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Testování identity oligoklonálních agamospermních drobných druhů – taxonomové versus mikrosatelity

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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.

K e y w o r d s: agamospermy, clonality, microsatellite variation, plant identification, population variation, *Taraxacum*, taxonomy

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 & 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, Vilgalys 2003, Hawksworth 2004, Holst-Jensen et al. 2004, Kristiansen et al. 2005, Záveská Drábková & 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-Crepidinae*), 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 & 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 & 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 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

& Štěpánek 1997). 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 & Štěpánek 1998, Uhlemann 2003, 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 (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 (Richards 1973, Ozias-Akins & van Dijk 2007), clonality (Kirschner & Štěpánek 1994), epigenetic heritability (Verhoeven et al. 2010a, b, Verhoeven & 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. numerous microspecies characterized by a limited within-species variation, was recognized more than a hundred years ago (Raunkiaer 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 (Electronic Appendix 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 & Lugon-Moulin 2002, Bhargava & 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.

Material and methods

Material

A detailed account of the plant material used is given in Electronic Appendix 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 & Štěpánek 1997), (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; Electronic Appendix 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.

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 & van Dijk 1999) and confirmed to be triploid.

DNA isolation

Total DNA was isolated from fresh leaf tissue, 1.2 cm^2, using the hexadecyl-trimethyl-ammonium-bromide (CTAB) procedure described by Rogstad (1992), with some modifications referred to as the "RETCH" method described by Vijverberg et al. (2004).

Microsatellite analysis

All plants were characterized by eight microsatellite loci (SSRs, Jarne & Lagoda 1996), which were distributed over two multiplex PCR reactions (mutliplex 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 folows: 15 min 95°C, 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, 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.

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, 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 2014). We used packages ade4 (Dray & 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 & 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 & 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 work-flow, software settings etc. are available from VZ upon request.

Genotype diversity was quantified according to Hughes & Richards (1988) as $G = 1–\Sigma x_i^2$, 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 alloploidy; 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 in Electronic Appendix 2.

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 (*T. alatum*), grouped genetically under the species name to which they were originally assigned on the basis of morphology. Sample pul1299 (*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 *T. alatum* were misidentified.

Bayesian clustering in BAPS revealed nine major clusters (Fig. 1) and three individuals were not included in the expected clusters. K-means clustering (Fig. 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 *T. hepaticum*) and ala11-103 (close to *T. piceatum*).

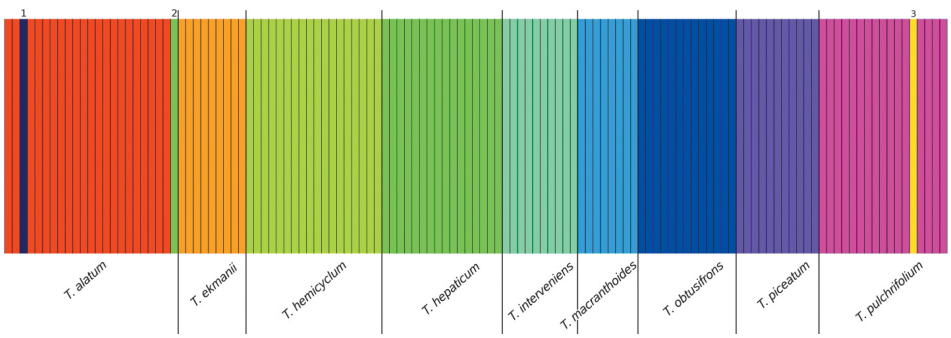

Fig. 1. – Bayesian clustering of individuals performed in BAPS. Each colour represents one inferred genetical cluster. The only three individuals not included in "their" clusters are two probably misidentified individuals of *Taraxacum alatum* (ala11-103 – marked "1" at the top of the figure, and ala5-2 – marked "2") and one of *T. pulchrifolium* (pul1299 – marked "3"; see Discussion). Compare with output of K-means clustering (Fig. 2).

