrastructure (https://metavo.metacentrum.cz, Falush et al. 2007). The R script (R Development Core Team 2008) Structure-sum-2009 (Ehrich et al. 2009) was used to summarize output files and determine the optimal K value (Evanno et al. 2005, Nordborg et al. 2005). The software CLUMP 1.1.2. (Jakobsson & Rosenberg 2007) and DISTRUCT (Rosenberg 2004) were used to make graphical outputs for individual Ks. Subsequent analyses used the same colour pattern for individual STRUCTURE groups based on the chosen K division.

Neighbour networks, first based on the data set without hybrid samples and second with all samples, were constructed in SplitsTree v.4.11.3 (Huson & Bryant 2006) using Jaccard's similarity coefficients (Jaccard 1908). Additionally, a neighbour-joining tree was constructed in PAUP 4.0 (Swofford 2002), and principal coordinate analysis (PCoA) was performed with the R package ade4 (Dray & Dufour 2007).

Amplification and sequencing

The amplification of the ITS region was performed with the following primers: ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). The PCR reactions were carried out in a total volume of 20 µl containing 10 ng of genomic DNA, 4 µl of 5× reaction buffer (Bioline), 12.5 pmol of each primer (Sigma Aldrich) and 1 U of MyTaq HS Red DNA Polymerase (Bioline). Amplification was performed on an Eppendorf Mastercycler Gradient with an initial denaturation for 1 min at 95°C, followed by 35 cycles of denaturation for 30 s at 95°C, 1 min of annealing at 55°C and 1 min at 72°C, and then by a 15 min final extension at

72°C. Two variable non-coding plastid DNA regions, *psb*A-*trn*H and *trn*C-*psb*MR, were chosen after a preliminary test of six regions and amplified with the following primers: *psb*A (5′-ACTTCTGGTTCCGGCGAACGAA-3′) (Sang et al. 1997), *trn*H (5′-ACGGGAATTGAACCCGCGCA-3′) (Tate & Simpson 2003), trnC^{GCA} (5′-CCAGTTCRAATCYGGGTG-3′) and *psb*MR (5′-ATGGAAGTAAATA-TTCTYGCATTTATTGCT-3′) (Shaw et al. 2005). The PCR reactions were carried out in a volume of 20 µl containing 10 ng of genomic DNA, 2 µl of 10× ImmoBuffer (Bioline), 0.2 mM dNTP, 2.5 mM $MgCl_2$, 6.25 pmol of each primer and 0.5 U IMMOLASE DNA Polymerase (Bioline). Amplification was performed on an Eppendorf Mastercycler Gradient with an initial denaturation for 10 min at 95°C, followed by 35 cycles of denaturation for 45 s at 95°C, 1 min of annealing at 59°C and 2 min at 72°C, and then by a 10 min final extension at 72°C. The PCR products were purified using the GenElute PCR Clean-up Kit (Sigma Aldrich) and sequenced in the DNA Sequencing Laboratory (Faculty of Science, Charles University, Prague) in both directions.

Data analysis

Sequences were assembled using the SeqMan program (Lasergene software, DNAStar ver. 5, 2001). Contigs were aligned in ClustalX 2.0.9 (Larkin et al. 2007) and manually edited using BioEdit 7.0.5.3 (Hall 1999). Phylogenetic analyses were performed with maximum likelihood (ML) using Garli 2.01 (Zwickl 2006), with the best substitution model (TIM1+G model for the ITS data set and TVM+G for the chloroplast data set) selected based on AIC in jModelTest v2.1.10 (Darriba et al. 2012). Further, a Bayesian approach was applied in MrBayes 3.2.4 (Ronquist & Huelsenbeck 2003), with the GTR+G model for both data sets and run for 10 million generations. Subsequently, bootstrap support was obtained from 1,000 replicates in ML analyses (Felsenstein 1985), and values higher than 50 were mapped on the trees. Although the genus *Schoenoplectus* is very genetically distant from the genus *Bolboschoenus*, we used three samples of *Schoenoplectus* as an outgroup to root the resulting trees, since other genera are even less related and less suitable for this purpose (Simpson et al. 2007, Jung & Choi 2010).

Morphometric analyses

The morphological variation of *Bolboschoenus* species and their differentiation were evaluated using seven inflorescence characters, three achene characters and six anatomical characters (Table 1, Fig. 1). The morphological characters were chosen based on previous morphometric studies (Browning & Gordon-Grey 2000, Hroudová et al. 1998a, Hroudová et al. 1998b, Píšová et al. 2017). Morphometric analyses of the two data sets were performed in order to reveal any correlation between morphological and genetic variation and to verify the current taxonomical concept of the genus *Bolboschoenus*. Part of the achenes were destroyed when obtaining their cross-sections; thus, it was necessary to analyse them separately. Consequently, population means of ratios (length and width, width and thickness) were added to each anatomical measurement of six characters as seventh and eighth characters. Therefore, only principal component analyses (PCA) were performed, as such data do not meet the criteria for discriminant analyses.

The first data set describing inflorescence structure consisted of seven morphological characters of 1390 inflorescences. The lengths of peduncles and spikelets were measured with a digital calliper. The length and width ratio was measured on 712 whole achenes using a dissecting microscope with a digital camera. To obtain the width and thickness ratio, 712 cross-sections of achenes were prepared by cutting the achenes with a razor at the widest part. Anatomical characters of the pericarp (second data set, width of exo-, meso- and endocarp in the narrowest and in the widest parts) were obtained from 20 µm thick cross-sections of achenes using a Shadon Cryotome® microtome (77200226, Shandon Sci., Astmoor Runcorn, UK). Final images were recorded with an Olympus BX50 microscope and an Olympus E 10 digital camera. All achene characters were measured from calibrated images using ImageJ software (National Institute of Health, USA).

Multivariate analyses were carried out on the first and second data set with the R script MorphoTools (Koutecký 2015) to evaluate the contribution of each character. All characters were tested for normality with the Shapiro-Wilk test, and correlations between characters were computed using non-parametric Spearman correlation coefficients in R version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria). The highly correlated character length of peduncles/length of spikelets ratio was excluded from subsequent analyses. Initially, two separate principal component analyses (PCAs) of the

inflorescence and achene characters were performed in order to obtain a general overview of the morphological variation. Subsequently, another five PCA analyses were applied for each of the four morphotypes and species with atypical achenes (*B*. *glaucus* and *B*. *schmidii*).

Table 1. – List of morphological characters.

Inflorescence characters
Number of peduncles
Number of spikelets
Length of peduncles
Length of spikelets
Number of spikelets/number of peduncles
Length of peduncles/number of spikelets
* Length of peduncles/length of spikelets
Achene characters
Length/width of achenes (L/W)
Width/thickness of achenes (W/T)
Anatomical characters of the pericarp
Width of exocarp at the widest part (exow)
Width of mesocarp at the widest part (mesow)
Width of endocarp at the widest part (endow)
Width of exocarp at the narrowest part (exon)
Width of mesocarp at the narrowest part (meson)
Width of endocarp at the narrowest part (endon)

* Highly correlated character that was excluded from the analyses.

Results

AFLPs

The AFLP analysis of 90 samples using four selective primer pairs resulted in 122 scored loci, of which 117 (96%) were polymorphic. The average number of loci per individual was 54.97. Scoring of the AFLP bands of replicate samples resulted in a 1.3% overall error rate.

According to similarity coefficients, the STRUCTURE analysis yielded consistent results for clusters K=2, K=5, K=9 and K=15. Four morphotypes were gradually differentiated as well as species from divisions K=2 to K=15 (Fig. 3). Solution K=2 differentiated the first morphotype (*B*. *yagara* from Europe and Asia) from the third and fourth morphotypes. Moreover, putative hybrids of the second morphotype (*B*. *laticarpus* from Europe and Asia, *B*.

medianus from Australia, and, partly, *B. fluviatilis* from North America) were assigned to the same group. Next, solution K=3 contributed to the separation of the third (*B. planiculmis* from Eurasia, *B. caldwellii* from Australia, and *B. paludosus* from North America) and the fourth morphotype (*B. maritimus* and *B. robustus*), but individuals of *B. glaucus* from Europe were included in the group with the third morphotype, and individuals from Africa, in the group of the third morphotype. This solution also showed admixture of the parental species (first and second morphotype) in hybrids of the second morphotype. Solution K=4 allowed North American *B. paludosus* to be distinguished from the rest of the species of the third morphotype. Further, European individuals of *B. glaucus* were differentiated as an independent group in solution K=5, whereas African individuals seemed to be an admixture of *B. glaucus* and *B. planiculmis*. Another two admixed individuals were found between *B. glaucus* and *B. maritimus* group. In solution K=6, species of the first morphotype were distinguished (*B. fluviatilis* from North America and *B. yagara* Eurasia), and two hybrids were detected with admixture of *B. fluviatilis* (*B. novae-angliae* and *B. fluviatilis* × *B. maritimus*). Solution K=7 separated individuals of the second hybrid morphotype, *B. laticarpus* from Europe. Furthermore, solution K=8 allowed differentiation of African *B. glaucus* and distinguished Asian and Australian individuals of the third morphotype (part of the *B. planiculmis* group and *B. caldwellii*). For solution K=9 (with the highest ΔK), Asian and Australian plants of the second hybrid morphotype (*B. laticarpus* and *B. medianus*) were admixed with Asian or Australian plants (part of the *B. planiculmis* group and *B. caldwellii*) rather than with European ones. Moreover, species of the fourth morphotype, *B. maritimus* and North American *B. robustus*, were separated in solution K=10, whereas *B. affinis* was separated as late as K=11. In addition, after differentiation of *B. robustus*, its admixture with *B. fluviatilis* was detected in a North American hybrid (*B. novae-angliae*). The next solutions, K=12 and K=13, enabled only slight differentiation within the Asian *B. planiculmis* group. The final two solutions, K=14 and K=15 (with the second highest ΔK), discriminated an individual of the Australian second morphotype (*B. medianus*) as an independent group. Despite the clear differentiation of these species, several samples (*B. schmidii*, *B. grandispicus* and a North American specimen described as *B. laticarpus*) had simultaneous admixture of more groups that was impossible to determine. In accordance with the STRUCTURE analysis, a similar pattern was observed in the neighbour-net diagrams and the neighbour-joining tree (Fig. 4A, 4B, 5). In those, *B. glaucus*

was divided into two closely related groups, one containing European samples and a second containing African *B*. *glaucus*, *B*. *grandispicus* and a hybrid sample (*B*. *glaucus* × *B*. *maritimus*). Both Iranian species, *B*. *affinis* (fourth morphotype) and *B*. *schmidii*, were clearly separated. European and Asian samples of each morphotype of *B*. *yagara*, *B*. *laticarpus* and *B*. *planiculmis* with *B*. *caldwellii* tended to be in the same clade.

Moreover, Australian hybrid *B*. *medianus* was situated in the *B*. *laticarpus* group (second morphotype). Species from North America were well separated from each other and the rest of the species, except individuals of the fourth morphotype (*B*. *robustus* and *B*. *maritimus*). Hybrid *B*. *fluviatilis* × *B*. *paludosus* was found in an intermediate position between its parental taxa. Another hybrid species of the second morphotype, *B*. *novae-angliae*, was assigned to *B*. *fluviatilis* instead of its second parent, *B*. *robustus*. A specimen described as *B*. *laticarpus* was not clearly placed in any clade of North American species.

The principal coordinate analysis (PCoA) illustrated a similar distribution of species into groups. The first three PCoA axes explained 10.19%, 6.84% and 5.54% of the variability, respectively (Fig. 6A, B). The species *B*. *yagara*, *B*. *laticarpus* and *B*. *maritimus* were separated along the first PCoA axis, whereas *B*. *planiculmis* from *B*. *maritimus*, along the second axis. The North American species, *B*. *fluviatilis*, *B*. *paludosus* and *B*. *robustus*, were placed into independent groups. In addition, the hybrid samples of *B*. *novae-angliae* and *B*. *fluviatilis* × *B*. *paludosus* were also found here in an intermediate position between their parental taxa, whereas hybrid *B*. *maritimus* × *B*. *robustus* was included in the *B*. *maritimus* group, and *B*. *laticarpus* from North America was not placed in any group. *B*. *affinis* was separated from *B*. *maritimus* along the second axis. Furthermore, the Australian species *B*. *caldwellii* and *B*. *medianus* were differentiated from the *B*. *planiculmis* group and the *B*. *laticarpus* group, respectively. A combination of all three axes was unable to distinguish the samples of *B*. *glaucus*, *B*. *grandispicus* and *B*. *schmidii* from the rest of the species, in contrast to the STRUCTURE analysis the neighbour-net diagram and the neighbour-joining tree.

