Taxonomic principles, reproductive systems, population genetics and relationships within selected groups of genus *Taraxacum* (Asteraceae)

Disertační práce— Doctoral thesis

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Part I

Preface
Chapter 1

Declaration — prohlášení

I declare I prepared this work independently. This thesis, neither any of its parts were not used to obtain any other academic degree. All used references are listed on the end of the thesis.

In Prague, 30th May 2018

Prohlašuji, že tuto práci jsem vypracoval samostatně. Tato práce ani její část nebyla použita k získání jiného akademického titulu. Všechny použité prameny jsou uvedeny v seznamu literatury.

V Praze dne 30. května 2018
Chapter 2

Acknowledgements

I would like to thank especially to my wife for her kind taking care of my welfare during writing the thesis. Big thanks go to my adviser for all his help and patience. I thank to all my colleagues participating to all of our shared projects for nice and inspirative co-work and their good hints in all phases of my work. Last but not least I thank to all authors of open-source software for their mostly volunteer investment of time in creation of great working tools.
Part I. Preface

Chapter 2. Acknowledgements

6

On the *Taraxacum* taxonomy etc. Vojtěch Zeisek (2018)
Chapter 3

Summary — shrnutí

English and Czech summaries (abstracts) of the thesis.

3.1 English abstract

Genus *Taraxacum* (Asteraceae), having ~60 sections and 2,800 species, is known for its complicated evolutionary relationships and taxonomy due to processes like frequent hybridization, polyploidization, asexual reproduction, clonality and low structural morphological variability. Various taxonomical concepts and approaches are reviewed, evaluated and discussed from point of view of their ability to deal with such a complicated genus as is *Taraxacum*. Various processes responsible for the complicated situation within *Taraxacum* are discussed and reviewed.

Section *Dioszegia*, comprising *T. serotinum* and its allies, are an exception because only sexuals are reported for all the members of this group. On the basis of the analysis of microsatellite (SSRs) variation, distribution and morphology, we addressed problems related to their mode of reproduction, among-population relationships, taxonomy and within-population variation. As a rule, outcrossing was the dominant mode of reproduction, with one notable exception: *T. serotinum* subsp. *tomentosum* (= *T. pyrrhopappum*) was autogamous and not heterozygous. A taxonomic revision of sect. *Dioszegia* recognizes *T. serotinum* subsp. *serotinum* (including an aberrant taxon, newly described as var. *iranicum*), *T. serotinum* subsp. *tomentosum* and *T. haussknechtii*.

There has been a decrease in the ability of biologists to identify their material correctly, particularly plants of complicated genera with common agamospermy, where old clonal entities are accorded the rank of species (microspecies), like *Taraxacum*. Agamospermous microspecies are taxonomic entities recognizable from one another by a set of minute morphological features. The knowledge of microspecies is confined to a few specialists. A selection of nine widespread, generally recognized agamospermous microspecies of *Taraxacum* sect. *Taraxacum*, which are characterized by means of eight microsatellite loci, were used to evaluate the ability of four European *Taraxacum* specialists to identify these microspecies consistently. With two exceptions (and one unclear result) for 125 plants coming from an area extending from Finland to central Europe, the experts identified the microspecies
consistently, exclusively on the basis of morphological differences. The within-species microsatellite variation corresponded to the mutational clone cluster hypothesis, with a single unclear result. Each microspecies consisted of one, more or less dominant, clone and several minority clones, each usually confined to a single plant.

The *Taraxacum* flora of the West Himalaya represents one of the dandelion diversity hotspots, with at least 17 sections and about 150 known species. A number of names published from that region were referred to *T.* sect.*Orientalia* Handel-Mazzetti in the literature. All these names are revised and newly interpreted, with emphasis on plants erroneously determined as *T. stenolepium*. An nrDNA ITS sequence analysis including the only sexual member of *T.* section *Squamulosa* and the other sexual taxa known in *Taraxacum* shows a separate position of *T.* sect. *Squamulosa*. The new section is compared with sections *Primigenia*, *Coronata* and *Orientalia*.

*Taraxacum koksaghyz*, dandelion from steppes of Kazakhstan, has been known for long time as potential rubber producer, possibly replacing currently the most popular rubber producing tropical tree *Hevea brasiliensis*. We evaluate its closely related congener, *Taraxacum bicorne*. Its taxonomy is reviewed, population genetic characteristic evaluated, and rubber content of the two species is compared. For the rubber extraction we modified existing method to require minimal amount of material. *Taraxacum bicorne* is shown to be outcrossing sexual diploid and its rubber content is about half of that of *T. koksaghyz* (~3.2% vs. ~7.2%), but because of relatively robust constitution of *T. bicorne* in comparison to *T. koksaghyz*, *T. bicorne* could be used as potential rubber source.

The taxonomy, micromorphology, karyology and evolutionary relationships of *Taraxacum bithynicum* DC. were studied using the original material and new samples from the summit area of Mt. Uludağ, Bursa Province, Turkey. It is sexual with 2n = 16, considerably isolated in outer phyllary and achene characters. The nrDNA ITS NeighborNet analysis shows relationships of *T. bithynicum* with members of sect. *Scariosa*. *Taraxacum bithynicum* is considered as a taxon endemic to the summit area of Uludağ.

All these case studies shed more light on the taxonomy, population genetics and undergoing mechanisms within genus *Taraxacum* — real touchstone of plenty of biological concepts, theories and methods.

**Keywords:** agamospermy, autogamy, clonality, Europe, evolution, Iran, isolation by distance, microsatellites, natural rubber, new section and species, nrDNA ITS, plant identification, population genetics, population variation, reproduction, reproductive systems, systematics, *Taraxacum, Taraxacum* sect. *Dioszegia, Taraxacum* sect. *Orientalia, Taraxacum* sect. *Squamulosa*, taxonomy, the West Himalaya.

### 3.2 Český abstrakt

Rod *Taraxacum* (pampeliška, hvězdnicovité), mající ~60 sekci a 2 druhů, je známý pro své komplikované evoluční vztahy a taxonomii díky procesům jako je častá hybridizace, polyploïdizace, nepohlavní rozmnožování, klonála a nízká strukturální morfologická va-
riabilita. Různé taxonomické koncepty a přístupy jsou v práci představeny, diskutovány a zdůrazněny z pohledu jejich schopnosti poradit si s tak komplikovaným rodem jako je _Taraxacum_. Různé procesy zodpovědné za tuto komplikovanou situaci v rámci pampelišek jsou diskutovány.


Jedním z kardinálních problémů současného přírodozpytu je klesající schopnost biologů správně určit rostlinný materiál. To zvláště platí pro rody s neobvyklými reprodukčními způsoby, např. koexistencí sexuality a agamospermie, kdy jednotlivé taxony jsou si značně podobné, jako je tomu v rodu _Taraxacum_. Jednotlivé klonální (oligoklonální) entity v takových skupinách jsou obvykle popisovány jako tzv. drobné druhy (mirospecie), navzájem rozeznatelné na základě souboru drobných morfologických rozdílů. Znalost takových mikrospecií je obvykle omezena pouze na úzkou skupinu specialistů. Vybrali jsme proto 9 široce rozšířených a běžně rozeznávaných druhů rodu _Taraxacum_ ze sekce _Taraxacum_ (pampeliška smetánka), určených čtyřmi specialisty z geograficky vzdálených oblastí (Finsko a střední Evropa). Soubor 125 rostlin jsme analyzovali pomocí 8 značně variabilních mikrosatelitových lokusů. Tyto molekulární markery rozčlenily použitý materiál na 9 shluků odpovídajících často nezajímavým druhům. Ukázal se, že u 122 rostlin z tohoto souboru identifikace expertů odpovídala geneticky charakterizovaným shlukům. Dva vzorky byly určeny mylně a jeden zůstal nejasný. Jednotlivé genotypy jsou určeny jednou, zvláště na různých lokalitách, jak ve Finsku, tak ve střední Evropě. Dalšího studovaný problém byla genetická variabilita v rámci geneticky i morfologicky charakterizovaných skupin, tj. mkrospecii, která odpovídala hypotéze, že mikrospecie jsou oligoklonální, obvykle s jedním dominantním klonem a několika přidruženými, velmi podobnými genotypy, zpravidla omezenými na jednu rostlinu a odvoditelnými pomocí mutací.

_Taraxacum_ ze západního Himálaje reprezentuje s minimálně 17 sekcemi a 150 známymi druhy jedno z vývojových center rodu. Množství jmen popsaných z této oblasti se v literatuře odkazuje ke sekci _Orientalia_ Handel-Mazzetti. Všechna tato jména byla zrevidována a nově interpretována, s důrazem na rostliny dříve chybějící. Toto _T. stenolepium_. Analýza nrDNA ITS sekvencí zahrnující jediného pohlavně se rozmnožujícího zástupce sekce _Squamulosa_ a ostatní pohlavně se rozmnožující druhy známé v rodu _Taraxacum_ uka-
zuji oddělenou pozici sekce Squamulosa. Tato nová sekce je porovnána se sekcemi Primigenia, Coronata a Orientalia.

*Taraxacum koksaghyz*, pampeliška z kazašských stepí, je již dlouho známa jako možný zdroj přírodního kaučuku, jako možná náhražka aktuálně nejpopulárnějšího zdroje přírodního kaučuku, tropického stromu *Hevea brasiliensis*. Zhodnotili jsme blízce příbuzný druh, *Taraxacum bicorne*. Zhodnotili jsme jeho taxonomii, populačně-genetické charakteristiky a produkci kaučuku a porovnali jsme oba druhy. Pro extrakci kaučuku jsme upravili existující metodiku tak, aby sho snadno pracovat i s minimálním množstvím materiálu. *Taraxacum bicorne* se ukázal být pohlavně se rozmnožující cizosprašný diploid, přičemž jeho obsah kaučuku je ve srovnání s *T. koksaghyz* asi poloviční (~3.2% vs. ~7.2%), nicméně díky robustější konstituci *T. bicorne* ve srovnání s *T. koksaghyz*, se *T. bicorne* jeví jako možný zdroj přírodního kaučuku.

Taxonomie, mikromorfologie, karyologie a evoluční vztahy *Taraxacum bithynicum* DC. byly studovány za využití originálního materiálu a nových sběrů z vrcholových partií Mt. Uludağ, provincie Bursa, Turecko. Jedná se o pohlavně se rozmnožující druhy s 2n = 16, značně izolovaný s ohledem na vnější zákrov a nažky. NeighborNet síť na základě nrDNA ITS ukazuje blízkou příbuznost *T. bithynicum* s druhy sekce Scariosa. *Taraxacum bithynicum* je vyhodnocen jako endemit vrcholových partií Uludağu.

Všechny tyto případové studie vnáší více světla na taxonomii, populační genetiku a související mechanismy v rámci rodu *Taraxacum* — opravdového prubířského kamene biologických konceptů, teorií a metod.

Chapter 4

Included papers

Individual papers are included as parts of this work, see citations below. All references from all articles are listed together on the end of whole work. Published as well as submitted papers were typeset in the same style as the introduction of the thesis (chapter II, page 17), the content was not changed\(^1\). Supervisor’s statements specify contribution of the student for the respective papers.


   Substantial contribution of the student. Complete work with material, laboratory work, statistics, programming, substantial part of the interpretation.


   Substantial contribution of the student. Innovative interpretation of SSRs data analysis (including work with three source genomes of triploids), complete statistics, programming and substantial part of the interpretation and good portion of the discussion.


\(^1\)Except for typography, styles, obvious typos, mistakes, etc.
Substantial contribution of the student. Complete analysis of novel evolutionary relationships among sexual members of *Taraxacum*, complete statistics, contribution to the discussion.


Substantial contribution of the student. Processing of the material for analysis, complete laboratory analysis (DNA anal, including population genetics, as well as analysis of rubber quantity in the roots), statistics, new modification of the rubber extraction procedure, processing of DNA results, significant contribution to the discussion.


Substantial contribution of the student. Considerable contribution to the text adjustment. Complete analysis of the evolutionary relationships within genus *Taraxacum*, complete statistics, contribution to the discussion.

I, as the supervisor, state, that without the contribution of the student, listed articles would not be created, or would be created with significantly worse quality and impact.

Signature of the supervisor, Průhonice, 30th May 2018 ........................................
Chapter 5

Author

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Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic, https://www.natur.cuni.cz/biology/botany/

Department of Taxonomy, Institute of Botany, Czech Academy of Sciences, Průhonice, Czech Republic, http://www.ibot.cas.cz/
5.1 Published papers


- Yvonne Němcová, Martina Pichrtová and Vojtěch Zeisek (2015). *Mallomonas alpestrina* sp. nov. (Synurales, Chrysophyceae, Stramenopiles) and its spineless relatives—*Mallomonas alata* group. In: *Phytotaxa* 222.2, pp. 111–120. ISSN: 1179-3163. DOI: 10.11646/phytotaxa.222.2.3. URL: https://biotaxa.org/Phytotaxa/article/view/phytotaxa.222.2.3


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14 On the *Taraxacum* taxonomy etc. Vojtěch Zeisek (2018)
Part II

Introduction
Scientists, and earlier philosophers, have been trying for epochs to sort out enormous diversity of life on the Earth. Probably the oldest such system, which had been widely used for most of antiquity and medieval, was created by famous Greek philosopher Aristotle from Stagira (384–322 BC), one of most influential philosophers of whole European history. He in his Τῶν περὶ τὰ ζώα ζώστοριων [Τον peri ta zoia zostorian] (in Latin Historia Animalium)\(^2\) classified animals into ‘animals with blood’ and ‘animals without blood’. Both categories contained sub-categories according to number of legs, if they lay eggs and so on. He also provided formal descriptions of the taxa, of individual species as well as of higher ranks. Unfortunately, we know his writings only from younger (commonly Latin or Arabic) transcriptions and translations and probably no comprehensive botanical writing has survived (if it was ever written). Despite all issues with this system seen by modern science, his work was probably the first systematic attempt to classify living organisms based on objective criteria.

With the development of natural science during modern history, ancient authorities started to be criticised and new methods and paradigms were needed. Medieval botanists used herbaria books containing many primarily medicinal plants (e.g. herbarium Commentarrii ... sex libros Pedacii Dioscoridis... by Pietro Andrea Mattioli (1501–1577) from mid 16\(^{th}\) century\(^3\)) and other species with practical usage. They sometimes included even magical and mythological ‘species’. Instead of simple names, they used short Latin sentences serving as name as well as brief description. Sorting of taxa used to be very variable. With European exploration of the World and bringing new and new specimens, this system was not sustainable any more.

Big methodological leap forward was introduced by Swedish botanist Carl Linné (1707–1778, in Latin Carolus Linnaeus)\(^4\) in his famous books Systema naturae (1735), Fundamenta botanica (1736), Genera plantarum (1737), Species Plantarum (1751) and Philosophia botanica (1753). He introduced binomial nomenclature (combination of the name of genus and species (and author of the description), e.g. Taraxacum serotinum (Waldst. et Kit.) Fischer) and hierarchical system of genera, classes, orders, families, kingdoms, etc. This system has been still used until nowadays (see McNeill et al. 2012). Linné classified plant species mainly according to floral structure and the methodology was well described. Every taxon had brief standardized Latin description and physical herbarium type as an etalon. These principles have been showing the robustness for 3\(^{rd}\) century.

Linné’s aim was to sort out diversity of living organisms to provide practical tool for scientists and other users (everyone knows what particular name means, e.g. for medical usage, gardening and agriculture, conservation and management), as well as celebrate

\(^2\)It can be downloaded from e.g. https://www.biodiversitylibrary.org/creator/16679, where several editions, translations and other writings are available.

\(^3\)Transcriptions of the name are variable. His writings in various editions and translations can be downloaded e.g. from https://www.biodiversitylibrary.org/creator/198141, https://www.biodiversitylibrary.org/creator/966 and https://www.biodiversitylibrary.org/creator/155852.

\(^4\)His writings can be downloaded from e.g. https://www.biodiversitylibrary.org/browse/collection/linnaeus.
God’s work. His theory did not include any evolution (all organisms were created). Species were seen as unchanging units according to the divine plan. Since mid 18th century, scientists like Pierre Louis Moreau de Maupertuis (1698–1759), Georges-Louis Leclerc de Buffon (1707–1788), Erasmus Darwin (1731–1802) or Jean-Baptiste de Lamarck (1744–1829) hypothesized about natural laws responsible for change of species in time. Probably the most advanced was Lamarck’s idea about gradual changes caused by usage of respective organ and inherited from parents (e.g. prolongation of giraffe neck by trying to eat leaves from higher and higher tree branches).

Revolutionary concept was introduced by Charles Robert Darwin (1809–1882) in his famous *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life* (1859). He came with an idea that there is overproduction of offspring with random variability in their adaptation to given conditions, and natural selection choosing the best adapted individuals. He was prompted to publish his most famous book by letter of Alfred Russel Wallace (1823–1913), founder of biogeography, who came with the similar concept and both gentlemen introduced the evolutionary theory together.

Principal weakness of Darwin’s theory was mechanism of heritability of the characters. Unfortunately, Darwin did not know work of his contemporary Gregor Johann Mendel (in Czech Řehoř Jan Mendel, 1822–1884), Moravian abbot, who experimented with *Pisum sativum* and found statistical rules (Mendel 1866), later known as Mendelian inheritance laws. He concluded, that the characters are not inherited directly, but there are some interacting precursors which are inherited and they cause the characters. His work slipped through the cracks and the laws as well as the writings were re-discovered on the beginning of 20th century by Hugo de Vries (1848–1935), Carl Erich Correns (1864–1933), Erich von Tschermak (1871–1962) and William Jasper Spillman (1863–1931).

Unification of Mendelian and Darwinian theory and of other inputs (e.g. molecular biology or population genetics) since early 20th century until nowadays use to be called a ‘modern synthesis’. It is not meaningful to list all players of this famous journey of human exploration of life here, some of them will be referenced in following chapters. Over 250 years after first modern classification we still use same hierarchical system of taxonomic ranks, we do not build our taxonomic systems on appearance of species, but according to evolutionary history of the taxa (Cellinese et al. 2012). Taxonomy uses findings of many natural scientific (not only biological) disciplines.

Taxonomy remains crucial biological discipline. Human mind requires things to be sorted and when we do any research on any species (e.g. ecological, physiological or pharmacological), we need to know delimitation of the species, otherwise we could every time work with something different and all our results would be misleading or unreliable. We must be able to determine any species safely and its name must be used consistently by various experts. Taxonomy tries to build the classification naturally according to evolutionary history of the species. What this statement means in practice, is subject of long lasting

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5His writings (in various versions and translations) are available e.g. from [http://darwin-online.org.uk/](http://darwin-online.org.uk/) and [https://www.biodiversitylibrary.org/creator/93](https://www.biodiversitylibrary.org/creator/93).

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discussions, a few pieces to the mosaic are added in the following chapters. Formal rules of nomenclature are described in the *International Code of Nomenclature for algae, fungi, and plants* (McNeill et al. 2012). The rules describe how to handle the names, not how to delimit the taxon — this depends on the taxonomic concept used by the taxonomist. Taxonomic concepts discussed in chapter 6.1 provide only vague methodological framework how to delimit species and higher taxonomic ranks.
Chapter 6

Species problem

How to define a species? Or better, how to define rules to delimit a species? This question is not so simple as it seems to be and it has been keeping biologists busy for many decades (de Queiroz 2007). It is obvious the answer can differ among various groups of organisms — according to reproduction (sexual or asexual), ploidy level (haploids, diploids, higher polyploids or possible changes of ploidies), life cycle, ecology, gene flow, etc. The problem has many levels: from philosophical (Do species really exist in nature or are they just human mental construct? See e.g. Kitcher 1984; Rieseberg et al. 2006) through theoretical (How can species continuously evolve while being distinguishable and distinct?) to practical (How to use given species/taxonomic concept and how useful it is?).

As I will show later, in genera like Taraxacum (see chapter 7, page 27) we can define up to thousands taxa differing by their morphology, ecology, distribution or limited gene flow (for any reason). Related questions are how did such richness evolved? How is it maintained? How detailed recognition makes sense for particular practical application (ecological study, conservation management, …)? Which species concept is the best for such situation? And why? Can we have one concept to fit whole diversity of life on the Earth?

6.1 Taxonomic concepts

As the Linnean taxonomy (name Taraxacum appears under Leontodon already in von Linné 1753) classified species solely according to their morphological similarity (phenetic approach), after prevail of evolutionary paradigm, biological classification started to reflect presumed evolutionary history and relationships among the taxa. This completely changed our view of species (Mayr 1968). Scientists stopped to see species as invariant entities, but rather as more or less continuously changing units (see e.g. debate of gradualism vs. punctualism in Eldredge and Gould 1972; Gould and Eldredge 1977; Gingerich 1984; Gould and Eldredge 1993). Nowadays, phylogenetic approach, based on evolutionary history and relationships among individuals and taxa, is the most common framework to build up the classification (de Queiroz and Donoghue 1988).

Taxonomy divides into alpha and beta taxonomy. Alpha taxonomy describes and delimits species, while beta taxonomy higher ranks (Mayr 1968). Alpha taxonomists are re-
sponsible for explorative description of new species found in the nature or during revision of samples (e.g. in herbarium). The classification must be not only natural, i.e. reflecting evolutionary history of the taxa, but also practical for usage (otherwise it’d be just an intellectual exercise). What this means in practice varies from approach to approach, see further. Complementary division of taxonomy is into microtaxonomy (works on individual species) and macrotaxonomy (works on higher ranks, Mayr 1982). In the study of *Taraxacum* genus, we stay on the finer end of the scale, working on lower ranks.

### 6.2 Plant species concepts

Taxonomy is a modern science defining and hierarchically sorting biological organisms. Taxonomic classification must be in agreement with evolutionary history of the species. Modern taxonomy approach uses combination of morphological characters (e.g. Kirschner et al. 2016), cytological characters (ploidy level, number of chromosomes, genome size, D. E. Soltis et al. 2003; Suda and Pyšek 2010; Weiss-Schneeweiss et al. 2013, and other articles in Preslia 82 (1)), ecological characteristics or geographic distribution (Marske et al. 2013), physiological characteristics (e.g. content of various alkaloids) and nowadays mainly many molecular methods analysing DNA, see chapter 8 (page 31).

Darwin (1859) was hypothesizing about the period of speciation, where two new species (splitting from one ancestral) are not distinct enough yet, but in his times, no tools able to test such hypotheses were available. Species problem *per se* was probably for the first time systematically addressed by Mayr (1942), who reviewed various approaches applicable to zoology. He used knowledges of recently developing field of genetics (e.g. Dobzhansky 1937) and introduced biological species concept, very natural idea that every species consist of populations, which are able to (at least potentially) mate and produce viable offspring, and are reproductively isolated from each other. Nowadays, this concept use to be credited to both Theodosius Dobzhansky as well as Ernst Mayer (Ayala and Fitch 1997; de Queiroz 2005).

Despite sessility of plants, making this concept slightly problematic, it is widely used also in botany (but see Donoghue 1985, criticizing it as nice theory, but hard to really use practically). This concept is very ’natural’, i.e. close to our common sense, but it requires huge number of information (including time-consuming crossing experiments) and does not deal with problem of hybridization, especially with species of hybridogenous origin or hybridization when parental species are still well distinguishable. These issues are very common in *Taraxacum* (e.g. Kirschner and Štěpánek 1996). Other issue is with the ’potentiality’ of the hybridization: what to do with the taxa successfully hybridizing in the experimental garden, but have no realistic chance to meet in the nature? In practice, decision is in the hands of respective taxonomist. So this concept is not so objective as it appears.

There are plenty of variants more or less derived from the biological species concept (de Queiroz 2005, 2007), commonly taking into account cladistics (Hennig 1950, 1966) and their
usage slightly varies from author to author (e.g. Baum and Donoghue 1995): main differences are if particular concept/author emphasizes history (evolution) or (particular) character or trait. Related concepts emphasize some particular principles like various reproductive isolation mechanisms (can be anything — e.g. geography, ecology or phenology; biospecies species concept, after Mayr 1942) or exclusivity of gene pool and genealogy (e.g. genic species, genetic species, genealogical concordance species G. G. Simpson 1943; Dobzhansky 1950; C. Wu 2001a,b; Hausdorf 2011).

These concepts fluently pass into concept emphasizing shared evolutionary history. Evolutionary significant unit is practically a synonymous concept emphasizing reproductive isolation from other species (lineages) and representation of significant evolutionary unit. Evolutionary species is defined as lineage evolving separately from other lineages. Similar ecospecies concept is slightly relaxing this criterion by emphasizing occupation of particular ecological niche, i.e. the species must form ecologically distinct unit (e.g. Wiley 1978).

Probably most ‘classical’ cladistic approach is Hennig’s species or cladospecies (Hennig 1950, 1966), where each terminal branch of cladogram (i.e. node between two speciations or from speciation to extinction) is one species. These concepts require only monophyletic taxa, i.e. all individuals must share same common ancestor and that ancestor does not have any offspring belonging to other species. Derived concept of phylodpecies calls ‘a species’ smallest monophyletic unit appropriate for phylogenetic analysis, including geographical lineages with some autapomorphy (unique character). Several concepts are derived from this approach (Eldredge and Cracraft 1980; Nelson and Platnick 1981; Cracraft 1983; Nixon and Wheeler 1990; Meier and Willmann 1997). These concepts commonly use coalescence theory (Schaal and Olsen 2000) to delimit species. Although, as shown recently (Sukumaran and Knowles 2017), coalescence can be well able to reveal structure (lineages), but it still can fail to delimit species (Sukumaran and Knowles 2017). Phylogenetic species concept and coalescence gave hope in finding objective criterion for species delimitation, but in fact, drawing border between ‘population’ and ’species’ is still problematic. Such concepts refuse polyphyletic (entities of multiple origin) or paraphyletic (within a monophyletic unit, some, typically small, monophyletic units are not part of the taxon, i.e. not all descendants of one common ancestors belong to same taxon) taxa, only reciprocally monophyletic (clades — all descendants of the common ancestors are members of the clade).\footnote{Well known examples of paraphyletic taxa are reptiles (birds and mammals are nested within them) or dicotyledons (monocotyledons are nested between basal dicotyledons and Eudicots; see https://www.mobot.org/MOBOT/research/Apweb/).}

In such cases, paraphyletic taxa are well understood and their usage is practical in some cases. On the other hand, strict following of cladistic paradigm can require to describe impractically high number of taxa of all ranks. This is main critical point of strict monophyly. During speciation, when some population(s) is/are splitting from the ancestral (meta)population, at least on the beginning the new species must necessarily make the old one paraphyletic. Cladistics is definitely useful framework, but it has many (practical) limitations. Even if we disregard this ‘theoretical backlog’, when we look closely e.g. to

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Taraxacum (Kirschner and Štěpánek 1996; Kirschner et al. 2003, 2015) we find many cases of paleo-hybridization (with alloplody and shift to agamospermy), current gene flow not leading to any stabilized progeny (coexisting sexuals and asexuals) and sexual × sexual hybridization. If we would strictly following cladistic/phylogenetic approach we would have basically two options: to lump nearly whole genus of *Taraxacum* into one species, or split it into nearly as many species as populations. These ‘options’ are not satisfactory.

Although most of the discussion is led by zoologists, botanists do not stay behind (Baum and Donoghue 1995; Hörandl 2006a). Rieseberg et al. (2006) found, that the lack of congruence between discrete phenotypic clusters of over 400 genera of plants and animals and taxonomic species is caused mostly by polyploidy, asexual reproduction and taxonomists-splitters, but not by recent hybridization. Results also show, that 70% of taxonomic species and 75% of phenotypic clusters in plants correspond to reproductively independent lineages, and, surprisingly, plant species represent reproductively independent entities more likely, than animals (Rieseberg et al. 2006). Statistically, plant genera like *Quercus* (Burger 1975), *Hieracium* (Chrtek et al. 2007), *Rubus* (Sochor et al. 2015) or *Taraxacum* (A. Richards et al. 1996; Kirschner et al. 2003) seem to be rather exception than rule.

‘Old good’ biological species concept does not require monophyly of the species, other so far mentioned concepts more or less do. Concepts requiring monophyly (usually somehow derived from Hennig 1950, 1966) work well only for strictly bifurcating lineages. It is not fully applicable for bacteria and other microorganisms (Rosselló-Mora and Amann 2001), which are known for intensive exchange of genes (horizontal gene transfer), hybridization, etc. From plants we also have plenty of examples of auto- (Rieseberg 1997) and alloplody and hybridization, e.g. in genera like *Hieracium* (Chrtek et al. 2007; Fehrer et al. 2007; Tyler and Jönsson 2013), *Taraxacum* (Záveská Drábková et al. 2009; Kirschner et al. 2015) or *Rubus* (Sochor et al. 2015). Such cases are problematic to fit into phylogenetic/cladistic species concepts: we would have to lump large groups into single species, or split some lineages into huge number of taxa. Following species concepts try to deal with that problem (Stace 1998).

Agamospecies (A. Richards 1973; A. Richards et al. 1996; Stace 1998) are asexual lineages, distinct from other lineages by their reproductive isolation (by any means) and distinct ecology and/or morphology. As such species are commonly of hybridogenous origin (e.g. allopolyploidy), application of phylogenetic/cladistic concept is very problematic here. Similar concept of microspecies is not requiring the asexuality, as, like in *Taraxacum* (A. Richards 1973; Hughes and A. Richards 1989; Kirschner and Štěpánek 1996), such distinct lineages do not have to be exclusively asexual. So that calling them ‘microspecies’ is more appropriate. Such concepts do not say much about genetic relationships. Genotypic cluster (Mallet 1995) is generalization of these ideas, going back to the biological species concept. A species is then monophyletic or polyphyletic distinct biological entity. This concept thus does not require reciprocal monophyly and can deal with hybrids (e.g. repeatedly arising allopolyploids). It also more or less lacks formal methodological criteria how to delimit species — it is responsibility of the taxonomist. Principal weakness is then lack of methodological framework what to do in case of disagreement among various experts. Following this
discussion, Dickinson (1998) emphasized role of metapopulation dynamics in such groups of clones as it can serve as methodological framework how to sort out the clonal lineages into natural genetic groups.

This returns us to the Linnean (1735; 1736; 1737; 1751), ‘classical’, morphological, taxonomic or according to Kitcher (1984) ‘cynical’ species concept: species is defined mainly by morphology (it is somehow morphological distinct) and species is just a group of organisms determined as a species by skilled and trained taxonomist, i.e. scientist with authority given by his education, knowledge, wisdom and scientific reputation. As species concepts derived after works of Dobzhansky (1937), Mayr (1942), Dobzhansky (1950), Hennig (1950, 1966) and Mayr (1968) and others do contain some objective criterion (or at least framework to develop one), the ‘classical’ Linnean concept is more or less explicitly abandoned, as it does not provide any testable hypothesis and it keeps open the question if species are only human mental construct or real biological entities in the nature (Kitcher 1984). Moreover, in such concept there is no way how to really decide in case of disagreement between two taxonomic experts.

The ‘cynical’ species concept is, implicitly or explicitly, the most common concept in botany. As polyploidy is extremely common among plants (D. E. Soltis et al. 2016) and hybridization is very common in some genera (e.g. Hieracium, Quercus, Rubus, Sorbus, Taraxacum), botanical taxonomists more or less openly more or less gave up trying to formalize their approach. As will be shown later on the example of genus Taraxacum (see chapter 7, page 27), when working with such complicated genera, we do not have much more options. Combination of recent as well as older hybridization, polyploidization, fragmented areal, common asexuality, etc. is simply impossible to be fitted into any simple formal framework. So we nowadays use the biological species concept with respect of phylogeny, and when needed, also concept of microspecies/agamospecies. This vague definition seems to be prevailing consensus among botanists, as it can fit everyone’s needs.

Hope given by the phylogenetic species concepts that we can find an objective methodological framework to delimit species was not fulfilled, they have too many theoretical and practical limitations do be widely used on low-level botanical taxonomy. Delimitation of species, at least in botany, still relies on expertise decision by skilled botanist-taxonomist, despite advances of modern genetic techniques (see Kirschner et al. 2016, chapter IV, page 101, and discussion there). This is critical point. In case of discrepancy between two taxonomists, despite all modern methods (see chapter 8, page 31), we often lack objective criterion how to solve the problem. Pessimists (and cynics) also use to say, that genera, where we do not see above-mentioned issues, just are not enough explored.

Groups with common apomixis are especially problematic for species concepts and their treatment is variable (Majeský et al. 2017). The above-discussed concepts use to start with need to deal with particular organismal group. This is well seen on concepts created for asexual lineages (A. Richards 1973; A. Richards et al. 1996) — they solve well given task, but they are not of much use for the others (fitting according to this concept obligatorily sexual species does not make much sense). This is good example why it is problematic to develop one model to fit them all. We should consider going one step further (or back?).
Contemporary biologists are sometimes ‘jealous’ on physicists as they use to have clear repetitive systems well-describable by mathematical models while living nature has been continuously refusing to fit into our simple distinct categories and behave according to our nice theoretical mathematical models.

We can see species more philosophically. Accept, that a ‘species’, as e.g. certain state of population-genetic processes, is more philosophical than technical/theoretical category and that species can have multiple different origins in the terms of various biological natures (‘natural histories’) of the organisms. It does not matter if a ‘species’ originated by quick hybridization and is apomictic, or if it is an old sexual lineage evolving separately for millions of years; it is still ‘species’ — an entity of certain level of (e.g. genetic or geographic) uniqueness and (e.g. ecological or morphological) distinctiveness. Rank of species remains basic for our thinking, but the word ‘species’ has different meaning in different types of organisms. Obvious drawback of such thinking is resignation to any (objective) methodological framework. On the other hand, ‘softer’ definition accepting high differences in possible pathways of species’ origin would be probably more realistic.

People have been always categorizing species into some ‘practical’ groups. An example from European region with one of the most traditional agricultures (remote parts of Romania, Molnár and Babai 2010) shows, that sometimes people very well distinguish even minute species (especially if they have particular usage), sometimes people group even very unrelated species into more ecological categories like ‘grasses’ or ‘weeds’. We are shifting this concept towards finding ‘natural’ groups somehow reflecting species’ evolutionary history, we just struggle with the enormous diversity of the life.

Conclusively we use modern genetic methods (see chapter 8, page 31) to delimit genetic clusters and lineages, but final decision relies on the experts, with respect to morphology, ecology, geography, etc. It is not the most optimistic conclusion of the first chapter, but it is not any tragedy. We can not rely on any ‘magic black box’, any software or molecular method, to think instead of us. Taxonomists are responsible for fair work and critical evaluation of all evidences.
Chapter 7

Taraxacum as a touchstone of the taxonomic and species concepts and methods

The genus Taraxacum Wigg. (Wiggers 1780, p. 56) is a species rich genus (about 2,800 species divided into about 60 sections) from family Asteraceae. According to modern phylogenetic analysis, it is placed within subfamily Cichorioideae (Mandel et al. 2014, 2017), tribe Cichorieae (Mandel et al. 2015), subtribe Crepidinae. Its closest relatives seem to be Askellia, Ixeris, Ixeridium and Youngia (Enke and Gemeinholzer 2008; J.-W. Zhang et al. 2011), but more data are needed to verify the placement (the above cited studies are not fully congruent).

Members of the genus Taraxacum are distributed worldwide, mainly in the Arctic and temperate zones of the Northern Hemisphere, with principal diversity in the Euroasian mountains (e.g. Kirschner and Štěpánek 2005; Kirschner et al. 2006; Kirschner and Štěpánek 2011; Kirschner et al. 2014, 2017). Only few species are in the temperate of the Southern Hemisphere (Uhlemann et al. 2004). Lineages of T. officinale are invasive worldwide.

Members of the genus Taraxacum have relatively uniform general appearance — they are perennial rosulate herbs (hemicryptophytes), with a taproot (can be sometimes covered by remnants of last-years leaves). Stem is usually one, hollow, leafless and unbranched. Plant indumentum consists of arachnoid hairs; leaf and scape hairs sometimes on low protuberances or ridges; hairs on floret tube often straight and simple. Leaves are entire or lobed, runcinate to pinnatisect. Capitulum points upward or downward after anthesis. Involucre has two distinct series of phyllaries. Some phyllaries are often cornicate or hooded at apex; outer phyllaries are variable in length and shape (imbricate) or almost uniform (not

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7Names Asteraceae and Compositae are synonymous. According to McNeill et al. (2012), article 18.5. The following names, of long usage, are treated as validly published: [...] Compositae (nom. alt.: Asteraceae; type: Aster L.) [...]. 18.6. The use, as alternatives, of the eight family names indicated as ‘nom. alt.’ (nomen alternativum) in Art. 18.5 is authorized.

8According to compilation made by IUCN French Committee and IUCN SSC Invasive Species Specialist Group, see http://www.iucngisd.org/gisd/speciesname/Taraxacum+officinale.

9The description is from B. Trávníček et al. (2010) and mainly Ge et al. (2011).
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*imbricate*), usually substantially shorter than inner ones, appressed to reflexed, glabrous to ciliate or with arachnoid surfaces, unbordered to variously pale to whitish bordered. Receptacles are naked, glabrous or sparsely arachnoid. Floral colour varies from yellow through white to pale or deep pink and (brownish) orange or reddish brown; ligules are flat, involute, or tubular, adaxial epidermal cell cuticle ± domed and transversely striate. Achene are whitish, straw-brown, ochraceous, reddish, reddish brown, deep brown, or ± black, usually composed of a body, which includes a narrowed but equally coloured cone, and apically with a beak but cone sometimes indistinct or not developed; body spinulose and/or squamulose in upper part (below cone), often tuberculate below or completely or almost smooth, or spinulose and tuberculate throughout, abruptly or gradually narrowing into cone (when cone developed); beak usually longer than achene body including cone or short, sometimes not developed at all, thin or thick. Pappus is with numerous scabrid bristles, white, yellowish, or light reddish brown. *Taraxacum* plants are self-incompatible, rarely self-compatible. Chromosome base number is \( x = 8 \) (diploids to dodecaploids) (B. Trávníček et al. 2010; Ge et al. 2011).

*Taraxacum* has been known for its problematic and complex taxonomy due to high number of low structural morphological diversity (e.g. B. Trávníček et al. 2010; Ge et al. 2011), loss of characters during evolution and precipitous changes during evolution, variation in reproduction system, agamospermy and common coexistence of agamospermous with sexual lineages at various levels, from individuals and populations to sections (summarized by Kirschner et al. 2003; Štěpánek and Kirschner 2012), hybridity and auto- as well as allopolyploidy (Kirschner et al. 2003, 2015), clonality (Kirschner and Štěpánek 1994) leading to high number of mutually similar and mostly hybridogenous species. Except for three tetraploid sexual species in the sect. *Piesis*, all known polyploid (triploids and higher ploidy levels) taxa are agamospermous (Kirschner et al. 1994). Diploids are always sexual. In particular, the repeated ancient or recent hybridization events are common in the evolutionary history of the majority of taxa (Kirschner et al. 2003, 2015).

Taxonomic principles reflecting the above-mentioned peculiar features and processes known in *Taraxacum* were summarized by A. Richards (1973), Kirschner and Štěpánek (1996), Kirschner et al. (2003) and Ge et al. (2011). The principles require different kinds of species to be recognized on the basis of the extent of variation and modes of reproduction, exploration of distribution of sexuality, study of variation within a family of siblings for each taxon (to detect autonomous aberrants and facultative sexuality); and exploration beginning with the study at the lowest variation level (within and among populations), are methodological principles to be followed to keep as much information as possible. The complexity of the genus, primarily the incommensurable variation patterns of species with different modes of reproduction, also requires a taxonomic rank placed between species and genus in the traditional hierarchy to make the population and taxonomic structure more easily understandable for non-specialists, and the rank of section is used in the *Taraxacum* literature. Sections are important also for classification of material from under-explored regions (e.g. many Asian countries), where general lack of material does not allow more accurate classification (Ge et al. 2011).
While there are about 10 sections for which sexuality is not recorded, in the majority of the sections both agamospermy and sexuality are recorded, with common geographical parthenogenesis (Hörandl 2006b). If we disregard monotypic sections with a single sexual species, such as sect. *Antarctica* (*T. gilliesii*), sect. *Biennia* (*T. nutans*) and sect. *Glacialia* (*T. glaciale*), there are only three sections in which all the members reproduce sexually: the Southern Hemisphere sect. *Australasica* and the three Northern Hemisphere sections, sects. *Dioszegia*\(^{10}\), *Primigenia* and *Piesis* (Kirschner et al. 1994; Kirschner and Štěpánek 1998b).