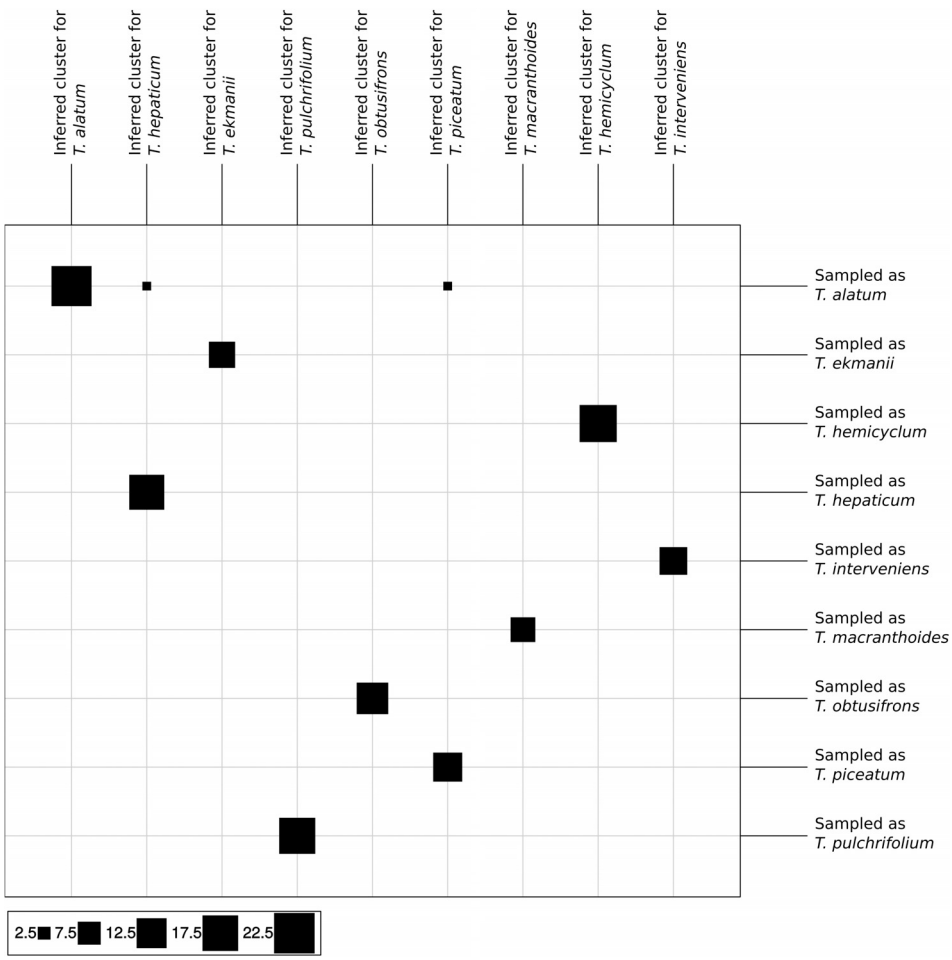

Fig. 2. – K-means Bayesian clustering showing an almost perfect match of inferred genetic clusters and originally sampled morphologically determined species. Sample ala5-2 groups with *Taraxacum hepaticum*, ala11-103 with *T. piceatum* and pul1299 with *T. pulchrifolium*. Size of squares is proportional to the number of individuals. Compare with output of BAPS (Fig. 1).

Trees (NJ and MSN) provide strong support for grouping these species together (regardless of technique and distance matrix), thus forming nine main branches (Fig. 3 and Electronic Appendix 3: Fig. 1). 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 & Øllgaard 1999). PCoA (Electronic Appendix 3: Fig. 2) of the original samples reveals generally well separated clusters. The most aberrant samples were probably misidentified.