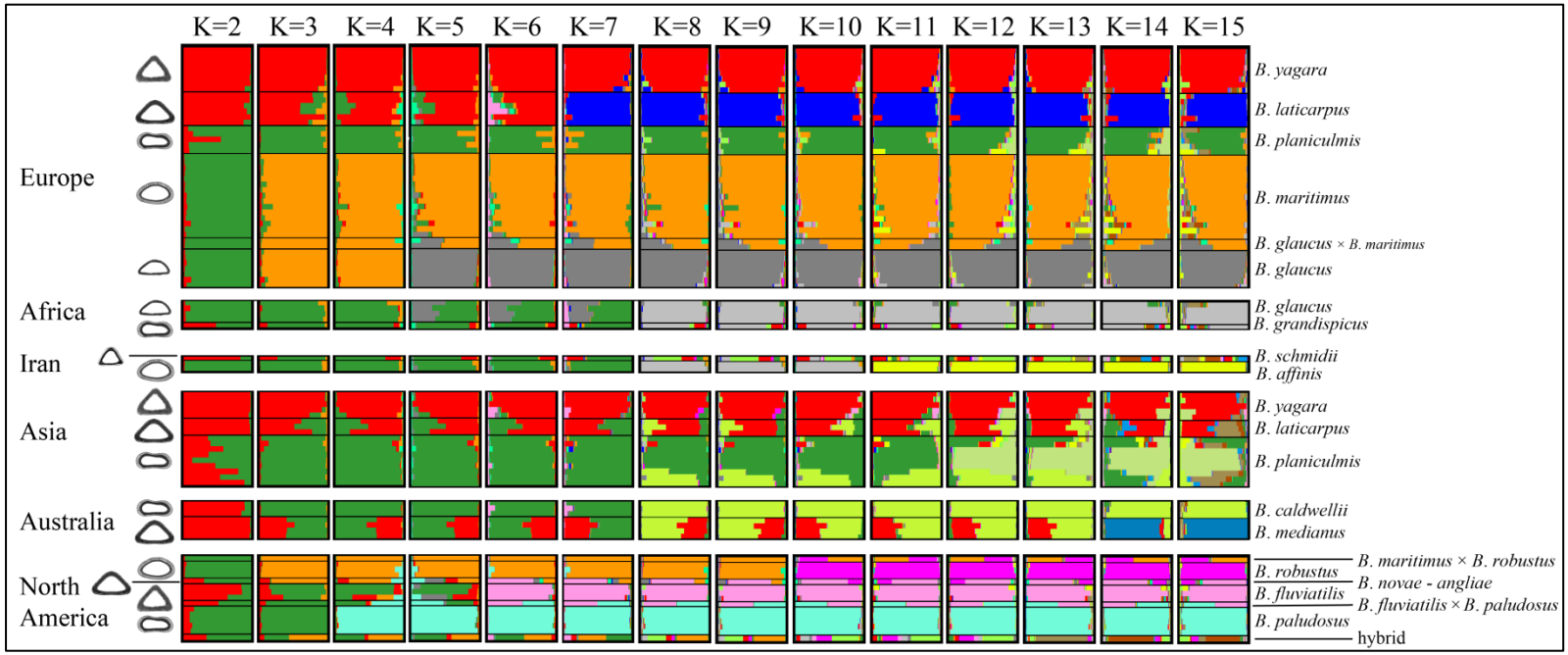

Fig. 3. – Bar plot showing Bayesian assignment probabilities from the software STRUCTURE for clusters (K=2-15) based on 122 AFLP loci and 90 individuals of *Bolboschoenus*.

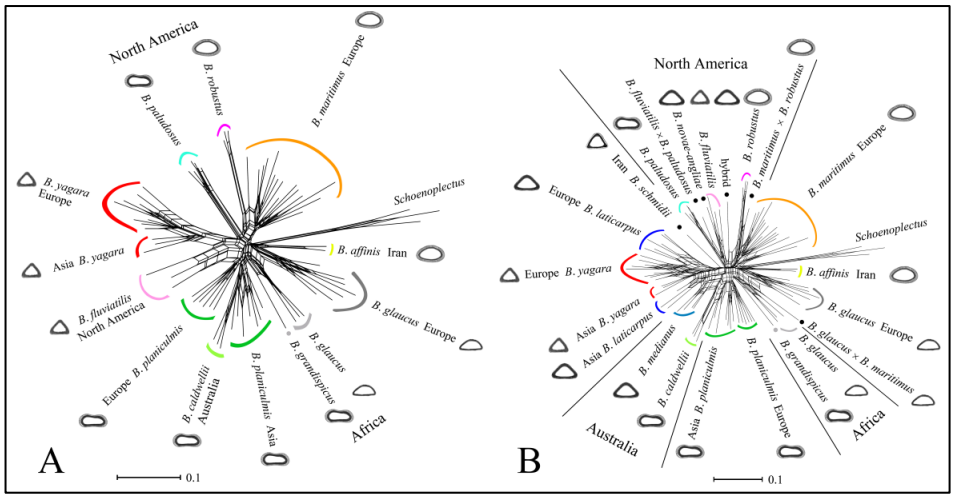

Fig. 4. – (A) Neighbour-net diagram of 70 individuals of *Bolboschoenus*. Only non-admixed samples are presented. (B) Neighbour-net diagram of 90 individuals of *Bolboschoenus*. All samples including admixed and hybrid samples are presented. Colours indicate AFLP groups detected by STRUCTURE.

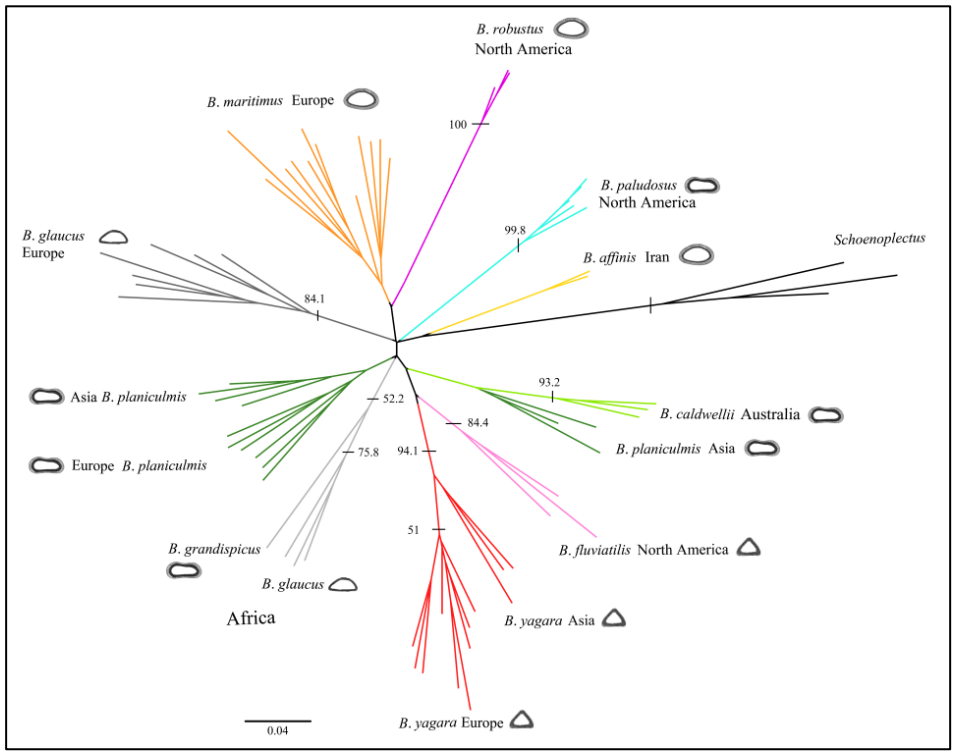

Fig. 5. – Neighbour-joining tree based on 70 *Bolboschoenus* samples and 122 AFLP loci. Bootstrap support values obtained with 1000 replicates and higher then 50 are shown. Only non-admixed samples are presented. Colours indicate AFLP groups detected by STRUCTURE.

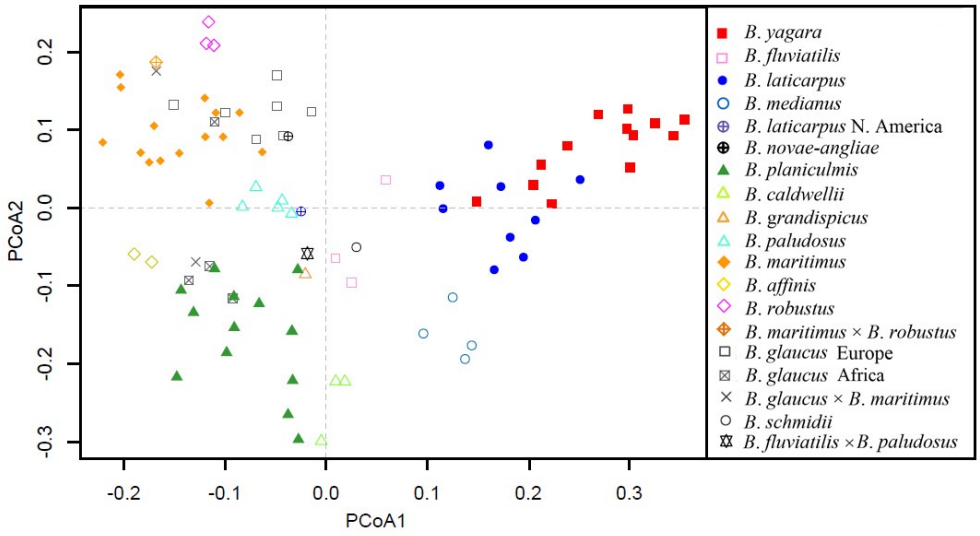

Fig. 6A. – Principal coordinate analysis (PCoA) of 90 *Bolboschoenus* samples based on 122 AFLP loci. The first two axes explain 10.19% and 6.84% of the variation, respectively. Colours indicate AFLP groups detected by STRUCTURE.

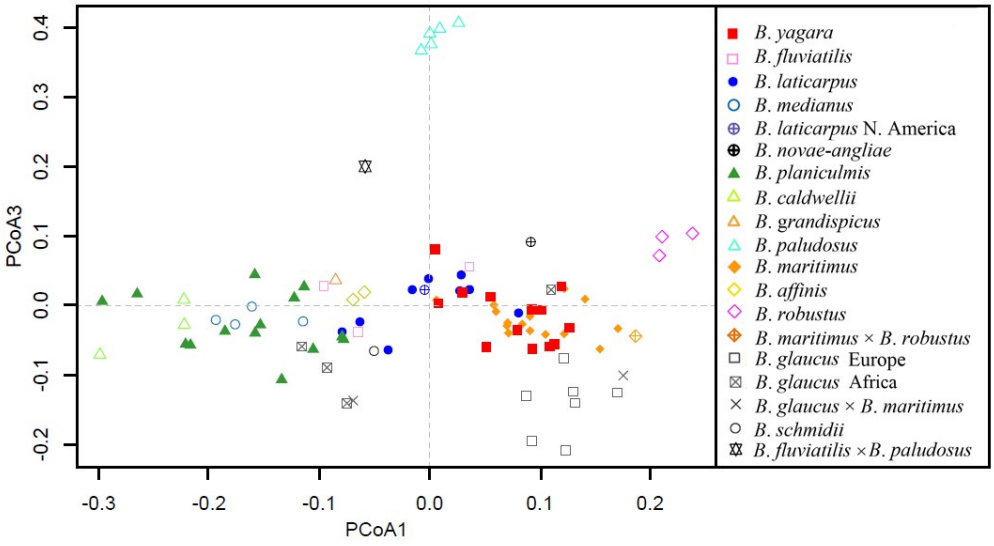

Fig. 6B. – Principal coordinate analysis (PCoA) of 90 *Bolboschoenus* samples based on 122 AFLP loci. The first and third axes explain 10.19% and 5.54% of the variation, respectively. Colours indicate AFLP groups detected by STRUCTURE.

Phylogenetic analyses

The phylogenetic analyses were performed on alignments with a length of 635 bp for the ITS region and 1761 bp for the chloroplast data set combining *trn*H-*psb*A and *trn*C-*psb*MR sequences. The ITS data set contained 120 variable sites, of which 114 were phylogenetically informative. The chloroplast data set consisted of 312 variable sites, of which 295 were phylogenetically informative. Both the maximum likelihood and Bayesian analyses resulted in similar tree topologies. The outgroup of *Schoenoplectus* samples was clearly separated from all the *Bolboschoenus* species. The next most distant clade presented a *B*. *glaucus* group with bootstrap support of 100% and Bayesian support of 100 (Fig. 7). In comparison, separate European and African subclades were distinguished in the chloroplast trees, both with support higher than 50 (Fig. 7B). The next clade included either only North American *B*. *fluviatilis* samples (first morphotype, chloroplast tree) or those samples and the hybrid species *B*. *novae-angliae* (second morphotype), indicating that *B*. *fluviatilis* is one of the parental taxa of *B*. *novae-angliae* (ITS tree, Fig. 7A). On the other hand, the same hybrid was placed in the *B*. *robustus* clade in the chloroplast tree. The next clade with higher support (80-100) consisted of *B*. *yagara* samples from both Europe and Asia. No subclade suggesting the geographical distribution was observed. The last supported clade with *B*. *robustus* (fourth morphotype) samples was differentiated in all trees. In addition, the hybrid sample *B*. *maritimus* × *B*. *robustus* was observed as a subclade of *B*. *robustus* in the ITS tree. The *B*. *paludosus* (third morphotype) group was not clearly distinguished and had low support in all analyses. The North American hybrid between *B*. *fluviatilis* and *B*. *paludosus* was observed in this group in both trees. The rest of the species from Europe, Asia and Australia of the second, third and fourth morphotypes were placed in an undifferentiated clade, except *B*. *schmidii*, *B*. *medianus* and *B*. *grandispicus*, which were distinguished only in some analyses and without high support. With regards to morphological patterns, all species of the first morphotype were clearly separated from each other; however, they had a common ancestral clade in the chloroplast tree. The rest of the morphotypes were not clearly distinguished, except for the North American species and hybrids.