Agamospermy tends to prevail, both geographically and in the number of species and individuals, and there are large areas where asexuality either totally predominates or is the only reproduction system present (Kirschner and Štěpánek 1996). Thus, the most common pattern found at a locality is a result of the coexistence of a few (rarely a single) to many microspecies. Asexual microspecies in *Taraxacum* are presumed to be entities, which in the majority of cases, came into being via multiple remote hybridizations, with hybridity ‘frozen’ by agamospermy, and the genotype diversity in the multiclonal agamic hybrid swarm reduced by subsequent strong selection. They differ in a number of autecological and morphological attributes (Kirschner and Štěpánek 1994, 1996).

The genus *Taraxacum* is a popular model for the study of diplosporous agamospermy (chapter 9.1, page 9.1), clonality (chapter 9.2, page 9.2), epigenetic heritability (chapter 9.5, page 9.5), potential germplasm for economic exploitation (chapter VI, page VI), polyploidy (combined auto- and allopolpoidy, chapter 9.3, page 9.3) and more. This usage is supported by other advantageous features, such as easy cultivation, unproblematic emasculation and efficient propagation.

### 7.1 *Taraxacum* modes of reproduction

Diploid species of Taraxacum are usually normally sexually reproducing outcrossing species with sporophytic multiallelic self-incompatibility. It is remarkable that the physiological mechanisms of incompatibility are retained in agamospermous dandelions. Although they are unimportant for apomicts, they offer a methodical tool for testing genetic identity (through pollen transfer and visual pollen germinability test (A. J. Richards 1997). Minority of diploids (e.g. *T. serotinum* subsp. *pyrrhopappum*, Zeisek et al. 2015) are sexual, but autogamous (self-fertilizing). Polyploid taxa (from triploids) are mostly (expecting few tetraploid species of sect. *Piesis*, Kirschner et al. 1994) asexual gametophytic diplosporous meiotic apomicts: normal reductional meiosis is replaced by a non-reductional division. Two unreduced megaspores (2n) are produced, of which one degenerates and the other develops into an unreduced gametophyte with an unreduced egg cell (van Baarlen et al. 2002; A. Richards 2003; Ozias-Akins and van Dijk 2007; Schön et al. 2009). Apomixis in *Taraxacum* is most probably controlled by duplicated *DIPLOSPOROUS* (*DIP*) gene, which is normally not transmittable to diploids (Tas and Van Dijk 1999; Van Dijk et al. 1999; van Zeisek et al. (2015) (see chapter III) showed that *T. serotinum* subsp. *tomentosum* reproduces autogamically (= *T. pyrrhopappum*).
Dijk and Bakx-Schotman 2004; Vijverberg et al. 2004, 2010; Majeský et al. 2012). There are ongoing debates what are (micro)evolutionary consequences of apomixis and its possible switches to sexual reproduction and back (see also chapter 9.1), here we can mention possible advantage in colonization of harsh conditions (e.g. higher altitudes or polar regions), but results here are not giving clear picture (Hörandl 2006b; Hörandl et al. 2011). State of (a)sexuality and ploidy level can be quickly and easily screened using flow cytometry (FCM, Krahulcová and Rotreklová 2010).
Chapter 8

Modern methods to study relationships among populations and species

Recent development of not only molecular methods gave us tools to test our hypothesis about species relationships and species concepts inferred previously mainly from morphology, geography, etc., and study and reconstruct evolutionary history and inter/intra-species relationships. The field of modern, mainly molecular, methods is very vast, so that I will stay only with methods relevant to the topic of this work — population genetics, low-scale taxonomy, (spatial) relationships between populations and closely related species, etc.

8.1 Molecular genetic methods

Older works relied mainly on usage of allozymes (alloenzymes) — alleles of genes (respectively proteins differing by structure, but not by functions), which are distinguishable on gel electrophoresis (Avise 1994). Sometimes they might be confused with isozymes (isoenzymes), enzymes having same function, but coded by different genetic regions. Isozymes can be used in same way. Some authors treat allozymes and isozymes as synonymous or write only about 'enzymatic' or 'electrophoretic' study. It is codominant marker and the technique is fast and simple (but it requires enough of fresh material). On the other hand, it uses to have only few (~2–4) alleles (bands on the electrophoretic gel) and it detects only little portion of the variability of respective genes. Moreover, scoring higher polyploids can be tricky due to high number of possible band combinations. Although studies using allozymes provided us with plenty of valuable information (as shown in Table 8.1), see also Kirschner and Štěpánek (1996), this method is nowadays more or less abandoned in favour of more modern tools working directly with DNA sequences and providing much more data, shortly reviewed in following paragraphs.

Random Amplification of Polymorphic DNA (RAPD) was one of the first PCR-based techniques used in population genetics, phylogeography and low-scale systematics (Avise 1994). It is very simple and fast method providing plenty of alleles and no prior knowledge about the species studied is required (usage example is in Table 8.1). RAPD uses one or more restriction enzyme(s) randomly cutting whole genome into fragments of variable
length. It can be applied only within one species or among very closely related species, because otherwise we can not be sure about homology of the alleles (bands on the gel electrophoresis). RAPD and AFLP (see following paragraph) loci are dominant — it is not possible to distinguish heterozygots and dominant homozygots, only presence or absence of the allele is available. RAPD suffers from serious technical problems like low repeatability and high sensitivity to PCR reaction conditions. Despite its cheapness and simplicity, due to its unreliability, it is not used any more at all.

Amplified Fragment Length Polymorphism (AFLP, Vos et al. 1995) is extremely variable method screening polymorphism randomly from whole genome. It can be applied to any organisms without need to prepare special primers. It cuts whole genome with several restriction enzymes. This step is followed by ligation of adaptors to sticky ends to amplify only subset of the fragments. Due to high variability of the anonymous alleles, it can be applied within one species or among several very closely related species. AFLP has been widely used by taraxacologists, see Table 8.1. It is very robust, although relatively expensive and labour-intensive method, so that it uses to be slowly replaced by modern next-generation/high-throughput sequencing (NGS/HTS) methods like genotyping by sequencing (GBS) and RAD-Seq and their variants (see further), as these newer methods can for similar money and wet-lab time provide much more data (not only dominant alleles). Some studies listed in Table 8.1 use methylation sensitive AFLP (MS-AFLP), as methylation is common form of epigenetic regulation, this technique allows us to detect epigenetic changes under variable (e.g. ecological) conditions. MS-AFLP uses methylation sensitive restriction enzymes. In all applications, AFLP proved to be very valuable tool to answer given questions.

Simple Sequence Repeats (SSRs, microsatellites, Jarne and Lagoda 1996) are very popular and highly variable (with high mutation rate) molecular markers used especially for studying population genetics, phylogeography and within-genus relationships. It requires species-specific primers (for Taraxacum e.g. Falque et al. 1998; Vašut et al. 2004, most of further cited studies use these primers on various species across genus Taraxacum), on the other hand, it is very quick and robust method. Previously, preparation of the primers required long laboratory work, nowadays, it is possible to use some HTS method and special software to develop the primers quickly and easily (e.g. Malausa et al. 2011; Wei et al. 2014).

Microsatellites are short tandem repeats (1–3 bp, in plants mostly AT) of non-coding DNA differing by the number of repeats (length of the allele) and scattered through whole genome. Modern PCR thermocyclers and sequencers allow to quickly score several loci from plenty of individuals (several primers can be usually multiplexed into one PCR reaction and sequenced together). It seemed that SSRs will be slowly replaced by advance of modern HTS methods (especially because of continuously decreasing price of HTS), but microsatellites have been remaining very popular tool (e.g. Hodel et al. 2016a,b). Taraxacum studies using microsatellites are listed in Table 8.1.

\[^{11}\text{We have set of unpublished SSRs primers developed according to Malausa et al. (2011) from sequences of Taraxacum koksaghyz, but we have not used them yet.}\]
Expressed sequence tags (ESTs) are short regions (ca. 200–800 bp) of cDNA prepared from mRNA. ESTs use to be used to find target regions by hybridization with respective genes, to design primers, or for direct sequence comparison in similar way as with sequences from Sanger sequencing (following paragraph). Generally it is popular marker\textsuperscript{12}, but Table 8.1 lists only few examples of the usage of ESTs in \textit{Taraxacum} research.

‘Classical’ method not only for phylogeny and phylogeography is usage of DNA sequences obtained by Sanger sequencing of coding or non-coding regions (Small et al. 1998; Shaw et al. 2005, 2007). Sequencing requires prior knowledge of the primers. Commonly, universal primers for nrDNA ITS1-5.8S rDNA-ITS2 nuclear region (White et al. 1990) and plastid cpDNA (Taberlet et al. 1991) genes are used. Especially nrDNA ITS1-5.8S rDNA-ITS2 region proved to be very valuable for usage in \textit{Taraxacum} studies (Kirschner et al. 2015). cpDNA has been commonly used in plant phylogeny and phylogeography, but its usage in \textit{Taraxacum} showed conflicting results with other data (Kirschner et al. 2003). Table 8.1 shows several studies using DNA sequences in \textit{Taraxacum} research. As cpDNA is inherited maternally (via seeds) in angiosperms (Taberlet et al. 1991), many studies use it with combination with other DNA techniques (nuclear genes, AFLP, SSRs, ...) to partitioning of gene flow through seeds and pollen. Important is selection of the region to study, with respect to their expected variability (Small et al. 1998; Shaw et al. 2005, 2007) and the question to be studied. Sanger sequencing can easily sequence genes longer than 1000 bp and it is one of the most important molecular techniques so far, but every gene must be processed separately and in case of internal variability of the sequences (if the individual contains more variants of the genes, commonly e.g. in nrDNA and/or allopolyploids), expensive and time-consuming cloning is required (Záveská Drábková et al. 2009). Sequencing higher number of genes from many accessions is time consuming and expensive, so that nowadays researchers, when possible, rather use NGS/HTS method (see further).

The field of so called next-generation/high-throughput sequencing (NGS/HTS) methods (e.g. Cronn et al. 2012) is very quickly developing and there are plenty of possible approaches (Levy and Myers 2016). Currently, most of sequencing methods use Illumina sequencing systems\textsuperscript{13} (they are also the most suitable for the topics of this work; I introduce here only relevant selection of the techniques), which are able to produce (depending on the model) up to ca. 1.5 Tbp (5 billion single reads) of data. Read length is variable, but usually 2×75–2×250 bp, which is significantly shorter than from Sanger sequencing. On the other hand, we get incommensurable more data when using Illumina. Other advantage of Illumina sequencing is that each site is sequenced multiple times (so called ‘coverage’) — this parameter is in the hands of the researcher: total capacity of the sequencing machine is divided by genome (or library) size and by required coverage. We then see how many samples we can process in one run. This is big advantage especially for studies including polyploids (Buggs et al. 2012; Oxelman et al. 2017) where we get (with higher coverage) all variants of the given genetic region. Unfortunately, there are so far only very few relevant studies using these techniques when studying \textit{Taraxacum} (Table 8.1). Hopefully, our team

\textsuperscript{12}See e.g. https://www.ncbi.nlm.nih.gov/dbEST/.

\textsuperscript{13}See e.g. https://www.illumina.com/systems/sequencing-platforms.html.
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will add some during upcoming years.

All methods using Illumina or any other NGS/HTS method require intensive bioinformatical processing of the raw data, usually involving scripting in UNIX command line (languages like BASH, Python or R). Large disk space and powerful computers are required to handle such data. This go hand in hand with continuous development of novel software.

Probably the most familiar Illumina-based method for the users of ‘old good’ nrDNA ITS1-rDNA-ITS2 and cpDNA sequences is method of genome skimming (Straub et al. 2012). It takes advantage of high abundance of regions like, ITS1-rDNA-ITS2, plastome and mitochondrial sequences. It is usually possible to recover whole plastome, chondriome and the nrDNA ITS1-rDNA-ITS2 region, commonly also other abundant genes. Due to the abundance of these regions, lower coverage use to be sufficient and it is possible to multiplex plenty of individuals in one Illumina sequencing run. It is relatively simple option how to obtain modest amount of data from many accessions. Although Kim et al. (2016a,b) and Y. Zhang et al. (2017) showed usefulness of complete plastomes on limited number of accessions, broader sampling (but using only few genes) by Kirschner et al. (2003) is not very encouraging for usage of plastome, especially for higher polyploids.

Unlike ‘classical’ Sanger sequencing where we sequence few long genes, with Hyb-Seq (Weitemier et al. 2014) (and other target enrichment methods) we can obtain shorter sequences of hundreds to thousands loci. Hyb-Seq requires probe sequences to sequence single-copy orthologous genes (COSII), prepared e.g. according to Schmickl et al. (2016). This can be relatively complicated, as closely related reference genome (typically species from the genus under study) is required. If it is not available, at least transcriptome sequencing, genome assembly and formation of a new reference genome is required. Alternatively, it is possible to use some existing set of probes, e.g. by F. Wu et al. (2006) for Euasterids. This method can be easily applied to phylogeny on the level of genus (Schmickl et al. 2016, and our data under preparation), but if population-genetic data are required, some variant of RAD-Seq (next paragraph) will probably be more suitable, as single-copy orthologous genes can be too conservative to show enough variability.

Restriction-site associated DNA (RAD-Seq, Peterson et al. 2012) has several variants, but in general, whole genomic DNA is cutted by restriction enzymes into fragments of required length (with respect to chosen Illumina sequencing protocol). The fragments are barcoded (to be able to separate the accessions) and sequenced. Similar in usage and aim is simpler genotyping by sequencing (GBS). RAD-Seq is very useful especially for population genetics and phylogeography (e.g. Kolár et al. 2016a,b). As most of the sites of the aligned RAD fragments are invariant, usually only single nucleotide polymorphism (SNPs) are retained during several in silico filtration steps. SNPs can be extracted also from Hyb-Seq or Sanger sequencing, depending on the requirements of subsequent analytical method.

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14 Although we (Roswitha Schmickl, Kenneth Oberlander, me and others) have Hyb-Seq data from multiple accessions of several South African Oxalis species (O. hirta, O. incarnata, ...) and we are able to recover some phylogeographic patterns. The data are now under preparation.
8.2 Flow cytometry and karyology

Apart of previously introduced genetic techniques, important role in *Taraxacum* research is played by flow cytometry (FCM, Krahulcová and Rotreklová 2010; Loureiro et al. 2010; Suda and Pyšek 2010), fast, simple and cheap method allowing us to quickly screen hundreds to thousands of samples and obtain their absolute or relative genome sizes. Genus *Taraxacum* shows high variability in this respect (Záveský et al. 2005; Lenka; Mártonfiová 2006; Lenka Mártonfiová et al. 2007). With flow cytometry we can quickly and easily distinguish ploidy levels (e.g. Šuvada et al. 2012). In ideal case, such data are accompanied by direct chromosome counting (e.g. following Krahulcová 1993).

8.3 Studies in *Taraxacum*

Due to the nature of *Taraxacum* described in chapter 7 (page 27), the genus became popular model to study various microevolutionary processes (see chapter 9, page 43), e.g. differentiation of sexual ancestors, hybridization, clonal structure, coexistence of various ploidy levels, autopolyploidy as well as allopolyploidy, epigenetic heritability of fine morphological and ecological traits. Table 8.1 starting on following page lists the studies and chapter 9 discusses them.
Table 8.1: Overview of Taraxacum studies using various molecular and cytogenetic techniques (see chapter 8, page 31) for the description of the methods. Nomenclature follows original works, some names are commented in footnotes. Sometimes it is unclear which T. officinale microspecies were sampled. In such case, we can ‘translate’ T. officinale as ‘probably sect. Taraxacum’.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method(s)</th>
<th>Section/species of Taraxacum</th>
<th>Main question(s), topic(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyman and Ellstrand (1984)</td>
<td>Allozymes, karyology</td>
<td>Triploid T. officinale</td>
<td>Clonal diversity within and among populations</td>
</tr>
<tr>
<td>Ford and A. Richards (1985)</td>
<td>Isozymes, karyology</td>
<td>T. officinale</td>
<td>Clonal diversity, microtaxonomy</td>
</tr>
<tr>
<td>Hughes and A. Richards (1985)</td>
<td>Isozymes, alkozymes</td>
<td>T. alacre, T. brevifloroides and hybrids</td>
<td>Breeding system</td>
</tr>
<tr>
<td>Mogie (1985)</td>
<td>Karyology, alkozymes</td>
<td>sect. Hamata</td>
<td>Genetic variation</td>
</tr>
<tr>
<td>Hughes and A. Richards (1988)</td>
<td>Isozymes, alkozymes</td>
<td>Outbreeding sexulas, obligate agamosperms and inbreeding sexuals</td>
<td>Reproductive systems, population structure, diversity</td>
</tr>
<tr>
<td>Menken and Morita (1989)</td>
<td>Allozymes</td>
<td>T. albidum</td>
<td>Clonal diversity within and among populations, spatial structure</td>
</tr>
</tbody>
</table>


17 T. aristum, T. bessarabicum, T. brachyglossum, T. pseudohamatum, T. pyropappum, T. serotinum, T. unguilobum, sect. Vulgaria. Name ‘Vulgaria’ (here and in Hughes and A. Richards (1989) and Menken et al. (1989) and elsewhere) is now considered invalid and name ‘Ruderalia’ is used instead, but Ruderalia is not one-to-one replacement of Vulgaria (see discussion in Kirschner and Štěpánek 1987).
<table>
<thead>
<tr>
<th>Reference</th>
<th>Method(s)</th>
<th>Section/species of <em>Taraxacum</em></th>
<th>Main question(s), topic(s)</th>
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<tbody>
<tr>
<td>Menken et al. (1989)</td>
<td>Allozymes</td>
<td>Sections <em>Mongolica</em> and <em>Ruderalia</em></td>
<td>Reproductive systems, clonal diversity</td>
</tr>
<tr>
<td>Battjes et al. (1992)</td>
<td>Allozymes</td>
<td>Sect. <em>Palustria</em></td>
<td>Clonal diversity, spatial structure</td>
</tr>
<tr>
<td>Akhter et al. (1993)</td>
<td>Allozymes, isozymes, karyology</td>
<td><em>T. hondoense</em></td>
<td>Clonal diversity, spatial structure</td>
</tr>
<tr>
<td>Lynn Martens King (1993)</td>
<td>Restriction analysis of rDNA and cpDNA</td>
<td>Polyploid agamospecies of sections <em>Celtica</em>, <em>Erythrosperma</em>, <em>Erythrocarpa</em>, <em>Mexicana</em> and <em>Ruderalia</em>(^\text{18})</td>
<td>Genotypic (clonal) diversity, hybridogenous origin of the species</td>
</tr>
<tr>
<td>Wittzell (1999)</td>
<td>cpDNA</td>
<td>237 species</td>
<td>Phylogeny, phylogeography, genotype variation</td>
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<td>van der Hulst et al. (2000)</td>
<td>AFLP, FCM</td>
<td>Apomictic <em>Taraxacum</em> spp.</td>
<td>Reproductive systems, genotype diversity</td>
</tr>
<tr>
<td>Mes et al. (2000)</td>
<td>cpDNA</td>
<td><em>T. aurantiacum</em>, <em>T. farinosum</em>, <em>T. perenne</em>, <em>T. serotinum</em> and sections <em>Borealia</em>, <em>Calanthodia</em>, <em>Mongolica</em> and <em>Naevosa</em></td>
<td>Structure of the plastomes</td>
</tr>
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</table>

### Table 8.1. Reference Method(s), Section/species of *Taraxacum*, Main question(s), topic(s)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method(s)</th>
<th>Section/species of <em>Taraxacum</em></th>
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<tr>
<td>Mes et al. (2002)</td>
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<td>Sect. <em>Naevosa</em></td>
<td>Clonal structure, genetic variability, clonal reproduction, spatial structure</td>
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<td>van der Hulst et al. (2003)</td>
<td>(AFLP)&lt;sup&gt;19&lt;/sup&gt;, SSRs, allozymes, cpDNA</td>
<td>Sections <em>Celtica</em>, <em>Hamata</em> and <em>Ruderalia</em></td>
<td>Population genetics, diversity</td>
</tr>
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<td>Kirschner et al. (2003)</td>
<td>cpDNA</td>
<td>Representatives of 44 sections</td>
<td>Taxonomy, evolution</td>
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<td>Brock (2004)</td>
<td>SSRs</td>
<td><em>T. ceratophorum</em> and <em>T. officinale</em></td>
<td>Hybridization, genetic assimilation</td>
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<td>van Dijk and Bakx-Schotman (2004)</td>
<td>SSRs</td>
<td><em>T. officinale</em></td>
<td>Genetic maps, reproduction</td>
</tr>
<tr>
<td>Vijverberg et al. (2004)</td>
<td>AFLP, SSRs</td>
<td><em>T. officinale</em></td>
<td>Genetic maps, reproductive systems, diplospory</td>
</tr>
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<td>Kashin et al. (2005)</td>
<td>Allozymes, isozymes</td>
<td><em>T. officinale</em> and <em>T. serotinum</em></td>
<td>Population structure and genetics</td>
</tr>
</tbody>
</table>

<sup>19</sup>Data from van der Hulst et al. (2000).

<sup>20</sup>*T. rubicundum*, *T. brachyglossum*, *T. tortilobum*, *T. lacistophyllum*, *T. parnassicum* and *T. scanicum.*
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<tr>
<th>Reference</th>
<th>Method(s)</th>
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<tr>
<td>Záveský et al. (2005)</td>
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<td>Sections <em>Coronata</em>, <em>Dioszegia</em>, <em>Erythrosperma</em>, <em>Glacialia</em>, <em>Kashmirana</em>, <em>Mongolica</em>, <em>Obovata</em>, <em>Palustria</em>, <em>Piesis</em>, <em>Ruderalia</em>, <em>Scariosa</em> and <em>Taraxacum (= Crocea)</em> and <em>T. pyrenciaicum</em> group</td>
<td>Genome size variation, taxonomy</td>
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<td>Hoya et al. (2007)</td>
<td>FCM</td>
<td><em>T. platycarpum</em> (2n), <em>T. venustum</em> (3m, 4n), and <em>T. albidum</em> (5n)</td>
<td>Differences of ecological and physiological traits among ploidy levels</td>
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<td>Uhlemann et al. (2009)</td>
<td>nrDNA (ITS1-5.8S rDNA-ITS2), karyology</td>
<td>Sect. <em>Arctica</em> s.l.</td>
<td>Phylogeny, taxonomy</td>
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<tr>
<td>Záveská Drábková et al. (2009)</td>
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<td>32 species from 11 sections</td>
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<td>Verhoeven et al. (2010b)</td>
<td>(MS-)AFLP</td>
<td>Triploid <em>T. officinale</em></td>
<td>Genetic and epigenetic variation and heritability</td>
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</table>
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... continued Table 8.1.

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<thead>
<tr>
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<td><em>T. officinale</em></td>
<td>Genetic maps, structure of the diplospory locus</td>
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<td>Majeský et al. (2012)</td>
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<td><em>T. officinale agg.</em>&lt;sup&gt;21&lt;/sup&gt;</td>
<td>Reproduction system, genotypic diversity</td>
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<td>McLeod et al. (2012)</td>
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<td><em>T. officinale</em></td>
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<td><em>T. officinale</em></td>
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<td>Kirschner et al. (2013)</td>
<td>AFLP, FCM</td>
<td><em>T. brevicorniculatum</em> and <em>T. koksaghyz</em></td>
<td>Taxonomy, species delimitation, identification</td>
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<td>P. Trávníček et al. (2013)</td>
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<td><em>T. stenocephalum</em></td>
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<td>Kirschner et al. (2015)</td>
<td>nrDNA</td>
<td>52 sexual accessions from 26 sections, and 13 agamospermous accessions</td>
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</tr>
<tr>
<td></td>
<td>(ITS1-5.8S rDNA-ITS2), cpDNA</td>
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<sup>21</sup>*T. sect. Taraxacum* (syn. *T. sect. Ruderalia*)
... continued Table 8.1.

<table>
<thead>
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<td>AFLP, SSRs, cpDNA, FCM</td>
<td><em>T. cristatum, T. prunicolor, T. pudicum</em> and <em>T. scanicum</em></td>
<td>Reproduction system, genotype diversity, species relationships</td>
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<td>Preite et al. (2015)</td>
<td>(MS-)AFLP</td>
<td><em>T. officinale</em></td>
<td>Genetic and epigenetic variation and heritability, clonal diversity, spatial structure</td>
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<td>Arias et al. (2016a)</td>
<td>AFLP, COS, SSRs, EST-SSRs</td>
<td><em>T. koksaghyz</em></td>
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<sup>22</sup>*T. alatum, T. ekmanii, T. hemicyclem, T. hepaticum, T. interveniens, T. macranthoides, T. obtusifrons, T. piceatum* and *T. pulcrifolium.*

<sup>23</sup>Here and elsewhere misspelled as *T. kok-saghyz.*
### Table 8.1

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Chapter 9

Evolution, microevolution and Taraxacum

Formation of new species must necessarily start on the level of populations by development of any reproductive isolation mechanism, progress of ecological, morphological and/or genetic distinctness. Although this process is more complex in Taraxacum due to at least occasional gene flow among taxa/lineages, if species are natural entities which we can anyhow distinguish in the nature (see also chapter 6.2), some processes responsible for the differentiation must necessarily be involved. As there are plenty of coexisting and closely related Taraxacum species on nearly every locality, population and metapopulation processes are crucial in Taraxacum mikroevolution, thus responsible for speciation. As summarized from various principal aspects by A. Richards (1973), Kirschner and Štěpánek (1996) and Štěpánek and Kirschner (2012), processes like coexistence of sexuality and agamospermy (chapter 9.1), clonality (chapter 9.2), polyploidization (chapter 9.3) and complex hybridization (chapter 9.4) are crucial for formation of new lineage within Taraxacum. Such new lineage, if it is enough stable and distinct, can in some time form a new species.

9.1 Sexuality and asexuality and their implications to Taraxacum microevolution

It has been shown many times that polyploidization in Taraxacum (chapter 9.3) goes hand-in-hand with asexuality. Earlier works were summarized by A. Richards (1996), who clearly demonstrated that even obligate apomicts can harbour high genetic variability, which is sort of ‘frozen’ and change mainly via individual mutations, but such lineages are still capable of evolution and further speciation (Klekowski 2003; Loxdale and Lushai 2003). Definitely, apomixis is not evolutionary death end (A. Richards 1973).

van Baarlen et al. (2000) studied microscopically microsporocytes of triploid apomictic T. officinale and found levels of chromosome pairing and chiasma formation at meiotic prophase I to be lower than in that of the sexual diploids, but still sufficient to assume recombination between the homologues. Incidental formation of tetrads was detected, suggesting
that hybridization can occur in triploid apomicts (van Baarlen et al. 2000). This explains occasional restoration of sexuality of the apomictic lineages.

Záveský et al. (2007) studied inheritance of apomixis. Apomictic *T. paludosum* (sect. *Palustria*) was used as pollen donor for crosses with various sections (*Alpina, Erythrosperma, Ruderalia*). Non-apomictic plants prevailed in F1 progeny, and a high incidence of sterility was observed. Triploid non-apomictic F1 hybrids were backcrossed with diploids (sects. *Ruderalia* and *Palustria*) and tetraploids (sects. *Palustria* and *Piesis*), and produced various types of progeny. The results indicate the independent genetic control of all apomixis elements in *T. paludosum*, and recombinations during a restitutional megasporogenesis in hybrids (Záveský et al. 2007).

Kirschner et al. (2016, chapter IV) showed, that distinct morphospecies are consisting of basically one genotype and several derived types differing by few mutations. Few cases of introgression of another genotypes were also detected.

As most of asexual species are able of at least occasional sexual reproduction, they can use advantages of both modes. Asexual lineages can be perfectly adapted to particular environment (von Hofsten 1954). This is bringing substantial competition advantage; and possibility of hybridization and sexual reproduction keeps opened door to ‘escape’ in case of sudden environmental change.

Similar principle was thoroughly documented in *Oenothera* sections *Oenothera* and *Calylophus* (Onagraceae), where asexual species occurred at higher latitudes, but did not differ in range size, compared with sexual species. Transitions to asexuality were associated with decreased investment in floral structures, including the length of petals, floral tubes and styles (Johnson et al. 2010). Asexuels were more capable of colonization of new environments (e.g. after deglaciation) and loss of sexuality was not a ‘blind alley’.

### 9.2 Clone clustering and diversity

As majority of *Taraxacum* species have prevailing asexual reproduction, so that question of clonal structure logically became one of important topics (Kirschner and Štěpánek 1994; A. Richards 1996), and the genus *Taraxacum* showed itself to be good model for such studies (see Table 8.1). Results of the studies are not uniform (see next paragraph). Some show no diversity at all, some very high diversity, some suggest recombination among lineages, some show star-like pattern.

Lyman and Ellstrand (1984) found in average 5 genotypes per population among 22 populations of North American *T. officinale*\(^{24}\). Ford and A. Richards (1985) found 10 agamospecies of *T. officinale* on 100 m\(^2\) and found any variability only in one of the three allozyme systems. Hughes and A. Richards (1988) tested isozyme variability within 3 sexual populations, 3 asexual populations and 6 hybrids of sexual and asexual *Taraxacum*. Some isozyme...
loci were uniform in sexual and/or asexual species, the results were comparable for both groups of species. Menken and Morita (1989) found nearly total uniformity in Japanese pentaploid obligate agamosperm *T. albidum*. On the other hand, Menken et al. (1989) found in the crosses between sexual dipooids of sections *Vulgaria* and *Mongolica* more or less pattern expected under Mendelian segregation. Battjes et al. (1992) found nearly no variability among samples of sect. *Palustria* from Czechia and Slovakia. Akhter et al. (1993) detected high heterozygosity (all triploids were heterozygots; all together, 21 clones were found using 3 alloenzymes) among population of *T. hondoense* from Honshu. Kirschner et al. (1994) found nearly no variability among populations of *T.* sect. *Piesis* ( *T. bessarabicum* and allies). Menken et al. (1995) revealed high clonal diversity (0.71–0.89) among sexual as well as asexual Central and Western European populations of *T.* sect. *Ruderalia*. Kashin et al. (2005) studied sexual *T. serotinum* and *Pilosella echioiides* and apomictic *T. officinale* and *P. officinarum*. Results of *Taraxacum* and *Pilosella* were comparable, in both cases, alloenzyme diversity was higher for the sexual species. All these studies use allozymes, which are known for low number of alleles. On the other hand, studies using other marker types or combination of markers use to show comparable results. Comparable allozyme study on species of *Hieracium* sect. *Alpina* (Štorchová et al. 2002) from Tatras (Slovakia) show big differences among species in diversity within populations as well as among populations.

van der Hulst et al. (2000, 2003) did not confirm clonal structure of triploid apomictic species of *Taraxacum* (in the region studied, also closely related sexual species occur and more dense sampling would be better to verify the clonality) from Northern Europe based on AFLP pattern, and found considerable diversity. View obtained by AFLP was generally supported by other data types (alloenzymes, SSRs and cpDNA, van der Hulst et al. 2003). The results did not show structure expected from nearly totally asexual species. Interesting study on *T.* sect. *Naevosa* from Norway (Mes et al. 2002) focused on diverging clone mates (genotypes of low abundance derived from ‘main’ genotype) using several markers. ITS sequences were probably showing ancient polymorphism pre-dating the origin of clones, but AFLP, isozymes and SSRs showed the expected star-like pattern, probably due to their faster evolution (Mes et al. 2002).

More detailed study focusing on Central European polyploid apomicts of *T.* sect. *Erythrosperma* (Majeský et al. 2015) showed occasional mating of apomictic males and sexual females while such occasional hybridization and mutations do not disband identity of the microspecies. AFLP, SSRs and cpDNA data show the microspecies to be consist of well defined clones (see also Majeský et al. 2012) with plenty of small derived lineages. Similar result was obtained in our study (Kirschner et al. 2016, chapter IV), where we also detected strong structure among 9 apomictic species of *T.* sect. *Taraxacum*. Among hybrids between native sexual *T. japonicum* and invasive apomictic *T. officinale* in Japan, Matsuyama et al. (2018) found high clonal diversity (within as well as among populations), suggesting multiple hybridization.

Conclusive summary can say, that within apomictic lineages, we commonly see one

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25In 1992 still Czechoslovakia, providing ~750 km transect from west to east.

26Unfortunately, authors are not very specific about the plant material used...
or few clonal lineages, accompanied by bunch of very closely related genotypes. As also asexual *Taraxacum* lineages are capable of at least occasional sexual reproduction, this picture is not absolute. Sexual diploids generally show high diversity and recombination rate. These features are well documented especially by Mes et al. (2002), Majesky et al. (2012, 2015) and Kirschner et al. (2016).

### 9.3 Autoploidy and allopolyploidy

Although majority of the *Taraxacum* species are polyploids, so far sect. *Piesis* is the only documented case of sexual tetraploid — all other polyploids are asexual apomicts, although usually capable of pollen production and occasional sexual reproduction. According to our data (Kirschner et al. 2003, 2015, 2017), sect. *Piesis* seems to be monophyletic, so that probably of autoploidal origin (e.g. *T. stenocephalum*, *T. stenolepium*, Kirschner and Štěpánek 1998b; Záveský et al. 2005; P. Trávníček et al. 2013). It is also relatively easy to obtain colchicine induced artificial tetraploids (e.g. Luo et al. 2018, and our unpublished data)

Studies of Kirschner et al. (2003, 2015, 2017) show plenty of cases of hybridizations among sections. As many of the analysed accessions were allopolyploids (i.e. polyploids of hybridogenous origin), we have a decent backbone for thinking about extend of role of (allo)polyploidization for the *Taraxacum* evolution. On the other hand, these studies rely on ‘old’ molecular data types and verification of these results by modern HTS method is required (see also chapter 8) as these techniques can shed more light and commonly even change our view of the evolution (e.g. Liston et al. 2014). Well documented case is allopolyploid hybridogenous origin of *Taraxacum* sect. *Borysthenica* (Kirschner and Štěpánek 2004, chapter 9.4). It is also good example, that polyploidization goes hand-in-hand with hybridization discussed in the following chapter.

### 9.4 Hybridity

Wittzell (1999) found among 237 sexual and apomictic accessions 46 haplotypes and 20 cpDNA lineages and detected several of these haplotypes in the advanced sections. More detailed study, including morphometrics, by Kirschner et al. (2003) showed, that particular, morphologically and otherwise well defined, sections (*Alpina*, *Borealia*, *Borysthenica*, *Confusa*, *Erythrosperma*, *Leucantha*, *Palustria*, *Ruderalia* and *Sinensis*) consisted of 2–3 different haplotypes. As the study Kirschner et al. (2003) contained samples of 44 of about 60 sections from Northern Hemisphere, including many polyploid apomictic accessions, it is, good demonstration of hybrid origin of some *Taraxacum* sections. Usage of cpDNA haplotypes was questioned by Kirschner et al. (2015), who showed big incongruence between nrDNA ITS1-rDNA-ITS2 and other data, as cpDNA. Nuclear genetic data also show evid-

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27We have plenty of data about rubber and inulin content in various hybrids and (artificial) polyploids of *T. koksaghyz* and its relatives. We suppose to evaluate last set of the data and submit the paper by the end of this year.
ences of hybrid origin of various *Taraxacum* sections. Similar result was obtained by (Kirschner et al. 2017) (also based on the ITS region) when analysing 105 sexual accessions from 26 sections. The results strongly suggest hybridization as the best explanation of the origin of some sections. The above-cited studies well document principles outlined by A. Richards (1973).

Polyploids containing several haplotypes (Wittzell 1999; Kirschner et al. 2003) are good signs of hybrid origin. The studies contain selected representation examples of the lineages. We at least see past events and we can suppose such events are common. On the other hand, it is hard to watch such events in real time. At least plenty of garden crossing experiments (remarkably decades of experience of Jan Kirschner and Jan Štěpánek; see also A. Richards 1970) show it is relatively easy to hybridize even distantly related *Taraxacum* (micro)species. These studies (as well as Kirschner et al. 2015, 2017, chapter V) document hybridization on rather ‘larger’ scale within *Taraxacum*. On the ‘finer’ end, van der Hulst et al. (2000, 2003) found strong disagreement between expect tree-like structure of the relationships among apomictic clones of *T. officinale* and genetic results obtained via AFLP. The most parsimonious explanation is hybridization among agamospecies, and possible switches from sexuality to asexuality and back.

Probably very common mechanism is hybridization of unreduced pollen (e.g. produced by agamosperous polyploid, still occasionally able of sexual reproduction) with haploid megaspore (of diploid plant). Although it is difficult to detect and prove such pathway, it can probably produce new polyploid hybrid combinations.

Well described case of hybrid origin of a section is a case of sect. *Borysthenica* (the section was formally described later, see Kirschner and Štěpánek 2004) to be of hybridogenous origin between *T. serotinum* (sect. *Dioszegia*) and *T. sect. Ruderalia* (Kirschner and Štěpánek 1996). All three sections are well distinct and sect. *Borysthenica* is extremely common in seminatural habitats of southern Ukraine and eastern Crimea (Kirschner and Štěpánek 1996). That preference for seminatural habitats might suggest young age of the section related to human activities.

Large study of Iaffaldano et al. (2018) was hybridizing *T. koksaghyz* and *T. officinale*, screened over 3.3 millions of plantlets and found only 219 plants with mixed or *T. officinale* phenotype. 360 controlled crosses produced only 25 true hybrids (15 inherited apomixis) out of 109 viable plantlets. Not really high succession rate, but could be sufficient in long term perspective to establish hybrid population under experimental conditions. Established natural population normally undergoes another set of checks: viability check, fecundity check, germinability check and a competition check, which makes the survival of a casual hybrid quite improbable (although, in a long-term perspective, many of the current agamospecies might have come into being through a similar process). Previous study (Y. Zhang et al. 2017) focussed on molecular delimitation of *T. koksaghyz*, *T. officinale* and *T. brevicorniculatum* and they found plenty of reliable markers in whole plastomes and SNPs from

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28 We have currently grant proposal under review, which should explore in detail ongoing formation of hybridogenous diplosporous allopoloids.

29 This is one of particular problems, which we would like to study more in detail using HTS.
entire multiple genomes.

Of course *Taraxacum* is not the only well documented case of hybridogenous origin of many taxa. E.g. Hodač et al. (2014) showed reticulation pattern in *Ranunculus auricomus* complex (hybridogenous origin of *R. variabilis*). Sochor et al. (2015) found 748 European species of *Rubus* subgen. *Rubus* to be probably descendants (after many events of reticulation and polyploidization) of only six (!) sexual species. Similar level of hybridization was found also in genus *Hieracium* (e.g. Fehrer et al. 2007). Insights into usage of HTS on evolution of hybrid a polyploid species of *Fragaria* was provided by Liston et al. (2014). We can now hope in soon application of such methods on *Taraxacum*.

### 9.5 Epigenetic changes

Epigenetic regulation has been more and more seen as an important mechanism responsible for regulation of complex traits (e.g. Cortijo et al. 2014, 60–90% heritability of flowering time and primary root length), influencing gene expression in polyploid plants (e.g. Adams and Wendel 2005), or human diseases (e.g. Birney 2011).

Table 8.1 lists relatively high number of studies dealing with epigenetic regulation. *Taraxacum* (in all cases microspecies from *T. officinale* s.l.) became popular model organisms here. Verhoeven et al. (2010a) proved by using MS-AFLP (chapter 8), that methylation of DNA can cause heritable phenotypic changes and the methylation can be caused by environmental stress, without changes of DNA sequences in apomictic *Taraxacum* lineages. Adding to this topic, Verhoeven et al. (2010b) showed in genetically identical offsprings of crosses of diploid and triploid *T. officinale* lineages modest level of variation in methylation. Source of variation in the methylation pattern was unclear, but it can obviously play an important role in ecological and/or morphological differentiation of the lineages. As the studies cited in this paragraph are from the same group, the story continues by Verhoeven and van Gurp (2012) who tested the above-mentioned effects in various experiments over several generations. The results were generally positive, i.e. showing continuous inheritance and effect on morphological and physiological traits of the methylation patterns. Preite et al. (2015) extended the studies to trace epigenetic variation among geographically distant populations (from southern Belgium to central Sweden) of several apomictic lineages. They found certain variation within lineages (partially correlating with genetic differences), including high heritability, but limited geographical structure of the epigenetic (methylation) variation. Wilschut et al. (2016) shown heritable differences in flowering times correlated with methylation patterns (revealed, as in other studies, by MS-AFLP) in single widespread apomictic lineage. Preite et al. (2018) observed stress-induced methylation patterns persisting over three generations of *T. alatum* and *T. hemicyclum* (*T. officinale* s.l.). They found high context-dependency and low predictability of the methylation patterns. It must be noted, that methylation of DNA is one, although very important, of several epigenetic regulation mechanisms. Slightly different approach was adopted by Ferreira de Carvalho et al. (2016), who tested using RNA-Seq of the mRNA differences within single apomictic lineage
and found about one third of the variability to be driven by activity of transposable elements. The differences were not explainable solely by genomic differences obtained from analysis of SNPs extracted from the transcriptomes. Such mechanisms can act as early steps in the evolutionary divergence of the apomictic lineages.