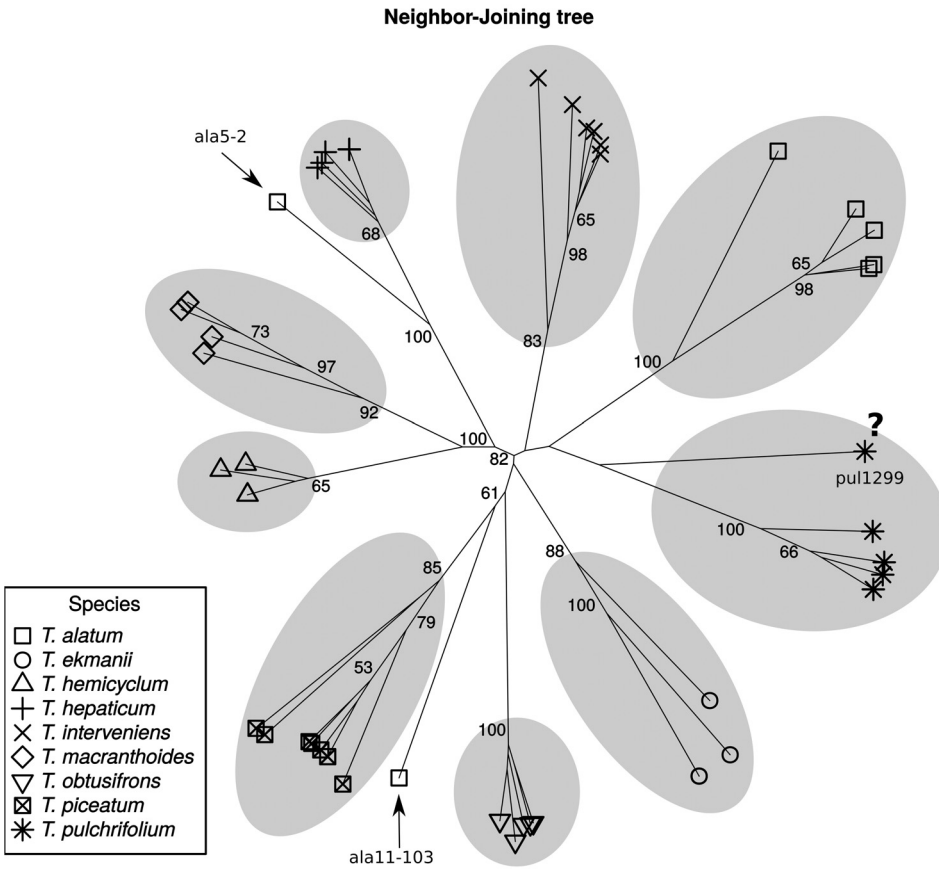

Fig. 3. – NJ tree based on Nei's distance. Grey ellipses mark supported clusters, while symbols indicate the original determinations by experts (arrows indicate identification mistakes and question mark the enigmatic sample pul1299). Bootstrap values higher than 50 are also displayed. Omitted are also numbers for crown clades with practically identical genotypes. As there are many samples with identical genotypes, their labels fully overlap.

The genotype diversity and distributions of MLGs within a microspecies clone cluster are given in Table 1. The diversity of G-values are comparatively high, much higher than those reported for agamosperms by Hughes & 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 Discussion), 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.

Table 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 Results and Discussion), so that for the *Taraxacum alatum* and *T. pulchrifolium* columns "Number of plants" does not include all the plants sampled under that name.

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

Discussion

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 1977) to vehement advocacy (Abbott 1979, see also Kirschner & 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 & 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 & Duyts 1981, Sterk et al. 1983, Sterk & Luteijn 1984, Roetmann & 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 & 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 south-western 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 & 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 King & 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 & Richards 1985, Kirschner & Štěpánek 1998, Van Baarlen et al. 2000, also Mogie 1985 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 & 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. hollandicum*, 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. hollandicum* 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 & Štěpánek 1998). A picture very similar to that of *T. hollandicum* is documented by Menken & 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 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.

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 & 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 a 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) and 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.

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 Gross et al. (2012) and Reisch (2004). 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. 1) gives it its own cluster and in distance-based methods (Fig. 3, Electronic Appendix 3, 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.

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 & 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.

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 & 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, Hollingsworth et al. 2009, Dong et al. 2012, Hollingsworth et al. 2014) and assortments of lineages of crop germplasm (e.g. 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 & Paulay 2005, Rubinoff et al. 2006, Seberg & 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. 2012), 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 & 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

distinquished 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.