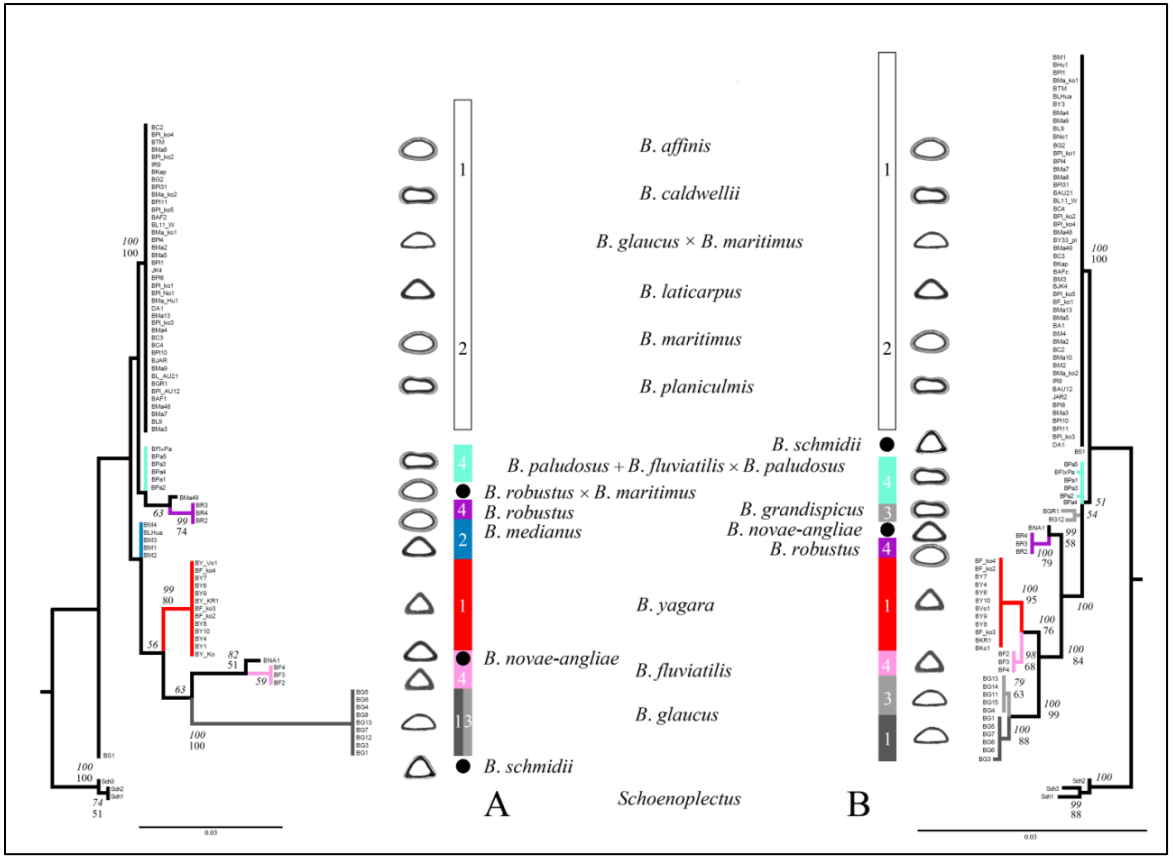

Fig. 7. – Analysis of relationships within the genus *Bolboschoenus*. (A) Best tree determined by maximum likelihood for the 86 ITS sequences. (B) Best tree determined by maximum likelihood for the 92 *psb*A and *psb*MR sequences. 1- Eurasia, 2- Australia, 3- Africa, 4- North America. Maximum likelihood bootstrap supports and Bayesian posterior probabilities (in italic) >50 are presented at nodes.

Morphometric analyses

A principal component analysis (PCA) performed on mean values of the inflorescence characters (1390 samples × 6 characters) resulted in overlapping groups (Fig. 8, 9). Groups of species belonging to the first (especially *B. yagara* and *B. fluviatilis*) and second morphotype (*B. laticarpus*, *B. medianus* and *B. schmidii*) were slightly separated along the first axis explaining 46.66% of the variability (due to the characters number of peduncles and length of peduncles). Groups of species belonging to the third (*B. planiculmis*, *B. grandispicus*, *B. caldwellii* and *B. paludosus*) and fourth morphotype (*B. maritimus*, *B. robustus*, and *B. affinis*) remained undifferentiated. Most of the *B. glaucus* samples also were not distinguished, but samples from the populations Košíře and Boquilobo were partially separated along the second axis (explaining 26.03% of the variability) due to the number of spikelets.

On the other hand, the PCA of achene characters yielded a better differentiation of the individual morphotypes and species. The first morphotype was clearly separated from the rest of the morphotypes along the first axis (explaining 66.94% of the variability) on account of the width of the mesocarp layer and narrow achenes (length and width ratio, Fig. 10A). Furthermore, North American *B. fluviatilis* and the Australian herbarium specimen of *B. fluviatilis* were differentiated along the first axis. In addition, they were separated from Eurasian plants of *B. yagara* along the second axis (explaining 11.20% of the variability, Fig. 10C) due to the width and thickness ratio. The second (hybrid) morphotype, with wide triangular achenes and a slightly thicker exocarp, was placed in an intermediate position between the first and third morphotype. *B. medianus* as well as Asian *B. laticarpus* overlapped with the European *B. laticarpus* group, whereas *B. laticarpus* from North America was slightly separated from it (Fig. 10D). In contrast, *B. schmidii* was clearly separated from the rest of the species on account of the width and thickness ratio. Moreover, the hybrid species *B. novae-angliae* was in an intermediate position between the first and fourth morphotype. A combination of the first and third axis also allowed *B. glaucus* to be distinguished (Fig. 10B). Moreover, an additional analysis was performed with species that were not placed in one of the four morphotypes, improving differentiation of B. glaucus, especially from its hybrid with *B. maritimus*. (Fig. 10G). The third morphotype was differentiated from the fourth morphotype along the second axis, mainly due to the width and length of achene ratio (Fig. 10A). The species *B. caldwellii*, *B. planiculmis* and

B. *paludosus* were slightly separated along the first axis in the analysis, whereas *B*. *grandispicus* remained undistinguished from *B*. *caldwellii* (Fig. 10E). An analysis of the fourth morphotype resulted only in the differentiation of B. affinis from *B*. *maritimus* along the first axis, but *B*. *robustus* and the hybrid *B*. *maritimus* × *B*. *robustus* were not separated from *B*. *maritimus* (Fig. 10F). In sum, the characters that contributed most to the overall separation of individual morphotypes along the first axis were the width of the mesocarp and endocarp layer; along the second axis, ratios describing the shape of achenes; and along the third axis, the width of the exocarp and endocarp layer (Table 2).

Table 2. – Results of morphometric analyses based on characters of achenes. The highest PCA eigenvectors are presented in bold.

Character	PCA1	PCA2	PCA3	PCA C1	PCA C2	PCA D1	PCA D2
Exow	-0.841	0.215	-0.433	-0.293	0.203	0.060	**0.913**
Mesow	**0.893**	0.209	-0.066	**-0.699**	-0.176	-0.434	0.058
Endow	0.745	0.251	-0.390	0.052	-0.251	-0.092	0.322
Exon	-0.801	-0.080	**-0.546**	-0.339	0.429	-0.392	0.752
Meson	**0.860**	0.358	-0.009	-0.626	-0.518	-0.750	-0.019
Endon	**0.854**	0.254	-0.160	0.008	**-0.760**	-0.533	-0.141
L/W	0.811	**-0.425**	-0.200	0.721	0.045	**0.822**	0.140
W/T	-0.727	**0.602**	0.200	-0.489	0.522	-0.512	-0.176

Character	PCA E1	PCA E2	PCA F1	PCA F2	PCA G1	PCA G2
Exow	0.399	**0.698**	0.739	-0.350	-0.851	0.278
Mesow	-0.807	0.253	0.610	0.646	-0.855	-0.220
Endow	-0.389	0.610	0.671	0.305	-0.094	**-0.737**
Exon	0.743	0.450	**0.819**	-0.238	-0.940	0.182
Meson	-0.784	0.230	0.581	0.595	-0.046	-0.646
Endon	-0.556	0.337	0.643	0.195	0.016	-0.631
L/W	-0.013	0.304	0.384	**-0.797**	**-0.947**	0.076
W/T	**-0.817**	-0.246	-0.595	0.522	0.340	0.595

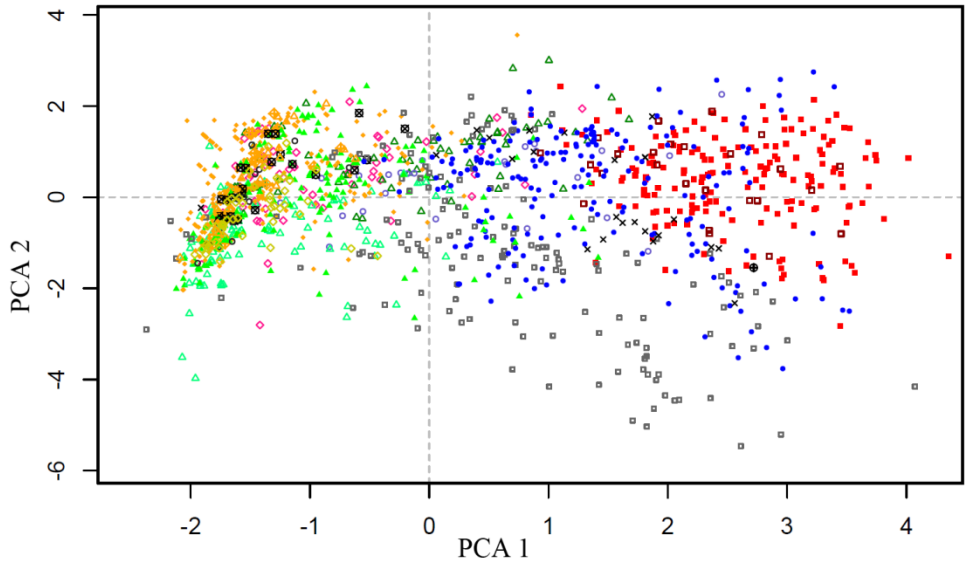

Fig. 8. – Principal component analysis (PCA) based on 6 morphological inflorescence characters and 1390 individuals of *Bolboschoenus*. The first two axes explain 46.66% and 26.03% of the variation, respectively.

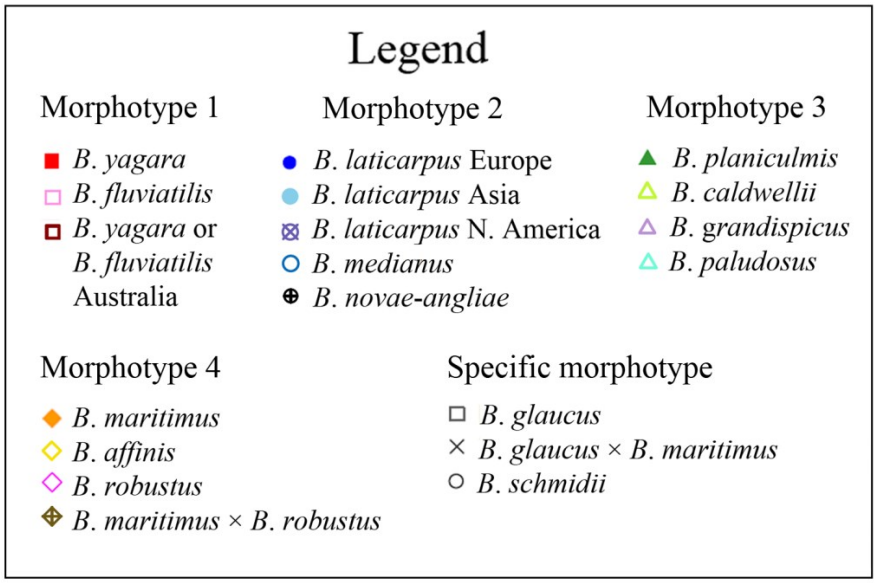

Fig. 9. – Legend for morphometrics analyses of inflorescence and achenes characters.

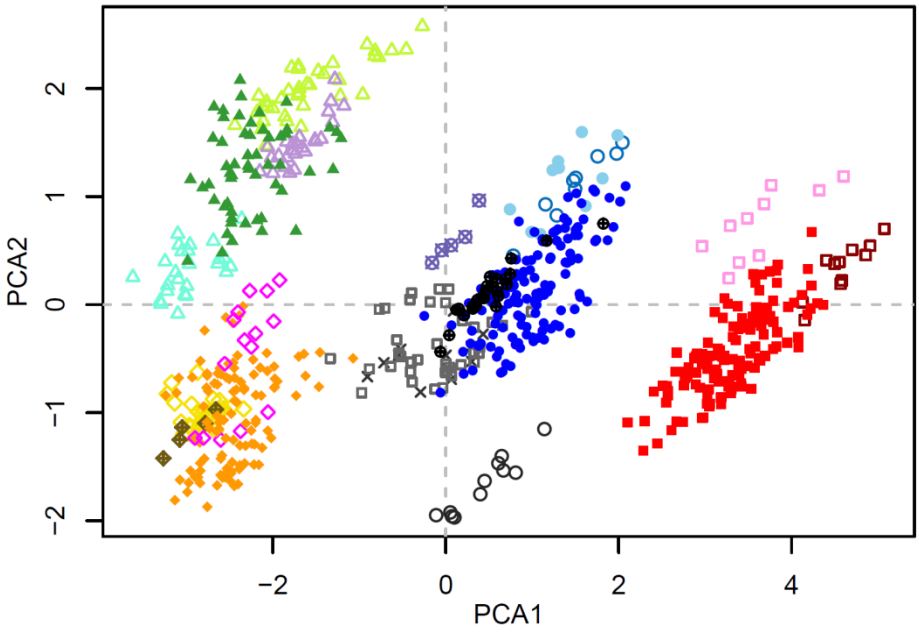

Fig. 10. – (A) Principal component analysis (PCA) based on 8 anatomical characters of 712 achenes. The first two axes explain 66.94% and 11.20% of the variation, respectively.