Cervigni et al. (2008) was studying differences in gene expression in diplosporous (tetraploids) and sexual (diploids and tetraploids obtained by colchicine treatment) of *Eragrostis curvula*. They found considerable differences in the gene expression among the EST cDNA libraries and suppose, that epigenetic regulation is responsible for at least part of the observed differences. Paun et al. (2010) found strong importance of epigenetic importance when studying three allotetraploid sibling orchid species (*Dactylorhiza majalis* s.s., *D. traunsteineri* s.l., and *D. ebudensis*). The epigenetic pattern is responsible for significant portion of the ecological differences among the species. As the species differ in geography and ecology, epigenetic differences open space for ongoing genetic differentiation by selection and/or drift.

Following years will probably bring more and more studies revealing so far unseen important role of epigenetic changes and regulation for ecological and morphological diversification and evolution. It will probably bring also need of altering of our evolutionary models. Epigenetic changes can obviously significantly alter ecological, morphological and physiological traits and contribute to formation of reproductive isolation mechanisms, and thus to the beginning of speciation. Interesting feature, hard to include in our current evolutionary theories, is its reversibility and possibility to be obtained during life of the individual (e.g. because of environmental stress) — it doesn’t depend on inherited, more or less random, mutations in DNA sequences.

### 9.6 Section as a group of species with common ancestry

Due to very high number of species (~2,800), some taxonomic rank between species and the genus is logical step, how to sort out the number of taxa. In *Taraxacum* research, level of `sections` is traditionally used. Most of about 60 sections (see Kirschner and Štěpánek 1997a, and onward) are relatively well distinct, so that they are also good basic determination unit for non-specialists. Commonly, it is not necessary to distinguish individual microspecies and determination into section level is sufficient. Concept of sections is not only practical for human users, but also for thinking about evolution of *Taraxacum* species (e.g. Kirschner and Štěpánek 1996). Sections usually contain sexual as well as apomictic species (see chapter 9.1). Our recent studies (Kirschner et al. 2003, 2015, 2017, chapter V) show sections also as important evolutionary units. Significant portion of *Taraxacum* evolution can be explained by processes within- and among-sections, typically hybridization (commonly related with polyploidization and switches of (a)sexuality) followed by e.g. geographical or ecological differentiation. On the basis of the sections we use to find several sexual diploid species and most of other members of the sections are then products of their hybridization (commonly including polyploidization and introgression from another sec-
9.7 **Plesiomorphic characters in *Taraxacum* sections**

The genus *Taraxacum* probably originated in West Himalayas during Cretaceous (A. Richards 1973). Discussion about plesiomorphic (ancestral) characters is limited by lack of comprehensive molecular study, which would deal with complex evolutionary relationships within the genus *Taraxacum*. Older works (e.g. A. Richards 1973; Doll 1982) relied on morphological data. Doll (1982) hypothesized section *Primigenia* to be ancestral, so that supposed plesiomorphic characters are simple achenes with short pappus, narrow oblong phyllaries, short leaves with several teeth in the middle, etc. (Doll 1982). Since that times we know some basic relationships evolutionary ancestral and advanced sections (Wittzell 1999; Kirschner et al. 2003; Uhlemann et al. 2009; Záveská Drábková et al. 2009; Kirschner et al. 2015): ancestral sections are primary diploid, sexual; and derived are polyploid or diploid, asexual or secondary sexual. Ancestral sections seem to be *Dissecta*, *Naevosa*, *Suavia*, *Dioszegia*, *Oligantha*, *Primigenia* or *Orientialia*. Sections *Leucantha* and *Stenoloba* seem to belong to so called precursor sections characteristics by mixture of ancestral and derived characters. Results here are not consistent as Uhlemann et al. (2009) see as presumably ancestral sections *Piesis*, *Antarctica*, *Arctica* and *Australasica*. It is hard to evaluate ancestral state of characters 30, but general conclusion from above-cited studies can be, that ancestral character states are probably (given our current limited state of knowledge) thick or medium thick short rostrum, smooth or very sparsely spinulose often red, brown or blackish achenes, not developed cones, coloured pappus, outer bracts appressed to ± erect and ± broadly bordered, with hairy surface, narrow cylindric involucres with few flowers, bracts corniculate to cor-nute, outer bracts few and broad, leaves usually not divided, or lobation simple, lobes not or sparsely dentate. There is significant concordance between morphological (e.g. Doll 1982) and modern molecular works (Kirschner et al. 2003, 2015).

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30 Especially as relationships among *Taraxacum* sections/species are not tree like and mathematical methods to reconstruct and test ancestral states generally do require bifurcating trees.
Chapter 10

Articles included in the thesis

Following parts contain original articles, published or submitted. All references from all articles are listed together on the end of whole work. The papers were typeset in the same style as the introduction of the thesis (chapter II, page 17), the content was not changed.


On the basis of the analysis of SSRs variation, distribution and morphology, we addressed problems related to mode of reproduction, among-population relationships, taxonomy and within-population variation within section Dioszegia (T. serotinum and its allies) using samples from southern France to the European part of southern Russia and Iran.


Agamospermous microspecies are taxonomic entities recognizable from one another by a set of minute morphological features. Specialists use microspecies names but there could be inconsistencies in the taxonomic concepts used by different experts. A selection of nine widespread, generally recognized agamospermous microspecies of Taraxacum sect. Taraxacum, were used to evaluate the ability of four European Taraxacum specialists to identify these microspecies consistently.


31Except for typography, styles, obvious typos, mistakes, etc.
The Taraxacum flora of the West Himalaya represents one of the diversity hotspots, with at least 17 sections and about 150 known species. All these names referred to T. sect. Orientalia Handel-Mazzetti are revised and newly interpreted, with emphasis on plants erroneously determined as T. stenolepium.


Taxonomy, description, distribution, population genetics and rubber content in T. bicorne (possible new source of natural rubber), including comparison with known rubber producer T. koksaghyz. Relationships of T. bicorne to its allies are evaluated. We developed novel method to extract rubber from the roots.


The taxonomy, micromorphology, karyology and evolutionary relationships of T. bithynicum DC. were studied using the original material and new samples from the summit area of Mt. Uludağ, Bursa Province, Turkey. The history of the original gathering of T. bithynicum and various concepts of this name in the literature are briefly discussed.
Part III

Microsatellite variation, sexual reproduction and taxonomic revision of *Taraxacum* sect. *Dioszegia*: relationships at a large spatial scale
This work was published as Vojtěch Zeisek, Jan Kirschner, Jan Štěpánek and Mohammad Amini Rad (2015). Microsatellite variation, sexual reproduction and taxonomic revision of Taraxacum sect. Dioszegia: relationships at a large spatial scale. In: Preslia 87.1, pp. 55–85. url: http://www.preslia.cz/2015.html#zeisek

The coexistence of agamospermy and sexuality characterizes most of the ~60 sections of the genus Taraxacum. Section Dioszegia, comprising T. serotinum and its allies, are an exception because only sexuals are reported for all the members of this group. On the basis of the analysis of microsatellite (SSRs) variation, distribution and morphology, we addressed problems related to their mode of reproduction, among-population relationships, taxonomy and within-population variation, using samples from populations in an area extending from southern France to the European part of southern Russia and Iran. We found strong isolation by distance and deep spatio-temporal structure among populations. As a rule, out-crossing was the dominant mode of reproduction, with one notable exception: T. serotinum subsp. tomentosum (= T. pyrrhopappum) was autogamous and not heterozygous. This subspecies is understood as a relic of a continental migration of T. serotinum in the late glacial/early post-glacial period, which became autogamous. Taraxacum haussknechtii is relatively highly heterozygous with a high degree of connectivity among populations, whereas populations of T. serotinum subsp. serotinum show high level of inter-population variability. A taxonomic revision of sect. Dioszegia recognizes T. serotinum subsp. serotinum (including an aberrant taxon, newly described as var. iranicum), T. serotinum subsp. tomentosum and T. haussknechtii. Full synonymy was compiled and lectotypes designated for six names. A list of the herbarium material studied is given for the latter three taxa, and a distribution map is provided for T. haussknechtii.

Keywords: autogamy, Europe, Iran, isolation by distance, microsatellites, population variation, reproduction, Taraxacum, taxonomy.
Mikrosatelitová variabilita, pohlavní rozmnožování a taxonomie *Taraxacum* sect. *Dioszegia*: vztahy na velké prostorové škále

Chapter 11

Introduction

The genus *Taraxacum* (Asteraceae-Cichorieae-Crepidinae), with about 60 sections and about 2,800 species, is generally considered to be a complicated example of a genus with coexisting agamospermy and sexuality, as summarized by Kirschner et al. (2003) and Štěpánek and Kirschner (2012). The problematic features are (i) mutual structural similarity, (ii) agamospermy and common coexistence of agamosperms with sexuals, (iii) hybridity and (iv) polyploidy (except for three tetraploid sexual species in the sect. *Piesis*), all known polyploid taxa are agamospermous). In particular, the repeated ancient or recent hybridization events are common in the evolutionary history of the majority of taxa.

While there are about 10 sections for which sexuality is not recorded, in the majority of the sections both agamospermy and sexuality are recorded, with common geographical parthenogenesis (Hörandl 2006b). If we disregard monotypic sections with a single sexual species, such as sect. *Antarctica* (*T. gilliesii*), sect. *Biennia* (*T. nutans*) and sect. *Glacialia* (*T. glaciale*), there are only four sections in which all the members reproduce sexually: the Southern Hemisphere sect. *Australasica* and the three Northern Hemisphere sections, sects. *Primigenia*, *Dioszegia* and *Piesis* (Kirschner et al. 1994; Kirschner and Štěpánek 1998b).

Plants belonging to the section *Dioszegia* (Heuffel) Heuffel are characteristically diploid and sexual (Doll 1975; Krahulcová 1993). They are perennial hemicryptophytes growing in well drained deep soils. Morphologically, this section is characterized by linear-lanceolate, imbricate (and apically arcuate) outer bracts, large subturbinate achenes very gradually narrowing into a subcylindrical to cylindrical cone, subcoriaceous leaves with hairs often growing on small ridges on the leaf surface and summer or late summer flowering. There are three geographical groups of populations usually recognized as separate species, sometimes subspecies, under the names *T. serotinum* (Waldst. et Kit.) Fischer, *T. pyrrhopappum* Boiss. et Reuter and *T. haussknechtii* Uechtr. While populations called *T. pyrrhopappum* and *T. haussknechtii* are restricted to relatively small areas, the former in southern France, Spain and northernmost Morocco, the latter in the Republic of Macedonia and adjacent regions in Albania, Greece and Bulgaria, *T. serotinum* has one of the largest distribution ranges in the genus, extending from the Czech Republic and Austria in the west through southern Ukraine and southern Russia to northern Kazakhstan and the westernmost part of Siberia () in the north-west, and through Anatolia and Caucasus to Afghanistan in the
south and south-east. The taxonomic positions of plants from Afghanistan remain to be determined. Its distribution overlaps that of *T. haussknechtii* (while it is allopatric with *T. pyrrhopappum*).

Gustafsson (1932) was the first to experimentally investigate sexuality in *T. serotinum* by castrating about 30 inflorescence at the bud stage and studying chromosome pairing during meiosis. His results were added to the text during proof reading in the form of the following footnote, which clearly documents sexuality in this species: „Nachdem Vorstehendes geschrieben war, habe ich Gelegenheit gehabt, Kastrationen in großem Massstabe (ungefähr 30 Körbe) von der obenerwähnten diploiden Art T. serotinum auszuführen, und es hat sich herausgestellt, dass sie wie erwartet sexuell war; auch waren bei der heterotypischen Metaphase 8 Doppelchromosomen zu sehen.“

Sexual reproduction and the diploid chromosome number of $2n = 16$ were confirmed by a study of new material by Poddubnaja-Arnoldi and Dianowa (1934). As for the possibility of the occurrence of spontaneous hybrids between *T. serotinum* and other diploids of different sections, sterile hybrids between *T. koksaghyz* and *T. serotinum* are recorded (Poddubnaja-Arnoldi 1939). These geographical entities share a diploid chromosome number ($2n = 16$, recently confirmed, e.g. by Krahulcová (1993) for *T. serotinum* and *T. haussknechtii*, and by Galán de Mera (2010) for *T. pyrrhopappum*), regular sized pollen grains, which is an important indicator of sexuality in *Taraxacum* (den Nijs et al. 1990) and the failure to produce progeny when the flower heads are emasculated and absence of matrocliny, i.e. their progeny vary and differ considerably from the maternal plant (in agamospermous *Taraxacum*, the offspring are very similar morphologically whereas those produced by sexual reproduction a very different).

Data and material collected for the section *Dioszegia* is to be used for a more detailed revision. In particular, the extent of the genetic differentiation among its members, the reproduction as a basis for the differentiation and variation across large areas occupied by this section are to be evaluated. A nomenclatural and taxonomic survey of the section logically follows the biosystematical analyses.

The following aspects of the intrasectional variation are dealt with in the present paper: (i) modes of reproduction recorded for this section (autogamy versus allogamy); (ii) the extent and character of the variation in populations and groups of populations; (iii) macrogeographical dimension of the genetic variation in *T. serotinum* subsp. *serotinum*; (iv) interspecific relationships within sect. *Dioszegia* based on microsatellite (SSRs) population data compared with the traditional classification; and (v) taxonomic revision of the section, typification of names and detailed morphological descriptions of all intraspecific taxa. Another aspect, not dealt with in the present paper, is the role of sect. *Dioszegia* as a parental group for the derived hybridogenous sections with agamospermous reproduction. This role is documented for sect. *Borysthenica* (Kirschner and Štěpánek 2004), but currently is not supported by molecular evidence.
Chapter 12

Material and methods

12.1 Plant material

We sampled the whole section aiming at a reasonable geographical coverage and including all the morphological variants of all taxonomic entities. Altogether, 115 plants from 20 localities were included in the analysis of SSRs, and each taxon (except T. serotinum var. iranicum) is represented by at least one sample bigger than six plants (the largest sample is 26 plants; see Table 12.1 for the samples used in the SSRs analysis). The geographical locations of where the samples were collected is displayed in Fig. 12.1. The samples, except the largest sample of 26 plants of T. serotinum, collected in the field, are the progenies of random field samples of achenes grown in cultivation at Experimental Garden of the Institute of Botany, The Czech Academy of Sciences, Průhonice, Czech Republic (49°59’41” N, 14°34’01” E, 318 m a.s.l.). Specimens used for morphological comparison are listed in the chapter 15.

12.2 Documentation and sources of information

Voucher specimens are deposited in the herbarium PRA, Institute of Botany, The Czech Academy of Sciences, Průhonice, Czech Republic. It is the largest collection of extra-European dandelions in the world, a result of expeditions to many regions of the Mediterranean, Europe and Middle and central Asia, cultivation of plants grown from seeds obtained from other botanists, seeds collected during expeditions and from cultivation of roots. Details of the cultivation methods are given in Kirschner and Štěpánek (1993). This study was supplemented by the examination of numerous herbarium collections. Those most relevant to the present study are BM, E, G, K, LE, PRC, PR, S, W32. Most of our revision labels are numbered and refer to the specimen to which they are attached (as 'no. det.', not necessarily to the duplicates).

32Abbreviation according to Index Herbariorum at http://sciweb.nybg.org/science2/IndexHerbariorum.asp.
Table 12.1: Samples of four taxa of *Taraxacum* sect. *Dioszegia*. **Pop.**.: population abbreviation. Abbreviations of taxon names (**Sp.**) are as follows: **TST**: *T. serotinum* subsp. *tomentosum* from France, **TH**: *T. haussknechtii* from the Republic of Macedonia, **TS-C**: *T. serotinum* subsp. *serotinum* from central Europe (Czech Republic and Austria), **TS-S**: *T. serotinum* subsp. *serotinum* from southern Europe (Bulgaria), **TS-E**: *T. serotinum* subsp. *serotinum* from eastern Europe (Ukraine and Russia) and **TSI**: *T. serotinum* var. *iranicum* from Iran. **No.**.: number of individuals. **Cult. no.**.: cultivation number of collections and cultivations of first three authors. **No. det.**.: Determination number of PRA herbarium. Some samples do not have determination number (NA).

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<td>Bitola</td>
<td>Heraklea Lyncestis</td>
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<td>Matka</td>
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<td>JŠ VODNO</td>
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<td>Ce</td>
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<td>26</td>
<td>Czech Republic</td>
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<td>Čejč</td>
<td>48°56'14.6&quot;</td>
<td>16°58'59.4&quot;</td>
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<td>16°42'24.8&quot;</td>
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<td>southern Moravia, Břeclav district</td>
<td>Sedlec</td>
<td>48°47'50.6&quot;</td>
<td>16°41'58.4&quot;</td>
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<tr>
<td>Ba</td>
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<td>southern Moravia, Břeclav district</td>
<td>Bavory</td>
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<td>Burgenland</td>
<td>Hainburg an der Donau</td>
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<td>16°57'</td>
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<td>Ch</td>
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<td>3</td>
<td>Bulgaria</td>
<td>Smolyan</td>
<td>Chvojna</td>
<td>41°52'</td>
<td>24°41'</td>
<td>3130</td>
<td>25351</td>
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<tr>
<td>Cp</td>
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<td>Smolyan</td>
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<td>Mi</td>
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<td>Ukraine</td>
<td>Mykolaivs'ka oblast', Pervomais'k</td>
<td>Between Migija and Semenivka</td>
<td>48°00'</td>
<td>30°59'</td>
<td>3486</td>
<td>27385</td>
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<td>Do</td>
<td>TS-E</td>
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<td>Ukraine</td>
<td>Mykolaivs'ka oblast', 15 km SE of Voznesens'k</td>
<td>Doroshivka</td>
<td>48°28'</td>
<td>31°28'</td>
<td>3527</td>
<td>27387</td>
</tr>
<tr>
<td>vPo</td>
<td>TS-E</td>
<td>6</td>
<td>Russia</td>
<td>Volgogradskaya oblast', Kumylzhenskiy region, Stantsia Kumylzhenskaya, Potapovskiy Khutor</td>
<td>Potapovskaya Dubrava</td>
<td>49°57'</td>
<td>42°43'</td>
<td>5321</td>
<td>27389</td>
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<tr>
<td>Ka</td>
<td>TSI</td>
<td>2</td>
<td>Iran</td>
<td>Golestan</td>
<td>Kalaleh</td>
<td>37°19'</td>
<td>55°53'30&quot;</td>
<td>11917</td>
<td>26456</td>
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<tr>
<td>Kl</td>
<td>TSI</td>
<td>4</td>
<td>Iran</td>
<td>Golestan</td>
<td>Kalaleh</td>
<td>37°22'30&quot;</td>
<td>55°56'</td>
<td>12296</td>
<td>26458</td>
</tr>
</tbody>
</table>
12.3 The nomenclature adopted in the present paper

Sectional nomenclature follows the previous nomenclatural and taxonomic accounts (Kirschner and Štěpánek 1997a), see also Kirschner and Štěpánek (1987, 2004). Plant names are in accordance with ICN (McNeill et al. 2012). In order to avoid confusion, we use the name *T. serotinum* subsp. *tomentosum* Lange consistently in what follows, instead of the homotypic *T. pyrrhopappum*.

12.4 Methods used in the taxonomic revision of the section and identification of the mode of reproduction

Principles used in the taxonomic evaluation of dandelions are summarized by (B. Trávníček et al. 2010) and (Ge et al. 2011) and are followed in the present paper. Identification of the reproduction system in *Taraxacum* is described in detail in Kirschner et al. (2006) and Gustafsson (1932).

12.5 Laboratory and statistical analyses

For microsatellite (SSRs) genotyping we used 14 published microsatellite primers MSTA145, MSTA131, MSTA101, MSTA105, MSTA143, MSTA102, MSTA93, MSTA133 and MSTA103 from Vašut et al. (2004) and primers MSTA44B, MSTA53, MSTA61, MSTA73, MSTA78 and MSTA85 from (Falque et al. 1998). Primers were originally developed for *T. officinale* agg. (sect. *Taraxacum*) and *T. laevigatum*, respectively. We isolated genomic DNA from leaf material stored in silica gel or herbarium specimens using Qiagen DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands) following the manufacturer’s instructions. Some samples were taken from plants grown in the experimental garden of the Institute of Botany, The Czech Academy of Sciences, Czech Republic in Průhonice. PCR was performed in a volume of 20 μl (multiplex of up to 9 primers) using Qiagen Multiplex PCR kit (Qiagen, Venlo, Netherlands) following the manufacturer’s instructions, with 10 μM of each primer. PCR conditions were: 95℃ for 15 min (hot start PCR polymerase) and then 30 cycles of 95℃ for 1 min, 54.5℃ for 1 min and 72℃ for 1 min and finally 72℃ for 4 min and a 10℃ hold. Alleles were scored and visualized in GeneMarker 2.4 (SoftGenetics LLC, State College, PA, USA) and rewritten into the data matrix. Most of the computations were performed in R 3.0 (R Core Team 2013–2018). We used packages ade4 (Dray and Dufour 2007), adegenet (Jombart 2008), ape (Paradis et al. 2004), pegas (Paradis 2010), PopGenKit (Paquette 2012), rworldmap (South 2011), sp (Pebesma and R. S. Bivand 2005) and spdep (R. Bivand 2013). We calculated the basic population statistics, i.e. observed and expected heterozygosity, F-statistics (Weir and Cockerham 1984), allelic richness (Paquette 2012), departure from Hardy-Weinberg equilibrium (HWE; Jombart 2008); only for populations with at least 6 individuals, and number of private alleles. We calculated Mantel test (Mantel 1967; Dray and Dufour 2007), Moran’s I (R. Bivand 2013),
principal coordinate analysis (PCoA, Dray and Dufour 2007) and neighbour-joining tree (Saitou and Nei 1987; Paradis et al. 2004; Popescu et al. 2012) of populations (tested by 10,000 permutations). For distance-based analysis we used Neil’s chord distance (Nei et al. 1983). Values of departure from HWE and for Moran’s I were tested using 100,000 bootstraps. Significance of Mantel’s test was tested using 1,000,000 permutations and F-statistics using 1000 permutations.

Spatially explicit Bayesian clustering of populations was computed in BAPS 6.0 (Corander et al. 2008; Cheng et al. 2013). We used K ranging from 2 to 30, 20 times each. Bayesian clustering of individuals was performed in STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003; Evanno et al. 2005; Nordborg et al. 2005; Falush et al. 2007; Hubisz et al. 2009). We used K ranging from 1 to 30, 20 times each. Length of burn-in was set to 1,000,000 and number of steps to 100,000,000. This analysis was performed at Bioportal of University of Oslo, Norway (Kumar et al. 2009). Outputs of independent runs were sorted using Structure.sum R script (Ehrich 2006) and CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) and visualized using distruct 1.1 (Rosenberg et al. 2002). Details about R workflow, software settings etc. are available from first author upon request.
Figure 12.1: Map showing the locations where the samples used in the present study were collected (see Table 12.1 for details) and a spatially explicit Bayesian clustering of populations performed in BAPS. Most of the populations form separated clusters, only some geographically closely related (Sk+Ma+So, Uv+Se+Ba, Mi+Po and Ka+Ki, respectively) occur in the same clusters. Upper part shows individuals plotted as vertical bars and lower shows Voronoi tessellation plot drawn over schematic map of Europe and the Near East. Clusters are the same in both. This result is very similar to the output of STRUCTURE for $K = 16$ (Fig. 13.2), which also shows an admixture.
Chapter 13

Results

13.1 Microsatellite analysis

We genotyped 115 individuals of *Taraxacum sect. Dioszegia* using 14 microsatellite (SSRs) primers. PCR product size ranged between 114 and 439 bp. Number of alleles ranged from 3 to 39 per locus and from 1 to 21 per locus per population. In total, we detected 243 alleles, of which 159 were private for species and/or a population (see Table 13.1 for population statistics). We detected 7 private alleles for *T. serotinum* subsp. *tomentosum*, 47 for *T. haussknechtii* and 105 for *T. serotinum* subsp. *serotinum* (incl. var. *iranicum*), respectively. All multi locus genotypes (MLGs) were unique for respective populations and not shared among species or populations. Basic diversity indices for populations with at least six individuals are given in Table 13.1. Number of alleles and heterozygosity varied considerably among loci and populations. For example, the 26 individuals of population Ce (*T. serotinum* subsp. *serotinum*) were very polymorphic, but monomorphic at locus msta102, which is polymorphic in other smaller populations. Most of the populations are highly heterozygous for most of the loci. Deviations from HWE usually were not statistically significant. Global $F_{ST}$ value was high for *T. serotinum* subsp. *serotinum* (0.26) and low for *T. haussknechtii* (0.14).

13.2 Relationships among species and populations

Bayesian spatial clustering of populations in BAPS revealed 14 clusters (Fig. 12.1). Most populations formed separate clusters, with only some geographically very close populations in the same clusters (for example some Czech populations of *T. serotinum* subsp. *serotinum*, or some populations of *T. haussknechtii*). Bayesian clustering performed in STRUCTURE (see Figs. 13.1 and 13.2) did not reveal a single most likely clustering pattern. Runs for respective K had a relatively low similarity coefficient and the lnP(D) curve revealed several possible more probable Ks. As the most probable we selected two results: 5 and 16 clusters (Figs. 13.1 and 13.2). Pattern of 16 clusters revealed by STRUCTURE (Fig. 13.2) is very similar to results of spatial clustering of populations from BAPS
Table 13.1: Basic population statistics for populations of at least six individuals. **Sp.**: species, **Pop.**: populations (see Table 12.1 for detailed information about species and populations), **No. of alleles**: total number of different alleles detected within population, **H\textsubscript{O}**: observed heterozygosity (average per all loci per population), **H\textsubscript{E}**: expected heterozygosity (average per all loci per population), **Allelic richness**, **No. of private alleles**: number of alleles unique for respective population, **P-value of departure from HWE**: significance of departure from HWE. Abbreviations of names: **TST**: *T. serotinum* subsp. *tomentosum*, **TH**: *T. haussknechtii*, **TS-C**: *T. serotinum* subsp. *serotinum* from central Europe and **TS-E**: *T. serotinum* subsp. *serotinum* from eastern Europe.

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<thead>
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<th>Sp.</th>
<th>Pop.</th>
<th>No. of alleles</th>
<th>H\textsubscript{O}</th>
<th>H\textsubscript{E}</th>
<th>Allelic richness</th>
<th>No. of private alleles</th>
<th>P-value of departure from HWE</th>
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</tbody>
</table>

(Fig. 12.1). STRUCTURE clearly separated species into their own clusters with a very restricted extent of the among-species ‘introgression’ (in the sense according to Corander et al. 2008), see Fig. 13.1. The ‘introgression’ was mainly observed between *T. haussknechtii* and *T. serotinum* subsp. *serotinum*. STRUCTURE clustering for K = 16 is in accordance with output of BAPS. In the STRUCTURE result for K = 5 (Fig. 13.1) in 9 runs of 20, the clusters were formed by *T. serotinum* subsp. *tomentosum*, *T. haussknechtii*, *T. serotinum* var. *iranicum* and two clusters of *T. serotinum* subsp. *serotinum*: central Europe and southern together with eastern Europe. Seven runs differed from the previous pattern in that *T. haussknechtii* formed two clusters (one for population He and the second for the other populations) and populations of *T. serotinum* subsp. *serotinum* from southern and eastern Europe formed a cluster together with *T. serotinum* subsp. *iranicum*. Remaining four runs resulted in a slightly different pattern, although generally similar to the two described. STRUCTURE for K = 16 (Fig. 13.2) resulted in a generally similar pattern for all runs: most of the populations retain their own ‘main’ clusters and there is extensive introgression (its level is the only difference among the runs) among populations within taxa — for *T. haussknechtii* (practically without population He) and *T. serotinum* subsp. *serotinum*, respectively.

PCoA analysis of all taxa (Fig. 13.3) shows *T. serotinum* subsp. *tomentosum* as distant
Figure 13.1: Results of STRUCTURE analysis for $K = 5$ (20 independent runs). *Taraxacum serotinum* subsp. *tomentosum* (TST: Fe) forms a well separated cluster, *T. haussknechtii* (TH: He, Oh, Pr, Ne, Sk, Ma and So) forms one or two clusters (depending on run) and shows a very limited introgression of *T. serotinum* subsp. *serotinum*. Remaining clusters are formed by *T. serotinum* subsp. *serotinum* (TS) from central (TS-C: Ce, Uv, Se, Ba and Ha), southern (TS-S: Ch and Cp) and eastern Europe (TS-E: Mi, Do and Po), respectively. Last cluster is formed by *T. serotinum* var. *iranicum* from Iran (TSI: Ka and Kl). Populations of *T. serotinum* var. *iranicum* seem relatively distantly related to the other populations of that species.
Figure 13.2: Results of STRUCTURE analysis for K = 16 (20 independent runs). *Taraxacum serotinum* subsp. *tomentosum* is forming one very well separated cluster. *Taraxacum haussknechtii* consists of several clusters, which are mostly exclusive for this species (there is limited, but detectable level of introgression of *T. serotinum* subsp. *serotinum*). All populations of *T. haussknechtii* and *T. serotinum* subsp. *serotinum* and var. *iranicum* show some level of admixture. Populations of *T. serotinum* subsp. *serotinum* from distantly related geographical regions show very limited genetic exchange. Results are very similar to spatial Bayesian clustering of populations performed in BAPS (Fig. 12.1).
from the other taxa, another cluster is formed by *T. haussknechtii* and there is a cluster of groups of *T. serotinum* subsp. *serotinum* and *T. serotinum* var. *iranicum* (i.e. populations from Iran, marginal to *T. serotinum* subsp. *serotinum* and adjacent to *T. haussknechtii*), with distinct sub-clusters of populations from southern Europe and central and eastern Europe, respectively.

Mantel test shows a clear isolation by distance pattern (Table 13.2) for *T. haussknechtii* and *T. serotinum* subsp. *serotinum* (including var. *iranicum*). The value of Moran’s I is positive and significant (i.e. the isolation by distance pattern) only for *T. serotinum* subsp. *serotinum* (including var. *iranicum*) and not significant for *T. haussknechtii* (not shown).

Neighbour-joining tree of all populations points to a very clear pattern (Fig. 13.4): the main branches of the unrooted tree are formed by respective taxa. Exact position of the root is unclear as branches leading to *T. serotinum* var. *iranicum* from Iran (and to *T. serotinum* subsp. *serotinum*), *T. serotinum* subsp. *tomentosum* and *T. haussknechtii* branch very rapidly.

### 13.3 Morphology and the mode of reproduction

**Table 13.2:** Mantel tests for *Taraxacum haussknechtii* and *T. serotinum* subsp *serotinum* (including var. *iranicum*). Observed values are significantly greater than expected values based on 1,000,000 permutations, which indicates significant isolation by distance.

<table>
<thead>
<tr>
<th>Species</th>
<th>Observation</th>
<th>P-value</th>
<th>Std. Obs</th>
<th>Expectation</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. haussknechtii</em></td>
<td>0.57741</td>
<td>0.000001</td>
<td>9.101255</td>
<td>-0.000003</td>
<td>0.004025</td>
</tr>
<tr>
<td><em>T. serotinum</em> subsp. <em>serotinum</em> (incl. var. <em>iranicum</em>)</td>
<td>0.33983</td>
<td>0.000001</td>
<td>8.244719</td>
<td>-0.000001</td>
<td>0.001699</td>
</tr>
</tbody>
</table>

Results of the morphological examination of the whole material are summarized in the taxonomic treatment below (chapter 15, see also Table 13.3 for comparison of diagnostic characters). We recognize *T. serotinum* subsp. *serotinum*, *T. s.* var. *iranicum*, *T. s.* subsp. *tomentosum* (*T. pyrrhopappum*) and *T. haussknechtii*. The separate specific status of *T. haussknechtii* is retained on the basis of morphological distinctiveness, geographical endemism and molecular analyses. Within *T. serotinum* subsp. *serotinum*, a similar magnitude of distinctiveness is found in the Iranian plants; we treat them as a newly described variety, var. *iranicum*. The limited number of Iranian specimens and extensive morphological variation of Iranian plants does not make it possible to draw safe conclusions about their taxonomic status and relationships. The allopatric *T. serotinum* subsp. *tomentosum* shows a very low level of morphological differentiation. The features characterizing subsp. *tomentosum* include non-specific attributes, such as short, non-elongated scapes, permanently closed or nearly closed capitula or often a well-developed petiole; it is therefore difficult to draw a line between subsp. *tomentosum* and subsp. *serotinum*. The former two
Figure 13.3: PCoA of *Taraxacum* sect. **Dioszegia** indicating that *T. serotinum* subsp. *tomentosum* is distantly related to the other taxa. Populations of *T. haussknechtii* form a well separated cluster. Remaining clusters are formed by populations of *T. serotinum* var. *iranicum* (most closely related to *T. haussknechtii*) and *T. serotinum* subsp. *serotinum* from southern, central and eastern Europe, respectively.
Figure 13.4: Unrooted neighbour-joining tree of populations of *Taraxacum* sect. *Dioszegia*. All taxa form distinct branches. Numbers show bootstrap support.
characters point to the fact revealed by the analysis of microsatellite data, the total homo-
zygosity associated with dominant autogamy (see also Hughes and A. Richards 1988, 1989,
and below). The statistical analysis of molecular data shows a more separate position of
subsp. *tomentosum* than expected from its morphology; the explanation of this discrep-
ancy is presented in the Discussion.

The analyses of microsatellite data reveal high variation and heterozygosity in *T. seroti-
num* subsp. *serotinum* and *T. haussknechtii*, pointing to their outcrossing sexuality. For
instance, the largest population of *T. serotinum* subsp. *serotinum* studied (Ce, 26 individuals)
has both a high mean heterozygosity and genotype diversity, a safe indicator of outcrossing.
Table 13.3: Characters distinguishing the taxa in *Taraxacum* section *Dioszegia*.

<table>
<thead>
<tr>
<th>Character</th>
<th><em>T. serotinum</em> subsp. serotinum (excluding Iran)</th>
<th><em>T. serotinum</em> var. iranicum (Iran)</th>
<th><em>T. serotinum</em> subsp. tomentosum (= <em>T. pyrrhopappum</em>)</th>
<th><em>T. haussknechtii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf posture</td>
<td>usually appressed to the ground</td>
<td>usually erect-patent</td>
<td>usually appressed to the ground</td>
<td>mostly erect-patent</td>
</tr>
<tr>
<td>Leaf shape</td>
<td>relatively broad, most often not divided (then having the shape of leaves of <em>Hypochaeris maculata</em> L.), if divided then lateral segments broad, ± obtuse</td>
<td>variable, relatively narrow, divided or undivided (segments similar to those of <em>T. serotinum</em>)</td>
<td>relatively broad, often not divided, not rarely divided (to pinnatisect) with ± broad rounded lateral segments (the shape similar to that of <em>T. obovatum</em>)</td>
<td>conspicuously narrow, almost always pinnatipartite to pinnatisect, lateral segments usually acute</td>
</tr>
<tr>
<td>Size of leaves</td>
<td>usually 12–20 × 2.5–5.5 cm</td>
<td>10–15 × 2–2.5 (–4) cm</td>
<td>usually 6–11 × 2.5–4 cm</td>
<td>usually 6–12 × 0.5–2 cm</td>
</tr>
<tr>
<td>Petiole length</td>
<td>1 (–2) cm</td>
<td>1.5–2.5 cm</td>
<td>1–4 cm</td>
<td>1.5–2 cm</td>
</tr>
<tr>
<td>Scape length in full blossom / in fruit</td>
<td>usually longer than leaves, 10–20 cm / to 35 cm</td>
<td>longer than leaves, 12–20 cm / to 25 cm</td>
<td>usually conspicuously shorter than leaves, often only 2–4 cm / usually shorter than leaves, rarely reaching 10–15 cm in fruit</td>
<td>variable, usually 3–12 cm / equalling to clearly overtopping leaves, often over 20 cm</td>
</tr>
</tbody>
</table>
Table 13.3.

<table>
<thead>
<tr>
<th>Character</th>
<th>T. serotinum subsp. serotinum (excluding Iran)</th>
<th>T. serotinum var. iranicum (Iran)</th>
<th>T. serotinum subsp. tomentosum (= T. pyrrhopappum)</th>
<th>T. haussknechtii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape and size of capitulum</td>
<td>small to medium-sized, ~3 cm in diam., ± flat, light to bright yellow</td>
<td>usually small (more material needed)</td>
<td>medium sized but remaining semi-closed, therefore only 1.5–2.5 cm in diam., ± light yellow</td>
<td>small to medium-sized, 1.5–2.5 (~3) cm in diam., ± flat, golden yellow to light yellow, rarely pale ochraceous-yellow</td>
</tr>
<tr>
<td>Outer phyllary number</td>
<td>30–46</td>
<td>26–35</td>
<td>26–38</td>
<td>(15–) 18–34</td>
</tr>
<tr>
<td>Outer phyllary margin</td>
<td>usually ± evenly densely ciliate</td>
<td>not available</td>
<td>relatively sparsely ciliate, usually only near phyllary apex</td>
<td>not ciliate or sparsely ciliate, if so, then more densely towards the apex</td>
</tr>
<tr>
<td>Rostrum length</td>
<td>(5–) 7–9 (~10.5) mm</td>
<td>9.5–11 (~13) mm</td>
<td>4–8 mm</td>
<td>(4–) 6–9 (~10.5) mm</td>
</tr>
<tr>
<td>Pappus length</td>
<td>6–8 (~10) mm</td>
<td>5.5–6 (~7) mm</td>
<td>4.5–7 mm</td>
<td>(5–) 6–8 mm</td>
</tr>
<tr>
<td>Pappus colour</td>
<td>variable, dirty whitish, dirty yellowish, pale brownish</td>
<td>pale brownish</td>
<td>invariably pale brownish with ± pinkish tinge (similar to T. bessarabicum)</td>
<td>pale yellowish-brownish, dirty brownish white, greyish-brownish or pale brownish with pinkish tinge</td>
</tr>
</tbody>
</table>
Chapter 14

Discussion

14.1 Sexuality and reproduction

The section *Dioszegia* was thought to be quite exceptional in the genus *Taraxacum* because all its members are exclusively sexual. The sexuality was tested previously by several authors (see chapter 11) and confirmed on the basis of studies on geographically and taxonomically representative samples and the molecular analyses in the present paper. Only another two sexual diploid *Taraxacum* species were studied from the viewpoint of their population genetic differentiation. *Taraxacum bessarabicum* (sect. *Piesis*) is almost completely autogamous and homozygous throughout its large geographical range and almost homogenous genetically in the western part of its range (Kirschner et al. 1994; Kirschner and Štěpánek 2008). *Taraxacum koksaghyz* (sect. *Ceratoidea*, see Kirschner and Štěpánek 2008; van Dijk et al. 2010; Kirschner et al. 2013) occupies a medium-sized geographical range in south-eastern Kazakhstan, comparable in size to that of *T. haussknechtii*. It is an obligate out crosser with the absolute predominance of within-population genetic variation (Kirschner et al. 2013). Thus, the sexual taxa in *Taraxacum* exhibit contrasting patterns of genetic variation. Within section *Dioszegia*, with the probable exception of the autogamous *T. serotinum* subsp. *tomentosum* (due to the limited sampling), there is remarkable among-population variation.

*Taraxacum serotinum* subsp. *tomentosum* exhibits an almost complete homozygosity, which indicates autogamy (also supported by structural adaptation of capitula, which remains partly closed during anthesis; and as a rule scapes do not elongate after anthesis). This view is also supported by its scattered distribution in small populations across southern France, northern Spain and (probably introduced) in northern Morocco. Our results corroborate the idea of self-compatibility introduced by Hughes and A. Richards (1988, 1989) who studied four plants of *T. serotinum* subsp. *tomentosum* (under the name ‘*T. pyropappum*’) and 14 plants of their progeny using 15 isozyme loci and did not find any heterozygosity. We therefore tentatively treat this subspecies as an autogamous taxon on the basis of two independent investigations covering two distribution centres of this taxon, France and Spain.