See www.preslia.cz for Electronic Appendices 1–3

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Souhrn

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. Jednotlivé klonální (oligoklonální) entity v takových skupinách jsou obvykle popisovány jako tzv. drobné druhy (mikrospecie), navzájem rozeznatelné na základě souboru drobných morfologických rozdílů. Znalost takových mikrospecií je obvykle omezena pouze na úzkou skupinu specialistů, kteří dosti často úzce navazují na znalosti předcházejících generací expertů. Specialisté používají publikovaná jména mikrospecií, avšak může existovat pochybnost, zda pod stejným jménem je vždy uváděn stejný taxon a zda jeden taxon pokaždé nese stejné jméno. 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 očekávaným druhům. Ukázalo se, že u 122 rostlin z našeho souboru identifikace expertů odpovídala geneticky charakterizovaným shlukům. Dva vzorky byly určeny mylně a jeden zůstává nejasný. Jednotlivé genotypy jsme zachytili na různých lokalitách, jak ve Finsku, tak ve střední Evropě. Dalším studovaným problémem byla genetická variabilita v rámci geneticky i morfologicky charakterizovaných skupin, tj. mikrospecií, 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. Kombinace tradiční identifikace experty na základě morfologických znaků a charakterizace mikrospecií pomocí vhodných molekulárních markerů může otevřít využití mikrospecií pro mnohem širší skupinu uživatelů v biologickém výzkumu.

References

Abbott R. J. (1979): Janzen's dandelions: a criticism. – Am. Nat. 114: 152–156.

Balloux F. & Lugon-Moulin N. (2002): The estimation of population differentiation with microsatellite markers. – Mol. Ecol. 11: 155–165.

Battjes J., Menken S. B. J. & den Nijs J. C. M. (1992): Clonal diversity in some microspecies of *Taraxacum* sect. *Palustria* (Lindb. fil.) Dahlst. from Czechoslovakia. – Bot. Jahrb. Syst. 114: 315–328.

Bhargava A. & Fuentes F. F. (2010): Mutational dynamics of microsatellites. – Mol. Biotechnol. 44: 250–266.

Bridge P. D., Roberts P. J., Spooner B. M. & Panchal G. (2003): On the unreliability of published DNA sequences. – New Phytol. 160: 43–48.

Bruvo R., Michiels N. K., D'Souza T. G. & Schulenburg H. (2004): A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. – Mol. Ecol. 13: 2101–2106.

Chrtek J., Tonková M., Mráz P., Marhold K., Plačková I., Krahulcová A. & Kirschner J. (2007): Morphological and allozyme diversity in the *Hieracium nigrescens* group (*Compositae*) in the Sudety Mountains and the Western Carpathians. – Bot. J. Linn. Soc. 153: 287–300.

Corander J., Sirén J. & Arjas E. (2008): Bayesian spatial modeling of genetic population structure. – Comput. Stat. 23: 111–129.

Cruz V. M. V., Nason J. D., Luhman R., Marek L. F., Shoemaker R. C., Brummer E. C. & Gardner C. A. C. (2006): Analysis of bulked and redundant accessions of *Brassica* germplasm using assignment tests of microsatellite markers. – Euphytica 152: 339–349.

Dong W., Liu J., Yu J., Wang L. & Zhou S. (2012): Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. – PLoS One 7: e35071.

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

Esselink G. D., Nybom H. & Vosman B. (2004): Assignment of allelic configuration in polyploids using the MAC-PR (microsatellite DNA allele counting-peak ratios) method. – Theor. Appl. Genet. 109: 402–408.

Falque M., Keurentjes J., Bakx-Schotman J. M. T. & van Dijk P. J. (1998): Development and characterization of microsatellite markers in the sexual-apomictic complex *Taraxacum officinale* (dandelion). – Theor. Appl. Genet. 97: 283–292.

Fang D. Q. & Roose M. L. (1997): Identification of closely related citrus cultivars with inter-simple sequence repeat markers. – Theor. Appl. Genet. 95: 408–417.

Ford H. & Richards A. J. (1985): Isozyme variation within and between *Taraxacum* agamospecies in a single locality. – Heredity 55: 289–291.

Gross C. L., Nelson P. A., Haddadchi A. & Fatemi M. (2012): Somatic mutations contribute to genotypic diversity in sterile and fertile populations of the threatened shrub, *Grevillea rhizomatosa* (*Proteaceae*). – Ann. Bot. 109: 331–342.