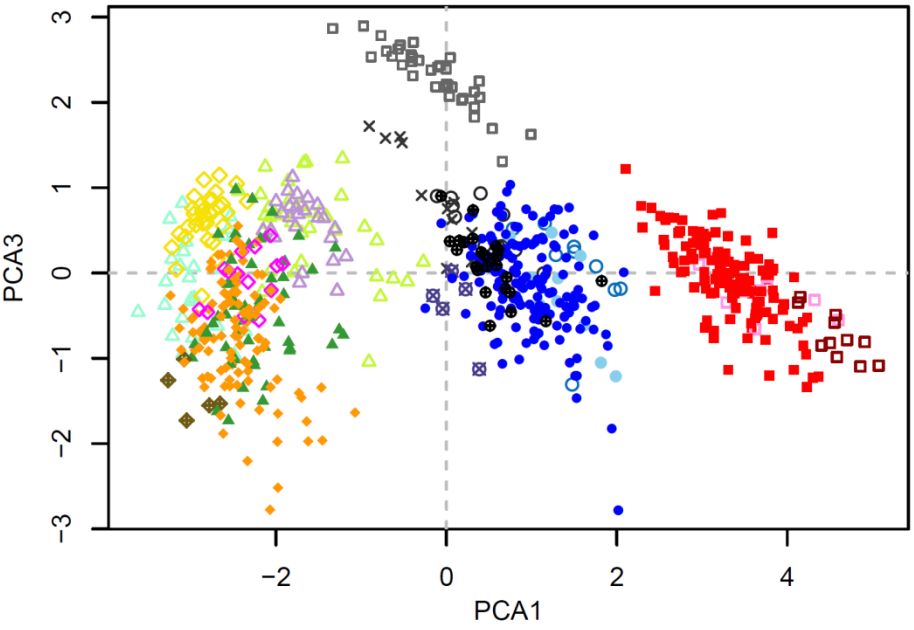

Fig. 10. – (B) Principal component analysis (PCA) based on 8 anatomical characters of 712 achenes. The first and third axis explain 66.94% and 9.34% of the variation, respectively. Colours indicate AFLP groups detected by STRUCTURE.

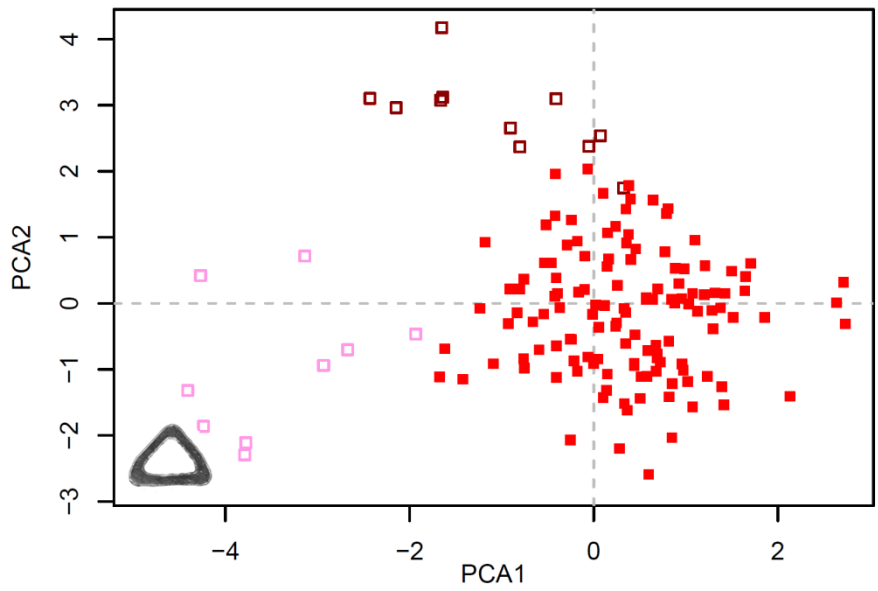

Fig. 10. – (C) Principal component analysis (PCA) of first morphotype based on 8 anatomical characters of 151 achenes. The first two axes explain 23.03% and 17.99% of the variation, respectively.

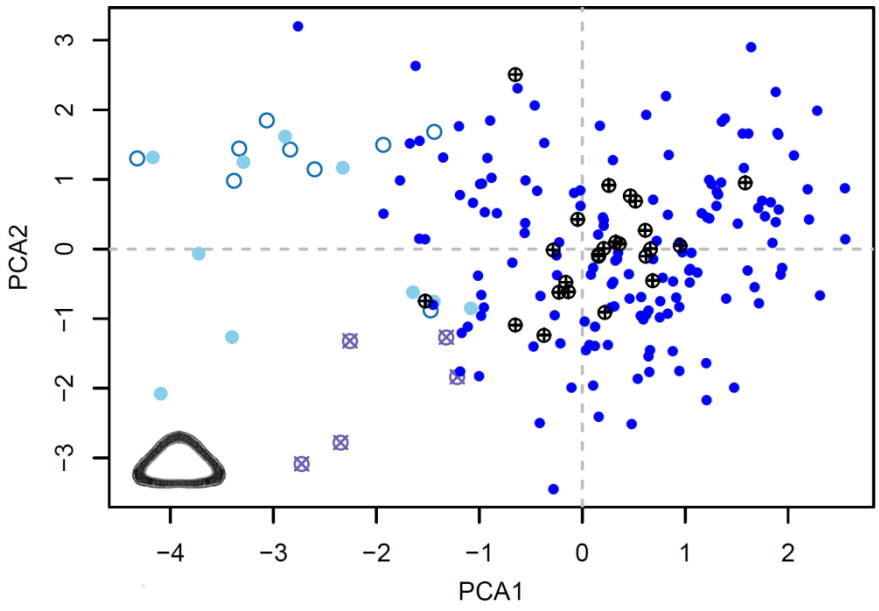

Fig. 10. – (D) Principal component analysis (PCA) of second (hybrid) morphotype based on 8 anatomical characters of 199 achenes. The first two axes explain 26.75% and 19.71% of the variation, respectively.

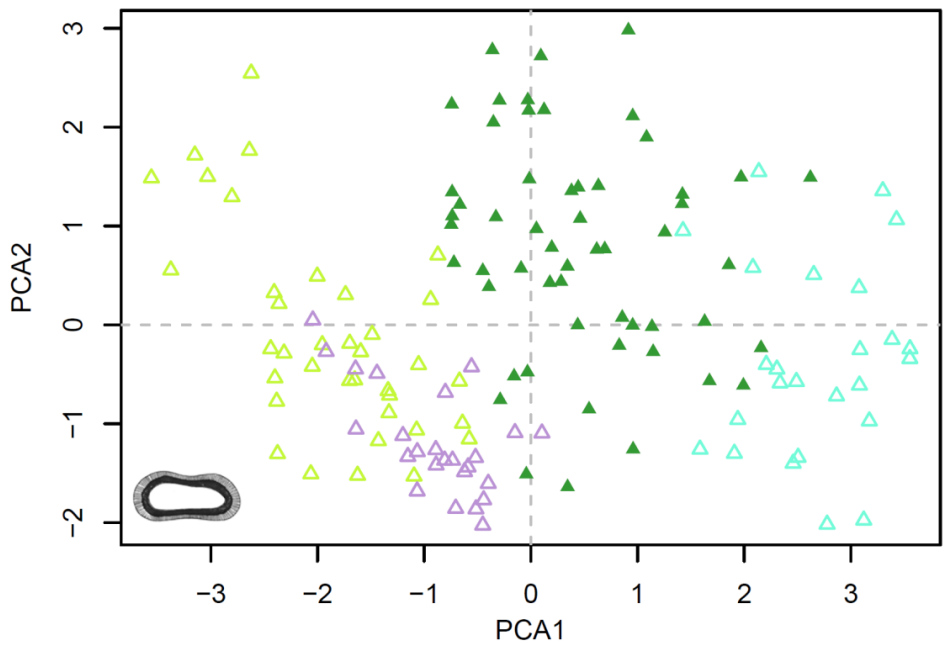

Fig. 10. – (E) Principal component analysis (PCA) of third morphotype based on 8 anatomical characters of 142 achenes. The first two axes explain 38.83% and 18.06% of the variation, respectively.

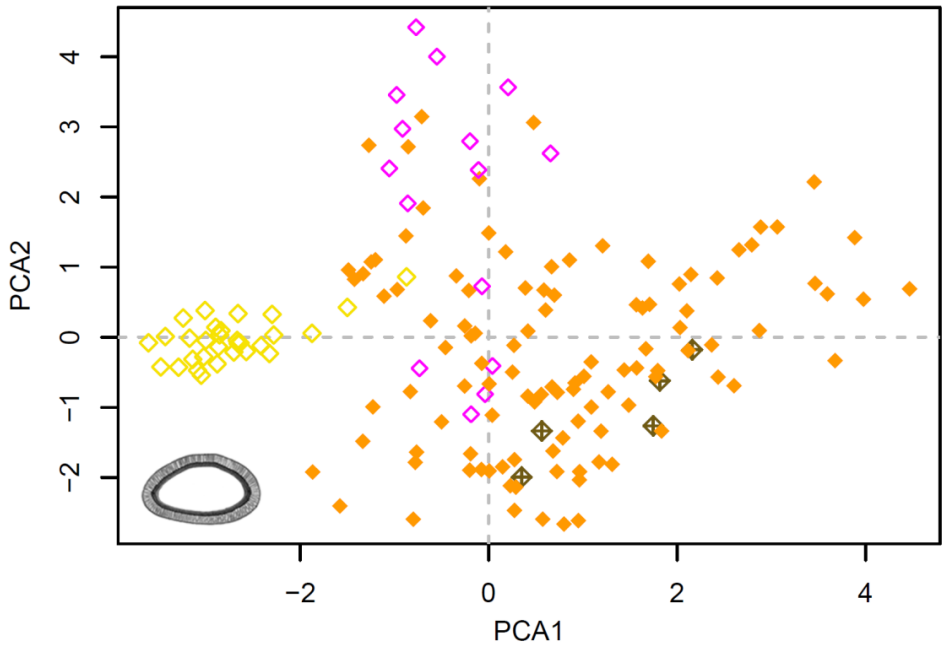

Fig. 10. – (F) Principal component analysis (PCA) of fourth morphotype based on 8 anatomical characters of 160 achenes. The first two axes explain 41.14% and 24.89% of the variation, respectively.

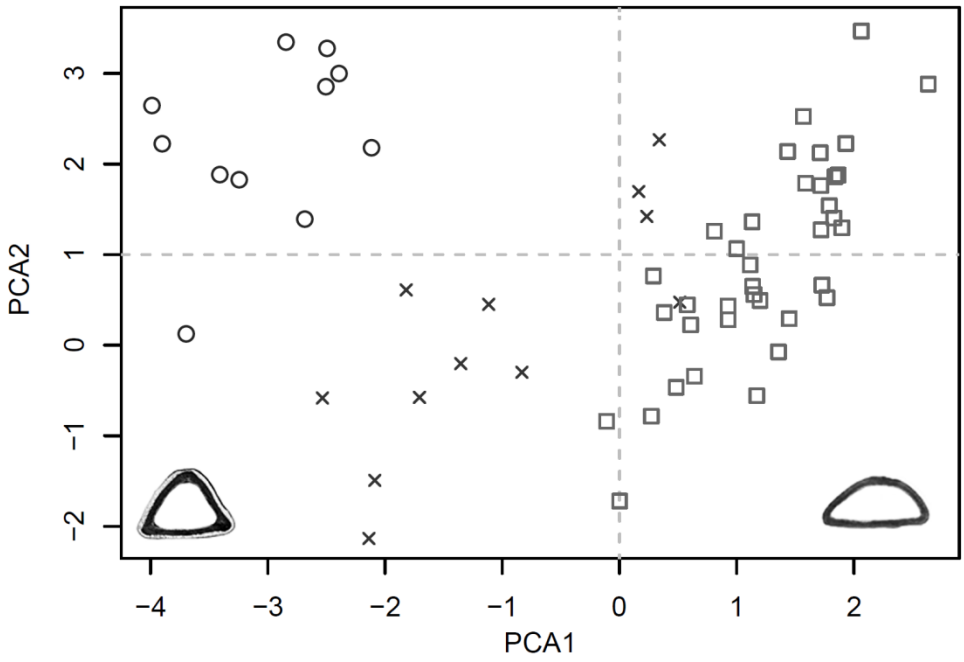

Fig. 10. – (G) Principal component analysis (PCA) of *Bolboschoenus glaucus*, *B. schmidii* and *B. glaucus* × *B. maritimus* samples based on 8 anatomical characters of 60 achenes. The first two axes explain 42.03% and 23.46% of the variation, respectively.

Discussion

In this study, genetic and morphological variation of *Bolboschoenus* species from all over the world was examined to evaluate which evolutionary scenario; either convergence or migration, more strongly influenced the current distribution pattern of the same morphotypes on different continents. All available species from Eurasia, Australia, Africa and North America were included. A combination of the AFLP method, Sanger sequencing of the ITS region of rDNA and two chloroplast regions (*trn*H-*psb*A and *trn*C-*psb*MR), and morphometric analyses of inflorescences and achenes was used to compare the genetic structure with the current morphological concept of the species (Browning et al. 2000). We present the first information about the intrageneric phylogeny, genetic relationships of species and morphotypes, and hybridization between species.

Intrageneric genetic structure

Although the low variability of the ITS and chloroplast data allowed more distant species from North America and Africa to be distinguished, a complex of closely related species from Eurasia and Australia remained unsolved. As the AFLP method proved to be a suitable molecular marker in a previous detailed study of central European species (Píšová et al. 2017), we applied this marker in a further investigation of closely related species at a worldwide scale.

The most distant species of all was *B. glaucus*, which appeared to be a stable taxon well differentiated from the other *Bolboschoenus* species. Although the African plants differ slightly in genotype from the European ones, they all represent a special morphotype distributed in a subtropical-temperate belt in the Mediterranean region through the Near East to Central Asia, Iran, Pakistan and India (Browning et al. 1998, Wollstonecroft et al. 2001, Hroudová et al. 2007).