The biggest population of *T. serotinum* subsp. *serotinum* (Čejč, Czech Republic, code: 75
Ce) shows a high level of heterozygosity, but for eight of 14 loci also shows significant departures from HWE. A similar pattern is recorded in other populations of this subspecies (see Table 13.1), which is expected for a sexual species with small populations and limited dispersal ability. Most samples came from random progenies from seeds collected in nature, therefore representing the potential variation of the parental populations. We therefore treat the widely distributed subspecies, in accordance with the literature (Gustafsson 1932; Poddubnaja-Arnoldi and Dianowa 1934), as a sexual outcrossing taxa.

Similarly, all the molecular and morphological results for *T. haussknechtii*, which was relatively densely sampled over an area covering a substantial part of its geographical range, support outcrossing sexuality.

### 14.2 Population analysis of widespread diploid outcrossing sexual species

A comparison of the population genetic parameters of *T. serotinum* subsp. *serotinum* and *T. haussknechtii* reveal that they both correspond to the isolation by distance model (highly significant results of the Mantel test). Global $F_{ST}$ for *T. serotinum* (including var. *iranicum*) is as expected high, 0.26; the global $F_{ST}$ value for *T. haussknechtii* (0.14) also points to a strong population substructure. The greater values for global $F_{ST}$ for these two species indicate a high microevolutionary potential, particularly for marginal populations (such as var. *iranicum*). On the other hand, departure from HWE was usually not statistically significant. This can be caused by small sample sizes, despite the isolation of the populations indicated by high number of private alleles and high uniqueness of the MLGs. Most of the case studies of similar situations compare the population genetic diversities of a widespread/rare species pair, which is not meaningful in the case of the isolated *T. serotinum* subsp. *tomentosum* characterized by a shift to autogamy. A heuristic search of genetic differentiation within widespread outcrossing sexual species reported in the literature shows that taxa with limited seed dispersal and entomogamy with specialised pollinators and large, sometimes discontinuous geographical ranges tend to develop a substantial population substructuring (e.g. Gitzendanner and P. S. Soltis 2000; He et al. 2000; Twyford et al. 2014), while many of the widespread anemogamous trees or plants that are efficiently pollinated by insects (diverse widespread pollinators, e.g. Gonela et al. 2013) are characterized by the absence of geographical population structure (Gitzendanner and P. S. Soltis 2000; García-Gil et al. 2003; Kado et al. 2003; Palmé et al. 2003; Neale and Savolainen 2004; Bloomfield et al. 2011). It should be added, however, that there are many departures from this rule (e.g. Ingvarsson 2005) and the examples listed above usually do not cite other circumstances important for evaluating population genetic diversity, such as (paleo)polyploidy, migration history or possible introgression.
14.3 New assessment of the taxonomy and relationships in *Taraxacum* sect. *Dioszegia*

In spite of the relatively extensive genetic diversification, all the taxa recognized in the section *Dioszegia* are very similar to one another in terms of the important morphological characters (achenes, outer phyllaries). The lowest level of morphological differentiation is found between the isolated *T. serotinum* subsp. *tomentosum* and *T. s. subsp. serotinum* (see Table 13.3). The main microevolutionary event, the shift from allogamy to autogamy is accompanied by rather inconspicuous structural morphological changes, mainly abbreviated scapes, incompletely opening capitula and usually a well-developed petiole in *T. serotinum* subsp. *tomentosum*, although the difference in the reproduction system, high homozygosity and the genetic make-up of the population studied might support its specific status, the morphological similarity with *T. s. subsp. serotinum* points to a lower rank.

The analysis of molecular data indicates that the main qualitative difference between subsp. *tomentosum* and subsp. *serotinum* is the missing alleles in the former. The mean number of alleles per locus in subsp. *tomentosum* is 1.071 while that of the other populations varies from 2.714 to 8.429. Populations are small and autogamous and the events of fixation of rare or new alleles may be quite frequent, which accounts for the other aspect of the molecular differentiation between the two subspecies. The lack of substantial structural morphological divergence between the two subspecies and the nature of the above molecular differences are the main arguments in favour of the subspecific treatment of this autogamous taxon within *T. serotinum*.

Another isolated, morphologically aberrant group of populations is confined to the northern part of Iran, which is genetically rather remote from subsp. *serotinum*, requires further study (some *T. serotinum* plants in Iran do not seem to belong to the same phenetic group, but that is based on a relatively limited amount of material). The plant specimens listed are so distinctive that we recognize them as a separate variety, var. *iranicum*, which is confined to north-central Iran and is relatively isolated from the nearest regions where *T. serotinum* subsp. *serotinum* occurs (the north and south Caucasus or Anatolia). Plants from Iran were known to be aberrant morphologically (the general habit deviating from the most common pattern of *T. serotinum* in other areas, see e.g. van Soest (1977), who mentions that ‘the oriental forms ... are mostly similar to *T. haussknechtii* by smaller size, smaller flower heads, more divided leaves’). The degree of differentiation between var. *iranicum* and var. *serotinum* is not easy to evaluate because of the relatively limited material available and, more importantly, because the characters diagnostic of var. *iranicum* are not equally clearly developed in all the Iranian specimens studied. Pending further population research and depending mostly on the basis of the results of the SSRs analyses we treat the Iranian plants as a variety.

Last, the specific status allotted to *T. haussknechtii* requires comment. The material analyzed genetically and the specimens seen are fully representative of this taxon, which has a small geographical range centred in the Republic of Macedonia, Greek Macedonia and
adjacent regions of neighbouring countries (Bulgaria, Albania, Serbia). In the north-east, it is parapatric with *T. serotinum* subsp. *serotinum*. Although we have seen much rich Bulgarian material of both taxa, there is no trace of hybridization or morphological transition between them. The isolating mechanisms are not known and may involve a different habitat and a shift in phenology (*T. haussknechtii* flowers earlier). Moreover, *T. haussknechtii* has several features making it possible to draw a line between it and the rest of the section (narrow leaves up to 2 cm wide, leaf lateral segments acute, outer phyllaries only distally sparsely ciliate or subglabrous, quite narrow, usually 0.6–1.2 mm wide).

### 14.4 Microevolution through shifts from allogamy to autogamy: *Taraxacum serotinum* subsp. *tomentosum* is a special case

If we disregard hybridization and polyploidy, there are three main evolutionary situations in which a homoploid shift from self-incompatibility or prevailing allogamy towards autogamy may be associated with instant advantages:

First, is a founder situation often followed by adaptive radiation, documented recently, for instance, for Hawaiian species of *Schiedea* Cham. et Schltdl. (Sakai et al. 2006), with three independent origins of obligate autogamy during island colonizations.

The second situation involves cases of sympatric evolution with the development of reproductive barriers through a shift to autogamy; a habitat shift is often also involved. A classic example is the evolution of *Stephanomeria malheurensis* Gottlieb, a descendant of the widespread *Stephanomeria exigua* subsp. *coronaria* (Greene) Gottlieb, which near its northern limit is confined to volcanic hilly sites (Gottlieb 1973, 2003). While the progenitor species has a sporophytic multiallelic self-incompatibility, the descendant species is almost completely autogamous. Another couple of similar cases are those of *Epipactis helleborine* subsp. *neerlandica* (Verm.) Buttler as an allogamous progenitor and the local autogamous derivative described as *E. renzii* Robatsch (Pedersen and Ehlers 2000) or *Aquilegia vulgaris* L. and *A. paui* Font-Quer as a progenitor-derivative pair (Martinell et al. 2011).

The last situation is more complicated: it includes cases of (repeated) migrations and retreats of a widespread outcrossing species during late glacial and early post-glacial periods, often leaving ‘witness’ populations in the formerly colonized areas. As the time available for possible microevolutionary changes was relatively limited, most of these isolated populations of continental migrants are not recognized as separate taxa. As examples, we can cite *Taraxacum bessarabicum* (Hornem.) Hand.-Mazz. (with an enormous continental distribution ranging from northern China and southern Siberia to eastern Austria and south-eastern Czech Republic, and an isolated locality in Auvergne, south-western France) or *Krascheninnikovia ceratoides* (L.) Gueldenst. (occupying a gigantic continental range from China and Mongolia to Austria and south-eastern Moravia (Czech Republic), with isolated sites in Spain and Morocco). At isolated sites, these migratory species may have undergone
speciation processes associated with genetic drift, which might have included the shift to autogamy.

The present case of *Taraxacum serotinum* subsp. *tomentosum* involves both continental migration, isolated ‘witness’ populations and a shift towards autogamy. In terms of morphology, the degree of divergence between subsp. *tomentosum* and subsp. *serotinum* is relatively small. At the SSRs level, however, the differentiation is more remarkable (see Fig. 13.4).
Chapter 15

Taxonomic treatment: a revision of *Taraxacum* sect. *Dioszegia*


For details of the sectional nomenclature and comments see Kirschner and Štěpánek (1987, 1996, 1997a).

Description: Rosulate hemicryptophytes. Flowers and leaves develop simultaneously. Main flowering season: summer to early autumn. Main habitat: secondary dry, semi-steppe sites or xeric grasslands. Plants medium-sized to robust, plant base densely brownish hairy. Leaves subcoriaceous, usually densely hairy, at least beneath, swollen at hair base (often forming low protuberances or ridges), rarely ± flat, shallowly to deeply lobed, lobation pattern usually uncomplicated, lobes usually subpatent to recurved, leaves usually flat, midrib without striatulate pattern, leaf blade unspotted, petioles winged or broadly winged. Scapes erect during flowering, unbranched, growing from the centre of leaf rosette, densely aranose. Involucre with ± rounded base, usually of medium width. Flowers yellow, florets usually very numerous, ligules flat to canaliculate. Interior involucral phyllaries usually callose to corniculate at the apex. Exterior bracts 15–46, relatively regularly arranged, usually imbricate, ± appressed at base, often arcuate to arcuate-recurved at the apex, narrowly linear-lanceolate, usually 5–8 mm long, pale greenish, often suffused red or pinkish, usually faintly bordered or with narrow paler or reddish margins, ciliate or sparsely ciliate to glabrous. Pollen always present, stigma pure yellow. Receptacle glabrous. Achenes sub-turbinate, usually 4.5–7 mm long, usually 0.8–1.1 mm thick, very gradually narrowing into the cone, achene body usually pale greyish straw brown, sparsely spinulose above, cone
subcylindrical to cylindrical (length difficult to measure because of the indistinct transition between achene body and cone), usually 0.8–1.5 mm long. Rostrum thin, usually 6.0–8.0 (–12) mm long, pappus usually 6.5–8.5 mm long, whitish-yellowish to brownish-pinkish, not deciduous. Reproduction: Sexual (diploid).

Diagnostic notes: A group of four taxa, here accepted as species, subspecies and a variety. Characteristic features include linear-lanceolate, imbricate (and apically arcuate) outer bracts, large achenes very gradually narrowing into the cone, subcoriaceous leaves with hairs often growing on small ridges on the leaf surface, and summer or late summer flowering. See Table 13.3 for comparison of morphological characters among all four taxa.

Comments on habitats: In contrary to the sites occupied by the sect. Piesis, Dioszegia typically occurs in areas of dry, semisteppe to steppe, often naturally disturbed places.

Distribution: Mainly distributed in SC and SE Europe and from Turkey to Afghanistan and NW Kazakhstan, isolated occurrence in SW Europe; formerly introduced into Morocco.

   a. *Taraxacum serotinum* subsp. *serotinum*
      — Lectotypus, hic designatus: ‘3459 Leont. Libanus. m. Aucher Eloy 1837’, [the date is probably that of accession in DC’s herbarium], Aucher Eloy 3459 (G-DC, no. det. 18921); isolecototype: (G-BOIS, no. det. 18827; P 691557, photo! — only upper left plant).

Icon.: Fig. 15.1.
Note: The type herbarium sheet of *T. voronovii* consists of numerous smaller plants on the lower half of the sheet, and a bigger specimen in the upper right corner of the sheet, most of them might belong to *T. serotinum*; the only plantlet (A) safely determinable as *T. serotinum* is selected as the lectotype. There is a bigger plant on the same sheet that probably belongs to a taxon close to *T. stenolepium* Hand.-Mazz. The original description combines the characters of both taxa but the achene description is closer to *T. serotinum*.

Description: Plants medium-sized to robust, 12–35 cm tall, densely brownish aranose at base (Fig. 15.1). Leaves subcoriaceous, usually appressed to the ground (most often erect-patent in var. *iranicum*), densely tomentose-aranose beneath, relatively densely aranose above, leaf surface often swollen at hair base (forming a low protuberance or a short ridge), not shiny, dark green to greyish green, not spotted, sometimes suffused purplish above; leaf blade oblong, elliptical to obovoid, usually 12–20 × 2.5–5.5 cm (10–15 × 2–4 cm in var. *iranicum*), either entire, obtusely acute to rounded at apex, densely irregularly denticulate, or pinnatifid to pinnatisect; terminal leaf segment triangular to broadly ovate; terminal leaf segment triangular to broadly ovate, rounded to obtusely acute, distal margin convex, denticulate, proximal margin straight to concave, denticulate, patent to recurved, lateral segments (3) 4–6, large, ± triangular, patent to subhamate, both margins denticulate, distal one convex, proximal one ± straight or concave; interlobes ± short, irregularly denticulate, margins raised, mid-vein pale green to pale brownish pink; petiole short, usually 1 (–2) cm long, usually light pink-purple. Scapes over
topping leaves, irregularly densely floccose-aranose. Capitulum small to medium-sized, ca. 3 cm in diam., ± flat, light to bright yellow; involucre cylindrical (Fig. 15.2), outer phyllaries 30–46 (26–35 in var. iranicum), linear-lanceolate, 6–8 × (1.0–) 1.3–1.8 mm, imbricate, appressed or loosely appressed at base, arcuate-recurved in upper half, green to grey-green, usually suffused pink or light brownish purple, middle part darker, bordered pale green, usually ± evenly densely ciliate, ± flat. Ligules flat, outer ones abaxially striped brown-purple. Pollen developed, pollen grains of ± uniform size; stigmas yellow. Achenes of various colours, usually pale greyish straw-brown, but also yellowish straw-coloured, olivaceous greyish, pinkish pale brown, medium brown or silvery whitish, sometimes also pale ochraceous, narrowly turbinate, gradually narrowing at both ends, 4.4–6.8 (–7.2) mm long (incl. cone), (0.8–) 0.85–1.1 mm wide, wider in upper 3/5–2/3 of achene length, achene body very gradually, almost indistinctly narrowing into the cone; cone ca. (0.7–) 0.8–1.4 (–2.0) mm long; rostrum (5–) 7–9 (–10.5) mm (to 11–13 mm in var. iranicum), pappus 6–8 (–10) mm (5.5–6 (–7) mm in var. iranicum), pale brownish, yellowish-brownish or dirty whitish. Flowering optimum late summer.

Reproduction: As proven by Gustafsson (1932), T. serotinum is not an autonomous apomict. Numerous experiments (carried out also by T. Černý and J. Štěpánek, not presented here) with isolated capitula showed that there is no tendency to unassisted autogamy, either. The character of self-incompatibility remains to be tested; the absolutely prevailing allogamy seems to be proven.

Distribution: The largest region of the continuous distribution of T. serotinum subsp. serotinum extends from eastern Romania and Bulgaria through Ukraine and SE European Russia to NW Kazakhstan (in the NW and near the Caspian coast); there is another large part of its range in the NW Pannonian region (reaching Moravia and NE Austria) and Anatolia, Lebanon and Syria. Smaller, more or less isolated regions of its occurrence are in other parts of the Balkans, in Transcaucasia, N Iran and adjacent parts of Turkmenistan and Afghanistan.

Material studied: We have seen over 800 herbarium specimens of subsp. serotinum from all the regions listed above. The lists of specimens are available upon request from JK or JŠ.

Variation: As may be expected for a sexually reproducing taxon with a large geographical range, the variation is relatively extensive, lower near the NW limit of its range, higher in some regions, such as Bulgaria and adjacent countries (a variation involving even achene colour). With a single exception, the variation does not form any population units.

The exception is T. serotinum in Iran where the plants studied are distinct in having shorter pappus and longer rostrum (much higher values of the rostrum/pappus ratio). We recognize plants with such aberrant achenes as a separate variety:

*Taraxacum serotinum* var. *iranicum* Kirschner, Štěpánek, Zeisek et Amini Rad, var. nova


Diagnosis: *A varietate typica rostro longiore et pappo breviore differt.*

Brief description: Plants less robust, with slightly narrower, erect-patent leaves, usually
deeply divided into denticulate lateral lobes, slightly smaller capitula and involucre, lower number of outer phyllaries (26–35) and achenes with rostrum usually 9.5–11 (–13) mm long and pappus 5.5–6 (–7) mm long.

Comments: Both the genetic relationships (inferred from SSRs analyses) and the morphology of the specimens analyzed indicated a relatively distinct position for the Iranian plants. However, because there are overlaps between the ranges in the variation in the diagnostic characters, and the material used does not show any population variation, we treat these plants as a variety of *T. serotinum*.

Distribution: The material studied is confined to the N to NE part of Iran, provinces of Mazandaran, Semnan and Khorasan. It is a region quite isolated from the nearest area where *T. serotinum* occurs.

Part III. Section Dioszegia

Chapter 15. Taxonomic treatment: a revision


Note: There are certain doubts about the priority of the various publications by O. Debeaux that appeared in 1891. The Bull. Soc. Bot. Fr. publication cites the Toulouse description (without page reference) and there is a separate booklet publication [Debeaux O. (1891): Note sur plusieurs plantes nouvelles ou peu connues de la région méditerranéenne et principalement des Pyrénées-Orientales.— Paul Klincksieck, Paris, 8 vol, 53 pp., n. v., Tax. Lit. 29344, probably Jul. 1891, corresponding to the Rev. Bot. publication] also cited in Rev. Bot. For the time being, we ascribe the priority to the Toulouse publication but a detailed bibliographic search might change this conclusion. It is important that all the three publications cite the material collected by J. Neyraut, first in June 1888 and then in August 1890.

≡ *Taraxacum serotinum* var. *spathulifolium* Rouy, [scheda, exs.:] Société Rochelaise 1891, no. 3101 (1892) ut ‘*spathulaefolium*’, see also Rouy, Fl. Fr. 9: 192 (1905) [the excisicate seen in MPU, no. det. 20358 & 20350; P 4304008 & 4304986, photo!].
Secunda, no. 460 (lectotypus, hic designatus: P 3726134, photo!; isolecotype: G, no. det. 22590; PRC 403195, no. det. 26948). — Residual syntypes: 'In argillosis sterilibus prope Dasoon in Arragonia.', Jul 1850, Willkomm (P 3726135, photo!). — 'In argiloso-arenosis in silvis Juniperi Sabinae inter Pozondón et Celda in Arragonia australi.', Aug 1850, Willkomm (P 3726135, photo!).

Icon.: Fig. 15.3.

Figure 15.3: *Taraxacum serotinum* subsp. *tomentosum*. Habit (A), scale bar = 5 cm, and achene (B), scale bar = 1 mm. Drawn by J. Štepánek.

Description: Plants small to medium-sized, usually 5–12 cm tall, densely brown aranose at base, the base also covered with remnants of old petioles (Fig. 15.3). Leaves subcoriaceous, leaf rosettes similar to those of *T. obovatum*, smaller than those of *T. serotinum*, with narrower, leaves usually appressed to the ground, light greyish-green, initially ± densely whitish tomentose-aranose, later glabrescent, leaf surface usually swollen at hair base; leaf blade broadly oblong-spatulate, usually 6–11 × 2.5–4 cm, margin (including that of lateral segments) usually irregularly denticulate, blade often undivided, frequently pinnatifid to pinnatisect, rarely pinnatisect, terminal segment usually large, dominant, ± obtuse, often with a minute mucro, lateral segments usually 2–4 (~5), broadly triangular to triangular-lingulate, short, patent to subrecurved, rarely subhamate; blade gradually narrowed into 1–4 cm long, narrow, pale to pink-brown, densely hairy petiole; mid-vein pale to paled pinkish-brownish, initially densely floccose-aranose. Scapes short at anthesis, usually con-
spicuously shorter than leaves, often only 2–4 cm long, usually remaining short during fruit ripening, light green, often suffused bronze, wholly tomentose-aranose or densely floccose-aranose. Capitulum small to medium-sized, imperfectly opening and therefore only 1.5–2.5 cm wide, flat, ± pale yellow; involucre rounded to subtruncate at base, ca. 7–8 mm in diam., light olivaceous-green, usually suffused pinkish, slightly pruinose; outer phyllaries 26–35 (–38), linear-lanceolate to linear, (4–) 5–8 × (0.8–) 1.0–1.7 (–2.3) mm appressed at base, upper half arcuate-recurved, in general very similar to those of *T. serotinum* in shape, size and coloration but relatively sparsely ciliate, usually only near phyllary apex, inner phyllaries to ca. 10 mm long, coriiculate, longitudinally striped purplish, pruinose. Ligules flat, outer ones abaxially striped grey-pink to grey orange, apical teeth yellow. Pollen developed, pollen grains uniform in size; stigmas yellow. Achenes (yellowish) light greyish pale straw-brown, narrowly turbinate, 4.6–5.9 mm long including cone, 0.8–1.05 mm wide, achene body with upper 1/3–1/4 covered with sparse to subdense short spinules (less often squamules), otherwise smooth, very gradually narrowing into subconical to subcylindrical cone ca. 0.8–1.5 mm long; rostrum 4–8 mm long, pappus 4.5–7 mm long, ± invariably yellowish light brown. Flowering optimum: summer to late summer.

Note: It is not easy to distinguish from *T. serotinum* subsp. *serotinum* (for details, see Table 13.3). In general, plants of *T. serotinum* subsp. *tomentosum* are smaller, leaves have a relatively longer petiole, leaf lateral lobes are less rounded (more 'angled'), scapess shorter, not elongating, capitulum not fully opening.

Reproduction: Both the imperfectly opening capitula and the almost complete homoyzogosity indicate autogamy.

Distribution: Widely distributed in CE and SE Spain, in S France and, probably introduced, at a single site in N Morocco (e.g. van Soest 1954).

Neyraut (MPU, no. det. 20359; P 4121414, photo!). — Aude, Montagne d’Alaric entre Hour et Moux, 11 Sep 1910, L. Marty 1720. (MPU, no. det. 20346; P 4272367, photo!). — Aude : mont Alaric, 12 Jul 1891, [E. J. Neyraut] (MPU, no. det. 20352). — Aude : Laroque-de-Fa, coteaux secs, bords des chemins., Aug-Sep, J. Delpont (P 4131406, photo!).

Spain: Province of Teruel. Sierra de Valacloche, pelouses arides, sur le calcaire, 1,600 mètres, 1893, E. Reverchon, Pl. Espagne, prov. Teruel, no. 836 (E, no. det. 11848; P 3726148, photo!); PR, no. det. 449 & 28610; PRC, no. det. 21148; UPS, no. det. 24226 & 24232; WU, no. det. 22120; P 4304090, 3726152 & 3726155, photo!; Z, no. det. 24514). — Castille : Bujedo, chemins, 7 Aug 1907, H. Elías (BRNU 29253, no. det. 921; MPU, photo!; P 3726144, photo!). — Castille : Obarenes, dans le bois près d’une bergerie, 11 Sep 1907, H. Elías (P 3726154, photo!).

≡ Taraxacum serotinum subsp. haussknechtii (Uechtr.) Gajić in Fl. SR Srbije 7: 298 (1975).

Part III. Section Dioszegia

Chapter 15. Taxonomic treatment: a revision


Icon.: Fig. 15.4.

Figure 15.4: Taraxacum haussknechtii. Habit (A), scale bar = 5 cm, leaves of two different morphotypes (B) and two achenes (C), scale bar = 1 mm. Drawn by J. Štěpánek.

Description: Plants small to medium-sized, usually 8–15 cm tall (in cultivation, they sometimes reach 25 cm); plant base densely brownish aranose, base not covered with remnants of old petioles (Fig. 15.4). Leaf rosettes large (leaves much more numerous than in the other taxa of this section) with numerous scapes, leaves usually erect-patent, some of them may be appressed to the ground, conspicuously narrow, usually 6–12 × 0.5–2 cm, subcoriaceous, light green, not spotted, less often with little brown-purple spots, sparsely to ± densely aranose; leaf blade linear-lanceolate, linear-oblanceolate to linear-elliptical in outline, almost always pinnatisect, rarely ± pinnatilobed; terminal segment relatively small, usually 7–15 × 4–10 mm, triangular to broadly so in outline, or helmet-shaped, often with a sagittate base, acute to acuminate, sometimes mucronate, distal margin ± straight or concave or sigmoid, entire or with several acute teeth, rarely with an incision, proximal margin
± straight or concave or sigmoid, entire or with 1–2 minute acute teeth; lateral segments 5–8 (–9) pairs, opposite or alternate, triangular to narrowly so, relatively small, usually 4–8 × 3–10 mm, slightly recurved to patent, acute; interlobes of variable length, usually narrow, (0–) 5–10 × 1–4 mm, not densely dentate to lobulate, margins raised, often bordered brown-purple; mid vein pale or brownish; petiole narrow or narrowly winged, pale greenish or suffused brownish pink, usually 1.5–2 cm long. Scapes very numerous (over 20 even in small plants), sub-equal, leaves, densely aranose, pale greenish to suffused bronze. Capitulum small, 1.5–2.5 (–3) cm in diam., ± flat, golden yellow to light yellow (rarely to light ochraceous yellow). Involucre very small, narrowly cylindrical, 4–5 (–6) mm wide, rounded to ± truncate at base; outer phyllaries (15–) 18–34, linear to linear-lanceolate, 4–7 × 0.6–1.2 (–1.4) mm, imbricate, relatively regularly arranged, appressed in lower part, distal part of phyllary arcuate to arcuate-recurved, flat to callose near apex, light green to pale pinkish to light purplish, paler towards margins, with 0.05–0.15 mm wide whitish-membranous border, not ciliate or sparsely ciliate, if so, then more densely towards the apex; inner phyllaries 13–25, usually 8–11 mm long, of equal width, light olivaceous green, later becoming pink. Outer ligules flat, striped pale to very pale greyish-pinkish, greyish-purplish to greyish olivaceous outside, apical teeth usually deep yellow or ± reddish, inner ligules canaliculate, with apical teeth yellow; stigmas deep yellow, pale yellow pubescent outside; pollen abundant, pollen grains uniform in size. Achenes usually (yellowish) light greyish pale straw-brown, narrowly turbinate, (4.2–) 5.0–6.2 (–7.0) mm long including cone, 0.9–1.1 mm wide, achene body with upper 1/3–1/4 covered with sparse to sub-dense short spinules, otherwise ± smooth, very gradually narrowing into subconical to subcylindrical cone 0.8–1.5 (–1.7) mm long; rostrum (4–) 6–9 (–10.5) mm long, pappus (5–) 6–8 mm long, yellowish light brown to pale greyish-brownish, sometimes with a pinkish hue. Flowering optimum: summer to late summer.

Distribution: T. haussknechtii is centered in the Republic of Macedonia and adjacent parts of Greek Macedonia and Thessaly, rarely in other parts, and marginally reaches Albania, Serbia and Bulgaria. See Fig. 15.5 for map of distribution of the species.

Reproduction: The results of population analyses point to the absolutely prevailing allogamy. Isolated capitula (tested by J. Štěpánek and T. Černý, not presented here) did not yield well developed achenes without forced autogamy.

Figure 15.5: Distribution of *Taraxacum haussknechtii* based on herbarium specimens (see the text).
Kaďmaktchalan (Macédonie), Ostrovo, Sep 1938, H. Humbers & S. Topali H396 (P 4278442, photo!).


On the Taraxacum taxonomy etc. Vojtěch Zeisek (2018)


Kosovo: M. Scardo or., ad basin montis Ljubatrin [Ljuboten] prope Katschanik, 23. VII. 1918, J. Bornmüller, Pl. Maced., no. 4248 (WU, no. det. 21915; B, no. det. 22195).

15.1 Excluded name

There is a name referred to the sect. Dioszegia (as sect. Serotina) by Arrigoni (2012). It is Taraxacum vallis-nibulae Arrigoni, a name undoubtedly belonging to sect. Taraxacum (= sect. Ruderalia Kirschner, H. Øllgaard et Štěpánek) but represented by plants collected at the stage of second, summer flowering.
Chapter 16

Acknowledgements

The authors are grateful to the keepers of the following herbarium collections for making their material available for the present study: B, BM, BRNM, E, G, IRAN, K, LE, MPU, PRC, PR, S, W, WU. Thanks are due to J. Molina (MPU), for the material of *T. serotinum* subsp. *tomentosum*, to F. Černý for a great help with experimental crosses and to Ms. V. Matějovičová and E. Ničová for technical assistance. The work was supported by the following grants: Ministry of Education grant (Czech-Chinese Collaboration Scheme KONTAKT), no. ME10143, long-term research & development project, no. RVO 67985939 and support provided by the Czech National Grant Agency, grant no. GA13-13368S and the EU Framework Programme 7, grant DRIVE4EU, no. 613697. We thank Tony Dixon for editing English of the accepted manuscript.
On the *Taraxacum* taxonomy etc.  
Vojtěch Zeisek (2018)
Part IV

Identification of oligoclonal agamospermous microspecies: taxonomic specialists versus microsatellites

There has been a decrease in the ability of biologists to identify their material correctly, particularly plants of complicated genera with common agamospermy, where old clonal entities are accorded the rank of species (microspecies). Agamospermous microspecies are taxonomic entities recognizable from one another by a set of minute morphological features. The knowledge of microspecies is confined to a few specialists. Specialists use microspecies names but there could be inconsistencies in the taxonomic concepts used by different, geographically remote experts. A selection of nine widespread, generally recognized agamospermous microspecies of Taraxacum sect. Taraxacum, which are characterized by means of eight microsatellite loci, were used to evaluate the ability of four European Taraxacum specialists to identify these microspecies consistently. With two exceptions (and one unclear result) for 125 plants coming from an area extending from Finland to central Europe, the experts identified the microspecies consistently, exclusively on the basis of morphological differences. Another problem studied was within-species variation. The within-species microsatellite variation corresponded to the mutational clone cluster hypothesis, with a single unclear result. Each microspecies consisted of one, more or less dominant, clone and several minority clones, each usually confined to a single plant. A combination of the traditional microspecies identification by experts and the characterization of microspecies by a set of molecular markers opens the field of microtaxonomy to a wider group of researchers.

**Keywords:** agamospermy, clonality, microsatellite variation, plant identification, population variation, Taraxacum, taxonomy.
Testování identity oligoklonálních agamospermních drobných druhů — taxonomové versus mikrosatelity

Chapter 17

Introduction

One of the cardinal issues of current botany is the decrease in the ability of biologists to identify their material correctly and verify previously published taxonomic data (Kirschner and Kaplan 2002). The high proportion of incorrect data, mistakes and misinterpretations in important databases and publications may prove to be a hindrance in the development of experimental botany and modern genomic studies (Bridge et al. 2003; Hawksworth 2003; Holst-Jensen et al. 2003; Vilgalys 2003; Kristiansen et al. 2005; Záveská Drábková and Kirschner 2013). A conclusion drawn on the basis of the literature offers two complementary methods of how to achieve a reliable means of identifying plant material: expert determination assisted by a specific combination of molecular markers.

In studies on Taraxacum, the problem of identification is even more important as the probability of misidentification is higher than in most other taxa, as the taxonomic knowledge is far from complete and the extent of the variation in taxa largely remains unexplored. Common dandelions thus represent a suitable model for testing the accuracy of identification and determining the intraspecific variation in autonomous agamosperms.

The genus Taraxacum Wigg. (Asteraceae-Cichorieae-Crepidae), a well-known example of biological and taxonomic complexity, is characterized by the coexistence of sexuality and agamospermy at various levels, from individuals and populations to sections. Agamospermy tends to prevail, both geographically and in the number of species and individuals, and there are large areas where asexuality either totally predominates or is the only reproduction system present (Kirschner and Štěpánek 1996). Thus, the most common pattern found at a locality is a result of the coexistence of a few (rarely a single) to many microspecies (e.g. von Hofsten 1954). Asexual microspecies in Taraxacum are presumed to be entities, which in the majority of cases, came into being via multiple remote hybridizations, with hybridity ‘frozen’ by agamospermy, and the genotype diversity in the multiclonal agamic hybrid swarm reduced by subsequent strong selection. They differ in a number of autecological and morphological attributes (Kirschner and Štěpánek 1994, 1996). There are two major issues associated with the coherence and individuality of Taraxacum microspecies: the ability of taxonomists to recognize and name the microspecies consistently and the existence and character of the variation within agamospermous microspecies.

As in many complicated plant groups, a detailed knowledge of the taxonomy of nu-
numerous *Taraxacum* microspecies and the ability to identify them in the field is confined to a very narrow community of taxonomists. A taraxacologist usually uses a general ‘imprint’ of a microspecies (a combination of characters perceived as a unity) to spot it among dozens of other dandelions; technically, only a simultaneous use of a series of characters can lead to a safer identification. Because of the complex nature of the problem, most *Taraxacum* taxonomists ‘inherited’ their knowledge from one or several founders of modern taraxacology, usually during joint excursions. There is an uncertainty whether a binomial in *Taraxacum* always covers the same clone or clone cluster when used in geographically remote parts of Europe, or by taxonomists of different taraxacological schools. There are several methods used by the taraxacologists to unify the taxon/name concepts and to disseminate new results: regular joint excursions and workshops and, importantly, the distribution of a standard exsiccate series, Taraxaca Exsiccata (distributed since 1986, now having reached over 1000 in number, Kirschner and Štěpánek 1997b). Thus, all the specialists involved in the present study have a similar background: field knowledge of the *Taraxacum* flora of a particular country, at least partly inherited from the previous generation of experts, a repeated joint field training and herbarium material serving as a standard collection (Kirschner and Štěpánek 1998a; Uhlemann 2003; B. Trávníček et al. 2010; Räsänen 2013). However, the very fact that several specialists jointly use species names for a group of similar individuals cannot serve as proof that a clone cluster within a microspecies always bears the same name, or that a name is always applied to the same clone cluster and therefore it is important to obtain an external data set to resolve this problem.

The majority of *Taraxacum* have a very uniform general appearance of rosulate short-lived hemicryptophytes with scapes, two series of involucral bracts, numerous yellow florets in the capitulum and the popular beaked cypselas. As regards the number of characters used to diagnose and describe *Taraxacum*, we can give an example of the detailed *Taraxacum* treatment in the Flora of the Czech Republic (B. Trávníček et al. 2010), in which the *Taraxacum* flora lacks much of the structural diversity of the genus. In spite of this fact, there are almost 90 characters used to describe the species, and the number of character states exceeds 400 (the flora includes more than 180 species, the majority of which are agamospermous microspecies). These characters, when taken separately as in a dichotomous key, are insufficient to distinguish more than a few very distinct taxa, and multiaccess keys or computer-generated identification tools are needed to increase the probability of correct identification. Because of the limited availability of material identified at the microspecies level, some authors refrain from recognizing these basic units and perform their experiments on mixtures of clones, which is an approach that may often lead to rather controversial results (Taylor 1987; van der Hulst et al. 2000, 2003).

The genus *Taraxacum* is a popular model for the study of diplosporous agamospermy (A. Richards 1973; Ozias-Akins and van Dijk 2007), clonality (Kirschner and Štěpánek 1994), epigenetic heritability (Verhoeven et al. 2010a,b; Verhoeven and van Gurp 2012) and potential germplasm for economic exploitation (Kirschner et al. 2013). This usage is supported by other advantageous features, such as easy cultivation, unproblematic emasculation and efficient propagation. The pattern in variation of agamospermous *Taraxacum*, i.e. numer-
ous microspecies characterized by a limited within-species variation, was recognized more than a hundred years ago (Raunkiær 1903) and, particularly European microspecies have often been accorded species names.

We selected nine widespread, generally recognized microspecies of *Taraxacum* sect. *Taraxacum*, sampled by four *Taraxacum* specialists (JK, IU, BT, JR) at geographically distant localities across northern and central Europe (Table 18.1); possible misidentifications may be cross-checked using voucher material reexamination. We tested the conformity of the species concepts of the experts by using presumably selectively neutral microsatellite markers (Balloux and Lugon-Moulin 2002; Bhargava and Fuentes 2010); any pattern associated with the person identifying the material should be detected.

The other main objective of this paper is to evaluate the character of variation within microspecies. During the evolution of an agamospermous microspecies, the originally (potentially) high ancestral multiclonality gradually decreases by means of selection; at the same time, the variation is gradually enriched by mutations. In regions where *Taraxacum* is almost exclusively asexual and most plants belong to stable obligately agamospermous species (i.e. where most of the samples used in the present study came from), the major source of variation is somatic mutation (Majeský et al. 2012). In non-uniclonal apomictic *Taraxacum*, therefore, the expected picture would be a cluster of closely related clones (Normark et al. 2003).

There are therefore three concepts to be evaluated: (i) a conformity of the microspecies concepts of experienced taraxacologists from different regions, (ii) a pattern of dominant mutational clone clusters within microspecies, and (iii) a statistical evaluation of clone clusters showing the expected agreement between expert opinions and the entities characterized by molecular means.
Chapter 18

Material and methods

18.1 Material

A detailed account of the plant material used is given in Table 18.1. The selection of microspecies was done based on the following criteria: (i) stabilized agamospermous triploids, (ii) distribution covering both northern and central parts of Europe, (iii) names safely typified, (iv) names issued in a standard exsiccate series (Taraxaca Exsiccata, cf. Kirschner and Štepánek 1997b), (v) species recognized by several Taraxacum specialists (i.e. J. Räsänen, B. Trávníček, I. Uhlemann and J. Kirschner). Four specialists collected achenes and usually also herbarium specimens of these species in six countries (mainly Czech Republic, Finland and Germany and less so also Austria, Poland and Slovakia). The material is deposited in the herbaria of the collectors and in the herbarium PRA. One to four samples were collected at each locality for each species of the selection present, and usually one, less often two were analysed. The following species were included: T. alatum Lindb. fil., T. ekmanii Dahlst., T. hemicyclum G. Hagl., T. hepaticum Railons., T. interveniens G. Hagl., T. macranthoides G. Hagl., T. obtusifrons Markl., T. piceatum Dahlst. and T. pulchrifolium Markl. As controls, two couples of samples were included each of a single parental plant (T. piceatum, samples pin2-1, pin2-2, and T. hepaticum, samples hepa8-1, hepa8-2; Table 18.1). It should be added that all the species included in the present study were described on the basis of plant material from Scandinavia and the Baltic countries, and only later recognized and recorded in central Europe.

We used up to two plants per locality to germinate seeds directly on a potting soil and pumice mixture (80:20), three per pot. After germination the pots were weeded back to one plant per pot. Leaf tissue for DNA isolation was collected when the plants were 4 weeks old.
Table 18.1: Localities of achene samples of common clonal apomictic species of *Taraxacum sect. Taraxacum*. Collector abbreviations: Juhani Räsänen (JR), Jan Kirschner (JK), Bob Trávníček (BT) and Ingo Uhlemann (IU).

<table>
<thead>
<tr>
<th>Name of taxon</th>
<th>Code</th>
<th>Date and collector</th>
<th>Locality</th>
<th>Coordinates and altitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>alatum</em></td>
<td>ala11-101</td>
<td>8.6.11 JR</td>
<td>FI, Karelia borealis, Joensuu, Raatekangas, by the side of Pankakoskentie</td>
<td>62°37'42&quot;N, 29°44'08&quot;E, 81 m</td>
</tr>
<tr>
<td><em>alatum</em></td>
<td>ala11-103</td>
<td>9.6.11 JR</td>
<td>FI, Karelia borealis, Joensuu, Linnunlahti, Pajutie</td>
<td>62°36'10&quot;N, 29°43'37&quot;E, 80 m</td>
</tr>
<tr>
<td><em>alatum</em></td>
<td>ala4-1</td>
<td>21.5.11 JK</td>
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<td>SK, Lazy pod Makytou</td>
<td>49°14'54&quot;N, 18°13'11&quot;E, 425 m</td>
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# Chapter 18. Material and methods

## Part IV. Identification of microspecies

... continued Table 18.1.