Hawksworth D. (2004): 'Misidentifications' in fungal DNA sequence databanks. – New Phytol. 161: 13–15.

Hebert P. D. N., Cywinska A., Ball S. L. & deWaard J. R. (2003): Biological identifications through DNA barcodes. – Proc. Biol. Sci. 270: 313–321.

Hollingsworth M. L., Andra Clark A., Forrest L. L., Richardson J., Pennington R. T., Long D. G., Cowan R., Chase M. W., Gaudeul M. & Hollingsworth P. M. (2009): Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. – Mol. Ecol. Resour. 9: 439–457.

Hollingsworth P. M., Forrest L. L., Spouge J. L., Hajibabaei M., Ratnasingham S., van der Bank M., Chase M. W., Cowan R. S., Erickson D. L., Fazekas A. J., Graham S. W., James K. E., Kim K.-J., Kress W. J., Schneider H., van Alphen Stahl J., Barrett S. C. H., van den Berg C., Bogarin D., Burgess K. S., Cameron K. M., Carine M., Chaco J., Clark A., Clarkson J. J., Conrad F., Devey D. S., Ford C. S., Hedderson T. A. J., Hollingsworth M. L., Husband B. C., Kelly L. J., Kesanakurti P. R., Kim J. S., Kim Y.-D., Lahaye R., Lee H.-L., Long D. G., Madriña S., Maurin O., Meusnier I., Newmaster S. G., Park C.-W., Percy D. M., Petersen G., Richardson J. E., Salazar G. A., Savolainen V., Seberg O., Wilkinson M. J., Yi D.-K. & Little D. P. (2014): A DNA mini-barcode for land plants. – Mol. Ecol. Res. 14: 437–446.

Holst-Jensen A., Vralstad T. & Schumacher T. (2004): On reliability. – New Phytol. 161: 11–13.

Hughes J. & Richards A. J. (1988): The genetic structure of populations of sexual and asexual *Taraxacum* (dandelions). – Heredity 60: 161–171.

Hughes J. & Richards A. J. (1989): Isozymes, and the status of *Taraxacum* (*Asteraceae*) agamospecies. – Bot. J. Linn. Soc. 99: 365–376.

Janzen D. H. (1977): What are dandelions and aphids? – Amer. Nat. 112: 58–59.