A species of the first morphotype, *B. yagara*, represents a well-defined genotype that differs clearly from the morphologically similar North American *B. fluviatilis*. However, the plants from Japan considered to be *B. fluviatilis* (Hayasaka & Ohashi 2002) and resembling this species by their taller robust shoots belong genetically to *B. yagara*, of which the Eurasian area of distribution was reported by Hroudová et al. (2007) and Tatanov (2003).

An additional well-defined taxon seems to be *B. affinis* (fourth morphotype). The plants from western China appeared to be identical with those from Iran, with both types being different from other *Bolboschoenus* genotypes. This result also corresponds with the different chromosome number of *B. affinis* (Jarolímová et al. in prep.) and to the strong ecological dissimilarity of *B. affinis* and the other *Bolboschoenus* species (temporary wet depressions in saline steppe habitats, warm continental climate) resulting from special selective processes during evolution.

In addition, two taxa appear to be genetically heterogeneous: *Bolboschoenus grandispicus* and *B. schmidii*. *B. grandispicus* is distributed only in Senegal, mostly in anthropogenically influenced habitats. Evidently, hybridization with *B. glaucus* contributed to the development of *B. grandispicus* during phylogenesis, but the influence of other species is difficult to guess – some species could have been introduced by shipping, growing in Senegal only temporarily. However, genetic analyses showed a strong difference between *B. grandispicus* and *B. affinis*. We can conclude that *B. grandispicus* is neither morphologically nor genetically identical to *B. affinis* as Tatanov (2007a) suggested.

Convergent evolution versus migration

The *Bolboschoenus* species occurring in North America are genetically different from the corresponding morphotypes in other continents. This indicates the early separation of American species from the original genotype group and subsequent convergent evolution in the American continent. *B. fluviatilis* is genetically clearly separated from Eurasian *B. yagara*. The triangular achenes with a thin exocarp resemble those of *B. yagara* but are larger and grey instead of black. Unfortunately, we do not have available plant material suitable for genetic analyses of *B. fluviatilis* from Australia. Thus, we are not able to determine whether Australian plants of *B. fluviatilis* are truly genetically identical to *B. fluviatilis* or might belong to Eurasian *B. yagara*. Nevertheless, the only available old herbarium specimen of a plant from Australia had smaller black achenes, similar to *B. yagara*.

Although *B. paludosus* was described as *B. maritimus* subsp. *paludosus* by Koyama (1980), no genetic relationship with *B. maritimus* was found. In contrast, this species was well differentiated from the other species and belongs instead to the morphotype with lenticular achenes, *B. planiculmis*. Nevertheless,

a partial relationship with *B. maritimus* can be found in another North American species, *B. robustus*, with similar convex achenes and a thick exocarp. Moreover, both species were reported from coastal areas, where they occurred in saline habitats (Browning et al. 1995). In addition, several hybrids were reported between these species, including *B. novae-angliae* (described below).

Similarities between continents were also found within the *B. planiculmis* morphotype, between East Asian *B. planiculmis* and Australian *B. caldwellii* (which was also reflected in *B. medianus*). The transfer of plants or seeds of *Bolboschoenus* from East Asia (China or Japan) to Australia seems to be very probable. Moreover, the next division of Eurasian *B. planiculmis* to the smaller geographical subgroups (European, Asian and *B. caldwellii*-like) suggested a more complex evolutionary history of this species. On the other hand, samples of *B. maritimus* collected throughout its Eurasian distribution area formed a compact group without geographical meaning. Plants with narrow, triangular achenes from both Europe and Asia (formerly described as *B. fluviatilis* subsp. *yagara*, Koyama 1980) were determined to be *B. yagara* and were not related to North American *B. fluviatilis*.

Plant disjunction between North America and Eurasia was also investigated in the genus *Vitis* subg. *Vitis*. Although broad morphological variation was observed in this complex of closely related species, their genetic differentiation was limited (Péros et al. 2011). Moreover, in this study, Asian species were also less diversified than North American species, and it seems that their diversification arose after the dispersal of plants to the new areas.

Convergent morphology between continents was also observed in the genus *Sparganium*. Pairs of closely related species with similar morphologies were reported from North America and Eurasia: *S. angustifolium* and *S. emersum* (Ito et al. 2016), *S. fluctuans* and *S. gramineum*, and *S. eurycarpum* and *S. erectum* (Sulman et al. 2013). The origin of this convergence is more likely a result of migration between continents in the past or of recent long-distant dispersal than of diversification as a consequence of continental drift. Frequent transoceanic dispersal was also found in the tribe Schoeneae (Cyperaceae, Viljoen et al. 2013). In addition, it seems that many aquatic angiosperms have dispersed more recently and after continental drift (Les et al. 2003).

Homoploid hybrid speciation

In this study, putative stable hybrids that have been described as independent species were examined along with admixture between species indicating recent hybridization. Hybrid species with an intermediate morphotype between the first and third or first and fourth morphotype were reported from Europe, Asia, Australia and North America (Browning et al. 1995, 1997a). In Europe, a stable hybrid species, *B. laticarpus*, was described (Marhold et al. 2004a) that exceeded both parental taxa by inhabiting a wider range of ecological niches and exhibiting a tolerance of high nutrient levels (Hroudová et al. 2014). Its hybrid origin was later confirmed with the parental taxa *B. yagara* and *B. planiculmis* (Píšová et al. 2017). In this study, plants from eastern China (Wuhan, Huangzhong) formerly called "*B. yagara*" were revealed to be a hybridogenous taxon equivalent to European *B. laticarpus*. This corresponds to its morphological characteristics and supports the theory of a tentative polyphyletic (multi-local) origin of *B. laticarpus*. The suggested parentage of such plants is *B. yagara* and Asian plants of *B. planiculmis*, but in contrast to European *B. laticarpus*, these plants seem to be younger hybrids. Another hybrid species with a similar parentage combination and a later origin is Australian *B. medianus*. Although *B. caldwellii* and *B. fluviatilis* were proposed to be its parental taxa (Browning et al. 1997a), the genotype of *B. medianus* did not show any part of the *B. fluviatilis* genotype but had a small part of the *B. yagara* genotype. Nevertheless, this may, along with the similarity of *B. yagara* and *B. planiculmis* plants, suggest the connection (common origin) of Australian *Bolboschoenus* species and plants of East Asia.

An additional hybrid was recorded in Europe between *B. glaucus* and *B. maritimus* (Azambuja, Portugal, and Lake Vrana, Croatia). The hybrid origin of plants from Azambuja was also reflected in their intermediate morphology, in accordance with the previous description of a similar plant by Browning from Pakistan and an introduced plant in North America (Browning et al. 1995).

Several other hybrids were recorded in North America, most notably, *B. novae-angliae*, which is known only from Atlantic coastal estuaries, where it is sympatric with its parental taxa, *B. fluviatilis* (occupying freshwater habitats) and *B. robustus* (occupying saline habitats, Browning et al. 1995). Additionally, the hybrids *B. fluviatilis* × *B. paludosus* and *B. maritimus* × *B. robustus* were identified. With only one sample from each parentage combination to

investigate, we can only suggest the parentage of these hybrids, and further research is needed to confirm it.

In addition, we are unable to estimate the age of all these hybrids without molecular dating of phylogenetic trees. They might be stable hybrid species such as *B. laticarpus* with their own ecology and intermediate morphological characters or the result of recent hybridization. In previous studies of genetic structure of central European species (Píšová et al. 2017) and their ecology (Hroudová et al. 2007, 2014), we found recent hybrids between *B. maritimus* and *B. planiculmis*. Moreover, these introgressants seem to be more tolerant of saline habitats or less saline habitats compared to their parental species.

Conclusions

After early separation from their morphological counterparts in Eurasia, the North American species evolved independently, and geographical isolation further enhanced their differentiation and hybridization. Nevertheless, they probably have common ancestry within morphotypes (as *B. fluviatilis* and *B. yagara* or *B. robustus* and *B. maritimus*). On the other hand, the Eurasian species are closely related, and a geographical pattern was observed only at the intraspecific level in *B. planiculmis*. Similarly, the Australian species are somewhat genetically related to the Asian plants, due to migration between continents in the past (e.g., *B. caldwellii* to *B. planiculmis*). Consequently, the hybrid species *B. medianus* resembles the Asian plants of *B. laticarpus* that are both younger hybrids in comparison to the European stable hybrid *B. laticarpus*. Whether the *Bolboschoenus* representatives in Australia are well-differentiated species or belong to the same species as the Asian plants within the same morphotype is a subject for further investigation.

The two Iranian species, *B. affinis* and *B. schmidii*, are clearly separated and defined species. The results suggest that *B. affinis* is not related to *B. grandispicus* from Senegal. Additionally, *B. schmidii* seems to be of a hybrid origin that remains unresolved. Further, Mediterranean *B. glaucus* is well separated from the European species, falling geographically into the European and African subgroup. Lastly, although *B. grandispicus* belongs to the third morphotype, genetic admixture of African *B. glaucus* and other species was found that raised questions about the origin of *B. grandispicus*.

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References

Alix K., Gérard P. R., Schwarzacher T. & Heslop-Harrison J. S. (Pat). (2017): Polyploidy and interspecific hybridization: partners for adaptation, speciation and evolution in plants. – Annals of Botany 120: 183–194.

Baldwin B. G. (1992): Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. – Mol. Phylogenet. Evol. 1: 3–16.

Barrett S. C. H., Echert C. G. & Husband B. C. (1993): Evolutionary processes in aquatic plant populations. – Aquat. Bot. 44: 105–145.

Bonin A., Bellemain E., Eidesen P. B., Pompanon F., Brochmann C. & Taberlet P. (2004): How to track and assess genotyping errors in population genetics studies. – Mol. Ecol. 13: 3261–3273.

Browning J. & Gordon-Gray K. D. (1992): Studies in Cyperaceae in southern Africa. 19: The genus *Bolboschoenus*. – S. Afr. J. Bot. 58: 380–385.

Browning J. & Gordon-Gray K. D. (1993): Studies in Cyperaceae in southern Africa. 21: The taxonomic significance of the achene and its embryo in *Bolboschoenus*. – S. Afr. J. Bot. 59: 311–318.

Browning J. & Gordon-Gray K. D. (1999): The inflorescence in southern African species of *Bolboschoenus* (Cyperaceae). – Ann. Bot. Fenn. *36 (2): 81–97.*

Browning J. & Gordon-Gray K. D. (2000): Patterns of fruit morphology in *Bolboschoenus* (Cyperaceae) and their global distribution. – S. Afr. J. Bot. 66: 63–71.

Browning J., Gordon-Gray K. D. & Smith S.G. (1995): Achene structure and taxonomy of North American *Bolboschoenus* (Cyperaceae). – Brittonia 47 (4): 433–445.

Browning J., Gordon-Gray K. D. & Smith S. G. (1997a): Achene morphology and pericarp anatomy of the type specimens of the Australian and New Zealand *Bolboschoenus* species (Cyperaceae). – Austral. Syst. Bot. 10: 49–58.

Browning J., Gordon-Gray K. D., Smith S. G. & van Staden J. (1996): *Bolboschoenus yagara (Cyperaceae)* newly reported for Europe. – Ann. Bot. Fenn. 33: 129–136.

Browning J., Gordon-Gray K. D., Smith S. G. & van Staden J. (1997b): *Bolboschoenus maritimus s.l.* in The Netherlands: a study of pericarp anatomy based on the work of Irene Robertus-Koster. – Ann. Bot. Fenn. 34: 115–126.

Browning J., Gordon-Gray K. D., Smith S. G. & van Staden J. (1998): *Bolboschoenus glaucus* (Cyperaceae), with emphasis upon Africa. – Nord. J. Bot. 18: 475–482.

Buerkle C. A., Morris R. J., Asmussen M. A., Rieseberg L.H. (2000): The likelihood of homoploid hybridization. – Hederity 84 (4): 441–451.

Darriba D., Taboada G.L., Doallo R. & Posada D. (2012): "jModelTest 2: more models, new heuristics and parallel computing". – Nat. Methods. 9 (8): 772.

Despéres L., Gielly L., Redoutet B. & Taberlet P. (2003): Using AFLP to resolve phylogenetic relationships in a morphologically diversified plant species complex when nuclear and chloroplast sequences fail to reveal variability. – Mol. Phylogenetics Evol. 27(2): 185–196.

Donovan L. A., Rosenthal D. R., Sanchez-Velenosi M., Rieseberg L. H. & Ludwig (2010): Are hybrid species more fit then ancestral parent species in the current hybrid species habitats? – J. Evol. Biol. 23: 805–816.

Dray S. & Dufour A.B. (2007): The ade4 package: implementing the duality diagram for ecologists. – J. Stat. Softw. 22 (4): 1–20.

Cronk J. K. & Fennessy M. S. (2016): Wetland plants: biology and ecology. CRC press.

Egorova T. V. & Tatanov I. V. (2003): O sistematicheskom polozhenii *Bolboschoenus planiculmis* i *Bolboschoenus koshewnikowii* (Cyperaceae) [On systematic position of *Bolboschoenus planiculmis* and *B. koshewnikowii* (Cyperaceae)]. – Bot. Zhurn. 88 (4): 131–142.

Ehrich D. (2009): Documentation for Structure-sum Version 2009. A series of R functions for summarizing the outputs of the program Structure ver. 2.2.

Ellstrand N. C., Whitkus R. & Rieseberg L. H. (1996): Distribution of spontaneous plant hybrids. – Proc. Natl. Acad. Sci. USA 93: 5090–5093.

Evanno G., Regnaut S. & Goudet J. (2005): Detecting the number of clusters of individuals using the software structure: a simulation study. – Mol. Ecol. 14: 2611–2620.

Falush D., Stephens M. & Pritchard J. K. (2007): Inference of population structure using multilocus genotype data: dominant markers and null alleles. – Mol. Ecol. Notes 7: 574–578.

Felsenstein J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. – Evolution 39 (4): 783–791.