<table>
<thead>
<tr>
<th>Name of taxon</th>
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<td>SK, Lazy pod Makytou</td>
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... continued Table 18.1.

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### Table 18.1.

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### Table 18.1

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<td>CZ, Kojetin</td>
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<td>SK, Lazy pod Makyto hovered</td>
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<td>DE, Saxony, Liebenau</td>
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</table>
18.2 Ploidy analysis

Leaf tissue was homogenized and cell nuclei were stained with DAPI to visualize the DNA-content and a leaf sample of a confirmed diploid sexual accession of the section *Taraxacum* was added as a reference. All accessions were analysed using a Partec flow cytometer (Tas and Van Dijk 1999) and confirmed to be triploid.

18.3 DNA isolation

Total DNA was isolated from fresh leaf tissue, 1.2 cm², using the hexadecyl-trimethyl-ammonium-bromide (CTAB) procedure described by Rogstad (1992), with some modifications referred to as the ‘RETCR’ method described by Vijverberg et al. (2004).

18.4 Microsatellite analysis

All plants were characterized by eight microsatellite loci (SSRs, Jarne and Lagoda 1996), which were distributed over two multiplex PCR reactions (multiplex 1: MSTA 31, 44B, 78, 58 and multiplex 2: MSTA 143, 67, 72, 61). Seven microsatellite loci and relevant primers (MSTA 31, 44B, 58, 61, 67, 72, 78) were taken from Falque et al. (1998) and one microsatellite locus (MSTA 143) comes from Vašut et al. (2004). The PCR reaction was performed using the QIAGEN Multiplex PCR kit (Qiagen, Venlo, Netherlands) according to manufacturer’s protocol in a final volume of 10 μl containing 200 nM of each primer and 30–50 ng of DNA. PCR protocol was as follows: 15 min 95℃, 30× (30s 94℃, 90s 57℃, 60s 72℃) and 30 min 60℃. Final PCR products were analysed using a 3130 ABI Genetic Analyser (Life Technologies, Carlsbad, CA, USA) and allele numbers and sizes were subsequently scored using the Genemapper v4.0 (Life Technologies, Carlsbad, CA, USA). Of the original set of 131 samples we excluded six samples because of amplification failure.

18.5 Statistical analyses

As all the taxa included in the analyses are agamospermous triploids, and the expected allelic configurations are simple, we used a method of identifying the gene dosage based on the peak size and area following Esselink et al. (2004).

For the purposes of the evaluation of the clone cluster data, we consider statistical techniques based on Bayesian clustering as very effective because they do not involve a priori hypotheses about sample clustering. As we lack any reference library for the taxa under study, nor for related taxa, Bayesian clustering is expected to reveal ‘natural’ genetic clusters as well as to show possible hybridization and/or influence of other genotypes. For this purpose we selected widely used software, BAPS 6.0 (Corander et al. 2008). We used a number of clusters (K) ranging from 2 to 25, 20 times each. We also used Bayesian K-means clustering as described for Discriminant Analysis of Principal Components (DAPC, Vojtěch Zeisek (2018) On the *Taraxacum* taxonomy etc. 121
Jombart et al. 2010). It works with data ‘cleaned’ by PCA and maximizes the manifestation of the major pattern involved in the data.

Most computations were performed in R 3.1 (R Core Team 2013–2018). We used packages ade4 (Dray and Dufour 2007), adegenet (Jombart 2008), APE (Paradis et al. 2004), pegas (Paradis 2010) and Poppr (Kamvar et al. 2014). We calculated the distribution and diversity of multi locus genotypes (MLGs) within species, Principal Coordinate Analysis (PCoA Dray and Dufour 2007), K-means clustering (with 100,000,000 iterations and maximal K = 15, Jombart et al. 2010), Minimum Spanning Network (MSN) and Neighbour-Joining (NJ) tree (Saitou and Nei 1987; Paradis et al. 2004; Popescu et al. 2012). NJ was tested using 10,000 permutations. For distance-based analysis we used Nei’s chord distance (Nei et al. 1983) based on frequency of shared alleles. For MSN we used Bruvo’s distance reflecting number of microsatellite repeats (Bruvo et al. 2004). Details about R workflow, software settings etc. are available from VZ upon request.

Genotype diversity was quantified according to Hughes and A. Richards (1988) as $G = 1 - \sum x_i^2$, where $x_i$ is the frequency of $i$-th MLG. This parameter is useful for population sets with expected variation in reproduction systems (i.e. a substantial departure from the Hardy-Weinberg expectations) and for situations where recombination is partially suppressed as a consequence of alloplody; it reasonably reflects both richness and evenness and closely approaches the modified Simpson’s index (Widén et al. 1994). The R function we used to calculate the values of genotype diversity is given below:^33

```r
## Calculates index of genetic diversity according to Hughes and Richards 1988 defined as "G.mlg = 1~- sum(X[I]^2)"
## where X is frequency of genotype I

## The functions requires as an input (variable MLG) a~genind object.
## For information about genind objects see poppr's manual or 
## "?genind".

# When the genind object contains several populations of one 
# species.
# Calculations are performed on every population,
# resulting index is for whole dataset.

G_mlg_pop <- function (MLG) {

# Function requires package poppr 
# http://grunwaldlab.cgrb.oregonstate.edu/poppr-r-package-
# population-genetics

require(package=poppr)
```

^33The function was updated to work with up-to-date R, adegenet and poppr.
X <- mlg.table(MLG, plot=FALSE, total=FALSE, quiet=FALSE)

# Initialize variable
ans <- c(0)

for (L in 1:length(X[,1])) {
    # Initialize variable
    freqs <- c(0)
    # Calculate frequencies of each MLG
    for (I~in 1:length(X[1,])) {
        freqs[I] <- (X[L,I]/length(X[1,]))^2
    }
    # Calculate the index
    G <- 1 ~ sum(freqs)
    ans[L] <- G
}

# Result
names(ans) <- popNames(MLG)
ans

# When the genind object contains several species.
# Calculations are performed on every species separately.

G_mlg_sp <- function (MLG) {

    # Function requires package poppr
    # http://grunwaldlab.cgrb.oregonstate.edu/poppr-r-package-
    # population-genetics
    require(package=poppr)

    # Initialize variable
    ans <- c(0)

    for (L in 1:length(levels(pop(MLG))))) {
        X <- mlg.table(MLG, sublist=L, plot=FALSE, total=FALSE, quiet=FALSE)
        # Initialize variable
        freqs <- c(0)
        # Calculate frequencies of each MLG
        for (I~in 1:length(X)) {

Vojtěch Zeisek (2018) On the Taraxacum taxonomy etc. 123
freqs[I] <- (X[I]/summary(pop[MLG])[L])^2

# Calculate the index
G <- 1 - sum(freqs)
ans[L] <- G

# Result
names(ans) <- popNames(MLG)
ans
Chapter 19

Results

In 125 individuals we detected a total of 44 MLGs. None of them were detected in more than one species. Within most species, samples from Finland and from central Europe shared some multilocus genotypes. All individuals except two, ala11-103 and ala5-2 (\textit{T. alatum}), grouped genetically under the species name to which they were originally assigned on the basis of morphology. Sample pul1299 (\textit{T. pulchrifolium}) showed only partial affinity to its morphology-based species and its position is ambiguous. Thus, in seven species out of nine, and in 122 samples out of 125, there is full agreement between the genetic grouping and the expert identification. The two samples of \textit{T. alatum} were misidentified.

![Bayesian clustering of individuals performed in BAPS. Each colour represents one inferred genetical cluster. The only three individuals not included in 'their' clusters are two probably misidentified individuals of \textit{Taraxacum alatum} (ala11-103 — marked '1' at the top of the figure, and ala5-2 — marked '2') and one of \textit{T. pulchrifolium} (pul1299 — marked '3'; see chapter 20). Compare with output of K-means clustering (Fig. 19.2).](image)

Bayesian clustering in BAPS revealed nine major clusters (Fig. 19.1) and three individuals were not included in the expected clusters. K-means clustering (Fig. 19.2), similar to BAPS, revealed nine very well separated clusters one for each of the respective species. The only individuals not included in the clusters, as expected, were ala5-2 (close to \textit{T. hepaticum}) and ala11-103 (close to \textit{T. piceatum}).

Trees (NJ and MSN) provide strong support for grouping these species together (re-
Table 19.1: Genotype diversity (G) calculated for microspecies samples, and the percentage of the within-species dominant multi locus genotype (MLG) in each sample. The three aberrant accessions are not included in this analysis (see chapters 19 and 20), so that for the *Taraxacum alatum* and *T. pulchrifolium* columns ‘Number of plants’ does not include all the plants sampled under that name.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of MLGs</th>
<th>Number of MLGs restricted to a single plant</th>
<th>% of plants belonging to the dominant MLG</th>
<th>Genotype diversity G</th>
<th>Number of plants</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. alatum</em></td>
<td>6</td>
<td>4</td>
<td>68</td>
<td>0.462</td>
<td>21</td>
</tr>
<tr>
<td><em>T. ekmanii</em></td>
<td>3</td>
<td>2</td>
<td>77</td>
<td>0.370</td>
<td>9</td>
</tr>
<tr>
<td><em>T. hemicyclum</em></td>
<td>2</td>
<td>1</td>
<td>93</td>
<td>0.290</td>
<td>18</td>
</tr>
<tr>
<td><em>T. hepaticum</em></td>
<td>4</td>
<td>2</td>
<td>75</td>
<td>0.414</td>
<td>16</td>
</tr>
<tr>
<td><em>T. interveniens</em></td>
<td>6</td>
<td>4</td>
<td>40</td>
<td>0.760</td>
<td>10</td>
</tr>
<tr>
<td><em>T. macranthoides</em></td>
<td>4</td>
<td>3</td>
<td>62</td>
<td>0.562</td>
<td>8</td>
</tr>
<tr>
<td><em>T. obtusifrons</em></td>
<td>6</td>
<td>4</td>
<td>46</td>
<td>0.639</td>
<td>13</td>
</tr>
<tr>
<td><em>T. piceatum</em></td>
<td>7</td>
<td>5</td>
<td>27</td>
<td>0.809</td>
<td>11</td>
</tr>
<tr>
<td><em>T. pulchrifolium</em></td>
<td>5</td>
<td>1</td>
<td>53</td>
<td>0.657</td>
<td>16</td>
</tr>
</tbody>
</table>

Regardless of technique and distance matrix, thus forming nine main branches (Figs. 19.3 and 19.4). NJ tree based on Nei’s distance provides very good resolution and high bootstrap support. MSN of MLGs reveals the star-like pattern typical of recently radiating groups. These species are usually formed by one central dominant MLG with several rare derived MLGs. In both cases, relationships among species remain unclear as we sampled only a small subset of all the species in this section (e.g. Lundevall and Øllgaard 1999). PCoA (Fig. 19.5) of the original samples reveals generally well separated clusters. The most aberrant samples were probably misidentified.

The genotype diversity and distributions of MLGs within a microspecies clone cluster are given in Table 19.1. The diversity of G-values are comparatively high, much higher than those reported for agamosperms by Hughes and A. Richards (1988, 1989). In most microspecies, the proportion of the dominant MLGs exceeds 50% (in *T. hemicyclum* it reaches 93%), and, with one exception, the majority of MLGs within microspecies are each confined to a single individual (26 MLGs of 44).

Genetic analysis leads to the recognition of groups corresponding to species, with two exceptions out of 125 individuals (and one unclear case, see chapter 20), and the accuracy of identification was nearly perfect, i.e. all the *Taraxacum* experts use the microspecies names in the same way and there is no identification bias associated with the person responsible for the determination. Within species samples we failed to detect any geographical pattern.
(analyses not shown). Results also indicate the existence of microspecies clone clusters.

**Figure 19.2**: K-means Bayesian clustering showing an almost perfect match of inferred genetic clusters and originally sampled morphologically determined species. Sample ala5-2 groups with *Taraxacum hepaticum*, ala11-103 with *T. piceatum* and pul1299 with *T. pulchriorifolium*. Size of squares is proportional to the number of individuals. Compare with output of BAPS (Fig. 19.1).
Figure 19.3: NJ tree based on Nei’s distance. Grey ellipses mark supported clusters, while symbols indicate the original determinations by experts (arrows indicate identification mistakes and question mark the enigmatic sample pul1299). Bootstrap values higher than 50 are also displayed. Omitted are also numbers for crown clades with practically identical genotypes. As there are many samples with identical genotypes, their labels fully overlap.
Figure 19.4: Minimum Spanning Network (MSN) showing relationships among multi locus genotypes (MLGs) based on Bruvo’s distance (arrows indicate identification mistakes and question mark enigmatic sample pul1299). Thickness of lines connecting the MLGs is reversely proportional to Bruvo’s distance (the thicker the stronger connection). Enigmatic sample originally identified as *T. pulchrifolium* is relatively distantly related to that species. Size of circles corresponds to the number of plants belonging to individual MLG.
Figure 19.5: PCoA based on Nei’s genetic distance showing individuals labeled according to original morphological groups. Species-specific clusters are generally distinct, only the aberrant samples of *T. alatum* do not fit their presumed cluster (see chapter 20).
Chapter 20

Discussion

20.1 Elucidation of the nature of *Taraxacum* microspecies

The opinions of theorists about the nature of agamospermous entities treated as microspecies range from their total denial (Janzen and D. H 1977) to vehement advocacy (Abbott (1979), see also Kirschner and Štěpánek (1994)). This discussion, however, was not based on reliable experimental data. The first person to study the population biology and ecology of microspecies thoroughly was (von Hofsten 1954). As regards the clonal identity of his material, he took advantage of the detailed knowledge of the *Taraxacum* flora of Sweden; at many places he pointed out the biological differences among microspecies of the *T. officinale* group, usually on the basis of careful cultivation experiments. His work, however, is seldom used or cited as it is written in Swedish. An important contribution to the knowledge of the competitive behaviour of individual *Taraxacum* sect. *Taraxacum* microspecies (defined as biotypes characterized by isozyme patterns) is presented by Solbrig and B. B. Simpson (1974, 1977). There is an array of papers elucidating various aspects of the biological and ecological differentiation among *Taraxacum* microspecies published by Dutch authors (van Loenhoud and Duyts 1981; Sterk et al. 1983; Sterk and Luteijn 1984; Roetman and Sterk 1986), all documenting the multidimensional identity of *Taraxacum* microspecies. The discussion on the clonal character of microspecies and the various pathways of *Taraxacum* evolution are summarized by Kirschner and Štěpánek (1994, 1996). Microspecies are seen as entities resulting from unique evolution and adaptation processes, able to generate variation, which exhibit a variety of clone-specific biological, reproductive and distribution features.

The problem of microspecies coexistence at a locality has been addressed several times using various molecular markers. In spite of relatively scanty material (26 plants of six species from 14 localities), the most important study is that of Reisch (2004). Three taxa of sect. *Erythrosperma*, *T. parnassicum*, *T. lacistophyllum* and *T. tortilobum*, each exhibit a RAPD variation corresponding to the clone cluster model. There is significant variation in *T. rubicundum*, which partly can be attributed to the nature of the RAPD markers and partly,
and very probably, to the fact that plants very similar to *T. rubicundum* are sexual in southwestern Europe, and the variation may be a consequence of either residual sexuality or the multiclonal character of the agamospermous species recently derived from a closely related sexual ancestor. Another two molecular studies also reliably document the coexistence of up to 10 species of sect. *Erythrosperma* (Ford and A. Richards 1985; van Oostrum et al. 1985).

Another aspect of the nature of *Taraxacum* microspecies is the existence of variation within progeny or, generally, the non-maternal offspring. Rather limited or not fully reliable data exists that indicates the extent of the variation in the progeny of *Taraxacum* apomicts. The variation reported by Lynn Mertens King and Schaal (1990) may, at least partly, be attributed to the intraindividual variation in nrDNA and *Adh* copies. However, the very fact of occasional variation in the progeny is undeniable (Ford and A. Richards 1985; Mogie 1985; Kirschner and Štěpánek 1998a; van Baarlen et al. 2000, but see below) and has been documented by various methods including karyology, isozymes and DNA markers. On the contrary, the enormous intraspecific clonal diversity reported by Lyman and Ellstrand (1984) in *T. officinale* refers to the whole section *Taraxacum* in the USA, i.e. to an assemblage of agamospermous microspecies.

The hypothesis of the uniclonal, or nearly uniclonal, nature of *Taraxacum* microspecies has received a considerable amount of attention. There are several examples documenting the uniclonality of *Taraxacum* microspecies, usually they are for morphologically very distinct species. Based on allozyme profiles, several species of sect. *Palustria* in the Czech Republic and Slovakia, *T. uliginosum* (28 plants from two sites) and *T. subalpinum*, are uniclonal (Battjes et al. 1992). This study mainly focused on another two species, *T. hollanicum*, with 228 plants from The Netherlands and the Czech Republic, i.e. sampled across a large geographical range, and *T. vindobonense* (87 plants from four localities). The former species was shown to be uniclonal (225 plants belonging to a single multilocus genotype, and three single-plant genotypes easily derived from the dominant clone by a single mutation). In contrast, the sample of the latter species consisted of 64 clones, not a single clone of which was found at more than one locality. While *T. hollanicum* is a triploid relict of an early postglacial period, with a few similar taxa in France, *T. vindobonense* is a young and the westernmost agamospermous derivative of an assemblage of forms with agamospermous and facultatively agamospermous reproduction, centred in the Pannonian Basin of Hungary and Romania (Kirschner and Štěpánek 1998a). A picture very similar to that of *T. hollanicum* is documented by Menken and Morita (1989) on the basis of isozyme spectra: samples of *T. albidum* taken from populations across a 1000 km wide geographical range of this pentaploid agamosperm reveal almost strict uniclonality (19 localities, 109 plants, only a single aberrant plant with a single-allele mutation). It should be added that the recent study by Sato et al. (2011) revealed an extensive ploidy and karyotype variation within *T. albidum*, and it is plausible that they covered not only *T. albidum* but also another whitish-flowered taxon (or taxa) of sect. *Mongolica*. A combined SSR and AFLP study similar to the present one but based on material of the sect. *Taraxacum* collected by a single specialist (Majeský et al. 2012) showed a similar pattern: clone clusters ori-
Originating through the accumulation of mutations and occasional recombinants of unknown origin and age. The most recent case (Kirschner et al. 2013) is an AFLP analysis of a triploid apomict, T. brevicorniculatum, cultivated in botanical gardens or preserved in germplasm collections all over the world for more than 50 years (six collections sampled) under the name T. koksaghyz and found at a number of wild localities in Kazakhstan (16 populations sampled). Taraxacum brevicorniculatum is almost uniclonal, with three plants each deviating from the dominant clone by the absence of a single fragment.

## 20.2 Clonal organisms and intraspecific variation

Several works emphasize the dynamic and adaptive features of the genome of clonal, asexual plants (Lushai et al. 2003). While in clonal vegetative apomicts a high level of recessive lethals is recorded (e.g. in ferns, Klekowski 2003), agamospermous lineages are a result of more complex processes. Predicted long-term effects of the loss of sex and recombination on genomes of agamospermous taxa include phenomena such as genomic panvicariance, disconcerted evolution, mutation rate changes, decay of sex- and recombination-specific genes, disadaptation, etc. (Normark et al. 2003). Loxdale and Lushai (2003) even point out the rapid changes in clonal populations. The role of somatic mutations among various sources of variation in apomicts is proven, for instance, in Grevillea rhizomatosa Olde et Mariotti (Gross et al. 2012). We can conclude that there are various sources of within-population genetic differentiation of clonal microspecies, including activation of mutagenic activity, recombinations, mechanisms that involve transposable elements, epigenetic changes, and autosegregation. On the other hand, in populations, we can view the disadvantageous (and advantageous) mutations as side branches of the genotype trees (clone clusters) that undergo within-cluster competition and selection.

There is relatively little literature evaluating genetic variation within agamospermous microspecies in genera other than Taraxacum. (Mráz et al. 2001; Štorchová et al. 2002; Chrtek et al. 2007) show that the majority of the species of Hieracium L. studied are uniclonal or nearly so, while some taxa (such as H. alpinum L.) are multiclonal. A similar situation occurs in stabilised agamospermous taxa of Rubus L. (Kraft et al. 1996; Nybom 1996); one of the clonal microspecies studied, R. nessensis Hall, is uniclonal over a relatively large area in southern Scandinavia and Germany. (Lo et al. 2010) made an important attempt to elucidate the within-progeny variation in the tetraploid pseudogamous Crataegus crus-galli L. and compare it with that of C. punctata Jacq., a sexual diploid. The former species was characterized by a much higher within-progeny genetic resemblance and lower extent of among-progeny differentiation. Sources of variation in C. crus-galli might have been residual sexuality.
20.3 Species-specific clone clusters

A cluster of clones is expected pattern under strict agamospermy (Klekowski 2003; Normark et al. 2003); another feature of this in microspecies is a high proportion of population-specific alleles of those that deviate from the dominant multilocus genotype, which is shown by Reisch (2004) and Gross et al. (2012). The same pattern was recorded in our sample of microspecies of sect. Taraxacum, if the two misidentified plants of T. alatum are disregarded (probably because they were collected late in the season, when Taraxacum species are rather difficult to identify).

Two probably misidentified accessions of T. alatum (ala11-103 and ala5-2) show different genetic affinities. While the sample ala5-2 exhibits certain relationships with T. hepaticum, the other sample, ala11-103, appears in various positions depending on the technique used. BAPS (Fig. 19.1) gives it its own cluster and in distance-based methods (Figs. 19.3, 19.4 and 19.5, and not-shown results) it was always placed differently. We conclude that the latter sample belongs to another species, not included in this study.

The most aberrant MLG belonging to T. pulchrifolium (pul1299) is rather ambiguous as it is the only plant that was impossible to assign either to a case of misidentification or a remote member of a species clone cluster. There is a hypothetical explanation that takes into account the fact that this sample was collected, unlike the others, in a lowland region in the northern part of Moravia, Czech Republic, where a diploid Taraxacum sect. Taraxacum is commonly recorded, and our plant might be a hybrid between a sexual plant as a maternal parent and T. pulchrifolium as a pollen donor. However, a detailed examination of the voucher specimen (BRNM 763212) did not reveal any deviation from the typical pattern of this species, and other alternatives, including a sampling- or labelling mistake must be considered. Further analysis will be carried out to check the siblings of this aberrant plant.

20.4 Genotype diversity

The relatively high number of single-plant mutations within our sample of oligoclonal microspecies results in high values of the genotype diversity index, particularly compared to the values published by Hughes and A. Richards (1988, 1989). The difference may be attributed to the different markers, i.e. allozymes as products of functional genes versus microsatellites, the latter being highly variable.

20.5 Utilization of molecular markers to distinguish closely related lineages

There are numerous studies in which various molecular markers are used to identify species (Hebert et al. 2003; Kress and Erickson 2008). Various methods have been evaluated to find appropriate genetic markers, using regional samples (Lahaye et al. 2008), samples of a particular group of organism (often for conservation purposes, e.g. Sass et al. 2007),
samples covering all vascular plants (Kress et al. 2005; M. L. Hollingsworth et al. 2009; Dong et al. 2012; P. M. Hollingsworth et al. 2014) and assortments of lineages of crop germplasm (Núñez et al. 2004; Thomson et al. 2007). The concept of so-called ‘barcoding’ is not generally accepted (nor the terminology stabilized) and there are ongoing debates about its usability (Meyer and Paulay 2005; Rubinoff et al. 2006; Seberg and Petersen 2009). It follows from the discussion that the genome barcoding techniques are not universal, nor can they replace traditional taxonomy. On the other hand, a carefully developed and tested system of molecular markers can be useful in solving a number of questions.

Microsatellites have been successfully used in the analysis of germplasm of a number of crop species and cultivars (e.g. Cruz et al. 2006; Madhou et al. 2013), most frequently of material propagated vegetatively for a long time. The most similar case to that dealt with in the present study is the study of Citrus L. species and cultivars; the cultivars are usually reproduced by means of adventitious embryony and represent products of hybridization similar to microspecies. Alternatively, they have been propagated vegetatively for a long time. Fang and Roose (1997) used 22 ISSR primers to distinguish 68 varieties of Citrus cultivars. Some of them are extremely difficult to recognize morphologically, especially at a young age and/or without fruit. The five botanical species can be distinguished by each of the 22 primers. Most cultivars can also be distinguished from one another, usually with the exception of sister cultivars that came into being through a single mutation. It should be added, however, that, to our knowledge, the concept of barcoding (or fingerprinting) has not been methodically applied to characterize uni- or oligoclonal agamospermous wild plants.

It is therefore a positive message that microsatellite screening of the above material revealed the pattern of microspecies with a high fidelity of clone cluster — name relationships, even when several geographically distant specialists identify the material. The other side of the same coin is the fact that a microspecies can be characterized, or ‘barcoded’, and identified statistically using a set of molecular markers, and, therefore, the utilization of the stabilized agamospermous microspecies concept, not only in Taraxacum, is open to a wider community of non-specialists, e.g. experimental botanists or ecologists. There are two possible approaches: a microsatellite screening of no-name samples followed by a Bayesian analysis to identify and compare ‘natural’ clusters or, alternatively, a step-wise building of a library based on samples identified by specialists and characterized by microsatellites. On the basis of the results presented in this paper, the latter approach, although time consuming and laborious, seems to be promising.
Chapter 21

Acknowledgements

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Part V

Taraxacum sect. Orientalia (Compositae-Crepidinae) and the West Himalayan dandelions: A new interpretation
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The *Taraxacum* flora of the West Himalaya represents one of the dandelion diversity hotspots, with at least 17 sections and about 150 known species. A number of names published from that region were referred to *T.* sect. *Orientalia* Handel-Mazzetti in the literature. All these names are revised and newly interpreted, with emphasis on plants erroneously determined as *T.* *stenolepium*. The revision is based on both older herbarium collections and a new material from expeditions of the late L. Klimeš. A new section, *T.* sect. *Squamulosa*, is recognized. An nrDNA ITS sequence analysis including the only sexual member of *T.* section *Squamulosa* and the other sexual taxa known in *Taraxacum* shows a separate position of *T.* sect. *Squamulosa*. The new section is compared with sections *Primigenia*, *Coronata* and *Orientalia*. Amended descriptions, range extensions and new interpretations are presented for another seven species previously mistakenly referred to *T.* sect. *Orientalia*. The true *T.* sect. *Orientalia* is analysed and briefly characterized; it is shown to be absent from the West Himalaya.

Chapter 22

Introduction

The genus *Taraxacum* (Wiggers 1780, p. 56) is known for its taxonomic complexity. There are numerous regions, particularly in Asia, where even the basic α-taxonomic exploration of this genus is far from completion. Flora of China, with the majority of species from the East Himalayan area, may be given as an example: When the original treatment of *Taraxacum* in the Chinese Flora R. P. Sinicae (Ge et al. 1999) is compared with the new one (Ge et al. 2011), out of 77 taxa listed in the old treatment, 19 were excluded from the Chinese *Taraxacum* flora (i.e. about 25%, and another four names remain unclear). In the new treatment, 116 species in 23 sections are included; of these, 40 are newly described and 14 are newly reported to occur in China (and three sections are newly recognized). The overlap between the two treatments is 50% (based on the new treatment). There is a similar situation in the exploration of dandelions in the West Himalaya, a region defined in Brummitt (2001), and here including also the adjacent regions.

In order to make a taxonomic treatment of dandelions comparable to the modern standards, there are several principles to be followed. They were summarized by A. Richards (1973), Kirschner and Štěpánek (1996), Kirschner et al. (2003) and Ge et al. (2011), and reflect the peculiar features and processes known in dandelions, particularly the coexistence of agamospermy and sexuality, complex hybridity and polyploidy, low level of structural morphological differentiation and the high number of mutually similar and mostly hybridogenous species. The principles derived from the above features include (i) different kinds of species to be recognized on the basis of the extent of variation and modes of reproduction, (ii) distribution of sexuality is to be explored, (iii) variation within a family of siblings should be studied for each taxon (to detect autonomous aberrants and facultative sexuality), (iv) the study should be started at the lowest variation level (within and among populations).

The complexity of the genus, primarily the incommensurable variation patterns of species with different modes of reproduction, also requires a taxonomic rank placed between species and genus in the traditional hierarchy to make the population and taxonomic structure more easily understandable for non-specialists, and the rank of section is used in the *Taraxacum* literature.
22.1 Basic outline of *Taraxacum* in the West Himalaya

The term West Himalaya is used according to Brummitt (2001) and includes Gilgit, Baltistan and Ladakh (but the Aksai Chin, a part of Ladakh claimed by China, is cut off). In the present paper we also take the adjacent regions into account (the Chinese Karakoram, Chitral, other parts of northern Kashmir). The dandelion flora of the West Himalaya represents one of the diversity centres of the genus *Taraxacum*. Although several works analysed this diversity, and a number of new taxa were described (mainly by van Soest 1961, 1963, 1966a,b, 1977; Abedin 2007), the knowledge of dandelions in the vast mountainous territories of N Pakistan and NW India remains rather fragmentary.

In order to understand the taxonomy of dandelions and to classify individual taxa into species groups, sections, a systematical sampling coverage of the given area is required, followed by mass cultivation of samples. An attempt at such a coverage was made by the present authors in Yunnan and Sichuan, with the aid of numerous other collectors and travellers (Ge et al. 2011, and unpubl.). Nevertheless, the only Himalayan region with a really thorough coverage of *Taraxacum* samples is Ladakh, NW India. It was L. Klimeš who explored the Ladakh mountains during numerous expeditions. Regrettably, L. Klimeš tragically died during his last expedition (see Bezděčka et al. 2010). The ample *Taraxacum* material collected by him, also cultivated by him or by the present authors, and deposited in the PRA herbarium, shows the diversity of dandelions in that part of the W Himalayas.

Publications of the present authors dealt with *Taraxacum* in the Himalayan region and cover the diversity of a few sections (Kirschner and Štěpánek 2004; Kirschner et al. 2006; Kirschner and Štěpánek 2008, 2011; Kirschner et al. 2014, 2015). Several recent projects are focussed on the genus *Taraxacum* in mountain regions of Asia, e.g. Flora of Pakistan (Ali 2008), Checklist of the flora of India34, Flora of China (Ge et al. 2011), Flora of Pan-Himalayas35. As regards *Taraxacum*, most of the above flora projects are with at least editorial participation of the present authors, and the present paper is a preparatory step towards the *Taraxacum* flora of the Pan-Himalayas. The sectional taxonomy of *Taraxacum* in the W Himalayas and adjacent areas was outlined in the above works, with a few corrections and additions having been made recently (Kirschner and Štěpánek 2004; Kirschner et al. 2006; Ge et al. 2011; Kirschner and Štěpánek 2011; Kirschner et al. 2014).

If we disregard the *T.* sect. *Orientalia* records analysed in the present paper, the following sixteen *Taraxacum* sections are known to be represented in the West Himalayan flora:


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34 See [http://www.tropicos.org/Project/India](http://www.tropicos.org/Project/India).
Chapter 22. Introduction Part V. Section Orientalia

- T. sect. Coronata von Handel-Mazzetti (1907) [XI]
- T. sect. Emodensia Kirschner & Štěpánek in (Ge et al. 2011, p. 291)
- T. sect. Erythrocarpa von Handel-Mazzetti (1907) [XI]
- T. sect. Leucantha (van Soest 1963, p. 6)
- T. sect. Macrocornuta van Soest (1960, p. 304)
- T. sect. Oligantha (van Soest 1963, p. 8)
- T. sect. Parvula von Handel-Mazzetti (1907) [XI], incl. sect. Kashmirana Soest
- T. sect. Tibetana van Soest (1963, p. 41)

What remains to be elucidated is usually referred to as T. stenolepium or T. sect. Orientalia, a group found very problematic during the revision of the West Himalayan dandelion flora. There are three main reasons for this uncertain status: first, it is the lack of a monographic treatment of T. section Orientalia, secondly, there are controversial concepts of the name T. stenolepium, in some treatments included in T. sect. Orientalia, and last, the name T. sect. Orientalia was used as an umbrella for a diverse assemblage of Himalayan forms by van Soest (1963, 1977).

In what follows, we pursue three main tasks: (i) clarification of the taxonomy of T. sect. Orientalia, including a checklist of species names to be included in it, as a prerequisite for further tasks, (ii) the analysis of the W. Himalayan taxa hidden under the name T. stenolepium, and (iii) a complete revision of the other names based on plant material from the West Himalaya and referred to as members of T. sect. Orientalia in the literature.
Chapter 22. Introduction

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Chapter 23

Material and Methods

An important part of the material studied is deposited in the Leoš Klimeš Taraxacum collection of the herbarium PRA, Institute of Botany, Academy of Sciences, Průhonice, Czech Republic. It represents one of the largest collections of West Himalayan dandelions in the world, a result of numerous expeditions of the late Leoš Klimeš to many regions of Ladakh, Jammu & Kashmir, India. Cultivation of plants grown from achenes collected during expeditions provided the major part of material for our study. Details of the cultivation methods are given in Kirschner and Štěpánek (1993). The cultivation, especially repeated mass cultivation, reveals limits of morphological plasticity of individual taxa. Moreover, it provided material for the study of reproduction systems of plants under study. Determination of the reproduction system, an important background for taxonomic decisions, was performed according to Kirschner et al. (2006).

This study was supplemented by the examination of numerous herbarium collections, and, a minor part, only as digital images. Those most relevant to the present study are BM, E, K, KUH, L, LE, PE, PRC, RAW, W and WU (abbreviations according to the Index Herbariorum36), and collections of B. Dickoré, G. Miehe and S. Miehe and their collectors. Most of our revision labels are numbered and refer to the specimens to which they are attached (as 'no. det.', not necessarily to duplicates).

Sectional nomenclature follows previous nomenclatural and taxonomic accounts Kirschner and Štěpánek (1997a), see also Kirschner and Štěpánek (1987, 2004) and Ge et al. (2011). Plant names are in accordance with the ICN (the latest edition, McNeill et al. 2012); when name authors are not given, the nomenclature follows Kirschner and Štěpánek (1997a, 1998b).

The micrograph of T. coronatum Handel-Mazzetti was taken in the low vacuum (LV) mode of the FEI Quanta 200 ESEM scanning electron microscope (SEM). The LVSEM method with its advantage of adjustable pressure in the sample chamber allowed to capture the surface of this non-conductive specimen in its natural state without any need of metal coating preparation and with minimized risk of artefacts. The sample was only carefully cleaned and placed on the aluminium stub in the microscope chamber using conductive adhesive carbon tape. Then the chamber was pumped to pressure of ca. 130 Pa and the

36See http://sciweb.nybg.org/science2/IndexHerbariorum.asp.
atmosphere inside was enriched by the (microscope controlled) addition of water vapor. The sample (scanned with a focused electron beam; HV 15 or 20 kV) was imaged by the special gaseous Large Field Detector (secondary electron detector with a little backscattered electron signal sensitivity).

For DNA analysis we used 93 sequences of ITS1-5.8S rDNA-ITS2 from Kirschner et al. (2015, see also Table 23.1) and remaining samples we newly sequenced (see Table 23.2 for details, including GenBank Accession Numbers). DNA was extracted from herbarium dried material with Qiagen DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands) following manufacturer’s protocol. The ITS1-5.8S rDNA-ITS2 region was amplified with primers ITS5 (forward) and ITS4 (reverse) from White et al. (1990) using Qiagen Multiplex PCR kit (Qiagen, Venlo, Netherlands) in 20 μl following manufacturer’s instructions. PCR conditions were 95°C hot start for 15 minutes and then 35 cycles of 95°C for 1 minute, 50°C for 1 minute and 72°C for 1 minute. We obtained PCR products of the length around 700 bp. For the GenBank Accession Numbers of these sequences see Table 23.1. Sequences were aligned with MAFFT 7.2 (Katoh and Standley 2013) and the alignment was manually edited in Geneious 9.1 (Kearse et al. 2012). Final alignment had length 466 bp. Neighbor Network was constructed in SplitsTree 4.14 (Huson and Bryant 2006) from uncorrected P-distances and tested with 1000 bootstraps (the values are not shown in the simplified figure).
Table 23.1: Samples from Kirschner et al. (2015) with revised nomenclature and sectional position. Arranged alphabetically according to the infrageneric classification.

<table>
<thead>
<tr>
<th>Species name</th>
<th>Infrageneric: Taraxacum section</th>
<th>GenBank Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. carpaticum</td>
<td>Alpestria</td>
<td>KF437445</td>
</tr>
<tr>
<td>T. paludosiforme</td>
<td>Alpestria</td>
<td>KF437411, KF437413</td>
</tr>
<tr>
<td>T. bulgaricum</td>
<td>Alpestria/Alpina</td>
<td>KF437412</td>
</tr>
<tr>
<td>T. gilliesii</td>
<td>Antarctica</td>
<td>AM946528</td>
</tr>
<tr>
<td>T. nigrocephalum</td>
<td>Arctica</td>
<td>KF437431</td>
</tr>
<tr>
<td>T. subalternilobum</td>
<td>Arctica</td>
<td>KF437432</td>
</tr>
<tr>
<td>T. aristum</td>
<td>Australasica</td>
<td>AM946527</td>
</tr>
<tr>
<td>T. zealandicum</td>
<td>Australasica</td>
<td>AF422138</td>
</tr>
<tr>
<td>T. nutans</td>
<td>Biennia</td>
<td>KF437460</td>
</tr>
<tr>
<td>T. sp.</td>
<td>Calanthondia</td>
<td>KF437424, KF437425, KF437427</td>
</tr>
<tr>
<td>T. koksaghyz</td>
<td>Ceratoidea</td>
<td>KF437406, KF437407</td>
</tr>
<tr>
<td>T. haussknechtii</td>
<td>Dioszegia</td>
<td>KF459942, KF459943</td>
</tr>
<tr>
<td>T. serotinum</td>
<td>Dioszegia</td>
<td>EU637252–EU637256</td>
</tr>
<tr>
<td>T. sp.</td>
<td>Emodensia</td>
<td>KF437426</td>
</tr>
<tr>
<td>T. minutilobum</td>
<td>Epyramidata</td>
<td>KF437409, KF437434–KF437437</td>
</tr>
<tr>
<td>T. pindicola</td>
<td>Erythrocarpa</td>
<td>KF437417, KF437428</td>
</tr>
<tr>
<td>T. erythrospermum</td>
<td>Erythrosperma</td>
<td>KF437416, KF437429, KF437430, KF437457</td>
</tr>
<tr>
<td>T. glaciale</td>
<td>Glacialia</td>
<td>KF437438</td>
</tr>
<tr>
<td>T. (aff.) dealbatum</td>
<td>Leucantha</td>
<td>KF437456</td>
</tr>
<tr>
<td>T. multiscaposum</td>
<td>Macrocornuta</td>
<td>KF437447–KF437452</td>
</tr>
<tr>
<td>T. (aff.) japonicum</td>
<td>Mongolica</td>
<td>KF437420–KF437423</td>
</tr>
<tr>
<td>T. cylleneum</td>
<td>not known</td>
<td>EU637135–EU637143</td>
</tr>
<tr>
<td>T. pyrenaicum</td>
<td>Obliqua</td>
<td>KF437454–KF437455</td>
</tr>
<tr>
<td>T. stevenii</td>
<td>Orientalia</td>
<td>KF437403, KF437404</td>
</tr>
<tr>
<td>T. tenuifolium</td>
<td>Palustria</td>
<td>EU637319–EU637328</td>
</tr>
<tr>
<td>T. bessarabicum</td>
<td>Piesis</td>
<td>EU637121–EU637128</td>
</tr>
<tr>
<td>T. stenocephalum</td>
<td>Piesis</td>
<td>EU637278–EU637286</td>
</tr>
<tr>
<td>T. primigenium</td>
<td>Primigenia</td>
<td>KF437405, KF437433</td>
</tr>
<tr>
<td>T. aphrogenes</td>
<td>Scariosa</td>
<td>KF437446</td>
</tr>
<tr>
<td>T. farinosum</td>
<td>Sonchidium</td>
<td>KF437418</td>
</tr>
<tr>
<td>T. linearisquameum</td>
<td>Taraxacum (= Ruderalia)</td>
<td>KF437414, KF437415, KF437419</td>
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Table 23.2: New DNA samples used in the present paper.