Jarne P. & Lagoda P. J. L. (1996): Microsatellites, from molecules to populations and back. – Trends Ecol. Evol. 11: 424–429.
Jombart T. (2008): Adegenet: a R package for the multivariate analysis of genetic markers. – Bioinformatics 24: 1403–1405.
Jombart T., Devillard S. & Balloux F. (2010): Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. – BMC Genetics 11: 1–15.
Kamvar Z. N., Tabima J. F. & Grünwald N. J. (2014): Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. – PeerJ 2: e281.
King L. M. & Schaal B. A. (1990): Genotypic variation within asexual lineages of *Taraxacum officinale*. – Proc. Natl. Acad. Sci. USA 87: 998–1002.
Kirschner J. & Kaplan Z. (2002): Taxonomic monographs in relation to global Red Lists. – Taxon 51: 155–158.
Kirschner J. & Štěpánek J. (1994): Clonality as a part of the evolution process in *Taraxacum*. – Folia Geobot. Phytotax. 29: 265–275.
Kirschner J. & Štěpánek J. (1996): Modes of speciation and evolution of sections in *Taraxacum*. – Folia Geobot. Phytotax. 31: 415–426.
Kirschner J. & Štěpánek J. (1997): Notes on the series of Taraxaca Exsiccata, Fasc. V–VII (Studies in *Taraxacum* 16). – Preslia 69: 35–58.
Kirschner J. & Štěpánek J. (1998): A monograph of *Taraxacum* sect. *Palustria*. – Botanický ústav AV ČR, Průhonice.
Kirschner J., Štěpánek J., Černý T., De Heer P. & van Dijk P. J. (2013): Available ex situ germplasm of the potential rubber crop *Taraxacum koksaghyz* belongs to a poor rubber producer, *T. brevicorniculatum* (*Compositae-Crepidinae*). – Genet. Resour. Crop Evol. 60: 455–471.
Klekowski E. J. (2003): Plant clonality, mutation, diplontic selection and mutational meltdown. – Biol. J. Linn. Soc. 79: 61–67.
Kraft T., Nybom H. & Werlemark G. (1996): DNA fingerprint variation in some blackberry species (*Rubus* subg. *Rubus*, *Rosaceae*). – Pl. Syst. Evol. 199: 93–108.
Kress W. J. & Erickson D. L. (2008): DNA barcodes: genes, genomics, and bioinformatics. – Proc. Natl. Acad. Sci. USA 105: 2761–2762.
Kress W. J., Wurdack K. J., Zimmer E., Weigt L. & Janzen D. H. (2005): Use of DNA barcodes to identify flowering plants. – Proc. Natl. Acad. Sci. USA 102: 8369–8374.
Kristiansen K., Cilieborg M., Drábková L., Jorgensen T., Petersen G. & Seberg O. (2005): DNA taxonomy: the riddle of *Oxychloë*. – Syst. Bot. 30: 284–289.
Lahaye R., van der Bank M., Bogarin D., Warner J., Pupulin F., Gigot G., Maurin O., Duthoit S., Barraclough T. G. & Savolainen V. (2008): DNA barcoding the floras of biodiversity hotspots. – Proc. Natl. Acad. Sci. USA 105: 2923–2928.
Lo E. Y. Y., Stafanović S., Ritland K. & Dickinson T. A. (2010): Fine-scale comparisons of genetic variability in seed families of asexually and sexually reproducing *Crataegus* (hawthorn; *Rosaceae*). – Am. J. Bot. 97: 1014–1024.
Loxdale H. D. & Lushai G. (2003): Rapid changes in clonal lines: the death of a 'sacred cow'. – Biol. J. Linn. Soc. 79: 3–16.
Lundevall C. F. & Øllgaard H. (1999): The genus *Taraxacum* in the Nordic and Baltic countries: types of all specific, subspecific and varietal taxa, including type locations and sectional belonging. – Preslia 71: 43–171.
Lushai G., Loxdale H. D. & Allen J. A. (2003): The dynamic clonal genome and its adaptive potential. – Biol. J. Linn. Soc. 79: 193–208.
Lyman J. C. & Ellstrand N. C. (1984): Clonal diversity in *Taraxacum officinale* (*Compositae*), an apomict. – Heredity 53: 1–10.
Madhou M., Normand F., Bahorun T. & Hormaza J. I. (2012): Fingerprinting and analysis of genetic diversity of litchi (*Litchi chinensis* Sonn.) accessions from different germplasm collections using microsatellite markers. – Tree Genet. Genomes 9: 387–396.
Majeský L., Vašut R. J., Kitner M. & Trávníček B. (2012): The pattern of genetic variability in apomictic clones of *Taraxacum officinale* indicates the alternation of asexual and sexual histories of apomicts. – PLoS One 7: e41868.
Menken S. B. J. & Morita T. (1989): Uniclonal population structure in the pentaploid obligate agamosperm *Taraxacum albidum* Dahlst. – Plant Spec. Biol. 4: 29–36.
Meyer C. P. & Paulay G. (2005): DNA barcoding: error rates based on comprehensive sampling. – PLoS Biol. 3: e422.