Guo Y. P., Vogl C., van Loo M. & Ehrendorfer F. (2006): Hybrid origin and differentiation of two tetraploid *Achillea* species in East Asia: molecular, morphological and ecogeographical evidence. – Mol. Ecol. 15: 133–144.

Hall T. (1999): BioEdit v5.0.9., Ibis Biosciences, North Carolina State University, NC, USA.

Hayasaka E. & Ohashi H. (2002): Achene gross morphology and pericarp anatomy of Japanese *Bolboschoenus* (Cyperaceae). – J. Jap. Bot. 77: 9–23.

Hroudová Z., Frantík T. & Zákravský P. (1998a): The differentiation of subspecies in *Bolboschoenus maritimus* based on the inflorescence structure. – Preslia. 70: 135–154.

Hroudová Z., Moravcová L. & Zákravský P. (1998b): Differentiation of the Central European *Bolboschoenus* taxa based on fruit shape and anatomy. – Thaiszia 8: 91–109.

Hroudová Z., Zákravský P., Ducháček M. & Marhold K. (2007): Taxonomy, distribution and ecology of *Bolboschoenus* in Europe. – Ann. Bot. Fenn. 44: 81–102.

Hroudová Z., Zákravský P. & Flegrová M. (2014): The tolerance to salinity and nutrient supply in four European *Bolboschoenus* species (*B. maritimus*, *B. laticarpus*, *B. planiculmis* and *B. yagara*) affects their vulnerability or expansiveness. – Aquat. Bot. 112: 66–75.

Huson D. H. & Bryant D. (2006): Application of phylogenetic networks in evolutionary studies. – Mol. Biol. Evol. 23: 254–267.

Ito Y., Tanaka N., Kim Ch., Kaul R. B., Albach D. C. (2016): Phylogeny of *Sparganium* (Typhaceae) revisited: non-monophyletic nature of *S. emersum* sensu lato and resurrection of *S. acaule*. – Plant Syst. Evol. 302(1): 129–135.

Jaccard P. (1908): Nouvelles recherches sur la ristribution florale. – Bull. Soc. Vaud. Sci. Nat. 44: 223–270.

Jakobsson M. & Rosenberg N. A. (2007): CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. – Bioinformatics 23: 1801–1806.

Jarolímová V. & Hroudová Z. (in prep.): Chromosome numbers within the genus *Bolboschoenus*.

Jarolímová V. & Hroudová Z. (1998): Chromosome numbers within the genus *Bolboschoenus* in Central Europe. – Folia Geobot. 33: 415–428.

Jung J. & Choi H. K. (2010): Systematic rearrangement of Korean *Scirpus* L. s.l. (Cyperaceae) as inferred from nuclear ITS and chloroplast rbcL sequences. – J. Plant Biol. 53: 222–232.

Kim S. C., Mejías J. A. & Lubinsky P. (2008): Molecular confirmation of the hybrid origin of the critically endangered western Mediterranean endemic *Sonchus pustulatus* (Asteraceae: *Sonchinae*). – J. Plant Res. 121 (4): 357–364.

Koutecký P. (2015): MorphoTools: a set of R functions for morphometric analysis. – Plant Syst. Evol. 301: 1115–1121.

Koyama (1980): The genus *Bolboschoenus* Palla in Japan. – Acta Phytotax. Geobot. 31 (4–6): 139–148.

Larkin M. A., Blackshields G., Brown N. P., Chenna R., McGettigan P. A., McWilliam H., Valentin F., Wallace I. M., Wilm A., Lopez R. & Thompson J. D. (2007): Clustal W and Clustal X version 2.0. – Bioinformatics. 23 (21): 2947–2948.

Les D. H., Garvin D. K. & Wimpee C. F. (1991): Molecular evolutionary history of ancient aquatic angiosperms. Proc. Natl. Acad. Sci. 88(22): 10119–10123.

Les D., Crawford D. J., Kimball R. T., Moody M. L. & Landolt E. (2003): Biogeography of discontinuously distributed hydrophytes: a molecular appraisal of intercontinental disjunctions. – Int. J. Plant Sci. 164(6): 917–932.

Marhold K., Hroudová Z., Ducháček M. & Zákravský P. (2004a): The *Bolboschoenus maritimus* group (Cyperaceae), in Central Europe, including *B. laticarpus*, spec. nova. – Phyton (Horn). 44: 1–21.

Marhold K., Lihová J., Perný J. & Beeker W. (2004b): Comparative ITS and AFLP analysis of diploid *Cardamine* (Brassicaceae) taxa from closely related polyploid complexes. – Ann. Bot. 93(5): 507–520.

Nordborg M., Hu T. T., Ishino Y., Jhaveri J., Toomajian C., Zheng H. G., Bakker E., Calabrese P., Gladstone J., Goyal R., Jakobsson M., Kim S., Morozov Y., Padhukasahasram B., Plagnol V., Rosenberg N. A., Shah C., Wall J. D., Wang J., Zhao K. Y., Kalbfleisch T., Schulz V., Kreitman M. & Bergelson J. (2005): The pattern of polymorphism in *Arabidopsis thaliana*. – PLoS Biol. 3: 1289–1299.

Péros J.-P., Berger G., Portemort A., Boursiqout J.-M. & Lacombe T. (2011): Genetic variation and biogeography of the disjunct *Vitis* subg. *Vitis* (Vitaceae). – J. Biogeogr. 38(3): 471–486.

Píšová S. & Fér T. (submitted): Homoploid hybrid speciation in *Sparganium erectum*: molecular, genome size and morphometric analyses.

Píšová S., Hroudová Z., Chumová Z. & Fér T. (2017): Ecological hybrid speciation in central-European species of *Bolboschoenus*: genetic and morphological evaluation. – Preslia. 89: 17–39.

Pritchard J. K., Stephens M. & Donnelly P. (2000): Inference of population structure using multilocus genotype data. – Genetics 155: 945–959.

R Development Core Team (2008): R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org.

Roalson E. H. (2008): A synopsis of chromosome number variation in the Cyperaceae. – Bot. Rev. 74: 209–393.

Ronquist F. & Huelsenbeck J. P. (2003): MRBAYES 3: Bayesian phylogenetic inference under mixed models. – Bioinformatics 19: 1572–1574.

Rosenberg N. A. (2004): DISTRUCT: a program for the graphical display of population structure. – Mol. Ecol. Notes 4: 137–138.

Rieseberg L. H. (1995): The role of hybridization in evolution: old wine in new skin. – Amer. J. Bot. 82 (7): 944–953.

Rieseberg L. H., Kim S. C., Randell R. A., Whitney K. D., Gross B. L., Lexer C. & Clay K. (2007): Hybridization and the colonization of novel habitats by annual sunflowers. – Genetica 129 (2): 149–165.

Riley H. P. (1938): A character analysis of colonies of *Iris fulva*, *Iris hexagona* var. *giganticaerulea* and natural hybrids. – Am. J. Bot. 25 (10): 727–738.

Sang T., Crawford D. J. & Stuessy T. F. (1997): Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). – Am. J. Bot. 84 (8): 1120–1136.

Santamaria L. (2002): Why most aquatic plants are broadly distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. – Acta Oecol. 23: 137–154.

Sculthorpe C. D. (1967): The biology of aquatic vascular plants. Edward Arnold, London. 610 pp.

Shaw J., Lickey E. B., Beck J. T., Farmer S. B., Liu W., Miller J., Siripun K. C., Winder C. T., Schilling E. E. & Small R. L. (2005): The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. – Am. J. Bot. 92, 142–166.

Simpson D., Muasya M., Alves M., Bruhl J., Dhooge S., Chase M., Furness C., Ghamkhar K., Goetghebeur P., Hodkinson T., Marchant A., Reznicek A., Nieuwborg R., Roalson E., Smets E., Starr J., Thomas W., Wilson K., & Zhang X. (2007): Phylogeny of Cyperaceae based on DNA sequence data–a new rbcL analysis. – Aliso 23: 72–83.

Soltis P. S. & Soltis D. E. (2009): The Role of Hybridization in Plant Speciation. – Annu. Rev. Plant Biol. 60: 561–588.

Stebbins G. L. (1959): The role of hybridization in evolution. – Proc. Am. Philos. Soc. 103(2): 231–251.

Sulman J. D., Drew B. T., Drummond C., Hyasaka E. & Systma K. J. (2013): Systematics, biogeography, and character evolution of *Sparganium* (Typhaceae): Diversification of a widespread, aquatic lineage. – Am. J. Bot. 100(10): 2023–2039.

Španiel S., Marhold K., Filová B. & Zozomová-Lihová J. (2011): Genetic and morphological variation in the diploid–polyploid *Alyssum montanum* in Central Europe: taxonomic and evolutionary considerations. – Plant Syst. Evol. 294: 1–25.

Tatanov I. V. (2003): Kriticheskie zametki o vidakh *Bolboschoenus desoulavii* (Drob.) A. E. Kozhevnikov i *Bolboschoenus yagara* (Ohwi) Y. C. Yang et M. Zhan (Cyperaceae) [Critical comments on the species *Bolboschoenus desoulavii* (Drob.) A. E. Kozhevnikov and *Bolboschoenus yagara* (Ohwi) Y. C. Yang et M. Zhan (Cyperaceae)]. – Novosti Sist. Vyssh. Rast. 35: 51–62.

Tatanov I. V. (2007a): Taksonomicheskiy obzor roda *Bolboschoenus* (Aschers.) Palla (Cyperaceae) [Taxonomic survey of the genus *Bolboschoenus* (Cyperaceae)]. – Novosti Sist. Vyssh. Rast. 39: 46–149.

Tatanov I. V. (2007b): Novyi mezhrodovoi gibrid × *Bolboschoenoplectus* Tatanov (Cyperaceae). [Hybrida intergenericus novus × *Bolboschoenoplectus* Tatanov (Cyperaceae)]. – Novosti Sist. Vyssh. Rast. 39: 150–158.

Tate J. A. & Simpson B. B. (2003): Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. – Syst. Bot. 28 (4): 723–737.

Vallejo-Marín M., Buggs R. J. A., Cooley A. M. & Puzey J. R. (2015): Speciation by genome duplication: Repeated origins and genomic composition of the recently formed allopolyploid species *Mimulus peregrinus*: speciation by genome duplication in monkeyflowers. – Evolution 69: 1487–1500.

Viljoen J.–A., Muasya A. M., Barrett R. L. Bruhl J. J., Gibbs A. K., Slingsby J. A., Wilson K. L. & Verboom G. A. (2013): Radiation and repeated transoceanic dispersal of *Schoeneae* (Cyperaceae) through the southern hemisphere. – Am. J. Bot. 100(12): 2494–2508.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. & Zabeau M. (1995): AFLP: a new technique for DNA fingerprinting. – Nucl. Acids Res. 23: 4407–4414.

Wang X. R., Szmidt A. E. & Savolainen O. (2001): Genetic composition and diploid hybrid speciation of a high mountain pine, Pinus densata, native to the Tibetan Plateau. – Genetics. 159 (1): 337–346.

Went F. W. (1971): Parallel evolution. – Taxon. 197–226.

White T. J., Bruns T. D., Lee S. B. & Taylor J. W. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: PCR protocols: a guide to methods and applications. [eds.] Innis M.A., Gelfand D. H., Sninsky J. J. & White T. J. Academic Press, Inc. New York. 315–322.

Whittall J.B., Hellquist C. B., Schnieder E. L. & Hodges S. A. (2004): Cryptic species in an endangered pondweed community *Potamogeton*, Potamogetonaceae) revealed by AFLP markers. – 91(12): 2022–2029.

Williamson P. S. & Schneiderin E. L. (1993): Nelumbonaceae. – In: K. Kubitzki, J.G. Rohwer, V. Bittrich [eds.], The families and genera of vascular plants, Springer-Verlag. New York. 470–473.

Winterfeld G., Schneider J., Perner K. & Röser M. (2014): Polyploidy and hybridization as main factors of speciation: complex reticulate evolution within the grass genus *Helictochloa*. – Cytogenet. Genome Res. 142: 204–225.

Wollstonecroft M. M., Hroudová Z., Hillman G. C. & Fuller D. Q. (2011): *Bolboschoenus glaucus* (Lam.) SG Smith, a new species in the flora of the ancient Near East. – Veg. Hist. Archaeobot. 20 (5): 459–470.

Yano O. & Hoshino. T. (2005): Molecular phylogeny and chromosomal evolution of Japanese *Schoenoplectus* (Cyperaceae), based on ITS and ETS 1f sequences. – Acta Phytotax. Geobot. 56: 183–195.

Zwickl D. J. (2006): Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion (Ph.D. thesis). The University of Texas at Austin.

Appendix 1 – List of the populations of the species of *Bolboschoenus* studied and their characteristics.AFLP – determination of plants (abbreviation for each species or × or ? for each hybrid) and their African (AF), European (E), Asian (AS) or North American (NA) classification based on the STRUCTURE analysis with K=15. ITS or cp – clade in which samples were found: Eurasian (EA), European (E), African (AF) or abbreviation of species. Ninf – number of measured inflorescences per population. Na – number of measured achenes per population.