<table>
<thead>
<tr>
<th>ITS</th>
<th>Species, <em>Taraxacum</em> section</th>
<th>Locality</th>
<th>Date, coordinates</th>
<th>Sampling number</th>
<th>No. det.</th>
<th>GenBank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta2</td>
<td><em>Taraxacum bithynicum</em> DC.</td>
<td>Uludağ</td>
<td>25.07.2015, 40.070556N, 29.223333E</td>
<td>B. Gürdal 893-16</td>
<td>NA</td>
<td>KY552475</td>
</tr>
<tr>
<td>41/5</td>
<td><em>Taraxacum bicorne</em> Dahlstedt, sect. <em>Ceratoidea</em></td>
<td>Kazakhstan, Kokpek</td>
<td>04.06.2014, 43.44578N, 78.67270E</td>
<td>J. Kirschner &amp; J. Štěpáněk</td>
<td>28161</td>
<td>KY552476</td>
</tr>
<tr>
<td>41/9</td>
<td><em>Taraxacum bicorne</em> Dahlstedt, sect. <em>Ceratoidea</em></td>
<td>Kazakhstan, Kokpek</td>
<td>04.06.2014, 43.44578N, 78.67270E</td>
<td>J. Kirschner &amp; J. Štěpáněk</td>
<td>28161</td>
<td>KY552477</td>
</tr>
<tr>
<td>41/16</td>
<td><em>Taraxacum bicorne</em> Dahlstedt, sect. <em>Ceratoidea</em></td>
<td>Kazakhstan, Kokpek</td>
<td>04.06.2014, 43.44578N, 78.67270E</td>
<td>J. Kirschner &amp; J. Štěpáněk</td>
<td>28161</td>
<td>KY552478</td>
</tr>
<tr>
<td>ITS</td>
<td>Species, <em>Taraxacum</em> section</td>
<td>Locality</td>
<td>Date, coordinates</td>
<td>Sampling number</td>
<td>No. det.</td>
<td>GenBank Accession No.</td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------------</td>
<td>----------</td>
<td>------------------</td>
<td>----------------</td>
<td>----------</td>
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</tr>
<tr>
<td>41/25</td>
<td><em>Taraxacum bicorne</em> Dahlstedt, sect. <em>Ceratoidea</em></td>
<td>Kazakhstan, Kokpek</td>
<td>04.06.2014, 43.44578N, 78.67270E</td>
<td>J. Kirschner &amp; J. Štěpánek</td>
<td>28161</td>
<td>KY552479</td>
</tr>
<tr>
<td>41/29</td>
<td><em>Taraxacum bicorne</em> Dahlstedt, sect. <em>Ceratoidea</em></td>
<td>Kazakhstan, Kokpek</td>
<td>04.06.2014, 43.44578N, 78.67270E</td>
<td>J. Kirschner &amp; J. Štěpánek</td>
<td>28161</td>
<td>KY552480</td>
</tr>
</tbody>
</table>
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Chapter 24

Results

24.1 Interpretation of the name *T. sect. Orientalia*

The name *Taraxacum* sect. *Orientalia* von Handel-Mazzetti (1923, p. 274) was published in a short footnote to the species name checklist. Sectional type was not designated by Handel-Mazzetti, and five species names were listed under *T. sect. Orientalia* (see Table 24.1). The protologue diagnosis characterized the section by large pale brownish or greyish to blackish grey achenes with body almost smooth or minutely tuberculate, rostrum very short or slightly longer than achene, white pappus, appressed outer phyllaries, and leaves not divided or only slightly so.

Most of the sections introduced by Handel-Mazzetti were divided into narrower groups later, which is also the case of *T. sect. Orientalia*. The sectional name was typified by Kirchner and Štěpánek (1997a) who designated *T. stevenii* (Sprengel) Candolle as the lectotype. Of the other species names listed in the protologue, only *T. paradoxum* Handel-Mazzetti (= *T. kurdicum* Handel-Mazzetti) belongs to *T. sect. Orientalia* (see Table 24.1).
<table>
<thead>
<tr>
<th>Name in von Handel-Mazzetti (1923)</th>
<th>Type</th>
<th>Section</th>
<th>Current name</th>
<th>Region of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. stevenii</em> (Sprengel 1826, p. 658) de Candolle (1838, p. 149)</td>
<td><em>Steven s.n.</em> (syn.: H, n. v.; K, no. det. 8799)</td>
<td><em>Orientalia</em></td>
<td><em>T. stevenii</em> (Sprengel) Candolle (syn.: <em>T. crepidiforme</em> Candolle)</td>
<td>The Caucasus and adjacent mountainous regions</td>
</tr>
<tr>
<td><em>T. heteroloma</em> von Handel-Mazzetti (1907, p. 120)</td>
<td>not designated</td>
<td><em>Emodensia</em></td>
<td>not in use</td>
<td>the Himalaya (protologue records)</td>
</tr>
<tr>
<td><em>T. porphyranthum</em> (Boissier 1867b, p. 790)</td>
<td><em>Ruprecht 22c</em> (lecto: LE, no. det. 6022; iso: G, no. det. 18797)</td>
<td><em>Porphyrantha</em></td>
<td><em>T. porphyranthum</em> Boissier</td>
<td>The Caucasus</td>
</tr>
<tr>
<td><em>T. stenolepium</em> (von Handel-Mazzetti 1907, p. 121)</td>
<td><em>Szovits 633</em> (lecto: LE, no. det. 6065)</td>
<td><em>Piesis</em></td>
<td><em>T. stenosephalum</em> Boissier &amp; Kotschy in (Boissier 1867b, p. 790) s. lat.</td>
<td>The Caucasus and adjacent mountainous regions, Turkey</td>
</tr>
</tbody>
</table>

37The type gathering represents an agamospermous taxon (on the basis of its very irregular pollen size).
38The majority of the six syntype herbarium sheets seen contain plants of *T. sect. Emodensia*.
39See the sectional revision in Kirschner and Štěpánek (1993).
40For nomenclatural discussion and further data see (Kirschner and Štěpánek 1998b).
The name *T. stenolepium* Handel-Mazzetti requires a special note. According to the protologue von Handel-Mazzetti (1907) and von Handel-Mazzetti (1923), this species has two centres of distribution, the Caucasus and the westernmost Himalaya. It was Schischkin and Tzvelev (1964, p. 536) who designated the lectotype of the latter name and restricted it to the plants of the Caucasus and neighbouring areas (see also Kirschner and Štěpánek 1997a). van Soest (1977) attempted to designate a new lectotype of *T. stenolepium* (Afghanistan, Griffith, herb. K) to replace the previous one, with the consequence that this name be confined to the West Himalaya and adjacent areas. However, this did not take any nomenclatural effect. It should be added that van Soest (1963) introduced a name to accommodate the Caucasian plants belonging to *T. stenolepium*, *T. szovitsii* Soest (based on duplicates of the Schischkin & Tzvelev’s lectotype of the name *T. stenolepium*). According to the original lectotype, *T. stenolepium* belongs to *T. sect. Piesis* (see the sectional revision Kirschner and Štěpánek 1998b), and, within it, to the complicated tetraploid complex referred to under the name *T. stenocephalum* Boiss. (see also P. Trávníček et al. 2013). The *T. sect. Piesis* has subsparsely minutely spinulose achenes gradually narrowing into an almost cylindrical cone, and pappus coloured, from tawny to yellowish, which is a combination of features not found among plants from N Pakistan. The W Himalayan forms referred to under the name *T. stenolepium* in fact do not belong to the taxon including the type of this name, and therefore a revision and a new interpretation is needed.

**Note:** For the sake of completeness, we provide an interpretation of the original syntype that was later, and mistakenly, designated as the lectotype of the name *T. stenolepium* by Soest. The specimen is deposited at K (Afghanistan, [W. Griffith, Herbarium of the late East India Company no. 3354 [no. det. 28168]). The label gives an unsatisfactory information on the origin of the plants, and a comparison of numbering in the Griffith’s diaries (McClelland 1848) with the East India Company herbarium numbers is needed. Most probably, the plant was collected in the E Afghanistan–W Pakistan border region. As regards taxonomy, the plant belongs neither to *T. sect. Orientalia* nor to the new Himalayan section described below; it is a member of *T. sect. Piesis* and is quite similar to the group of *T. jaschilkulense* Vainberg.

The true *T. sect. Orientalia* also deserves a revision, and according to our recent study, its diagnostic characters include achenes with sparsely scattered minute spinules (*T. stevenii*) to subdense small spinules, achene body very gradually narrowing into a relatively short cone tapering from its broader base, and outer phyllaries appressed, lanceolate to ovate-lanceolate, with narrow to broad pale or whitish borders. All what can be summarized from the currently available data is given below, particularly a newly compiled description and a list of species names referable to the section.


Type: *Taraxacum stevenii* (Sprengel) de Candolle (1838, p. 149) [= *Leontodon stevenii* Sprengel (1826, p. 658) = *Leontodon alpinus* Steven (1813, p. 100) non Jacq. 1773], selected by Kirschner and Štěpánek (1997a, p. 94).

Flowers and leaves develop simultaneously. Plants usually small, plant base subglabrous. Leaves usually glabrous, not swollen at hair base, usually undivided or shal-
lowly lobed, midrib without striatulate pattern, blade unspotted, petioles usually narrowly winged or ± unwinged. Scapes erect during flowering, unbranched, growing from the centre of leaf rosette, glabrous or subglabrous. Involucre with sub-obconical or rounded base, usually of medium width (ca 7–10 mm wide). Inner phyllaries flat, without corniculation at the apex, rarely callose. Outer phyllaries 10–14, of ± equal length, appressed to erect, lanceolate to ovate, with narrow to broad, distinct, white or paler border, rarely border ± absent, glabrous or sparsely minutely ciliate. Flowers yellow, florets usually ± numerous, ligules flat. Pollen usually present, stigma discoloured (usually greenish to almost black in dry condition). Receptacle glabrous. Achenes 3.9–4.8 mm long, slender to medium thick, usually 0.8–1.0 mm wide, gradually and often indistinctly narrowing into the cone, achene body usually pale greyish straw brown, sparsely spinulose, spinules minute, cone subconical, with a broader base, thicker, usually 0.4–0.6 mm long. Rostrum thin, sometimes thickened, 1–8 mm long, not breaking off, pappus 4–6 mm long, white, not deciduous. Main flowering season: summer (in high mountains). Main habitat: subalpine and alpine meadows. Sexual or apomictic.

This description is based on the material of *T. stevenii*, a sexual (studied on the basis of plants from our Caucasus expeditions and from numerous herbarium collections), and three closely allied agamosperms, see the list below.

A list of members of *Taraxacum* sect. *Orientalia* Handel-Mazzetti:

- *T. stevenii* (Sprengel) Candolle
- *T. kurdicum* Handel-Mazzetti in Nábělek
- *T. litvinovii* Schischkin in Grossgeim (1934, p. 250)
- *T. psychrophilum* Boissier (1849, p. 48)

These four taxa are distributed from Inner Anatolia and NW Anatolia to the Caucasus, the northernmost mountain Iraq and the western and central parts of the northern Iran.

However, van Soest (1963, 1966a, b, 1977) referred to this section another eight names from the region of the West Himalaya. The analysis of his material shows that these plants are far beyond the morphological limits of *T. sect. Orientalia* as set above (with the exception of *T. crepidiforme*, see Table 24.1). The following names were reported from West Himalaya as members of the latter section:

- *T. amblylepidocarpum* van Soest (1963, p. 12)
- *T. canum* Soest in Rechinger (1977: 245)\(^{41}\)
- *T. crepidiforme* de Candolle (1838, p. 149)
- *T. baltistanicum* van Soest (1963, p. 14)

\(^{41}\)See van Soest (1977).
• *T. melleum* van Soest (1963, p. 14)

• *T. pubens* van Soest (1966a, p. 367)  
  Note: Richards in Löve (1969) gave $2n = 24$ as the chromosome count for *T. pubens*. Later, A. J. Richards (1972) referred the count to his new species, *T. nigricornis* A. J. Richards, so that the count and species record are not relevant for our study.

• *T. stenolepium* Handel-Mazzetti

• *T. tricolor* van Soest (1966b, p. 75)

• *T. wendelboanum* van Soest (1966b, p. 77)  
  Note: A. J. Richards (1972) reported *T. calciphilum* A. J. Richards & Soest from Afghanistan as a member of *T. sect. Orientalia*. Kirschner and Štěpánek (1998b) included this species name into the variable group of *T. stenocephalum* Boiss. It undoubtedly belongs to *T. sect. Piesis* but further study is needed as to its possible separate status. Another two names, *T. hydrophilum* Soest and *T. pallidipapposum* Soest, from Iran and Afghanistan were also mistakenly referred to *T. sect. Orientalia* (van Soest 1977).
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Chapter 25

Taxonomic treatment

25.1 A new section to accommodate the West Himalayan *T. stenolepium*

The Himalayan plants reported to belong to *T. stenolepium* (van Soest 1963, 1977) represent a conspicuous group with usually undivided and often entire leaves, turbinate or subturbinate involucre base, very narrow outer phyllaries with distinct whitish borders; they also share important achene characters: achene body ± thin to medium thick, with distinct, not numerous broad thin squamules in the upper 1/5–1/6, sometimes forming a ‘collar’, cone subconical to subcylindrical. This character combination is not known from other dandelion groups, and we consider it as a good basis for the recognition of these plants as a new section.

*Taraxacum* sect. *Squamulosa* Kirschner & Štěpánek, sect. nova

Type: *Taraxacum tenuiculum* Kirschner & Štěpánek

Diagnosis: Plantae graciles sed altitudine variabili, tenues, foliis angustis, linearibus vel lanceolato-linearibus indivisis, saepe integris, raro lobulis lateralibus triangulari-linearibus praeditis, involuco basi subturbinato vel obconico-subrotundato, phyllariis involucralibus exterioribus linearibus vel lineari-lanceolatis, adpressis, marginibus albis vel albidis, acheniis angustis, supernae saepissime sparse sed distincte squamulosis, in pyramidem subconicam vel subcylindricam, 0.6–1.2 mm longam subsensim vel subabrupte abeuntibus, rostro tenui.

25.1.1 Description

Flowers and leaves develop simultaneously. Plants slender, plant base subglabrous to aranose. Leaves subglabrous to sparsely aranose, surface not swollen at hair base, blade linear to linear-oblanceolate, usually undivided or sometimes with a few lateral lobules or lobes, often entire; petiole long, narrow, unwinged, usually purplish. Scapes erect during flowering, unbranched, growing from the centre of leaf rosette, subglabrous to aranose. Involucre with obconical base, narrow (3–)4–6 (–7) mm wide. Outer phyllaries 6–15, of ± equal or unequal length, appressed, linear, linear-triangular or linear-lanceolate, with a distinct whitish or
white border. Receptacle glabrous. Flowers yellow, florets numerous, outer ligules ± flat. Stigmas discoloured (usually blackish). Pollen present. Achenes 4.4–6.2 mm long, greyish stramineous, ochraceous, light reddish orange or pale fulvous-greyish, body slender, 0.9–1.1 mm thick, with distinct, subsparse broad thin squamules often in rows and sometimes forming a thin collar above (Figs. 25.1 and 25.4), partly covering the transition into cone, body subgradually to subabruptly narrowing into subconical to subcylindrical cone 0.6–1.2 mm long, ca. 0.3–0.4 (–0.5) mm thick. Rostrum thin, 5–6.5 mm long; pappus 5–7 mm, white to yellowish white, not deciduous. Flowers in summer (in the mountains). Main habitat: subalpine and alpine meadows. Sexual or agamosperous.

Figure 25.1: A detail of the characteristic achene squamulosity in T. section Squamulosa. T. persquamulosum (G. Miehe & S. Miehe 2606, no. det. 28118). Structures that appear to be long spinules are in fact broad squamules in side view.

25.1.2 Phylogenetic position of T. sect. Squamulosa

Kirschner et al. (2003, 2015) analysed the phylogeny of the genus Taraxacum using various approaches and techniques. No unequivocal pattern could be inferred from the data available, in all likelihood because even some of the sexuals studied came into being through hybridization and/or polyploid resexualization. The latter work, however, a nrDNA study of sexually reproducing dandelions, provided a framework showing the major coherent groups or, on the contrary, defining sections or their groups clearly unrelated to one another. We therefore used the published pattern involving sexual representatives of 24 sections and compared it with the new material of T. stenotegulatum, the only known sexual member of T. sect. Squamulosa. The result of our comparison is displayed on Fig. 25.2. Three samples of T. stenotegulatum form a separate group without clear relationships with any Taraxacum section included in the analysis. This supports our conclusion that T. stenotegulatum and the two (or three) related apomicts form a separate section. In particular, we can safely exclude closer relationships between Taraxacum sections Squamulosa and Orientalia.
25.1.3 Comparison with morphologically similar taxa

On morphological and geographical grounds, we compare *T. sect. Squamulosa* with several groups:


*Taraxacum primigenium* von Handel-Mazzetti (1907, p. 17) (type of *T. sect. Primigenia*) is based on Bornmüller 5131 (lecto: G, fide Soest 1975, as 'holo.'). It is quite similar to *T. sect. Squamulosa* in having narrow, linear, narrowly whitish-bordered outer phyllaries. We have seen a number of duplicates of the lectotype and one of them (W, no. det. 9050) has relatively well developed achenes. They are up to 4 mm long, minutely sparsely spinulose or tuberculate above or almost smooth, with a very short smooth zone above the tubercles, and a very thick and short, ca. 0.5 mm long rostrum. These characters document both the distinctiveness of *T. primigenium* (sometimes mistakenly equated with *T. assemanii* Boiss.) and the low degree of relatedness to *T. sect. Squamulosa*. Together with the results of the molecular analysis (see Fig. 25.2), we can exclude *T. primigenium* from our considerations.


The type and the only member of *T. sect. Coronata* is based on a rather scanty material. We have seen two specimens, the lectotype (C. B. Clarke 30774B, LE, no. det. 11279, fide Kirschner and Štěpánek 1997a, from Tilail, Gurez, N of Srinagar, Kashmir) and R. R. Stewart, E. Nasir & M. A. Siddiqi 1280 (KUH, no. det. 19462, from Lowari Top, Dir Dis-
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trict, Pakistan). A SEM image of an achene of the latter specimen is provided on Fig. 25.3.
It is obvious that the thick, robust achenes, covered with coarse spinules and squamules,
with body very abruptly narrowed into a thick subcylindrical cone, decisively deviate from
achene characters of T. sect. Squamulosa. Although little is known about the relationships
and distribution of T. coronatum, we can exclude it from the closest relatives of our newly
described section.
The circumscription and members of T. sect. Orientalia are provided above. We repeat that most taxa, including the type species, are characterized by achenes with sparse
to subdense minute spinules, sometimes only tubercles, rarely smooth, very gradually, almost indistinctly narrowing into a subconical, sometimes not discernible cone, and beak
usually not thin. The distinctly squamulose pattern known from T. sect. Squamulosa was
not observed in T. sect. Orientalia. Furthermore, outer phyllaries of members of T. sect.
Orientalia are generally broader and ± lanceolate, with a variably wide, usually very broad,
paler border. These important characters show that T. sect. Squamulosa cannot be equated
According to the type material from Chitral, Pakistan (holotype: O, no. det. 11706, and
paratype: O, no. det. 11707), Taraxacum obtusum (van Soest 1966b, p. 73) Doll (1982, p. 531),
the only known member of the section, is characterized by features substantially deviating
from those of T. sect. Squamulosa: receptacle aranose, outer phyllaries imbricate, usually
ovate, light green with short dark horns and a narrow whitish border, often denticulate
and ciliate distally, stigmas yellow, achenes 5–5.5 × 0.8–1.0 mm, almost smooth (only very
sparse and minute spinules above), achene body ± turbinate, slender, very gradually narrowing into the beak, cone almost not developed, beak thickened, 1.5–2 mm long, pappus
whitish, ca. 2.5 mm long. Most of these characters point to the relationship with T. subsect.

25.1.4 A key to the members of Taraxacum sect. Squamulosa
1. Outer phyllaries 9–15; achenes permanently light pure grey to light fulvous greyish . . .
. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 3. T. tenuiculum
— Outer phyllaries 6–10; achenes light stramineous-brown to yellowish-greyish or variously ochraceous-orange . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 2
2. Outer phyllaries distally arcuate-recurved; cone thick, conical, ca. 0.5 mm thick at base.
. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 4. T. amblylepidocarpum
— Outer phyllaries appressed; cone medium thick to thin, subcylindrical to subconical, up
to 0.3–0.4 mm thick at base . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 3
3. Achenes light stramineous-brown to yellowish-greyish; pollen regular in size (sexual
plants); cone 0.7–0.9 mm . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 1. T. stenotegulatum
– Achenes ochraceous when immature, later pale reddish-orange to pale cinnamon-orange;
pollen irregular in size (agamosperms); cone 0.9–1.2 mm . . . . . . . . . . . 2. T. persquamulosum
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Vojtěch Zeisek (2018)


1. *Taraxacum stenotegulatum* Kirschner & Štěpánek, sp. nov. (Fig. 25.5).

Type: PAKISTAN. N Pakistan, Iter Karakorumense I (1990), Shinghai Gah to Pahot Gali, 4000 m, 35°48’55”N, 74°10’17”E, relatively humid subalpine/alpine meadow ... with Kobresia schoenoides, veg. rec. 82, 31 Jul 1990, G. Miehe & S. Miehe 1399 (holotype: herb. Miehe, no. det. 28114).

Etymology: With narrow involucre.

Diagnosis: Plantae sexuales tenues foliis indivisis linearibus vel lineari-lanceolatis, saepissime integris, involuco ad basin turbinato vel subturbinato phyllariis exterioribus paucis (6–9) adpressis linearibus vel triangulare-lanceolatis, floribus luteis, stigmatibus pallide viridibus vel griseo-flavis, achenis pallide brunneo-stramineis corpore superne distincte squamuloso, pyramide subconica 0.7–0.9 mm longa.
Slender plants 12–22 cm tall. Petiole long, thin, narrow, unwinged, usually suffused purple, sometimes greenish, plant base ± glabrous, petiole bases not persistent; leaves mid-green, linear to linear-lanceolate, 5–22 cm × 4–11 mm, glabrous, blade margin usually entire, sometimes with 1–2 remote pairs of short teeth, inner leaves sometimes with elongated terminal segment 3–5 cm × 2–5 mm and 1–2 pairs of linear, subrecurved lateral segments to 6–7 mm long. Scapes brownish green, ± equalling leaves, most often ± glabrous, sometimes sparsely to densely aranose above. Capitulum 1.5–2 cm wide. Involucre 4–7 mm wide, base turbinate to slightly rounded-obconical. Outer phyllaries 6–9, unequal but not imbricate, appressed, linear, linear-lanceolate or seldom triangular-lanceolate, usually (4–) 5–6 (–8) mm long, 1.2–2.4 mm wide, with a black to black-green middle strip ca. 0.5–1 mm wide and white to whitish-membranous border 0.3–0.6 mm wide, transition into middle strip abrupt, margins glabrous, entire or with 1–2 minute teeth, apex flat, not corniculate; inner phyllaries (10–) 13–15 (–16) mm long, grey-green to blackish green, often with a black middle line, apex flat, often purplish. Ligules yellow, outer ligules almost flat, striped greyish or greyish-pinkish outside, inner ligule teeth long, ± grey-pink. Stigmas greyish pale green or yellowish grey. Anthers polliniferous, pollen grains regular in size.
**Taraxacum stenotegulatum** grows in moderately wet sub-alpine and alpine grasslands within the span of 3600 to 4000 m. Its distribution extends from the SW Karakorum to the Dras Region of Ladakh (Fig. 25.6).


2. **Taraxacum persquamulosum** Kirschner & Štěpánek, *sp. nov.* (Fig. 25.7).

**Type:** PAKISTAN. N Pakistan, Iter Karakorumense II (1991), Hunza Valley, Rakaposhi N flank, above Nilt, 36°12’14”N, 74°26’E, 3950–3970 m, relatively humid alpine Cyperaceae mats dominated by *Carex* and *Kobresia* spp. on alpine turf, veg. rec. 670–671, G. Miehe & S. Miehe 6527 (holotype: herb. Miehe, no. det. 28129).

**Etymology:** Very conspicuously squamulose.

**Diagnosis:** Plantae agamospermae tenues foliis linearibus vel lineari-oblanceolatis acutis indivisis, saepissime integris, involucro ad basin turbinato vel subturbinato phyllariis exterioribus paucis (6–10) adpressis, linearibus vel lineari-triangularibus usque ad lineari-lanceolatis, late albo-marginatis, floribus luteis stigmatibus saturate viridibus vel nigrescentibus, antheris polliniferis, achenis maturescentibus ochraceis, maturis aurantiaco-rubentibus vel cinnamo-meo-aurantiacis, corpore superne subsparse squamuloso, pyramide subconica, 0.9–1.2 mm longa.

Plants slender, to 18 cm tall. Petiole dirty purplish to dirty greenish brown, long, narrow, unwinged, plant base ± glabrous; leaves mid-green, linear or linear-oblancoleate, ± acute, usually 6–10 cm × 5–9 mm, usually undivided and often entire, sometimes with 2–3 pairs of acute patent teeth 1–2 mm long, less often with a large terminal segment and 1–3 pairs of patent, linear, acute lobules 2–4 mm long. Scapes brown-green, usually overtopping leaves, sparsely aranose, densely so below capitulum. Capitulum 2–3 cm wide. Involucre 5–6 mm wide, turbinate to slightly rounded-obconical. Outer phyllaries 6–10, unequal but not imbricate, appressed, linear to linear-triangular or linear-lanceolate, (3.5–) 5–7 (–9) × (1.2–) 1.5–1.8 (–2.1) mm, with a narrow to broad black middle strip and a ± white border 0.3–0.5 mm wide, margins glabrous or sparsely ciliate near apex, apex flat to callose; inner phyllaries blackish green, 12–17 mm long, flat. Ligules yellow, outer ligules ± flat, striped light grey to grey outside, inner ligule teeth long, grey-yellow. Stigma green to blackish. Anthers polliniferous; pollen grains irregular in size. Achenes (Fig. 25.4) ochraceous when immature, later pale reddish-orange to pale cinnamon-orange or cinnamon-brown, (4.7–)
Figure 25.5: *Taraxacum stenotegulatum* (PRA, no. det. 28114). Scale bar = 3 cm.
4.9–5.0 (–5.4) × 0.9–1.1 mm, body ± irregularly, not densely covered with variously broad squamules in the upper 1/4–1/5, otherwise smooth, ± gradually narrowing into a subconical cone 0.9–1.2 mm long, ca. 0.4 mm thick at base; beak thin, ca. 6 mm, pappus white, ca. 6 mm long. Agamospermous.

Ecology and distribution: *Taraxacum persquamulosum* grows in relatively humid sub-alpine and alpine pastures and grasslands, often dominated by several *Kobresia* and other Cyperaceae species, between 3800 and 4500 m. It is probably the most common species of this section; it is known from various regions of the Karakorum and the adjacent parts of Ladakh (Fig. 25.8).

**Specimens seen (Fig. 25.8):** PAKISTAN. Karakorum, 14 500 [ft.], C. B. Clarke 30196B (K, no. det. 28169, one of the original syntypes of the name *T. stenolepium*). — Iter Karakorumense II (1991), Hunza Valley, Rakaposhi N flank, above Nilt, 36°12’14”N, 74°26’E, 4230–4300 m, 31 Aug 1990, relatively humid alpine Cyperaceae mats dominated by *Carex* and *Kobresia* spp. on alpine turf, veg. rec. 681–683, G. Miehe & S. Miehe 6629 (holo: herb. Miehe, no. det. 28121). — N Pakistan, Iter Karakorumense I (1990), Shinghai Gah to Pahot Gali, 4240 m, 35°48’55”N, 74°10’17”E, relatively humid alpine Cyperaceae mats dominated by *Carex* and *Kobresia* spp. on alpine turf, west-facing slopes, veg. rec. 99–100, G. Miehe & S. Miehe 1620 (herb. Miehe, no. det. 28119). — N Pakistan, Iter Karakorumense I (1990), Khaibar / Upper Hunza, 36°35’N, 74°43’E, 4000 m, subalpine *Juniperus macropoda* dwarf-
Figure 25.7: *Taraxacum persquamulosum* (PRA, no. det. 28129). Scale bar = 3 cm.

3. **Taraxacum tenuiculum** Kirschner & Štěpánek, sp. nov. (Fig. 25.9).

Type: INDIA. NW India, [Ladakh], Watakul Valley, 3840–4250 m, 34°23’N, 75°49’E, 13 Sep 2004, L. Klimeš 6642 (holotype: PRA, no. det. 28127; isotype: PRA, no. 28138).

Etymology. Diminutive of *tenuis*.

Diagnosis: Plantae agamospermae tenues foliis linearibus vel lineri-oblanceolatis indivisis, interdum integris, saepissime remote dentatis vel lobatis, lobis lateralibus 2–3 integris acutissimis, scapis araneosis, involucris angustis, basi subturbinatis, phyllariis exterioribus numerosis (9–15) linearibus vel lineari-lanceolatis, adpressis, conspicue albo-marginatis, floribus luteis.
stigmatibus nigrescentibus, antheris polliniferis, achenis pallide fumosis vel pallide fumosofulvescentibus, corpore angusto superne conspicue sparse squamuloso, pyramide subcylindrica, saepissime 0.7–0.9 mm longa.

Figure 25.9: Taraxacum tenuiculum (PRA, no. det. 28127). Scale bar = 3 cm.

Plants slender, usually 8–22 cm tall. Petiole purple to purplish green, long, narrow, un-winged, plant base sparsely aranose. Leaves mid-green with pale green or purplish midvein, linear to linear-oblanceolate, usually 6–12 × 0.6–1.2 cm, subglabrous to sparsely aranose, not divided and entire or with 2–3 pairs of short recurved teeth or with 2–3 pairs of narrowly linear-triangular, acute, usually entire, recurved lobes 2–4 mm long. Scapes brownish green, aranose, densely so below capitulum, overtopping leaves. Capitulum ca. 1.5 cm wide. Involucre narrow, cylindrical, (3–) 4 (–5) mm wide, obconical at base. Outer phyllaries (9–) 10–13 (–15), ± not imbricate, linear to linear-lanceolate, usually 5–8 × 1.0–1.8 mm, appressed, often some arcuate-recurved distally, with a distinct white to whitish-pinkish border 0.2–0.4 mm wide, with an abrupt transition into almost black middle part, margins sparsely ciliate below apex, apex often reddish, ± flat; inner phyllaries blackish green, 13–
17 mm long, apex flat. Ligules yellow, outer ligules usually not very exserted, canaliculate to cucullate, striped faintly grey-purplish outside, inner ligules with blackish apical teeth. Stigmas blackish. Anthers polliniferous, pollen grains irregular in size. Achenes (Fig. 25.4) uniformly light pure grey to light fulvous greyish, 4.9–5.2 (–6.2) × 0.9 mm, body relatively slender, smooth, only with 1–3 horizontal rows of, or scattered, broad, erect-patent squamules in the upper 1/5, seemingly subabruptly narrowing into subcylindrical cone (0.6–) 0.7–0.9 (–1.1) mm long, 0.3 (–0.4) mm thick; beak 5–6 mm long, ± thin; pappus yellowish white to white, 5–6 mm long. Agamosperm.

Ecology and distribution: *Taraxacum tenuiculum* grows in medium humid subalpine and alpine pastures, from 3800 to 4500 m. It is known from Ladakh (Fig. 25.10).

![Figure 25.10: Distribution of Taraxacum tenuiculum.](image)

**Specimens seen (Fig. 25.10):** INDIA. NW India, Umbo village to the Watakul Valley [Ladakh, Umba], 4200–4500 m, 34°21’N, 75°53’E, 12 Sep 2004, L. Klimeš 6641 (PRA, no. det. 28128 and duplicates). — NW India [Ladakh], Tongul, valley S of the village, 3800–4100 m, 27 Aug 2005, L. Klimeš 7436 (PRA, no. det. 18463 and duplicates).


Plants small, to 12 cm tall, plant base sparsely aranose. Petiole long, narrow, unwinged,
± pinkish pale green. Leaves ± mid-green, ± oblanceolate in outline, 9–12 × 0.9–2 cm, ± pinnatifid, lateral segments in 3–5 pairs, narrowly triangular-deltoid, recurved, distal margin straight to convex, usually entire or with a single minute tooth, proximal margin ± straight, entire, interlobes distinct, ca. 0.5–1 × ca. 0.2 cm, entire or with 1–2 filiform teeth, terminal segment triangular-sagittate with basal lobules recurved, apex acute. Scape greenish-brownish, sparsely aranose, densely so below capitulum, ± subequalling leaves. Capitulum ca. 1.5 cm wide. Involucre narrow, ca. 5–6 mm wide, narrowly rounded at base. Outer phyllaries ca. 7–9, appressed at base, recurved or arcuate in the distal half, narrowly linear, 7.5–8.5 × 1.4–1.7 mm, border distinct, whitish to whitish-membranous, 0.3–0.4 mm wide, with abrupt transition into a blackish green middle strip, margins glabrous, apex flat or slightly callose. Inner phyllaries ca. 12 mm long, to 2 mm wide. Ligules yellow, outer ligules ± flat, striped grey-pink outside, inner ligule teeth black-purple. Pollen absent. Stigmas black-green. Achenes [not fully ripe] light stramineous brown, ca. 5 mm long, 1.1–1.2 mm thick, body coarsely squamulose in the uppermost part and sparsely spinulose-squamulose below the main squamulosity, with a subabrupt transition into a robust, thick, conical cone 0.9–1.0 mm long, beak ± thin, ca. 5 mm long (unripe), pappus white, ca. 5 mm long.

This species is known from a single gathering from Kashmir (Fig. 25.11) consisting of a single small individual. Its relatively thick, robust achenes, with a thick, conical cone represent a safe diagnostic character distinguishing the core species of *T.* sect. *Squamulosa* and *T.* amblylepidocarpum. The character of outer phyllaries, sculpture of achenes and the narrow involucre is not far from the pattern of *T.* sect. *Squamulosa*, however. We therefore treat *T.* amblylepidocarpum as a marginal species of *T.* sect. *Squamulosa*.

### 25.2 Taxa with broadly ovate outer phyllaries and yellow stigmas

In the W Himalayas, there are two species quite distinct in having round-ovate to broadly ovate short outer phyllaries, stigmas almost yellow to dirty yellow, and achenes (known from a single sample of each species) with a short beak to 3–4 mm long and body sparsely minutely squamulose, indistinctly narrowing into a thick cone. This character combination is not known from other Himalayan plants nor was it observed in the dandelion flora of Middle Asia. The material available, however, is too scanty to allow any conclusion as to the sectional position of these plants. There is a range extension to be reported for both species involved, as well as further morphological measurements to complete the descriptions.


Type: PAKISTAN. Baltistan, Ghondokoro Glacier, 4050 m, 14 Jul 1955, Nasir & Webster 6104 (holotype: RAW, n. v.; isotype: GH 13009, photo !)

Plants small, slender, to 10 cm tall. Petiole pale green to pinkish, narrow, unwinged, plant base glabrous; leaves dull to deep green, linear to linear-oblanceolate, 5–8 cm × 3–5 mm, undivided, entire, apex obtuse to subacute. Scapes brownish green to purplish green,
Figure 25.11: Distribution of *Taraxacum amblylepidocarpum*.

glabrous or with very sparse aranose hairs below capitulum. Capitulum small, ca. 1.5 cm wide. Involucre ca. 4 mm wide, ± rounded at base. Outer phyllaries 5–9, unequal, most often broadly ovate to round-ovate, the outermost ones ca. 3.5–4 × 2.1–2.5 (~2.8) mm, the others to 5.2 mm long and narrower, ca. 1.5–1.8 mm wide, appressed, with grey to black-green middle part and white to whitish border to 0.6 mm wide, apex flat to callose, margins glabrous; inner phyllaries 7–10 mm long, flat to callose, blackish green. Ligules yellow, outer ligules ± flat, striped light black-purple to greyish purple outside, inner ligule apical teeth blackish purple. Stigmas yellow to slightly greenish yellow. Anthers polliniferous, pollen grains irregular in size. Achenes light straw-brown when immature, later ± light grey, 4.5–5 × 1.0–1.1 mm, body very minutely and sparsely appressed squamulose above, with a gradual and indistinct transition into a short, thick, conical-cylindrical cone 0.5–0.6 mm long, ca. 0.5 mm thick; beak thick (0.2–0.3 mm), 2.5–2.8 mm long; pappus white, 4.5–5 mm long. Agamosperm.

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Figure 25.12: Distribution of Taraxacum baltistanicum.


Type: PAKISTAN. Hazara N.W.F.P., North West Himalaya, Baltistan, Ghondokoro Glacier, 13,500’, 14 Jul 1955, Nasir & Webster 6117 (holotype: RAW, no. det. 28125; isotype GH 13010, photo !).

Plants ± small, ca. 7–10 cm tall. Petiole usually purplish, long, narrow, unwinged, plant base ± glabrous, with persistent remnants of old petioles; leaves mid-green to dull green, ± ob lanceolate in outline, 0.7–2.5 cm wide, ± glabrous, with 1–2 pairs of patent, triangular lateral lobes usually 3–5 mm long, terminal segment large, ± narrowly triangular, with short patent basal lobules and subobtuse apex. Scape brownish green, sparsely aranose, equalling or ± overtopping leaves. Capitulum to 1.5–2 cm wide. Involucre 5–8 mm wide, rounded at base. Outer phyllaries 8–10, black-green, ± imbricate, broadly ovate, the innermost ovate lanceolate, 2.8–3.5 (–5) × 2.0–2.5 mm, appressed, with a distinct whitish-membranous border 0.2–0.5 mm wide, margin minutely ciliate near apex, apex blackish to purplish, × flat; inner phyllaries blackish green, 9–11 mm long, flat. Ligules yellow, outer ligules ± flat, striped blackish green to blackish purple, inner ligule teeth very long, blackish. Stigmas ± yellow or with reddish tinge or dirty yellow. Anthers polliniferous, pollen grains irregular in size. Achenes (not fully ripe) 5.5 × 1.1 mm, body with sparse coarse spinules or laterally flat spinules in the upper part (and on cone base), gradually narrowing into subconical cone 1.1–1.3 mm long, ca. 0.3–0.4 mm thick at base, ca. 0.2 mm at apex; beak ca. 4 mm long.
slightly thickened; pappus white, ca. 5 mm long. Agamosperm.

Specimen seen (Fig. 25.13): INDIA, NW India, Jammu & Kashmir State, Ladakh, Indus Valley, Domkhar-Dha, Phatt River Valley, above upper Doksa, 4450–4490 m, 34°38.17′N, 76°43.72′E, 10 Sep 2006, L. Klimeš 7099 (PRA, no. det. 28126).

Figure 25.13: Distribution of Taraxacum melleum.

25.3 Further Himalayan taxa reported as belonging to T. sect. Orientalia

Taraxacum tricolor van Soest (1966b, p. 75).

Type: PAKISTAN. Chitrall, Owir An, ca. 4500 m, 30 Jul 1950, P. Wendelbo s. n., cultivated in BG Oslo, collected 17 Jun 1953 (holotype: O, no. det. 11913; isotype: L, no. det. 19584).

Plants 6–14 cm tall. Petiole purplish, relatively long, narrow to very narrowly winged (in outer leaves), plant base almost glabrous; leaves mid-green to dull deep green, subglabrous to sparsely aranose, narrowly oblanceolate in outline, usually 5–12 × (1–) 1.5 (–2) cm, pinnatisect, lateral segments in 4–6 pairs, usually linear-triangular, subrecurved to ± patent, entire or rarely with a single thin tooth, apex acute to lingulate, sometimes bent upwards, interlobes ± short, entire or subentire, margins often tar-coloured; terminal segment narrowly triangular to lingulate-triangular, sometimes elongated, not rarely sagittate, usually
1–3 cm long. Scapes green-purplish or brown-purplish, aranoise, densely so below capitulum. Capitulum 2–3 cm wide. Involucre usually 8–9 mm wide, rounded at base. Outer phyllaries (8) 9–10 (12), black-green, often pinkish apically, ± not imbricate, appressed, ovate to ovate-lanceolate (to lanceolate), 5–6 (–7) × 2–3 mm, with a distinct white to whitish-membranous or whitish-greenish border usually 0.5–0.8 mm wide, with an abrupt transition into the dark median part, margins sparsely ciliate, apex usually minutely blackish corniculate; inner phyllaries dark green with black apex, initially ca. 10–11 mm long, later elongating to reach 15 (–18) mm, ± callose to flat. Ligules yellow, outer ligules almost flat, striped grey-pink to blackish-purple outside, inner ligules with blackish apical teeth. Stigmas dark green to grey. Anthers without pollen, rarely a few pollen grains hidden in the anther tube. Achenes (Fig. 25.16) light greyish straw-brown, 4.7–5.4 × 0.9–1.0 mm, upper 1/3–1/5 of achene body (and cone base) with subsparse to medium dense, sometimes remote coarse spinules and smaller spinules (usually bigger spinules on achene ridges, smaller ones between ridges), spinules usually ± patent to erecto-patent, rarely some spinules subrecurved, body ± gradually narrowing into cylindrical cone 0.8–1.0 (–1.1) mm long, ca. 0.3 mm thick; beak 3.5–4 mm long; pappus white, 5–5.5 mm. Agamosperm.