Mogie M. (1985): Morphological, developmental and electrophoretic variation within and between obligately apomictic *Taraxacum* species. – Biol. J. Linn. Soc. 24: 207–216.
Mráz P., Chrtek J. & Kirschner J. (2001): Genetic variation in the *Hieracium rohacsense* group (*Hieracium* sect. *Alpina*). – Phyton 41: 109–116.
Nei M., Tajima F. & Tateno Y. (1983): Accuracy of estimated phylogenetic trees from molecular data. – J. Mol. Evol. 19: 153–170.
Normark B. B., Judson O. P. & Moran N. A. (2003): Genomic signatures of ancient asexual lineages. – Biol. J. Linn. Soc. 79: 69–84.
Núñez Y., Fresno J., Torres V., Ponz F. & Gallego F. J. (2004): Practical use of microsatellite markers to manage *Vitis vinifera* germplasm: molecular identification of grapevine samples collected blindly in D. O. "El Bierzo" (Spain). – J. Hort. Sci. B. 79: 437–440.
Nybom H. (1996): DNA fingerprinting: a useful tool in the taxonomy of apomictic plant groups. – Folia Geobot. Phytotax. 31: 295–304.
Ozias-Akins P. & van Dijk P. J. (2007): Mendelian genetics of apomixis in plants. – Ann. Rev. Genet. 41: 509–37.
Paradis E. (2010): Pegas: an R package for population genetics with an integrated-modular approach. – Bioinformatics 26: 419–20.
Paradis E., Claude J. & Strimmer K. (2004): APE: Analyses of Phylogenetics and Evolution in R language. – Bioinformatics 20: 289–290.
Popescu A.-A., Huber K. T. & Paradis E. (2012): Ape 3.0: new tools for distance based phylogenetics and evolutionary analysis in R. – Bioinformatics 28: 1536–1537.
R Core Team (2014): R: a language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, URL: http://www.r-project.org.
Räsänen J. (2013): A review of *Taraxacum* (*Asteraceae*) in eastern Finland (Ladoga Karelia and North Karelia). – Mem. Soc. Fauna Fl. Fenn. 89: 139–182.
Raunkiaer C. (1903): Kimdannelse uden Befrugtning hos Mælkebøtte (*Taraxacum*) [Reproduction without fertilization in *Taraxacum*]. – Bot. Tidsskr. 25: 109–139.
Reisch C. (2004): Molecular differentiation between coexisting species of *Taraxacum* sect. *Erythrosperma* (*Asteraceae*) from populations in south-east and west Germany. – Bot. J. Linn. Soc. 145: 109–117.
Richards A. J. (1973): The origin of *Taraxacum* agamospecies. – Bot. J. Linn. Soc. 66: 189–211.
Roetman E. & Sterk A. A. (1986): Growth of microspecies of different sections of *Taraxacum* in The Netherlands. – Acta Bot. Neerl. 35: 5–22.
Rogstad S. H. (1992): Saturated NaCl-CTAB solution as a means of field preservation of leaves for DNA analyses. – Taxon 41: 701–708.
Rubinoff D., Cameron S. & Will K. (2006): A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. – J. Hered. 97: 581–594.
Saitou N. & Nei M. (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. – Mol. Biol. Evol. 4: 406–425.
Sass C., Little D. P., Stevenson D. W. & Specht C. D. (2007): DNA barcoding in the Cycadales: testing the potential of proposed barcoding markers for species identification of cycads. – PLoS One 2: e1154.
Sato K., Yamazaki T. & Iwatsubo Y. (2011): Cytogeography of *Taraxacum albidum* (*Asteraceae*) in Japan. – Cytologia 76: 201–212.
Seberg O. & Petersen G. (2009): How many loci does it take to DNA barcode a crocus? – PLoS One 4: e4598.
Solbrig O. T. & Simpson B. B. (1974): Components of regulation of a population of dandelions in Michigan. – J. Ecol. 62: 473–486.
Solbrig O. T. & Simpson B. B. (1977): A garden experiment on competition between biotypes of the common dandelion (*Taraxacum officinale*). – J. Ecol. 65: 417–430.
Sterk A. A., Groenhart M. C. & Mooren J. F. A. (1983): Aspects of the ecology of some microspercies of *Taraxacum* in the Netherlands. – Acta Bot. Neerl. 32: 385–415.
Sterk A. A. & Luteijn M. M. (1984): A study of the flowering phenology of *Taraxacum* microspecies in some biotypes in the Netherlands as observed during three successive years. – Acta Bot. Neerl. 33: 39–59.
Štorchová H., Chrtek J. jun., Bartish I. V., Tetera M., Kirschner J. & Štěpánek J. (2002): Genetic variation in agamospermous taxa of *Hieracium* sect. *Alpina* (*Compositae*) in the Tatry Mts (Slovakia). – Pl. Syst. Evol. 235: 1–17.
Tas I. C. & van Dijk P. J. (1999): Crosses between sexual and apomictic dandelions (*Taraxacum*). I. The inheritance of apomixis. – Heredity 83: 707–714.