Acronym	Locality and date of collection	AFLP	ITS	cp	Ninf	Na
Bolboschoenus affinis						
BAF	Iran, Azerbaijan-E: Tabriz, Bandor-e Sharafkhaneh, N 38.176667, E 45.471667, 1300m, 6.7.2010, leg. Amini Rad M. & Torabi H., cultivated plants	AFF	EA	EA	25	5
BAFc	China, Xinjiang, Altay, Alahakezhen, N 47.75017, E 87.48318, 509mnm, 13.9.2013, leg. Mandák B. & Vít P., cultivated plants	AFF	EA	EA	24	25
Bolboschoenus caldwellii						
BC1	Australia, The valley E of Adelaide, S 34.926931, E 138.719194, 2006, leg. Ganf G., cultivated	CAL	EA	EA	25	25
BC2	New Zealand, Kaikoura, NE coast of the southern isle, S 42.419017, E 173.705700, 2m, 3.4. 2004, leg. Krahulec F., cultivated plants	CAL	EA	EA	25	5
BC3	Australia, New England, S 32.6, E 148.4, 2012, leg. Bruhl J. &Waterway M. J., cultivated plants	CAL	EA	EA	25	7
Bolboschoenus fluviatilis						
BF2	USA, Wisconsin, Dane County. Near the north side of Rutlend-Dunn Town Line Road just west of Hawkinson Road, about 4 miles east of the village of Oregon. N 42.931944, W 89.309722, 1.9.2008, leg. Smith S. G. & Sulman J., cultivated plants	FLU	FL	FL	-	5
BF3	USA, Indiana, Grant County. Shallow marsh 5 km S from the town Upland, N 40.432785, W 85.493331, 14.7. 2009, leg. Rothrock P. E., cultivated plants	FLU	FL	FL	25	5
BF4	USA, North Dakota, Stutsman County. In a wet prairie pothole, in a soybean field. Along 21th Street Southeast, 11.5 miles west of highways 52 and 281, ca. 24 miles northwest of Jamestown, N 47.109889, W 99.108889, 25.9. 2011, leg. Smith S. G. & Mushet D., cultivated plants	FLU	FL	FL	-	-

Acronym	Locality and date of collection	AFLP	ITS	cp	Ninf	Na
Bolboschoenus glaucus						
BG1	Czech Republic, Central Bohemia, Prague distr. Košíře, depression (the former brick-clay pit) by the Podbělohorská and U Klikovky street, N 50.075000, E 14.370556, 290 m, 14.8.1999, leg. Hroudová Z., Jarolímová V., Zákravský P., cultivated plants	GLA E	GL	GL E	25	5
BG2	Portugal, Emerged shore of the pool by the road near Azambuja ditch, ca. 6 km SSW of the town of Santarém, N 39.19, W 8.72, 10m, 23.9.1998, leg. Hroudová Z., & Zákravský P., cultivated plants	GLA E × MAR	EA	EA	25	5
BG3	Greece, Delta of the Aliakmon river, near the town of Nea Agathoupoli. N 40.46667, E 22.58333, 1996, cultivated plants	GLA E	GL	GL E	25	7
BG4	Iran, East Azerbaijan Province, the moat by the road 5 km W from the city Miyáneh, N 37.421198, E 47.671425, 18.5.1997, leg. Kaplan Z., Sádlo J., cultivated plants	GLA E	GL	GL AF	15	5
BG5	Greece, the Zakynthos island, southward from the village Kalamaki, ditch 0.5 km from the coast, N 37.739980, E 20.901019, 18.8.2005, leg. Moravcová L., cultivated plants	GLA E	GL	GL E	25	5
BG6	Portugal, Border of ploughed field in lower part of the Paúl do Boquilobo Natural Reserve, 20km NE of the town of Santarém, N 39.375291, W 8.533551, 15 m, 23.9.1998, leg. Hroudová Z. & Zákravský P., cultivated plants	GLA E	GL	GL E	25	5
BG7	Montenegro, Budva, village Lastva, Jaz, nearby seashore, sand dunes in marshy, subhalophyte places. N 42.283889, E 18.802778, 5 m, 10.6.2007, leg. Ducháček M., cultivated plants	GLA E	GL	GL E	25	5
BG8	Romania, field on the left side of the Danube river and the road between towns Moldova Veche and Cornini, N 44.707161, E 21.664797, 85 m, leg. Krahulec F. & Skálová H., cultivated plants	GLA E	GL	GL E	6	5
BG10	Croatia: Zadar County: NW shore of Lake Vrana (Vransko jezero) 2.7 km NNE of Pakoštane; sea level. N 43.938333, E 15.515833, 5.8.2014, leg. Kaplan Z., no. 3144, cultivated plants	GLA E × MAR	-	-	-	-
BG11	Africa, Senegal, Bas Senegal, Ross-Bethio, in rice field N N16.279167, W16.161111, 5 m, 8.9.2014, leg. Mesterházy A., cultivated plants	GLA AF	-	GL AF	-	-
BG12	Africa, Senegal, Niayes, Pikine (Dakar), N 14.769444, W17.403889, 6.9.2014, 8 m, leg. Mesterházy A., cultivated plants	GLA AF	GL	GRA	-	-
BG13	Africa, Senegal, Soudan, edge of swamp, Koutal, N 14.094722, W16.077778, 4 m, 24.9.2014, leg. Mesterházy A., cultivated plants	GLA AF × ?	GL	GL AF	-	-
BG14	Africa, Senegal, Bas Senegal, Richard Toll, in rice field, N 16.380000, W15.911944, 4 m, 9.9.2014, leg. Mesterházy A., cultivated plants	-	-	GL AF	-	-

Acronym	Locality and date of collection	AFLP	ITS	cp	Ninf	Na
BG15	Africa, Senegal, Bas Senegal, Saint Louis, edge of brackish swamp, N 15.956944, W16.477222, 3m, 8.9.2014, leg. Mesterházy A., cultivated plants	GLA AF	-	GL AF	-	-
Bolboschoenus grandispicus						
BGR1	Senegal, Niayes, Dakar: Rufisque, abandoned rice field at the edge of swamp, 3 m, N 14.751111, W 17.339167, 6.9.2014, leg. Mesterházy A., cultivated plants	GLA AF × ?	EA	GRA	16	3
Bolboschoenus laticarpus						
BL9	Canada, Ontario, Lambton, W. Darcy McKeough Floodway (St. Clair Region Conservation Authority) Roberts, North Sydenham River. Edge of reservoir and adjacent ditch; distributed ground, N 42.6936201, W 82.40332291, 19.7.2005, leg. Oldham M. J. (No. 31755), cultivated p	?	EA	EA	25	5
BLHua	China, Qinghai, Xining, Huangzhong, N 36.50, E 101.57, cultivated plants	LAT AS	ME	EA	25	5
BL11_W	China, Wuhan, N 30.58, E 114.29, 2008, Mei Yang, cultivated plants	LAT AS	EA	EA	25	5
BL_AU2	Austria, Lower Austria, the fishpond shore near railway bridge at eastern border of the town Bernhardsthal, N 48.692783, E 16.877217, 160 m, 4.8.2011, leg. Píšová S., Hroudová Z., Fér T., field samples	LAT E	EA	EA	25	25
Bo	Czech Republic, North Moravia, field depression near north-western border of the village Bohuslavice, ca. 6 km N of the town Mohelnice, N 49.827783, E 16.936117, 260 m, 5.8.2011, leg. Píšová S., Hroudová Z., Fér T., field samples	LAT E	-	-	25	25
DVl	Czech Republic, South Moravia, wet depression in field 0.5 km W of the village Dolní Věstonice, N 48.886383, E 16.630283, 170 m, 4.8.2011, leg. Píšová S., Hroudová Z., Fér T., f.s.	LAT E	-	-	25	25
Nt	Czech Republic, Central Bohemia, Prague city, wet depression in field near Netluky farm-house, near the road Uhříněves – Koloděje, N 50.044450, E 14.615550, 270 m, 21.7.2010, leg. Píšová S., Hroudová Z., Zákravský P., field samples	LAT E	-	-	25	25
SK-MA	Slovakia, Záhorie lowland, wet depression in field N of the road Plavecký Štvrtok – Láb, ca. 800 m NE of the village Láb, N 48.369717, E 16.980833, 190 m, 3.8.2011, leg. Píšová S., Hroudová Z., Fér T., field samples	LAT E	-	-	25	25
SK4	Slovakia, Záhorie lowland, wet depression in field near the road Vysoká pri Morave – Záhorská ves, 2.4 km NW of the village Vysoká pri Morave, N 48.347217, E 16.891950, 180 m, 3.8.2011, leg. Píšová S., Hroudová Z., Fér T., field samples	LAT E	-	-	25	25

Acronym	Locality and date of collection	AFLP	ITS	cp	Ninf	Na
Bolboschoenus maritimus						
BMa2	Germany, Münzenberg, wet field depression near the railway station by NW edge of the town Münzenberg; about 9 km N from the town Bad Nauheim, N 50.464079, E 8.768846, 6.9.2003, leg. Hroudová Z., Zákravský P., Gregor T., cultivated plants	MAR	EA	EA	25	5
BMa3	Iran, Fárs province, Qaderabad, 8 km S of the town, near bridge, N 30.230022, E 53.271334, 1997, leg. Sádlo J., cultivated plants	MAR	EA	EA	25	5
BMa4	Iran, West Azerbaijan, Maku, Buralan wetland, N 39.72, E 44.58, 2007, leg. Amini Rad M., cultivated plants	MAR	EA	EA	25	25
BMa5	Germany, Sachsen-Anhalt, surroundings of saline "slagheap", Salzstelle Teutschenthal W of the town Halle, N 51.453056, E 11.815556, 100 m, cultivated plants	MAR	EA	EA	25	25
BMa7	France, salt marshes in the Laita estuary near the town of Concarneau, N 47.77, W 3.53, 27.8.2002, leg. Collias É., cultivated plants	MAR	EA	EA	25	5
BMa8	Sweden, the coast by the town of Barsebäckshamn, N 55.755, E 12.904, 1990, leg. Hroudová Z. & Vidén M., cultivated plants	MAR	EA	EA	25	5
BMa9	France, la Camarque, Rhône Delta, Tour du Valat Wildlife Reserve 1, marsh Emprunt Nord Tamarquiron, N 43.50, E 4.50, leg. Charpentier A., cultivated plants	MAR	EA	EA	25	25
BMa13	Kazakhstan, East Kazakhstan, the basin around the village of Sarydzhaz, between the mountains Gory Basulytau and Ketmen Tau, Karasaz: wet saline margins of muddy pools in broad ditch along the road from Sarydzhaz to Karasaz, 10 km NE of the village of Sarydzhaz, N 42.946778, E 79.704944, 1887 m, 2008, leg. Štěpánek J. & Kirschner J., cultivated plants	MAR	EA	EA	25	5
BMa48	Turkey SE, Iskenderun bay, seaside with the border of sand dunes, N 36.60, E 36.19, 3. 4. 1998, leg. Adamec L., cultivated plants	MAR	EA	EA	25	5
BTM	Turkey, Mersin, a lagoon 18.5 km S from the city Tarsus and 2.6 km N from Seyhan River estuary, N 36.742328, E 34.897939, 22. 3. 2013, 0 m, leg. Kubešová M., field sample	MAR	EA	EA	-	-
BDA	Denmark, Jylland (Jutland), Region Syddanmark (Region of Southern Denmark): Højer, along the drainage ditch ca 2,7 km SSW of the village, N 54.938028, E 8.684583, 3. 8. 2012, leg. Prančl J. et al., field samples	MAR	EA	EA	25	5
IR	Ireland, Aran Islands, Inishmore, sea marsh, N 53.137639, W 9.710361, 12 m, 29.7.2011, leg. Kolar F., Urfus T., Vit P., field samples	MAR	EA	EA	10	-

Acronym	Locality and date of collection	AFLP	ITS	cp	Ninf	Na
JAR	South Africa, Tienie Versfeld Wild Flower Reserve, 11 km from the town Yzerfontein, S 33.336722, E 18.275056, 26.8.20011, leg. Fér T., 24.9.2014, Suda J. & Chumová Z., field samples	MAR	EA	EA	-	-
BMa49	Canada, Ontario, Nova Scotia, Yarmouth, Roberts Island, salt marsh, N 43.76673915, W 65.90387123, 100m, 13.9.2010, leg. Oldham M J. & COSEWIC Vascular Plants SSC, cultivated plants	ROB × MAR	RO × ?	EA	25	5
BMa_ko1	South Korea, Ganghwa-gun, Donggeom-RI, N 37.589750, E 126.520111, 5 m, 11.6.2008, AJOU Herbarium 806004, leg. Jung J. D., herbarium speciemen	PLA AS	EA	EA	-	-
BMa_ko2	South Korea, Ganghwa-gun, Donggeom-RI, N 37.589750, E 126.520111, 6 m, 11.6.2008, AJOU Herbarium 806008, leg. Jung J. D., herbarium speciemen	PLA AS	EA	EA	-	-
Bolboschoenus medianus						
BM1	Australia, The valley E of Adelaide, S 34.926931, E 138.719194, 2006, leg. Ganf G., cultivated plants	MED	ME	EA	25	7
Bolboschoenus novae-angliae						
BNA1	U.S.A, Delaware, Delmarva peninsula, north side of Swan Creek, west side of New Wharf Rd., north New Wharf Rd. Bridge, northeast of Milford, brackish tidal marsh, N 38.938917, W 75.403983, 4.4.2010, leg. McAvoy W. A., cultivated plants	FLU × ROB	FL	RO	-	-
Bolboschoenus paludosus						
BPa1	U.S.A, Nebraska, Lincoln, shore of Oak Lake, N 40.83, W 96.717, 2008, leg. Kaul R. B., cultivated plants	PAL	PAL	PAL	25	5
BPa2	U.S.A, Nebraska, Lincoln, shore of Oak Lake, N 40.83, W 96.717, 2008, leg. Kaul R. B.,	PAL	PAL	PAL	25	5
BPa3	U.S.A, Nebraska, Lincoln, shore of Oak Lake, N 40.83, W 96.717, 2008, leg. Kaul R. B.,	PAL	PAL	PAL	25	5
BPa4	U.S.A, Nord Dakota, Stutsman County. Via a gravel road ca. one mile west of North Dakota Hwy, ca. 30 miles west of Jamestown, 25.9.2011, N 46.67, W 99.00, leg. Smith, D. Mushet S. G., cultivated plants	PAL	PAL	PAL	25	5
BPa5	U.S.A, Utah, the Utah lake 59 km S from the Salt Lake City, N 40.198417, W 111.886917, leg. Mandák B., cultivated plants	PAL	PAL	PAL	5	5