Fig. 25.14 and 25.16.

Figure 25.14: Taraxacum tricolor (PRA, no. det. 28142/5). Scale bar = 3 cm.
78°14.4'E, 12 Sep 2005, L. Klimeš 7473 (PRA, no. det. 28141). — NW India, Rulung River valley, 5100–5520 m, 33°13.5'N, 78°09.7'E, L. Klimeš 7470 (PRA, no. det. 28140). — NW India, Startsa Puk Tso, SE, to Polokonka La, 4550–4850 m, 33°15.6'N, 78°04.6'E, L. Klimeš 7468 (PRA, no. det. 28139).

*Taraxacum tricolor* is a very distinct species characterized by the character combination of anthers without pollen, dark grey stigmas, large, prominently spinulose achenes with body ± gradually narrowing into cylindrical cone, short beak, and leaves pinnatisect with 4–6 pairs of lateral segments having distal base broader and distal margin concave. The gap in its distribution range, i.e. between Chitral and Ladakh (Fig. 25.15), probably is a consequence of the incomplete exploration of the regions in N Pakistan, now difficult to access.

![Figure 25.15: Distribution of *Taraxacum tricolor*.](image)

As regards the sectional position of this species, its closer relationships with *T*. sect. *Orientalia* are improbable, mainly because of the prominently spinulose achene body in the upper 1/3–1/2, with spinules medium dense, acute and long, which is the character combination not found in *T*. sect. *Orientalia*. According to the evaluation of all decisive character groups, *T*. *tricolor* belongs to *T*. sect. *Suavia* Kirschner and Štěpánek (2004, p. 264) (see also Kirschner and Štěpánek 2005).

*Taraxacum wendelboanum* van Soest (1966b, p. 77).

Relatively slender plants to ca. 18 cm tall, sparsely aranose at base. Leaves light grey-green, usually 5–12 × 0.5–2 cm, outer leaves with a narrowly triangular, elongated terminal segment and 2–3 pairs of linear to narrowly triangular, patent to subrecurved lobes to 0.6–0.8 cm long (lobes and interlobes entire); middle or inner leaves (probably the most developed ones during full or late flowering) with a distinct, lingulate, linear-elongated terminal segment ca. (2–) 4–5 (–5.5) × 0.5–0.7 cm, entire or rarely with a pair of sub-basal obtuse teeth (or a single tooth), and 1–2 pairs of linear-triangular, subrecurved, entire or subentire lateral segments. Scapes usually 1–3, to ca. 15 cm long, sparsely aranose to aranose below capitulum. Involucre ± rounded at base, ca. 7–9 mm wide; inner phyllaries green to dark green, ca. 12–14 mm long. Outer phyllaries 10–14, lanceolate to linear-lanceolate, sometimes to ovate-lanceolate, (5–) 6.4–7.0 × 2.0–2.3 (–2.5) mm, margins sparsely ciliate, with a narrow black middle strip and a dark green-blackish middle part, with a gradual to subabrupt transition into greenish white (sometimes membranous to white) border usually 0.2–0.3 (–0.4) mm wide. Flowers yellow, often canaliculate, striped grey outside; ligule teeth ± blackish. Stigmas greenish to green, with blackish pubescence. Pollen present, irregular in size. Achenes (Fig. 25.16) pale greyish straw-brown, 4.5–4.6 (–4.9) × 0.8–0.9 (–1.0) mm, achene body very gradually narrowing into subcylindrical cone ca. 0.9–1 mm long and ca. 0.3 mm thick, with medium sparse squamules and some thin acute spinules in the upper 1/4–1/5, otherwise smooth, rostrum slightly thicker, 4–6 mm, pappus white to yellowish white, ca. 5 mm long. Agamosperm.

![Figure 25.16](image-url): Achenes of *T. tricolor* (A, no. det. 28111; B, LK3785, not fully ripened) and *T. wendelboanum* (C, no. det. 28144). Scale bar = 1 mm.

**Specimens seen:** INDIA. NW India, Lakong, 4560–4700 m, 32°56'N 77°13.5'E, 16 Aug 2004, *L. Klimeš 6652* (PRA, no. det. 28144, source of achenes).

*T. wendelboanum* is based on a single, relatively rich gathering (herb. O, L) but the interpretation of this name is complicated because of the lack of achenes. There is a single gathering in the L. Klimeš collection (PRA) with plants matching the type in the outer phyllary and flower characters, and having ripe achenes. According to the achene spinulosity traits, *T. wendelboanum* is not close to *T.* sect. *Squamulosa*. In this respect, it is comparable with *T. tricolor*.

*T. canum* van Soest (1977, p. 245).
Type: PAKISTAN. Batrasi Pass, 4000 ft., in pine forest, 20 Apr 1963, Nasir & Siddiqi 1635 (holotype: RAW, n. v.).

Plants dwarf, to 7 cm tall, plant base with a thick layer of remnants of old dry petioles. Petiole short, unwinged to narrowly winged, ± grey-green. Leaves conspicuously grey-green, linear-oblong in outline, 3.5–5 × 0.8–1.2 cm, ± pinnatisect, lateral segments in 3–5 pairs, triangular-deltoid to hamate, recurved, distal margin convex, usually with 1–2 teeth, rarely ± entire, proximal margin concave, entire to sparsely dentate, interlobes short, usually entire, sometimes with 1–2 minute teeth, terminal segment triangular-lingulate with basal lobules recurved, apex subobtuse. Scape greenish-brownish, sparsely aranose, ± equalling leaves. Capitulum ca. 1.5 cm wide. Involucre narrow, ca. 5–6 mm wide, obconical to rounded-obconical at base. Outer phyllaries 10–13 (often one rudimentary phyllary descending 1–2 cm below capitulum), of very unequal length, linear, 3.5–8 × 0.8–1.1 mm, border whitish-pinkish to whitish-membranous, 0.2–0.4 mm wide, with ± gradual transition into a blackish green middle strip, margins ciliolate near apex, apex callose to subcorniculate. Inner phyllaries 12–14 mm long. Ligules yellow to light yellow, outer ligules ± flat, striped greenish grey outside, inner ligule teeth long, black to black-purple. Pollen present, slightly irregular (ca. 80% bigger, invariable, about 20% smaller, also invariable). Stigmas pale yellowish light green, pubescence outside hyaline or slightly greyish. Achenes light stramineous brown, ca. 4.9–5.1 mm long, body coarsely squamulose in the upper half, with a few large spinules above, gradually narrowing into a thin cylindrical cone 1.9–2.0 mm long, beak thin, ca 4 mm long (unripe), pappus white, 5 mm long. Agamosperm (but a part of achenes undeveloped).


A relatively rich collection under three collection numbers forms the original material, surely belonging to a single species. There are serious discrepancies between the original description and that presented here, particularly in the size of achene parts. We suppose that Soest overlooked a part of the thin long cone but this question can be answered only after the examination of the holotype.

In most features of general habit, leaves and involucre, this species is close to T. sect. Parvula, while the achene characters do not support this relationship.

Taraxacum pubens van Soest (1966a, p. 367).


Plants medium-sized, 18–24 cm tall, plant base sparsely aranose. Petiole long, narrow, unwinged, ± pink purplish. Leaves ± light mid-green, aranose, ± linear-oblancoleate in outline, 8–15 × 1.3–2.1 cm, ± pinnatisect, lateral segments in (3–) 4–6 pairs, patent to patent-recurred, narrowly linear-triangular, distal margin ± straight, usually entire or with a single minute tooth, proximal margin ± straight, entire, interlobes distinct, usually ca. 0.5–0.7 × ca. 0.4–0.7 cm, entire, terminal segment elongated-triangular, apex acute. Scape brownish green, aranose, densely so below capitulum, overtopping leaves. Capitulum ca.
2.5 cm wide. Involucre ± narrow, ca. 6–7 mm wide, narrowly rounded at base. Outer phyllaries 10–13, not imbricate, appressed to loosely so, narrowly triangular-lanceolate to linear-triangular, 4.8–6.2 × 1.2–1.8 mm, light olivaceous-green with a narrow black-green middle strip and reddish apex, with a very gradual transition of the paler border from the light olivaceous-green part along the middle strip to pale greenish-membranous marginal parts (the membranous part of the border is narrow, distinct, ca. 0.2 mm wide), margins sparsely ciliate, apex flat to minutely corniculate. Inner phyllaries 13–16 mm long. Ligules (light ?) yellow, outer ligules ± flat, striped light grey-pink outside, inner ligule teeth grey. Pollen present; indistinctly irregular in size, perhaps regular. Stigmas green to grey-green. [Achenes absent from the material available.]. Probably agamosperm.

In the absence of achenes and with a rather scanty material available, it is difficult to speculate about the relationships of *T. pubens*. Outer phyllaries and its general habit point to the affinity with *T. tricolor*. 
Chapter 26

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Part VI

Analysis of *Taraxacum bicorne* (Compositae-Crepidinae) as a potential alternative natural rubber crop
This work is being finalised and formatted for submission as Vojtěch Zeisek, Jan Kirschner, Peter J van Dijk, Jan Štěpánek, Tomáš Černý and Jan Kotek (2018). ‘Analysis of Taraxacum bicorne (Compositae-Crepidinae) as a potential alternative natural rubber crop’. Finished draft being formatted for submission to Genetic Resources and Crop Evolution

Taraxacum koksaghyz, dandelion from steppes of Kazakhstan, has been known for long time as potential rubber producer, possibly replacing currently the most popular rubber producing tropical tree Hevea brasiliensis. In this work, we evaluate its closely related congener, Taraxacum bicorne. Its taxonomy is reviewed, population genetic characteristic evaluated, and rubber content of the two species is compared. For the rubber extraction we modified existing method to require minimal amount of material. Taraxacum bicorne is shown to be outcrossing sexual diploid and its rubber content is about half of that of T. koksaghyz (~3.2% vs. ~7.2%), but because of relatively robust constitution of T. bicorne in comparison to T. koksaghyz, T. bicorne could be used as potential rubber source.

Keywords: Natural rubber, population genetics, rubber content, rubber extraction, Taraxacum bicorne, Taraxacum koksaghyz, taxonomy.
Chapter 27

Introduction

Vulnerability of the current production and economy of Hevea brasiliensis Müll. Arg. natural rubber and the need for both emergency and economically viable alternatives were summarized by Mooibroek and Cornish (2000), see also van Beilen and Poirier (2007a,b) and Kirschner et al. (2013). In the past, only two alternative sources of natural rubber were exploited commercially or during the time of rubber shortage, Parthenium argentatum A. Gray, also known as guayule (D T Ray 1993), and Taraxacum koksaghyz Rodin, rubber dandelion (if we disregard shorter periods of industrial exploitation of Chondrilla ambigua Kar. et Kir. or similar attempts with Scorzonera tausaghyz Lipsch. & Bosse and Landolphia owariensis P. Beauv., see Ulmann 1951; Neuwinger 1996). However, at least 2100 plant species produce a certain amount of rubber. There are several, either regional or global lists or accounts of rubber producing plants, particularly that of Ulmann (1951), who also provided a digest of the relevant Soviet literature, and two more recent but rather rare catalogues (Vakhrusheva 1988, 1990).

Although current breeding programmes are concentrated on the most promising alternative rubber crops (Arias et al. 2016c), Parthenium argentatum and Taraxacum koksaghyz (and on hybrids of the latter), there are good reasons not to neglect other taxa, particularly the closest relatives of the above two plants (de Rodriguez et al. 2005; Kirschner and Štěpánek 2008; Dennis T Ray et al. 2010; Iiut et al. 2015). Inclusion of such taxa into breeding and hybridization schemes may broaden the genetic basis of the future rubber crops, including broader ecological and variation amplitudes.

27.1 Relatives of Taraxacum koksaghyz on the basis of morphology

The earlier studies dealing with T. koksaghyz included it in section (or subsection) Macrocornuta Soest (Schischkin and Tzvelev 1964; T. N. N 1987) or sect. Scariosa Hand.-Mazz. (Yu 1934; Orazova 1975). In the recent study, Kirschner and Štěpánek (2008) analyzed the morphology and ecology of what was originally called T. sect. Macrocornuta, and recognized a group of taxa morphologically very similar to one another, and deviating in a number
of features from the rest of the section *Macrocornuta* (Kirschner and Štěpánek 2008). The group was described as *T. sect. Ceratoidea* Kirschner & Štěpánek. The new section, when contrasted with sect. *Macrocornuta*, was primarily diagnosed by light green, appressed horned outer phyllaries, yellow stigmas and beak shorter than 7–8 mm; the two groups also differ in their habitats, sect. *Ceratoidea* preferring (temporarily) wet subsaline sites, while sect. *Macrocornuta* growing on drier, subsaline, often disturbed places.

The core of sect. *Ceratoidea*, i.e. *T. koksaghyz* and its closest relatives, is represented by four predominantly sexual species quite similar to one another:

- *T. neolobulatum* Soest ex Schischk. et Tzvelev (type of the section, growing mainly in Iran and adjacent regions),
- *T. koksaghyz* Rodin (growing in Kazakhstan and a narrow border area with Xinjiang, China),
- *T. monochlamydeum* Hand.-Mazz. (growing mainly in Uzbekistan and adjacent areas), and
- *T. bicorne* Dahlst. (for details, see below).

In accordance with the geographical parthenogenesismodel (Štěpánek et al. 2011), marginal parts of the section’s range are occupied by agamospermous species: *T. glaucanthos* (C. A. Mey. ex Ledeb.) DC. and *T. halophilum* Trautvetter ex Regel in the north, and *T. badachschanicum* Schischk. and *T. varsobicum* Schischk. in the southeast.

There is therefore a hypothesis that the core of sect. *Ceratoidea* is the source germplasm to search for further rubber producing dandelions. We selected *T. bicorne* Dahlst. from among the Kazakh dandelion relatives to be tested as a potential rubber plant in the present paper.

The reconstruction of phylogenetic relationships in *Taraxacum* is complicated by several factors, primarily by widespread and complex hybridity (the parental taxa often not being extant), frequent allopolyploidy (usually triploid but occasionally up to hexa- or even dodecaploidy) and a high number of taxa to be analysed (Kirschner and Štěpánek 1993, 2004; Kirschner et al. 2016). Attempts at the reconstruction of evolutionary relationships thus mostly failed when cpDNA was included as the major information source (Wittzell 1999; Kirschner et al. 2003), and only a few features of *Taraxacum* evolution could have been inferred from the analysis of nrDNA of sexual species of *Taraxacum* (Kirschner et al. 2015). The latter work, however, set up a framework to evaluate either the major coherent groups of sections, or on the contrary, to define sections or their groups clearly unrelated to one another. We therefore used the published sequences involving sexual representatives of 25 sections (including *T. koksaghyz* of sect. *Ceratoidea*) and compared it with the new nrDNA sequences of *T. bicorne* Dahlst.
Chapter 28

Material and Methods

28.1 Taxonomy

Our general strategy is to make our taxonomic treatments of Taraxacum uniform and comparable to the modern standards, and we therefore follow principles briefly outlined by A. Richards (1973), Kirschner and Štěpánek (1996), Kirschner et al. (2003) and Ge et al. (2011), and are derived from the peculiar features and processes known in Taraxacum, particularly the regional coexistence of apomixis (agamospermy) and sexuality, complicated hybridity and polyploidy, relatively low structural morphological differentiation and numerous similar and mostly hybridogonous species. The principles inferred from the Taraxacum general attributes include (i) different kinds of species to be recognized on the basis of the extent of variation and modes of reproduction, (ii) distribution of sexuality is to be explored, (iii) variation within a family of siblings should be studied for each taxon (to detect autonomous aberrant, facultative sexuality etc.), (iv) the study should be started at the lowest variation level (within and among populations).

The complexity of the genus, primarily the incommensurable variation patterns of species with different modes of reproduction, also requires a taxonomic rank placed between species and genus in the traditional hierarchy, to make the population and taxonomic structure more easily understandable for non-specialists, and the rank of section is used in the Taraxacum literature.

28.2 Plant material

Taraxacum bicorne Dahlst. was studied in the wild in two natural populations in the vicinity of Kokpek (District of Enbekshikazakhskiy Rayon, basin of Dolina Sogety, between hilly regions of Gory Sogety (Sogeti) and Gory Toraigyr, near the SE foothills of the Kungei Alatau Range, Fig. 28.1): the first site (Site I) was visited in 2008 (JK and JŠ), at saline, temporarily wet banks of dry brook beds, population centre at 43°26’38.58”N, 78°40’16.53”E (1115 m a.s.l.), the second site (Site II) was sampled in 2014 (JK and JŠ), at subsaline to saline, partly humid pastures and spring areas just below the village, population centre at
43°26′49.68″N, 78°40′26.04″E. The material from Site I was used for flow cytometry analyses and hybridization experiments, while that from Site II was analysed to get data about rubber production and genetic make-up of the population and of the species.

Another important source of plant material comes from herbarium collections, the most important ones having been those of TASH, LE and AA. The herbarium material was used to compile the description, to assess the variation limits of *T. bicorne*, and to describe the geographic range of this species.

**Figure 28.1:** The Kokpek region in the SE Kazakhstan, with Site I (yellow) and Site II (red) indicated. Scale bar marks 300 m. Data are from OpenStreetMap (accessed through Seznam.cz).

### 28.3 Cultivation and reproduction system

Details of the cultivation methods are given in Kirschner and Štěpánek (1993). The cultivation, especially repeated mass cultivation, reveals limits of morphological plasticity of individual taxa. Moreover, it provided material for the study of reproduction systems of plants under study. Determination of the reproduction system, an important background for taxonomic decisions, was performed according to Kirschner et al. (2006).

### 28.4 Hybridization

For both the breeding purposes and to have another information source on the relationships between *T. koksaghyz* and *T. bicorne*, we performed a series of reciprocal crosses between...
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Figure 28.2: A, flower beds with dandelion samples at the experimental garden. B, cages covered with a plexiglass, each with a half-sib family of cultivated plants.

these two species. All the experiments were carried out in the Experimental Garden of Institute of Botany, Academy of Sciences, Průhonice, Czech Republic. The half-sib families of experimental plants were cultivated in open beds, each in a separate wooden box embedded in garden soil. Mother plant families were isolated by caging (sides of cages with a mesh, each cage covered with plexiglass, see Fig. 28.2). Crosses were done by rubbing the flowering capitula twice during the full blossom (see Fig. 28.3), within one or two days of flowering. Each individual cross, i.e. the mother capitulum and the pollen donor plant, was identified by a unique number.

28.5 Molecular analysis

For taxonomic analysis we used 108 sequences of ITS1-5.8S rDNA-ITS2 from Kirschner et al. (2015, 2017).

KASP data record alleles of genes involved in rubber synthesis. Currently, respective sequences are not public and data in this form were kindly provided by Keygene.

All 32 plants were genotyped by 13 microsatellite loci (SSRs, Jarne and Lagoda 1996), which were distributed over two multiplex PCR reactions (multiplex 1: MSTA 44B, 73, 78, 93, 103, 105 and 131, and multiplex 2: MSTA 53, 61, 85, 102, 133 and 143). Six microsatellite loci and relevant primers (MSTA 44B, 53, 61, 73, 78 and 85) were taken from Falque et al. (1998) and seven microsatellite loci (MSTA 93, 102, 103, 105, 131, 133 and 143) from Vašut et al. (2004). Primers were originally developed for T. officinale agg. (sect. Taraxacum) and T. laevigatum, respectively. The PCR reaction was performed using the QIAGEN Multiplex PCR kit (Qiagen, Venlo, Netherlands) according to manufacturer’s protocol in a final volume of 20 μl containing 10 μM of each primer and 30–50 ng of DNA. PCR protocol was as follows: 95°C hot start for 15 min, 30× (30s 94°C, 90s 57°C, 60s 72°C) and 30 min 60°C. Final PCR products were analysed using a 3130 ABI Genetic Analyser (Life Technologies, Carlsbad,
Figure 28.3: The method of hand crossing.
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CA, USA) and allele numbers and sizes were subsequently scored using GeneMarker 2.4 (SoftGenetics LLC, State College, PA, USA) and rewritten into the data matrix.

28.6 Statistical evaluation

Neighbour Network from ITS sequences was constructed in SplitsTree 4.14 (Huson and Bryant 2006). The figure was modified from Fig. 25.2, page 161. Calculations based on SSRs as well as KASP loci were performed in R 3.1 (R Core Team 2013–2018). We used packages ade4 (Dray and Dufour 2007), adegenet (Jombart 2008), APE (Paradis et al. 2004), pegas (Paradis 2010) and Poppr (Kamvar et al. 2014). We computed the basic population statistics, i.e. observed and expected heterozygosity, departure from Hardy-Weinberg equilibrium (HWE; Jombart 2008), allelic richness (Paquette 2012), distribution and diversity of multilocus genotypes (MLGs), Shannon-Wiener Index of MLG diversity (H), E.5 (Evenness measuring distribution of genotype abundances ranging from 0 where population is dominated by single genotype to 1 where all genotypes are equally distributed), and Index of Association (Ia) and its standardized version (rbarD). Ia and rbarD detect clonal reproduction within populations. Calculation based on the ratio of the variance of the raw number of differences between individuals and the sum of those variances over each locus. It as the observed variance over the expected variance — if they are the same, then the index is zero (= prevailing clonal reproduction) after subtracting one — it rises with with increasing differences. For details see Kamvar et al. (2014). Genotype diversity was quantified according to Hughes and A. Richards (1988) \[ G = 1 - \sum x_i^2 \], where \( x_i \) is the frequency of i-th MLG. This parameter is useful for population sets with expected variation in reproduction systems (i.e. a substantial departure from the Hardy-Weinberg expectations) and for situations where recombination is partially suppressed as a consequence of alloplody; it reasonably reflects both richness and evenness and closely approaches the modified Simpson’s index \( \lambda \) (Kirschner et al. 2016). Details about R work-flow, software settings etc. are available from VZ upon request.

28.7 Flow cytometry (FCM)

The FCM analyses followed Záveský et al. (2005) and P. Trávníček et al. (2013); the PI measurements and Lycopersicon esculentum cv. ‘Stupické polní tyčkové rané’ as a standard having been used. The FCM screening was carried out on the material cultivated from wild roots (collected under no. 74/1 to 74/33) in 2008–2009, under no. JK 5276/1 through JK 5276/33, i.e. on 33 plants from the Kokpek locality.

28.8 Rubber content quantification

We modified method of Post et al. (2012) based on dilution of rubber in toluene, precipitation in methanol and weighting, a method feasible in lower volumes, and thus more efficient and
Roots were dried and grinded into fine powder. Into 10 ml glass vial flasks we added 0.5 g of the powder, 5 ml of toluene and magnetic mixer. The flasks were incubated in thermoblock in 85°C for 24 h. Everything was transferred to fine filter with fritted glass and filtered into 25 ml round-bottom flasks. Inside of the vials as well as of the filters were washed by 1–2 ml of toluene (also added into the flasks). The remaining powder was discarded. Liquid phase was removed on rotary evaporator (water bath had temperature 50°C). Rubber (on the walls of the flasks) was repeatedly (4×) diluted in 0.75 ml of toluene and the liquid was transferred into weighted 15 ml centrifuge tubes. The flasks were then repeatedly (2×) washed by 3 ml of methanol (it precipitates the rubber) and transferred into the same centrifuge tubes. The tubes were shaked and let overnight to precipitate. Next day, tubes were centrifuged for 1 h. Liquid phase was discarded and the tubes were gently cleaned by acetone and distilled water. Tubes were then dried overnight in the drier (50–60°C) and weighted. What remains on the walls of the tubes is pure rubber. We measured 4 samples of *T. bicorne* and 12 of *T. koksaghyz*. 

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Chapter 29

Results

29.1 Evolutionary relationships of *Taraxacum koksaghyz* and *T. bicorne*

The analysis of ITS region in SplitsTree (Fig. 29.1) unanimously shows *T. bicorne* as a very close relative of *T. koksaghyz*. The hypothesis set up on the basis of shared morphological characters was therefore supported from another data source. Furthermore, these results also support the taxonomic conclusions about the separate status of sect. *Ceratoidea* (Kirchner and Štěpánek 2008).

29.2 Population genetic statistics

Our aim was to characterize the heterozygosity (and the H-W equilibrium relationships) to infer reproductive attributes of *T. bicorne* populations on one hand, and on the other hand, genotype diversity and the overall level of genetic diversity in the population.

Results obtained by 13 microsatellite (SSRs) and 8 KASP loci (only particular genotype was recorded, no sequence) produced very different results. KASP data showed nearly non-existing difference of expected and observed heterozygosity (Fig. 29.2) and departure from Hardy-Weinberg equilibrium (p-value of significant departure 0.02 for locus CPT1 and 1 for remaining 7 loci). On the other hand, SSRs data showed high differences of expected and observed heterozygosity (Fig. 29.2) and departure from Hardy-Weinberg equilibrium (p-value of significant departure < 0.05 for 9 loci, > 0.05 for 3 loci and not available for 1 locus). The same difference is shown in estimated level of inbreeding: KASP loci show high level of inbreeding while SSRs exhibit low inbreeding (Fig. 29.3). These differences are addressed in Discussion section.

Table 29.1 shows population-genetic indices summed over all loci of KASP and SSRs. Shannon-Wiener (H) shows much higher allelic diversity for SSRs than KASP loci. Evenness (E.5) of proportional occurrence of genotypes is much higher in KASP than SSRs data. Index of association (Ia and its standardized version rbarD) is insignificant for KASP data while for SSRs data it shows significantly prevailing outcrossing. Genotype diversity (G) is extremely
Figure 29.1: Neighbour Net constructed from uncorrected P-distances in SplitsTree based on nrDNA ITS sequences of sexual members of 24 Taraxacum sections. The position of the section Ceratoidea is marked by dark grey; two species under study are in bold. Modified from Kirschner et al. (2017) where also lists of samples are given.
high for SSRs data.

According to the SSRs data, *T. bicorne* is an outcrossing sexual species, with a high SSR genotypic diversity. KASP data are not so conclusive in this aspect due to their low diversity. The conclusion on the mode of reproduction is supported by the absence of achenes in isolated inflorescences indicating the absence of autonomous autogamy.

### 29.3 FCM

According to flow cytometry measurements using PI, all our plants of *T. bicorne* are diploids (*2n = 16*). The sample size (33 plants) did not show substantial variation, the sample/standard ratio ranged from 1.29 to 1.399, with coefficient of variation (standard deviation/mean) being 2.31–5.07. These figures correspond to those ascertained in *T. koksaghyz* (but are much higher that those found in *T. stevenii*, a species with remarkably small genome, T. Černý, unpubl.).

### 29.4 Rubber content of *T. bicorne*

Mean content of rubber in dry root biomass was 3.2% for *T. bicorne* (4 measurements) and 7.1% for *T. koksaghyz* (12 measurements, Fig. 29.4). Although there is an obvious difference of the rubber content between these two species, because values of *T. bicorne* do not fit normal distribution (tested by Shapiro test), we had to use non-parametric Kruskal-Wallis
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Figure 29.3: Frequency histograms of level of inbreeding for SSRs (left) and KASP (right) markers. Horizontal axes show level (prevalence) of inbreeding and vertical respective frequency.

The experimental crosses yielded the following results (number of crosses is represented by the number of capitula used):

**TKS (M) × TBI:**
- overall number of crosses: 56
- successful crosses: 49
- total number of hybrid achenes: 2425
- mean number of achenes per capitulum: 51.6

**TBI (M) × TKS:**
- overall number of crosses: 81
- successful crosses: 54
- total number of hybrid achenes: 1499
- mean number of achenes per capitulum: 27.8

We can conclude that both reciprocal crossing experiments showed a high yield of hybrid achenes and were generally very successful, which is primarily due to the sexual diploidy of both parents and the evolutionary proximity of these two species.
Rubber content in T. bicorne and koksaghyz

<table>
<thead>
<tr>
<th>Species</th>
<th>T. bicorne</th>
<th>T. koksaghyz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.</td>
<td>1.381</td>
<td>2.792</td>
</tr>
<tr>
<td>1st Qu.</td>
<td>1.717</td>
<td>4.873</td>
</tr>
<tr>
<td>Median</td>
<td>3.264</td>
<td>6.545</td>
</tr>
<tr>
<td>Mean</td>
<td>3.225</td>
<td>7.124</td>
</tr>
<tr>
<td>3rd Qu.</td>
<td>4.742</td>
<td>7.500</td>
</tr>
<tr>
<td>Max.</td>
<td>5.050</td>
<td>18.645</td>
</tr>
</tbody>
</table>

**Figure 29.4:** Percentage of content of rubber in dry root mass of *T. bicorne* (left) and *T. koksaghyz* (right). Inset legend shows basic statistics of the measurements. Content of rubber is significantly different in both species (tested by Kruskal-Wallis test, $p = 0.029$).
Table 29.1: Population-genetic indices for the population of *T. bicorne* summed over all loci of KASP and SSRs respectively. See text for detailed explanations. ‘n.s.’ stands for non-significant results.

<table>
<thead>
<tr>
<th>KASP</th>
<th>Microsatellites (SSRs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of multilocus genotypes (MLG) among 32 genotyped individuals</td>
<td>5</td>
</tr>
<tr>
<td>H (Shannon-Wiener Index of MLG diversity)</td>
<td>0.87</td>
</tr>
<tr>
<td>E.5 (Evenness)</td>
<td>0.792</td>
</tr>
<tr>
<td>Ia (Index of Association)</td>
<td>-0.0483 (n.s.)</td>
</tr>
<tr>
<td>P-value for Ia</td>
<td>0.959 (n.s.)</td>
</tr>
<tr>
<td>rbarD (standardized Ia)</td>
<td>-0.0528 (n.s.)</td>
</tr>
<tr>
<td>P-value for rbarD</td>
<td>0.959 (n.s.)</td>
</tr>
<tr>
<td>G (Genotype diversity)</td>
<td>-13.84</td>
</tr>
</tbody>
</table>

29.6 Taxonomy of *T. bicorne*

The protologue of the name *T. bicorne* appeared in an early study of sect. *Borealia* Hand.-Mazz. (as the group of *T. ceratophorum*, Dahlstedt 1905). Since then, this species name was seldom mentioned, mostly as a member of *T. sect. Ceratophora* auct. (a synonym of the name *T. sect. Borealia*), sometimes (Russian authors, e.g. Schischkin and Tzvelev 1964) as belonging to sect. *Macrocornuta* Soest, but usually without indication of its relationships. Most importantly, it was T. N. N (1987) who listed *T. bicorne* among members of sect. *Ceratophora*.

As a result of the above confusion, *T. bicorne* was only seldom considered as a potential rubber crop. According to our records, only Il’in and Yakimov (1950) and Il’in (1953) mentioned *T. bicorne* (see also Vakhrusheva 1990).

Together with *T. koksaghyz*, *T. bicorne* was listed among members of the sect. *Ceratoidea* (Kirschner and Štěpánek 2008, with nomenclatural details). In order to put our study on a solid taxonomic basis, we give a full description of *T. bicorne*, with notes on its ecology and distribution, and with a selection of herbarium specimens studied.


Illustrations: Fig. 29.5 in the present paper; Dahlstedt, Ark. Bot. 5/9: Plate 17, 1906 (Fig. 29.6).

Description: Plants small, usually 7–10 cm tall at grazed sites, or up to 12 (–20) cm tall at sheltered places (e.g. among shrubs). Root not conspicuously thickened, usually branched above, when broken, the parts of root connected with thin threads of rubber.
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Figure 29.5: Flowering capitula of T. bicorne from the Kokpek locality (2014).

Petiole narrowly winged, usually ± green above with pale green to purple mid-vein, or suffused lividoid. Leaves vivid greyish green, usually with lividoid hue, linear ob lanceolate in outline, usually 4.5–8 × 0.5–1.3 cm, occasionally to 12 cm long, rarely undivided, usually pinnatilobed, with 2–4 pairs of ± patent triangular-deltoid, broadly triangular to linear-triangular lateral lobes, entire or with a few minute teeth on distal margin; terminal lobe usually larger, often elongated, sometimes acute with concave sides. Scapes pale green or suffused purple, aranose, usually overtopping leaves. Capitulum usually 2–2.5 cm wide, lighter yellow. Involucre light green, ca 6–8 mm in wide and ± rounded at base. Outer phyllaries appressed, loosely appressed or erect, usually 10–13, light yellowish green, with distinct, anastomozing venation, narrowly lanceolate to ovate, relatively short, usually 5–6.5 × 1.5–2.3 mm, with 0.2–0.4 mm wide whitish border, upper part of phyllaries usually suffused pink or dirty pink to purplish, with a thick obtuse grey-pink horn below apex, the apex itself bent perpendicularly and having an appearance of another horn (thus 'bicorne'), margin entire, not ciliate, occasionally denticulate at apex; inner phyllaries ca 9–11, pale green, pinkish above, initially 11–12 mm long, later conspicuously elongating to reach at least 16–17 mm. Outer ligules flat, lighter yellow inside, striped dirty pinkish below and grey-pink above, outer ligule teeth greyish-pinkish, inner ones ± yellow or pinkish yellow. Stigmas yellow. Anthers polliniferous, pollen grains of regular, ± equal size. Achenes light stramineous-brown to light greyish so, (3.2–) 3.5–3.8 × 0.8–0.9 mm, body with numerous conspicuous ridges, upper half of achene body, particularly on ridges, covered with
erect-patent to upwards sickle-shaped coarse spinules, body subgradually narrowing into ± subconical cone 0.7–1.0 mm long, with a few basal spinules; beak thin, usually 8–9.5 mm long, pappus ± pure white, 8–9 mm long. Sexual.

### 29.7 Distribution and ecology

Fig. 29.7 shows distribution range of *T. bicorne* based on herbarium specimens and literature records. It is distributed in Kazakhstan, Kyrgyzstan and Uzbekistan. Limited number of records come from Turkmenistan, Mongolia and China. It was also recorded in Pakistan, Afghanistan and northern Iran (the latter occurrence is based on literature record only).

As regards the ecology, *T. bicorne* grows under relatively harsh, continental conditions. The humidity and available ground water are only seasonal (not relatively permanent as *T. koksaghys*), the span of diurnal and annual temperature fluctuations is broader, and the salinity is high. *T. bicorne* grows in temporarily wet depressions in saline steppes or a degraded steppe, often in the vicinity of *Sophora alopecuroides* L. (as in the vicinity of Kokpek, Kazakhstan).

### 29.8 Selected specimens studied


Uzbekistan: Margelan [Margilon], Karl Marx Street, 19 Apr 1959, Kovalevskaya 90 (TASH, no. det. 15781). — Yad’yavanskiy r-n [district], kolkhoz Stalinabad, 19 May 1957, U.
Figure 29.6: A reproduction of the figure in the protologue of the name *T. bicorne* (Dahlstedt, Ark. Bot. 5/9: Plate 17, 1906).
Figure 29.7: Distribution of *T. bicorne* in Central Asia. Grey triangles mark literature records and white circles herbarium vouchers.

Turkmenistan: Herb. Horti botanici Turcomanici, near Charshanga, 193(?), Anonymus 1689 (LE, no. det. 20491).

Tajikistan: Leninabad [Khujand, Khudzhand], botanical garden, 16 Apr 1959, Kovalevskaya 33 (TASH, no. det. 15768).


Pakistan: Quetta, Pishin Forest, 7 May 1965, S.M.A. Kazmi 1442 (RAW, no. det. 33765).


29.9 Reliable literature records

According to the determination labels in the herbarium collections of LE, AA and TASH, we identified S. Kovalevskaya and T. Vainberg as reliable authors of *T. bicorne* records. We therefore add literature records of *T. bicorne* from the following sources: Kovalevskaya (1962) and Vainberg (1991, 1993). When only regions or districts are given in these sources, we place a map dot in the centre of the region (Fig. 29.7).
Chapter 30

Discussion

30.1 Population genetic analysis of sexual dandelions

Our molecular analysis of ITS sequences confirmed morphological placement of T. bicorne into section Ceratoidea (Fig. 29.1) as close relative of well-known rubber producer, T. kok-saghyz.

Genus Taraxacum has been known for high importance of clonality, hybridization, polyploidy and combination of sexual and asexual reproduction (e.g. Kirschner and Štěpánek 1994, 1996; Kirschner et al. 2003; Záveská Drábková et al. 2009; Kirschner et al. 2015). Combination of these features makes genetic studies of Taraxacum problematic, as the researcher must be aware plenty of possible problems.

Comparison of our resultswith other species of the genus Taraxacum is not straightforward as most of the Taraxacum species are agamospermous polyploids, and there are not many population-genetic studies on diploid sexual species of the genus done with variable genetic markers. Older studies use to use allozymes, e.g. Hughes and A. Richards (1988) found percentage of polymorphic loci 40–50 (mean 45.7) for sexual and 27–47 (mean 38) for triploid agamospermous populations. Genotype diversity (G in our study) was 0.14–0.17 (mean 0.16) for sexual and 0.27–0.40 (mean 0.38) for agamospermous populations. Menken and Morita (1989) found nearly no isozyme variability in pentaploid T. albidum from Japan. Similarly, Battjes et al. (1992) studied T. hollandicum, distinct triploid agamosperm, and found very low level of genotypic diversity: among 231 individuals sampled along a transect about 1000 km long, only three plants were found to differ in one locus of multilocus isozyme genotypes. On the other hand, Akhter et al. (1993) studied triploid, tetraploid and pentaploid agamospermous populations of T. hondoense from Japan and found clonal diversity ranging from 0 to 0.73 (mean 0.43). Distribution of allozyme phenotypes also showed geographical pattern through the island of Honshu. Kirschner and Štěpánek (1994) found in 20 sexual populations of T. bessarabicum and related species of T. sect. Piesis proportion of polymorphic loci 0–0.89 (mean 0.31) and heterozygote frequency per population over all loci 0–0.36 (mean 0.13). Menken et al. (1995) detected, although most of their results were not statistically significant, gene flow among sexual diploid and asexual triploids of T. sect. Ruderalia and western and central Europe. They found almost no departure
from Hardy-Weinberg equilibrium and geographical structuring of the populations. Kashin et al. (2005) detected by allozymes mean observed heterozygosity 0.62 for apomictic *T. officinale*, 0.52 for sexual *T. serotinum*, 0.46 for apomictic *Pilosella officinarum* and 0.46 for sexual *P. echioides*. As the above-cited studies used very similar methodological approach, their striking differences clearly reflect contrasting biological nature between sexual and asexual lineages of *Taraxacum*, and results obtained on sexual lineages are comparable to values found in our study.

van der Hulst et al. (2003) compared allozymes, AFLP, SSRs and cpDNA when studying population structure of triploid *Taraxacum* (mainly section *Ruderalia*) from northern Europe. Generally, they found high agreement with random segregation of alleles and low departure from Hardy-Weinberg equilibrium of most of the markers. They suppose presence of both sexual and asexual reproduction in the populations studied (van der Hulst et al. 2003). It must be noted that variability of apomictic lineages is of different character than in sexual species studied in the current work, due to their limited recombination and exchange of alleles (Reisch 2004; Kirschner et al. 2016).

Zeisek et al. (2015) characterized sexual diploids of *Taraxacum* sect. *Dioszegia* (when excluding probably autogamous *T. serotinum* subsp. *tomentosum*) observed heterozygosity 0.52–0.63 (mean 0.56) and expected heterozygosity 0.43–0.71 (mean 0.57). Departure from Hardy-Weinberg equilibrium was not significant. Study of Zeisek et al. (2015) used SSRs primers from same set as the study presented here (Falque et al. 1998; Vašut et al. 2004). *Taraxacum kokssaghyz* (Kirschner et al. 2013) occupies a medium-sized geographical range in south-eastern Kazakhstan, comparable in size to that of *T. haussknechtii* (Zeisek et al. 2015). Both are obligate out-crossers with the absolute predominance of within-population genetic variation (Kirschner et al. 2013) and their levels of genetic variabilities are comparable.