Taylor R. J. (1987): Populational variation and biosystematic interpretations in weedy dandelions. – Bull. Torrey Bot. Club 114: 109–120.

Thomson M. J., Septiningsih E. M., Suwardjo F., Santoso T. J., Silitonga T. S. & McCouch S. R. (2007): Genetic diversity analysis of traditional and improved Indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. – TAG 114: 559–568.

Trávníček B., Kirschner J., Štěpánek J. & Vašut R. J. (2010): *Taraxacum* Wiggers – pampeliška (smetánka) [*Taraxacum* – Dandelion]. – In: Štěpánková J., Chrtek J. & Kaplan Z. (eds), Květena ČR [Flora of the Czech Republic] 8: 23–269, Academia, Praha.

Uhlemann I. (2003): Die Gattung *Taraxacum* (*Asteraceae*) im östlichen Deutschland. – Mitt. Flor. Kart. Sachsen-Anhalt, Sonderheft 2003: 1–136.

Van Baarlen P., van Dijk P. J., Hoekstra R. F. & de Jong H. J. (2000): Meiotic recombination in sexual diploid and apomictic triploid dandelions (*Taraxacum officinale* L.). – Genome 43: 827–835.

Van der Hulst R. G. M., Mes T. H. M., den Nijs J. C. M. & Bachmann K. (2000): Amplified fragment length polymorphism (AFLP) markers reveal that population structure of triploid dandelions (*Taraxacum officinale*) exhibits both clonality and recombination. – Mol. Ecol. 9: 1–8.

Van der Hulst R. G. M., Mes T. H. M., Falque M., Stam P., den Nijs J. C. M. & Bachmann K. (2003): Genetic structure of a population sample of apomictic dandelions. – Heredity 90: 326–335.

van Loenhoud P. J. & Duyts H. (1981): A comparative study of the germination ecology of some microspecies of *Taraxacum* Wigg. – Acta Bot. Neerl. 30: 161–182

van Oostrum H., Sterk A. A. & Wijsman H. J. W. (1985): Genetic variation in agamospermous microspecies of *Taraxacum* sect. *Erythrosperma* and *T.* sect. *Obliqua*. – Heredity 55: 223–228.

Vašut R. J., van Dijk P. J., Falque M., Trávníček B. & De Jong J. H. (2004): Development and characterization of nine new microsatellite markers in *Taraxacum* (*Asteraceae*). – Mol. Ecol. Notes 4: 645–648.

Verhoeven K. J. F., Jansen J. J., van Dijk P. J. & Biere A. (2010a): Stress-induced DNA methylation changes and their heritability in asexual dandelions. – New Phytol. 185: 1108–1118.

Verhoeven K. J. F., van Dijk P. J. & Biere A. (2010b): Changes in genomic methylation patterns during the formation of triploid asexual dandelion lineages. – Mol. Ecol. 19: 315–324.

Verhoeven K. J. F. & van Gurp T. P. (2012): Transgenerational effects of stress exposure on offspring phenotypes in apomictic dandelion. – PLoS One 7: e38605.

Vijverberg K., Van der Hulst R., Lindhout P. & van Dijk P. (2004): A genetic linkage map of the diplosporous chromosomal region in *Taraxacum officinale* (common dandelion; *Asteraceae*). – Theor. Appl. Genet. 108: 725–732.

Vilgalys R. (2003): Taxonomic misidentification in public DNA databases. – New Phytol. 160: 4–5.

von Hofsten C. G. (1954): Studier över släktet *Taraxacum* Wigg. med särskild hänvisning till gruppen *Vulgaria* Dt i Skandinavien [Study of the genus *Taraxacum* Wigg. with special interest in the group *Vulgaria* Dt in Scandinavia]. – LTs Förlag, Stockholm.

Widén B., Cronberg N. & Widén M. (1994): Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants, a literature survey. – Folia Geobot. Phytotax. 29: 245–263.

Záveská Drábková L. & Kirschner J. (2013): *Oreojuncus*, a new genus in the *Juncaceae*. – Preslia 85: 483–503.

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