Acronym	Locality and date of collection	AFLP	ITS	cp	Ninf	Na
Bolboschoenus planiculmis						
BPl1	Czech Republic, Louny, Lenešice, former sandpit, southern bank of the western pond 500 m NE from the railway station, N 50.380556, E 13.779722, 185 m, 1.6.2001, leg. Ducháček M., cultivated plants	PLA E	EA	EA	25	5
BPl4	Japan, Aomori, Rokkasho, Obuchinuma lake, 2000, N 40.96, E 141.36, leg. Sudoh K. (seeds sent by Suzuki J. I.), cultivated plants	PLA AS	EA	EA	25	5
BPl8	China, Hangzhou, N 30.26, E 120.14, 2007, leg. Yang M., cultivated plants	PLA E?	EA	EA	25	5
BPl10	Kazakhstan, East Kazakhstan, distr. Iliyskii, the brook in the valley between Baiserke and Zhanaarna villages, E of the highway A3 from Almaty to Kapshagai, ca. 15 km N of the city of Almaty, N 43.527750, E 77.003833, 577 m, 2008, J. Štěpánek & J. Kirschner, cultivated plants	PLA E	EA	EA	25	5
BPl11	Poland, Krościna Mała, small fishpond at S border of the village, ca. 1 km W of the village of Prusice (near the road No. 5 from Trzebnica to Żmigród), N 51.371047, E 16.943072, 2003, leg. Hroudová Z. & Zákravský P., cultivated plants	PLA E	EA	EA	11	5
BPl_AU1	Austria, Lower Austria, shore of the small fishpond near the road Poysdorf – Herrnbaumgarten, near southern border of the village Herrnabumgarten, N 48.688617, E 6.672217, 190 m, 4.8.2011, leg. Píšová S., Hroudová Z., Fér T., field samples	PLA E	EA	EA	25	25
BPl31	China, Xiasha, Hanghzhou, N 30.30, E 120.355, 2008, leg. Yang M., cultivated plants	PLA E	EA	EA	25	5
BPl33	Russia, town Novosibirsk, village Karasevo, N 54.166083, E 83.002306, leg. Chrtek J., cultivated plants	-	-	EA	-	-
BJK	Czech Republic, South Moravia, wet depression in the meadow near the pool Kutnar, 3 km SW of the village Rakvice, N 48.836667, E 16.793617, 180 m, 29.6.2011, leg. Píšová S., Hroudová Z., Fér T., field samples	PLA E	EA	EA	25	25
BPl_ko1	Japan, Chiba, Sanmu, Hasunumaro, Hasunuma Garden House Marino, N 35.598889, E 40.516111, 8 m, 3.5.2007, AJOU Herbarium 821-2, leg. Ito Y., herbarium specimen	PLA AS	EA	EA	-	-
BPl_ko2	China, Yunnan, Yuxi, Zhuangzi, N 24.454056, E 102.858417,1708 m, 15.7.2010, AJOU Herbarium 1007153, leg. Jung J. D., herbarium specimen	PLA E	EA	EA	-	-
BPl_ko3	South Korea, Ganghwa-gun, Donggeom-RI, N 37.589750, E 126.520111, 5 m, 11.6.2008, AJOU Herbarium 806008, leg. Jung J. D., herbarium specimen	PLA AS	EA	EA	-	-

Acronym	Locality and date of collection	AFLP	ITS	cp	Ninf	Na
BPl_ko4	South Korea, Incheon, Ganghwado island, 2.3 km E from the town Gilsang-myeon, N 37.632278, E 126.532528, 11.6.2008, AJOU Herbarium 806014, leg. Jung J. D., herbarium specimen	PLA AS	EA	EA	-	-
Bolboschoenus robustus						
BR1	USA, California, China Camp State Park, San Pablo Bay, N 38.000598, W 122.461023, 2006, leg. Rejmánková E., cultivated plants	ROB	ROB	ROB	25	5
BR2	USA, Texas, Brazoria County, Brazoria National Wildlife Refuge. Roadside of auto tour loop near Big Slough. N 29.060875, W 95.236503, 28. 10. 2008, leg. Adams T., cultivated plants	ROB	ROB	ROB	25	5
BR3	USA, Texas, Chambers County. Roadside ditch on the north of roadside of Business Highway 146, 2.4 km east of its intersection with Highway 146 proper, in the town of Baytown. N 29.714833, W 94.981667, 23.10. 2008, leg. Rosen D. J., cultivated plants	ROB	ROB	ROB	9	5
Bolboschoenus schmidii						
BSch1	Iran, Semnan, Biarjomand, Touran protected area, Kuh-e Majerad, Kalat-Asbe, N 35.62, E 55.78, 27.6.2007, leg. Amini Rad M. & Torabi H., cultivated plants	?	SCH	SCH	16	11
Bolboschoenus yagara						
BY1	Japan, Honsu II, Yamagata pref., Murayama-shi, Tateoka, Lake Osawa-chosuichi (E shore), N 38.488639, E 140.400528, 110 m, 9.10.2007, leg. Hayasaka E., cultivated plants	YAG	YAG	-	25	5
BY4	Japan, Honsu I, Yamagata pref., Murayama-shi, Tateoka, Lake Osawa-chosuichi (N shore), N 38.482361, E 140.402528, 130 m, 9.10.2007, leg. Hayasaka E., cultivated plants	YAG	YAG	YAG	25	5
BY6	Czech Republic, South Bohemia, the fishpond Ostrý ca. 600 m S of the village Kolence, 5 km E of the town Lomnice n. Lužnicí, N 49.084450, E 14.784717, 420 m, 29.7.2010, leg. Píšová S., Hroudová Z., Zákravský P., field samples	YAG	YAG	YAG	17	25
BY7	Germany, small fishpond (Kleiner Forstteich) near the road Kamenz-Oßling, ca. 800 m N from the village of Milstrich, N 51.334917, E 14.158944, 141 m, 27. 8. 2004, leg. Hroudová Z. & Zákravský P., cultivated plants	YAG	YAG	YAG	4	5
BY8	Poland, fishpond SE of the village of Wola, 2 km N of the village of Brzeszcze, N 50.000139, E 19.142972, 233 m, 2003, leg. Hroudová Z., Zákravský P. & Wójcicki J., cultivated plants	YAG	YAG	YAG	5	5

Acronym	Locality and date of collection	AFLP	ITS	cp	Ninf	Na
BY9	Czech Republic, South Bohemia, Lipno dam reservoir, the bay near the village of Nová Pec, N 48.79038, E 13.95836, 727 m, 1999, leg. Sádlo J., cultivated plants	YAG	YAG	YAG	1	5
BY10	Slovakia, Orava reservoir, exposed shore in the bay S from the village Zubrohlava, N 49.418444, E 19.514139, 24.6.2011, Z. Hroudová et al., cultivated plants	YAG	YAG	YAG	25	5
BKO	Czech Republic, South Bohemia, north-eastern shore of the fishpond Koclířov, ca 1 km SW of the town Lomnice n. Lužnicí, N 49.077217, E 14.701383, 425 m, 16.6.2011, leg. Píšová S., Hroudová Z., Zákravský P., field samples	YAG	YAG	YAG	25	25
BKR	Czech Republic, South Bohemia, the fishpond Králek 3.2 km ESE of the town Kardašova Řečice, N 49.176269, E 14.896160, 470 m, 28.7.2011, leg. Píšová S., Hroudová Z., Zákravský P., field samples	YAG	YAG	YAG	25	25
BVO	Czech Republic, South Bohemia, the fishpond Velká Ochoz, 2 km S of the town Kardašova Řečice, N 49.164577, E 14.839472, 430 m, 20.9.2010, leg. Píšová S., Hroudová Z., Zákravský P., field samples	YAG	YAG	YAG	25	25
BF_ko2	South Korea, 10 km from the town Goseong, N 38.473028, E 128.430667, 10 m, 27.8.2008, AJOU Herbarium 808308, leg. Jung J. D., herbarium specimen	YAG	YAG	YAG	-	-
BF_ko3	China, Jilin, 21 km SW from the town Dunhua, N 43.211694, E 128.075778, 627 m, 15.7.2010, AJOU Herbarium 1007053, leg. Jung J. D., herbarium specimen	YAG	YAG	YAG	-	-
BF_ko4	South Korea, 9 km S from the town Boryeong, N 36.255028, E 126.623639, 15 m, 15.6.2008, AJOU Herbarium 806035, leg. Jung J. D., herbarium specimen	-	YAG	YAG	-	-
Australia	Australia, Victoria, PR Herbarium, herbarium specimen	-	-	-	-	6
Bolboschoenus fluviatilis* × *paludosus						
BFL×PA	Canada, British Columbia, Cariboo District, Dry Lake, 4 km southwest of bridge over Fraser River (Gang Ranch Bridge); seasonal lake, N 51.502778, W 122.329167, 635 m, 22.9.2016, leg. Steen O., cultivated plants	FLU × PAL	PAL	PAL	-	-

Curriculum Vitae – Soňa Píšová

* 13.8.1982, Varnsdorf, Czech Republic

Research interest:

- speciation of wetland and aquatic plants
- homoploid hybridization
- use of molecular markers in plant systematics (AFLP, SSR´s, Hyb-Seq)

Education and jobs:

since 2011: Laboratory of Flow Cytometry, Institute of Botany, ASCR (laboratory technician)

2009-2011: DNA laboratory at Department of Botany, Faculty of Science, Charles University in Prague (part-time job; laboratory technician)

since 2009: Ph.D. study in Botany at Department of Botany, Faculty of Science, Charles University in Prague; Ph.D. Thesis: "Homoploid hybrid speciation in closely related taxa of wetland plants". (supervisor: Mgr. Tomáš Fér, Ph.D.)

2006-2009: MSc. study in Vascular Plant Botany at Department of Botany, Faculty of Science, Charles University in Prague; MSc. Thesis: Evaluation of variability within *Sparganium erectum* using morphometrics, AFLP and flow cytometry. (supervisor: Mgr. Tomáš Fér, Ph.D.)

2001-2005: BSc. study in Ecology and Environment Conservation at Department of natural sciences, Faculty of Environment, Jan Evangelista Purkyně Univerzity in Ústí nad Labem; BSc. Thesis: Examination of distribution of genus *Utricularia* in phytogeographical districts No. 52 Ralsko-Bezděská tabule and No. 53 Podještědí. (supervisor: RNDr. Iva Machová, Ph.D.)

Awards:

Second best poster presentation at BioSyst.EU 2013 Global systematics conference in Vienna.

Stays abroad:

November 2012 and 2013: University of Natural Resources and Life Science, Vienna, Participation in the project: "Polyploidy, ecological niche and demographic development of the wetland annual plant species *Cyperus fuscus*", supervised by Dr. Karin Tremetsberger.

Grant projects

Principal researcher:

2011–2013: Parallel evolution or migration in the genus *Bolboschoenus* (Cyperaceae) at worldwide scale? (GAUK 428311/2011)

Member of the team:

2016-2018: Old wine in new skin: the role of polyploidization and hybridization in an adaptively-radiated plant group using high-throughput sequencing methods. (GAČR, GJ16-15134Y, principal investigator R. Schmickl)

2013–2015: Remarkable cytotype co-existence of *Pilosella echioides*: the only known sexual system with triploid dominance. (GAČR, GP13-18610P, principal investigator P. Trávníček)

2010–2013: Immigrated vs. in situ created diversity in postglacial areas: A story of the polyploid complex *Galium pumilum* agg. (GA ČR, P506/10/0704, principal investigator J. Suda)

SCI – Publications:

Píšová S. & Fér T. (submitted): Homoploid hybrid speciation in *Sparganium erectum*: molecular, genome size and morphometric analyses.

Píšová S., Hroudová Z., Chumová Z. & Fér T. (2017): Ecological hybrid speciation in central-European species of *Bolboschoenus*: genetic and morphological evaluation. – Preslia. 89: 17–39.

Kolář F., **Píšová S.**, Záveská E., Fér T., Weiser M., Ehrendorfer F. and Suda J. (2015): The origin of unique diversity in deglaciated areas: traces of Pleistocene processes in north-European endemics from the *Galium pusillum* polyploid complex (Rubiaceae). Molecular Ecology, 24: 1311–1334.

Non-SCI publications:

Píšová S., Hroudová Z., Fér T., Ducháček M., Zákravský P. (2015): Do we know our *Bolboschoenus* species? Morphological, ecological and genetic interests. Živa 4: 165–168.

Píšová S. & Fér T. (2017): Intraspecific Variability and Crossbreeding of the Branched Bur-reed. Živa. 3:108–111.

Abstracts and posters:

Píšová S., Fér T. & Hroudová Z. (2013): Genetic variation and hybridization in the genus *Bolboschoenus* in Central Europe. BioSyst.EU 2013, Vienna, Austria, 18-22.2.2013 [poster].

Píšová S. & Fér (2012): Genetic variation and hybridization within the genus *Bolboschoenus* in Central Europe. International Conference on Polyploidy, Hybridization and Biodiversity, Průhonice, Czech Republic, 7-10.5.2012 [poster].

Fér T. & **Píšová S.** (2012): Speciation and hybridization within branched bur-reed (*Sparganium erectum* L.): subspecific concept based on AFLP, morphometrics and genome size. International Conference on Polyploidy, Hybridization and Biodiversity, Průhonice, Czech Republic, 7-10.5.2012 [poster].

Píšová S. & Fér (2011): Evaluation of variability within *Sparganium erectum* L. using morphometrics, AFLP and flow cytometry. BioSystematics Berlin 2011, 21-27.2.2011 [poster].