Majority of newer *Taraxacum* population-genetic studies still focus more or less on apomictic taxa. E.g. Majeský et al. (2012) studied 187 individuals of two morphological series of apomictic accessions of *T. officinale* agg., and reported gene diversity 0.81–0.89 (mean 0.83) with 6 SSRs. Similar pattern was revealed by van Baarlen et al. (2000) and van der Hulst et al. (2000). In a similar study, Majeský et al. (2015) obtained for 9 apomictic taxa of *T. sect. Erythrosperma* gene diversity of individual species 0.43–0.89 (mean 0.68) using 6 SSRs loci (exploiting the same set of SSR primers as in our study). AFLP data of Majeský et al. (2015) showed much lower gene diversity 0.04–0.18 (mean 0.11). Both markers revealed comparable genotype diversity, with exception of one species, 0.48–1 (mean 0.83). Matsuyama et al. (2018), using SSRs, studied the hybridization between the native *T. japonicum* (sexual diploid) and an introduced *T. officinale*. They reported clonal diversity of *T. japonicum* 0.98, *T. officinale* 1 and 3× and 4× hybrids 0.7–0.9 (mean 0.85); and evenness of *T. japonicum* 0.98, *T. officinale* 1 and 3× and 4× hybrids 0.51–0.92 (mean 0.79). Similar problem was addressed by Iaffaldano et al. (2018), who studied possible hybridization between *T. kokssaghyz* and *T. officinale* and they found only very little introgression of *T. officinale* pollen into *T. kokssaghyz*. Some apomictic hybrids were able to produce viable seeds, non-apomictic hybrids were sterile (Y. Zhang et al. 2017; Iaffaldano et al. 2018).
Regarding *T. koksaghyz*, large population sampling (175 individuals) was genotyped by McAssey et al. (2016) by 17 EST-SSRs primers. They found FST (depending on the loci) 0.10–0.19 (mean 0.11) and GST 0.03–0.11 (mean 0.06). Population observed heterozygosity 0.28–0.47 (mean 0.37) and unbiased expected heterozygosity 0.28–0.50 (mean 0.43). PCoA did not reveal any structure among their 17 populations (they were sampled from relatively small region in Kazakhstan). In our study, we detected in *T. koksaghyz* slightly higher observed (mean 0.64) as well as expected (mean 0.63) heterozygosity.

Differences among population-genetic statistics obtained by SSRs and KASP loci in our study can be explained by different nature of the genetic markers. Microsatellites are extremely variable, while we detected only 5 MLGs derived from from KASP data (Table 29.1). It must be noted, that none of the indices used evaluates the number of mutational steps separating SSRs alleles; and for KASP data, only two alleles are available (and thus three genotype states for each locus). KASP loci are functional genes with possible strong impact on fitness, possibly being under strong selection pressure. KASP loci are by their nature closer to allozymes (van der Hulst et al. 2003, see e.g. comparison of the loci in).

*Taraxacum bicorne*, as well as *T. koksaghyz* (Kirschner et al. 2013), are diploids with normally developing pollen and seeds and our genetic data show values typical for outcrossing sexual organisms.

### 30.2 Rubber content in *T. bicorne* and *T. koksaghyz*

Rubber is stored mainly in outermost layer in roots, protecting inulin (storage polysaccharide) from herbivores, thus physiological state and sampling season must inevitable play an important role regarding yield of rubber from the roots. A surprisingly low proportion of dandelion rubber percentage reports gives a satisfactory description of methods and circumstances of sampling to allow comparability or even repeatability of the data. The following factors should be taken into account in order to have interpretable data:

- The age of cultivated plants, particularly when cultivated as a winter crop (the rubber yield in the second year is much higher (e.g. Suomela 1950, pp. 64–66);
- storage of harvested roots (much increased amount of rubber after storage, B and D. N. N 1940);
- ecological conditions, or agronomy in the case of planted material (e.g. Arias et al. 2016b; Kreuzberger et al. 2016; Hodgson-Kratky et al. 2017a; Eggert et al. 2018);
- levels of natural variation in individual rubber production (e.g. Arias et al. 2016c);
- breeding success and cultivars available (e.g. Hodgson-Kratky et al. 2017b);
- health and physiological condition of plants;
- allelic make up regarding the rubber-relevant loci, including (poly)ploidy (e.g. Warmke 1945; Luo et al. 2018);
• accuracy of the analytical methods of rubber quantification and the liability of results to vary (e.g. Sikandar et al. 2017); and

• homogeneity of the dry root sample.

In most works that involve rubber quantification, the above factors are not properly considered, which has a negative impact on the reliability of rubber quantity figures. Some works referenced in the following paragraphs show substantial variability of rubber content according to season of sampling, planting conditions, etc. Another issue, not addressed here or elsewhere, is exactness of the used analytical methods, their comparisons and limitations.

So far, *T. bicorne* was overlooked as potential rubber source (if we disregard possible misidentification or taxonomical confusion). Il’in and Yakimov (1950) briefly mentioned *T. bicorne* among rubber producing plant and reported it to contain 1.3–5% of rubber in roots (probably DW); later (Il’in 1953), he gave slightly higher figures repeated by Vakhruševa (1990): 1.3–8.1%. Our laboratory tests showed a similar DW content of rubber as that reported in the literature: 1.4–6.2%.

As regards the comparison of our measurements of rubber content in roots of *T. kok-saghyz*, Eggert et al. (2018) reported around 4–5%, but the number was strongly varying among years and higher concentrations were obtained when plants were planted in ridges than on the flat bed. Plant density also played role (Eggert et al. 2018). Luo et al. (2018) reported average concentration of rubber in roots around 3% in natural diploids, 5.8% in colchicine-induced tetraploids and 3.5% in colchicine-treated diploids. Similarly, Warmke (1945) found 2.95% of rubber content in roots of colchicine-treated tetraploids and 2.14% in diploids. Kreuzberger et al. (2016) found significant variability (ca. 4–9%) depending on planting and harvest season and trials. Season also influenced degree of polymerization of inulin. Arias et al. (2016a) measured 2.14–6.5% (in one case even 11.5%). They found slightly higher content of rubber in plants planted under lower irrigation dose. No significant variability was found among populations or in planting date. Bobkov (1939) reported 1.65% of rubber in fresh roots, Ignatiev (1939) 4–7% in dry root weight, Brandes (1941) 2–2.5% in fresh roots and up to 7% in dry roots on the end of the season. According to Kolaciov (1941), rubber content may reach 26% of dry weight. Reichert (1942) reported around 12% of dry root weight and Drobov (1945) 4–5% after one season and up to 12% after second season. These records show high variability, obviously depending on a number of factors (see above). Moreover, majority of available ex site germplasm of *T. kok-saghyz* belongs to closely related, but asexual poor rubber produce *T. brevicorniculatum* (Kirschner et al. 2013), making many recent reports problematic. These results are comparable to our own (mean rubber content in root dry weight 3.25% for *T. bicorne* and 7.12% for *T. kok-saghyz*) obtained from wild plants sampled in the field (Fig. 29.4).

Percentage of rubber in root dry weight of *T. bicorne* is on average about twice smaller than that of *T. kok-saghyz*, and plants of the latter are rather slender. However, Both species can be easily cultivated in many temperate regions and it is more question for practical field experiment which of these two species would be finally better rubber producer in practice. Our results are to be taken as preliminary, firstly because we used roots of *T. bicorne* collected

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in the wild (with variable age and health condition), characterized by a quite low weight. Also our method of rubber quantification gives better results with increasing number of repeated samples. Thus, a direct comparison between the rubber percentage of *T. koksaghyz* and that of *T. bicorne* is difficult on the basis of our results. Both species can be easily cultivated in many temperate regions and it is a question for practical field experiment which of them would be, eventually, a better rubber producer in practice. However, the *T. bicorne* figures show that this species cannot be excluded from the basic germplasm of rubber producers, either as a new source of natural rubber, or a source of germplasm for selection and breeding.
Chapter 31

Acknowledgments

This study was supported by a long-term research & development project, no. RVO 67985939 and the funding was also provided by the Czech Science Foundation (grant no. GA13-13368S) and the EU Framework Programme 7 (grant DRIVE4EU, no. 613697). We are grateful to our great technician Kateřina Moravcová, curators of all visited herbariums and all people helping us when forking in the field.
On the *Taraxacum* taxonomy etc. Vojtěch Zeisek (2018)
Part VII

What is and what is not *Taraxacum bithynicum* (Compositae, Crepidinae)
This work was accepted as Bahar Gürdal, Jan Štěpánek, Vojtěch Zeisek, Jan Kirschner and Neriman Özhataý (2018). ‘What is and what is not Taraxacum bithynicum (Compositae, Crepidinae)’. Accepted in Phytotaxa

The taxonomy, micromorphology, karyology and evolutionary relationships of Taraxacum bithynicum DC. (Compositae, Cichorieae, Crepidinae) were studied using the original material and new samples from the summit area of Mt. Uludağ, Bursa Province, Turkey. It is sexual with 2n = 16, considerably isolated in outer phyllary and achene characters. The nrDNA ITS NeighborNet analysis shows relationships of T. bithynicum with members of sect. Scariosa. Taraxacum bithynicum is considered as a taxon endemic to the summit area of Uludağ. The exploration of the latter area also revealed another, probably related but agamospermous and triploid (2n = 24) species that is described as T. pseudobithynicum, also confined to Uludağ. The endemism in the Mt. Uludağ flora is briefly characterized, and the two species studied are expected to be related to other similar plants restricted to mountain areas of SW and S Anatolia. The history of the original gathering of T. bithynicum (Aucher-Éloy, no. 3540) and various concepts of this name in the literature are briefly discussed.

**Keywords:** Taraxacum pseudobithynicum; chromosome numbers; M. R. Aucher-Éloy; taxonomy.
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Chapter 32

Introduction


The summarized goals of the present study are (i) taxonomic and nomenclatural characterization of *T. bithynicum*, (ii) accommodation of *T. bithynicum* in the modern sectional system of this genus, and (iii) taxonomic analysis of a sympatric agamospermous relative of *T. bithynicum*. 
Chapter 33

Material and methods

Details of the cultivation methods are given in Kirschner and Štěpánek (1993). Determination of the reproduction system, an important background for taxonomic decisions, was performed according to Kirschner et al. (2006). We emphasize the pollen size uniformity as a reliable indicator of sexuality in *Taraxacum* (den Nijs et al. 1990), see also Fig. 36.1.

Achene length measurements include cone; achene body width represents the widest dimension of the achene.

Sectional nomenclature follows previous nomenclatural and taxonomic accounts (Kirschner and Štěpánek 1987, 1997a, 2004; Ge et al. 2011). Plant names are in accordance with the ICN (latest edition, McNeill et al. 2012).

Voucher specimens are deposited in ISTE and PRA (see Index herbariorum for the abbreviations). JK & JŠ use numbered determination labels (‘no. det.’ in the text).

The SEM analysis was performed using gold coating and FEI Quanta 450 FEG-EDS.

For chromosome counting, root tips were pretreated with α-bromonaphthalene at 4°C for 24 h, and fixed in Carnoy’s solution. Material was hydrolyzed with 1 N HCl for 15 min at 60°C and stained in Schiff reagent. At least five metaphase plates were examined from different individuals.

We used 105 sequences of ITS1-5.8S rDNA-ITS2 from Kirschner et al. (2015, 2017). See Kirschner et al. (2017) for respective methodology.

Chapter 34

Results

34.1 Identity of Taraxacum bithynicum

We studied the original material of T. bithynicum which comes from the summit area of the Uludağ (seven syntype herbarium specimens seen, see chapter 35) and compared it with our material from the same macrolocality. The gatherings collected by BG there as T. bithynicum proved to be taxonomically identical with the original material (Fig. 34.1).

34.2 Problems associated with the name Taraxacum bithynicum

The protologue of the name T. bithynicum does not leave any doubt about the identity of the original material, confined to a single gathering. However, von Handel-Mazzetti (1907, pp. 33–34) included it in his sect. Scariosa and extended it to cover plants from a vast region from Crete, through Anatolia and Lebanon to Transcaucasia, and since then, both the circumscription and the sectional position remain unclear. An extremely broad T. bithynicum concept within sect. Scariosa was presented by Doll (1976), with several characters not found in the original material (achenes longer, densely spinulose, cone twice longer, pappus brownish white etc.), and the distribution covering the whole Balkan Peninsula, Italy, Turkey, Lebanon, Crete and the Transcaucasia. This situation was not much clarified in A. J. Richards and Sell (1976) as T. bithynicum in Flora Europaea was not interpreted at the species level but was used as an aggregate covering most of what is generally understood as sect. Scariosa. As regards the morphology, closer relationships between T. bithynicum and sect. Scariosa are doubtful. Also the alpine habitats and aestival phenology of T. bithynicum substantially differ from those of sect. Scariosa (lower elevation rocky or sandy sites, rock-steppe, frequently along the coast; sect. Scariosa plants usually avoid the hot and dry late spring and summer season as regards their phenology).

The following taxa were found under the name of T. bithynicum in herbarium collections: T. assemanii Boissier (1867b, 791, herb. JE, G, G-BOIS, ZT), T. stenocephalum Boissier (1867b, p. 790) s. lat. (herb. JE, G, ZT, PR), T. bulgaricum van Soest (1976, 179, herb. B,
34.3 Collections of R. Aucher-Éloy and *T. bithynicum*

The name *T. bithynicum* DC., treated in detail below, is based on a gathering of Pierre Martin Rémi Aucher-Éloy (1793–1838), an outstanding plant collector in the region of modern Turkey, but also in other areas of the Near East. We have attempted to find details of his journey to the Bithynian Olympus (Uludağ, Bursa) in order to identify an exact site of the type gathering (Baytop 2005). The diaries of Aucher-Éloy were published by Jaubert (1843), a contemporary of Aucher-Éloy, together with a biography and selected letters. It turns out that plants to become *T. bithynicum* were collected in 1833, a year spent by Aucher-Éloy with his family in Constantinople (Istanbul). Occasional trips to the regions not far from Istanbul were not included in the diaries, so that details of his visit to Uludağ are not extant. The diaries describing the 1834 journey from Istanbul via Ankara to Iran clearly show that Uludağ was not visited that year, and 1833 is the most probable collection year;

Figure 34.1: A detail of the lectotype of the name *T. bithynicum* (G-DC, no. det. 18919).

KRAM), *T. aleppicum* Hugo Dahlstedt (1926, 14, herb. G), and sect. *Scariosa* members from Crete.
Chapter 34. Results  Part VII. What is and is not *T. bithynicum*

*T. bithynicum* plants reached De Candolle in 1837, as given on the label in G-DC.

It remains to be stated that Jaubert (1843) also mentioned Bithynian Olympus only for the journeys undertaken in 1833. However, the exact location on Uludağ remains unknown.

Another problem associated with the type gathering of *T. bithynicum* is the number cited with the name. In most works, including von Handel-Mazzetti (1907) and van Soest (1975), it is cited as a collection number. However, in fact it is a species number added to the specimens in 1837 when the whole collection was prepared for distribution by Brongniart in Paris as ‘Aucher-Éloy-Herbier d’Orient No.’ and accession species numbers were accorded to each species (Ghazanfar 1996). The species number (referred to *T. stevenii*) was cited in two ways: de Candolle (1838), Boissier (1867b) and von Handel-Mazzetti (1907) and others read the Herbier d’Orient number as 3540, while van Soest (1975) decided that the species number was 3940 and explicitly stated that 3540 was a reading mistake. It was relatively easy to solve this problem, as the numbered species list of the Herbier d’Orient did not exceed the number of 3860 (Ghazanfar 1996), and therefore the *T. stevenii* number (i.e. that of *T. bithynicum*) is 3540. We can add that the type gathering of the name *T. crepidiforme* de Candolle (1838, p. 149) (‘Aucher-Éloy-Herbier d’Orient No. ’ 3546), originally as Crepis, was unanimously interpreted as 3546, even by van Soest (1975, 1977) himself.

![Figure 34.2: *Taraxacum bithynicum*, 2n = 16. A, somatic chromosomes (with line contours added); B, karyotype (ISTE 107391). *T. pseudobithynicum*, 2n = 24. C, somatic chromosomes; D, karyotype (ISTE 102911).](image-url)
### 34.4 Chromosome number of *T. bithynicum*

Chromosome number of 2n = 16 for *T. bithynicum* was published by Doll (1976) with a reference to the unpublished thesis of A. J. Richards (1969), see also A. Richards (1973). The locality of plant material originally referred to as *T. bithynicum* in the thesis was Skopje, Republic of Macedonia, a region where *T. bithynicum* surely does not occur. The *T. bithynicum* chromosome record was not included in any later paper published by A. J. Richards. We therefore conclude that the chromosome number published in the present paper is the first for *T. bithynicum*. *T. bithynicum* is diploid with 2n = 16 chromosomes; its karyotype can be summarized as 8 MC. In *Taraxacum* this karyotype is considered as relatively ancestral (Fig. 34.2).

### 34.5 Achene micromorphology of *T. bithynicum*

The achene characters (Fig. 34.3) show how distant is *T. bithynicum* from sect. *Scariosa* in terms of morphology. In particular, the sparse spinules scattered in the uppermost part of the achene body which gradually narrows into the cone are diagnostic (in sect. *Scariosa*, achenes are usually covered with ± dense squamules).

![Figure 34.3: SEM micrographs of achenes. A. *T. bithynicum* (ISTE 107391); B. *T. pseudobithynicum* (PRA, no. det. 31334). Scale bar equals 1 mm.](image)
34.6 Relationships of *T. bithynicum* on the basis of the nrDNA sequence variation

von Handel-Mazzetti (1907), van Soest (1975) and Doll (1976) included *T. bithynicum* in sect. *Scariosa*. However, the morphology (as summarized in the detailed description below) does not indicate any closer relationships between *T. bithynicum* s. str. and members of sect. *Scariosa* (see Table 34.1). The main differences consist in the coloration, border and corniculation of outer phyllaries, and shape and sculpture of achenes, all characters of sectional importance. It should be emphasized that *T. bithynicum* does not exhibit any attribute generally considered as plesiomorphic in *Taraxacum*. The NeighborNet analysis of nrDNA ITS sequences of *T. bithynicum* and the other *Taraxacum* sexuals was used to find probable relationships, taking into account the hybridogenous origin of some sexual taxa in *Taraxacum* (Kirschner et al. 2015). The result is displayed on Fig. 34.4. Surprisingly, *T. bithynicum* is shown to be relatively close to the sect. *Scariosa* sexuals and, moreover, one of the *Scariosa* sexual samples (*T. aegeum* ined.) is extremely close to *T. bithynicum*. It is therefore possible that *T. bithynicum* might be an old mountain hybridogenous derivative of sect. *Scariosa*. However, the sectional position of *T. bithynicum* remains unclear because of the conflict between different data sets (morphology and ITS sequences) and the probable hybridogenous origin of *T. bithynicum*. 
Figure 34.4: NeighborNet constructed from uncorrected P-distances in SplitsTree based on nrDNA ITS sequences of sexual dandelions of 24 sections. The position of *T. bithynicum* is marked dark grey. Species names with sample number (e.g. *T. minutilobum*-5) belong to species with sample majority in another branch of the graph. Modified from Kirschner et al. (2017, chapter V, Fig. 25.2, page 161).
Table 34.1: A comparison of morphological characters of *T. bithynicum*, *T. pseudobithynicum* and *T. sect. Scariosa*.

<table>
<thead>
<tr>
<th><strong>Taraxacum</strong></th>
<th><strong>bithynicum</strong></th>
<th><strong>pseudobithynicum</strong></th>
<th><strong>sect. Scariosa</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>habitat</strong></td>
<td>subalpine</td>
<td>subalpine</td>
<td>lower elevation, dry rock crevices, often coastal habitats or xeric grasslands</td>
</tr>
<tr>
<td><strong>phenology</strong></td>
<td>summer flowering</td>
<td>summer flowering</td>
<td>summer rest</td>
</tr>
<tr>
<td><strong>outer phyllaries: border</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>paler but indistinct, 0.1–0.2 mm wide</td>
<td>paler green to whitish, 0.2–0.3 mm wide</td>
<td>broad, distinct, white or whitish pale green</td>
<td></td>
</tr>
<tr>
<td><strong>outer phyllaries: corniculation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>flat</td>
<td>flat</td>
<td>corniculate</td>
<td></td>
</tr>
<tr>
<td><strong>pollen</strong></td>
<td>regular</td>
<td>irregular</td>
<td>regular or irregular, or absent</td>
</tr>
<tr>
<td><strong>achene colour</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>greyish straw-brown</td>
<td>medium greyish straw-brown</td>
<td>straw brown, ochraceous or pale reddish</td>
<td></td>
</tr>
<tr>
<td><strong>achene size</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5–4 × (0.7–) 0.8–0.9 mm</td>
<td>4.4–4.9 × 0.9–1.0 mm</td>
<td>3.8–5.0 × ca. 1.0–1.2 mm</td>
<td></td>
</tr>
<tr>
<td><strong>achene cone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subconical to subcylindrical, ca. 0.5 mm</td>
<td>cylindrical to subcylindrical, 0.7–1.0 mm</td>
<td>subcylindrical, thin, 0.8–1.5 mm</td>
<td></td>
</tr>
<tr>
<td><strong>achene body to cone transition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gradual</td>
<td>gradual</td>
<td>subgradual to ± abrupt</td>
<td></td>
</tr>
<tr>
<td><strong>achene body spinulosity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sparsely minutely spinulose in the upper 1/4, otherwise ± smooth</td>
<td>with short spinules in upper 1/4, otherwise almost smooth</td>
<td>densely squamulose and spinulose in upper 1/3 or squamulose, spinulose and tuberculate throughout</td>
<td></td>
</tr>
<tr>
<td><strong>chromosome number (2n)</strong></td>
<td>16</td>
<td>24</td>
<td>16, 24, 32</td>
</tr>
</tbody>
</table>
Chapter 35

Taxonomic treatment: *T. bithynicum*

*Taraxacum bithynicum* de Candolle (1838, p. 149), as 'Bithynicum' (Figs. 34.1, 34.3 A and 35.1.)

Type: [TURKEY] Alpes Olymp. Byth., [1833], R. M. Aucher-Éloy. [Distributed as Aucher-Éloy, Herb. Orient., no. 3540 as *Leontodon stevenii*] (lectotype: G-DC, no. det. 18919, designated by van Soest (1975, p. 798); isolectotypes: G-BOIS, no. det. 18800; MPU, no. det. 20323; BM, no. det. 8451; FI, no. det. 17935; JE !; P 691586 [web]).

Further illustrations: von Handel-Mazzetti (1907, Plate I, 12a, b, Plate IV: 7).

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Figure 35.1: A representative specimen of *T. bithynicum* DC. (ISTE 107391).

Plants small, usually 3–5 cm tall, often with many-headed root system; plant base with subsparse brownish hairs and black-brown remnants of old petioles. Leaves ± numerous, variously erect-patent, 2–4 (~6.5) × 4–7 cm, ± glabrous, vivid green, leaf blade narrowly spatulate in outline, usually shallowly pinnatifid to almost pinnatisect, or undivided and then with remote short teeth; terminal lobe helmet-shaped, 5–11 × 5–7 mm, apex rounded to broadly subacute, with distal margin convex to slightly sigmoid, entire to remotely minutely denticulate, with basal lobules ± patent and the proximal margin ± straight, entire; lateral lobes 3–4, short and broad, usually 2–3 × 3–5 mm, ± patent, broadly subacute to almost rounded, distal margin convex to slightly sigmoid, entire to remotely denticulate, proximal
margin convex to straight. Interlobes often distinct, narrow, usually 2–3 (–4) × 1–1.5 mm, with raised margins, usually entire to sparsely denticulate. Petiole narrow to narrowly winged, light green. Scapes thin, equalling leaves, subglabrous (sometimes brownish hairy distally) to glabrous. Involucre narrow, ± oblong, broadly obconical and ca. 3 mm wide at base. Outer phyllaries 7–13, short, reaching only 1/4–1/3, rarely to 1/2 of the length of the inner ones, loosely appressed, narrowly lanceolate to broadly ovate, (3–) 4–5 × 1–2.5 mm, ± dark olivaceous-green, later (and when dry) blackish-olivaceous to almost black, often with black mid-vein and narrow submarginal black strips, usually with an indistinct, 0.1–0.2 mm wide paler border, sometimes with a blackish middle part and gradually paler to membranous borders to 0.5 mm wide, margin distinctly ciliate, apex ± obtuse and paler, ± flat; inner phyllaries 8–9, of ± equal width, deep green, blackish green and sometimes with a callosity near apex. Capitulum very small, to ca. 1.5 cm in diameter, yellow, with low floret number; outer ligules ± flat, subcucullate near apex, striped pale greyish outside, apical teeth probably brownish; stigmas long, ± yellow to pale greyish-greenish yellow, with pale pubescence outside. Pollen present, pollen grains of ± equal size (25.5–25.7 μm). Achenes greyish straw-brown, 3.5–4 × (0.7–) 0.8–0.9 mm, achene body sparsely minutely spinulose in the upper 1/4, otherwise ± smooth, gradually narrowing into subconical to subcylindrical short cone ca. 0.5 mm long, beak ca. 3.5–4 mm long, pappus ca. 4–5 mm long, pure white. Fl. June to August.

35.1 Specimens examined

TURKEY. [NW Anatolia, A2(A), Bursa Province] Bursa vicinity, ascent to the Bithynian Olympus, alpine region, 25 Jul 1912, B. A. Fedtschenko 119 (LE, no. det. 6011; WU, no. det. 9004); Olympus [Uludağ], Aug 1841, Thuret (G-BOIS, no. det. 18851); Olym. Bithyn. [Uludağ], s. dato, Pauli 54 (JE, no. det. 30230); Uludağ, Kara Tepe [partly illegible], 2 Aug [19]45, Heilbronn & M. Baṣarman (G, no. det. 18868); Olympus Bithynus. [Uludağ], Aug 1842, s. coll. (G-BOIS, no. det. 18799); Bursa, Ulu Dag [Uludağ], Nordhang und Gipfel Zirve Tepe, 2000–2400 m., 27 Jun 2004, L. Meierott & Elsner LZ 04/606 (herb. L. Meierott, no. det. 27984); Bursa, Ulu Dag [Uludağ], W/NW-Hang Zirve Tepe, 2300–1900 m, Felsschutt, steinige Rasen, 26 Jun 2004, L. Meierott & Elsner LZ 04/582 (herb. L. Meierott, no. det. 27982); Uludağ, Zirve-Karatepe, 30 Jun 1944, M. Baṣarman (ISTF 3782); Uludağ milli parkı, Keşiş’in evine çıkarken, 2202 m, 25 Jul 2015, B. Gürdal 893-16, M. Koçyiğit, Ç. Ocak (ISTE 107391).

35.2 Distribution, endemism and ecology

The material of T. bithynicum studied shows that this species represents an endemic of the Uludağ, located in the Bursa province, southeast of Marmara Sea. It is the highest mountain in the northwestern Anatolia, reaching 2543 m. A combination of outcrops of acid granite, gneiss, schist and crystalline limestone has given rise to a wide range of habitats. The flora is exceptionally rich with its diversity of unique vegetation types and richness in rare
species (including many local endemics). Owing to the rich biodiversity, different habitats, zonation of its plant communities at various altitudes, Uludağ was declared as a Centre of Plant Diversity by the World Conservation Union (IUCN) and World Wide Fund for Nature (WWF-International). The area of 11,336 ha. of the Uludağ Important Plant Area was declared as a national Park on September 20, 1961, as one of the first NP established in Turkey. Uludağ is the Important Plant Area and Important Bird Area (Özhatay et al. 2003).

Uludağ can be regarded as one of the most important single mountain sites in floristic terms not only in Turkey but also in the whole of Europe. According to the floristic surveys, a total of 1309 taxa belonging to 102 families have been recorded, with 169 taxa endemic, including 31 taxa confined to this single site (Daşkı̈n and Kaynak 2010a,b).

*T. bithynicum* has a specific habitat and an elevation span distinct from most western Anatolian dandelions. It is a small mountain species and grows above 2000 m around snowbeds, alpine open stony and rocky slopes and mountain lakes, often relatively sheltered. As regards the IUCN conservation categories, we recommend the VU status (i.e. vulnerable). This type of habitat is very different from the sites occupied by members of sect. *Scariosa*.

According to Daşkı̈n and Kaynak (2010a,b), there are 31 taxa endemic to Uludağ, the majority of them confined to the subalpine and alpine vegetation, i.e. in the Alpinetum zone (from 1900 to 2543 m). However, a more detailed search and new literature comparisons show that a part of these taxa are not confined to Uludağ only, and there are other taxa to be considered. A selection of species endemic to Uludağ or subendemic to that region (see Davis et al. 1988; Davis 1965–1985) was used to identify probable florogenetic relationships of the Uludağ flora and to elucidate the origin of *T. bithynicum*. There are the following main types of the Uludağ endemism or subendemism:

1. Taxa associated with the flora of the Caucasus (and NW Iran), e.g. *Scorzonera pygmaea* Smith (1813, p. 123) subsp. *pygmaea*, a taxon very close to *S. seidlitzii* Boissier (1867b, p. 775) of the Transcaucasia, *Juncus anatolicus* Sven (1978, p. 194), most closely related to *J. alpinus* K. C. Koch (1848, p. 627) of the Caucasus and adjacent regions, less so to the widespread *J. atratus* Krocker (1787, p. 562), or *Silene olympica* Boissier (1843, p. 24), closely related to *S. lasiantha* K. C. Koch (1842, p. 712) of the Transcaucasia and NW Iran.

2. Probably the most common relationships of the Uludağ (sub)endemics are those with the flora of the mountains of the Balkan Peninsula, particularly Greece, sometimes also Aegean Islands or the northwesternmost Anatolia (allied species in brackets): *Allicium sibthorpiannum* Schultes & Schultes fil. in Roemer & Schultes 1830, p. 1027 [A. frigidum] Boissier & Heldreich in Boissier (1854, p. 34), *Linum olympicum* Boissier (1843, p. 56) [L. pathulatum] (Halácsy & Baldacci in von Halácsy 1893, p. 576) von Halácsy (1900, p. 258), *Senecio olympicus* Boissier (1844, p. 13) [S. macedonicus Grisebach (1846, p. 221), S. castagneanus de Candolle (1838, p. 354)], *Achillea multifida* (de Candolle 1838, p. 295) Grisebach (1846, p. 213) [close to A. clusiana] Tausch (1821, p. 551) of the Balkans which also reaches the E Alps, or *Arabis drabiformis*
Boissier (1842, p. 55) related to A. bryoides Boissier (1842, p. 55) of Greece. Sometimes these relationships go farther to the central Mediterranean: Galium olympicum Boissier (1843, p. 41), a member of the group of relicts, most closely related to the Italian G. paleoitalicum Ehrendorfer in Ehrendorfer & Krendl 1974, p. 271\(^{43}\) of Italy and G. pyrenaicum Gouan (1773, p. 5) of the Iberian Peninsula, or Gagea bithynica Pascher (1904, p. 121), endemic to NW Anatolia, derived from the central Mediterranean G. chrysantha Schultes & Schultes fil. in Roemer & Schultes 1829, p. 545.

3. Several species are probably derived from widespread variable congeners, e.g. Rumex olympicus Boissier (1844, p. 45) from R. patientia von Linné (1753, p. 333), Ornithogalum nudaniae Y. Bağci & Savran in Bağci et al. (2009, p. 165), probably a descendant of O. oligophyllum E. D. Clarke (1816, p. 555), or Ranunculus fibrillosus K. C. Koch (1847, p. 47), derived from the widely distributed and variable R. constantinopolitanus (Candolle 1818: 281) D’Urville (1822, p. 317) extending from the Balkans to Iraq and Syria, and Allium flavum var. minus Boissier (1867c, p. 255), a derivative of the variable and widespread group of taxa of A. flavum von Linné (1753, p. 299) s. lat.

4. Important for our considerations about T. bithynicum are taxa endemic or subendemic to Uludağ and related to other taxa confined to small mountain areas in other parts of Anatolia, their centre of diversification. For instance, Sideritis dichotoma Huter (1903, p. 360), a remarkable plant of Uludağ, is very closely related of the limestone taxon endemic to Crimea, Ukraine [S. taurica (Willdenow 1800, p. 66) and another limestone plant of N and C Anatolia, S. amasiaca Bornmüller (1932, p. 137)]. Another case is Aubrieta olympica Boissier (1867a, p. 251), related to A. canescens (Boissier 1867a, p. 252) Bornmüller (1836: 44) subsp. canescens of Ak Dağ, and to A. anamasica P. H and Güner (1978, p. 35) confined to the Isparta region, see also M. A. Koch et al. (2017). Dianthus leucophaeus Smith (1809, p. 288) subsp. leucophaeus is the closest relative of D. goerkii Hartvig and Strid (1987, p. 321) of the Cilician Taurus (mainly the Adana Province).

Our relatively detailed knowledge of the Taraxacum floras of the Caucasus (e.g. Kirschner and Štěpánek 1993), the Balkan Peninsula and Turkey makes it possible to exclude the florogenetic groups 1, 2 and 3 from considerations on the origin of T. bithynicum. Further study to support the idea of T. bithynicum as belonging to the fourth group is needed, which includes the exploration of the mountains of the south-central Anatolia to find possible relatives of the T. bithynicum group (comparatively similar but not well preserved specimens of dandelions are known from several sites in Ak Dağlar, Antalya).

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Chapter 36

Evaluation of an apomictic congener of *T. bithynicum* from Uludağ

Among *Taraxacum* plants in the summit area of Uludağ, in addition to the sexual *T. bithynicum*, there are also similar agamospermous plants with a number of distinctive characters. On the basis of newly collected and/or cultivated material, the evidence regarding its possible separate status, particularly in the fields of karyology, micromorphology and gross morphology clearly shows that the agamospermous plants are to be considered as a separate species, and are described under the name of *T. pseudobithynicum*. The main differences of this new species from *T. bithynicum* are wider and more distinct whitish borders to exterior phyllaries, discoloured stigmas and much longer achenes and beak (although the achene shape and spinulosity is very similar).

36.1 Chromosome number and karyotype

These plants aberrant form *T. bithynicum* proved to have 2n = 24 and are therefore triploid. Karyotype is 3 × 8 MC, i.e. all chromosomes are metacentric. It has satellites observed on chromosomes 1, 2, 3 and 4, respectively (Fig. 34.2). Judging from the karyotype, similarly simple as that of *T. bithynicum*, one can hypothesize that our agamosperm is close to *T. bithynicum* and might have come into being through hybridization of *T. bithynicum* with a taxon having a very similar karyotype.

36.2 Micromorphology of achenes

Achenes of *T. pseudobithynicum* are similar to those of *T. bithynicum* in their shape and the character of spinulosity (Fig. 34.3). They differ from achenes of *T. bithynicum* in much bigger size and substantially longer beaks. Achenes have shallow longitudinal ribs. It has short, acute spinules, covering about 1/4 of achene body surface above. The achene size is therefore the most conspicuous achene difference from *T. bithynicum*. As regards pollen, pollen grains are very variable in size (Fig. 36.1), pollen grains are ranging from 14 to 32 μm
in diameter, oblate-spheroidal and echinolophate. The irregularity of pollen is a good proof of agamospermy when found consistently.

![Pollen size variation](image)

**Figure 36.1:** Pollen size variation in sexual and agamospermous *Taraxacum* taxa. Left: *T. pseudobithynicum*, with variable pollen grain size (scale bar = 0.1 mm); voucher: ISTE 102911. Right: *T. bithynicum*, a sexual taxon with ± uniform pollen grain size (scale bar = 0.05 mm); voucher: ISTE 107391.
Chapter 37

Taxonomic treatment:

*T. pseudobithynicum*

_Taraxacum pseudobithynicum_ B.Gürdal, Štěpánek, Zeisek, Kirschner & N. Özhatay, sp. nov. (Figs. 34.2 C, 34.2 D, 34.3 B, Fig. 37.1.)

Type: TURKEY. A2(A) Bursa, Uludağ, Volfram çevresi, 2176 m, 11 Aug 2014, B. Gürdal 835-16, M. Koçyiğit (holotype: ISTE 102911).

Diagnosis: Plantae parvae oreophyticae, habitu et acheniis forma cum Taraxaco bithynici optime congruentes sed foliis saturate viridibus pinnatisectis, lobis lateralibus triangulari-deltoides vel falcatis, phyllariis exterioribus adpressis ovatis usque ad lanceolatis, atroviridis, phyllariis conspicuis pallidis 0.2–0.3 mm latis, ecorniculatis, stigmatibus viridescentibus, antheris polliniferis, acheniis longioribus breviter spinulosis, in pyramidem cylindricam vel subcylindricam subsensim abeuntibus.

Plants small, usually 6–10 cm tall. Root head with several paucifoliate rosettes, tunic absent, plant base with medium dense brownish hairs. Leaves variously erect-patent, usually 4–7 × 1–1.5 cm, deep green to darker green, sparsely arachnoid, mainly on mid-vein abaxially; leaf blade narrowly ob lanceolate in outline, distinctly pinnatisect with bigger terminal lobe and relatively small lateral ones; terminal lobe helmet-shaped in outline, 1–1.8 × 0.7–1.2 cm, obtusely subacute to acute, with distal margins convex to subsigmoid, entire or with 1–2 acute teeth, often also with 1–2 ± deep incisions, basal lobules recurved, acute, proximal margins subconcave, entire; lateral lobes 2–5 pairs, deltoid-triangular to sickle-shaped, sometimes of a bird-wing shape, recurved with patent proximal part, acute, usually 3–7 × 3–5 mm, entire or with a single tooth, proximal margin straight or slightly concave, usually entire; interlobes long and narrow, usually 3–6 × 1–2 mm, with raised and brown-purple coloured margins, usually with (1)2 short acute teeth, rarely several unequal teeth; mid-vein pale or brownish-pinkish; petioles narrow to narrowly winged, broadly so at the very base, faintly greyish purplish. Scape subequaling leaves, relatively densely arachnoid (particularly below capitulum), purplish at base or later almost entirely purplish. Capitulum ca. 3 cm in diameter, flat, yellow; outer ligules flat, striped dark purplish grey-olivaceous outside, apical teeth black-purple, inner ligules canalicate, with apical teeth deep yellow. Involucre rounded to subtruncate, ca. 4–5 mm wide at base; outer phyllaries
Figure 37.1: Cultivated specimen of *T. pseudobithynicum* (PRA, no. det. 31334).
10–13, tightly appressed, some with erect tips, outer of them ovate, short, reaching 1/3–2/5 of the length of inner phyllaries, ca. 4 × 2.5–3 mm, the others longer, lanceolate, to 8 mm long, relatively dark, dark olivaceous green (to almost black-green when dry), the middle dark part with abrupt transition into distinct, paler to whitish border 0.2–0.3 mm wide, suffused pinkish in upper 1/2, flat and black-purple below apex, margins subglabrous to ciliate, inner phyllaries 10–12, light olivaceous, ca. 10 mm long, of equal width. Stigmas light greenish/greyish yellow, outside with pubescence of short black-tipped hairs. Pollen abundantly present, variable in size (14–32 μm). Achenes medium greyish straw-brown, relatively slender, 4.4–4.9 × 0.9–1.0 mm, with short spinules in upper 1/4, otherwise almost smooth, subgradually narrowing into cylindrical to subcylindrical cone 0.7–1.0 mm long, often with a few spinules at base, beak (4–) 5.5–7 mm long, thin, pappus 5–5.5 mm, pure white. Agamosperm. Fl. July–August.

37.1 Chromosome number

Agamosperm. Triploid (2n = 24), see Fig. 34.2, det. B. Gürdal sub no. sit-19, 20 (ISTE 102911, no. det. 31327; 102912, no. det. 31336), also JŠ sub no. 29/99.

37.2 Habitat and distribution

The new species, *T. pseudobithynicum*, is currently known only from one macrolocality on Mt. Uludağ. It grows on high mountain steppe and around Tungsten Mine at an elevation of 2100–2200 m, whilst *T. bithynicum* grows above 2000 m on snowbed slopes, alpine stony and rocky sites but generally more sheltered places. The two species are not in contact in the summit area.

37.3 Additional specimens examined

On the *Taraxacum* taxonomy etc.  

Vojtěch Zeisek (2018)
Chapter 38

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