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Biosystematic studies in the family *Cyperaceae*

Ph.D. Thesis

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České Budějovice 2013

This thesis should be cited as:

Košnar J., 2013. Biosystematic studies in the family *Cyperaceae*. Ph.D. Thesis Series, No. 7. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, 139 pp.

Annotation

The thesis was focused on the microevolutionary mechanisms that contribute to morphological diversity in selected members of the sedge family (*Cyperaceae*). Natural hybridization, evidenced from both morphological characters and molecular markers, was revealed to be a potentially important source of diversification in the tropical spikerushes of *Eleocharis* subgenus *Limnochloa*. High levels of phenotypic plasticity of clonal growth but rare genetic (ecotypic) differentiation among contrasting morphotypes were found in the polymorphic species *Carex nigra*, which implied that taxonomic splitting of the species was unreasonable.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

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České Budějovice, 9.2.2013 .....

Jan Košnar

Financial support

The research presented in this thesis was supported by the Ministry of Education, Youth and Sports of the Czech Republic (grant MSM6007665801), the Academy of Sciences of the Czech Republic (grant AV0Z60050516), the National Science Foundation (grants 0089211 and 0516159), and the Mattoni Awards for Studies of Biodiversity and Conservation Biology 2004 and 2005.

Acknowledgements

I thank to everybody who helped me during the years of work on my thesis. For interest in my work, wise advice, patience, and selfless support I am especially grateful to Milan Štech, Jiří Košnar, Ester Ekrtová, Libor Ekrt, Miroslava Herbstová, Eliška Rejmánková, Petr Šmilauer, Marie Šmilauerová, Jiřina Laburdová, Jitka Laburdová, my mother Miloslava Košnarová, my father Jan Košnar, my grandfather Bohuslav Šauer, my grandmother Marie Šauerová, and my grandaunt Jaroslava Sílová.

I dedicate this thesis to the memory of my grandfather Bohuslav Šauer.

List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

I. **Košnar J.**, Košnar J., Herbstová M., Macek P., Rejmánková E., Štech M., 2010. Natural hybridization in tropical spikerushes of *Eleocharis* subgenus *Limnochloa* (*Cyperaceae*): evidence from morphology and DNA markers. American Journal of Botany 97, 1229–1240 (IF=2.664).

Jan Košnar collected part of samples in the field, carried out morphometric analysis, participated in molecular analyses, analysed most of the data, wrote the draft of the manuscript, and edited the comments of the co-authors and reviewers.

II. **Košnar J.**, Štech M., Koutecký P., 2012: Environmental control of clonal growth in *Carex nigra:* What can be masked under the name *Carex nigra* subsp. *juncella* in the Czech Republic. Flora 207, 294–302 (IF=1.639).

Jan Košnar collected data and plant material in the field, participated on isozyme analyses, carried out the cultivation experiments, analysed data, wrote the draft of the manuscript, and edited the comments of the co-authors and reviewers.

III. **Košnar J.**, Košnar J., Štech M.: Morphological and DNA variability of *Carex nigra* in Czechia and northern Europe provides no support for taxonomic splitting [manuscript].

Jan Košnar collected most of the plant material in the field, carried out morphometric analysis, participated on molecular analyses, analysed data, wrote the draft of the manuscript, and edited the comments of the co-authors.

Contents

General introduction

General introduction

With more than 5000 species so far described, sedge family (*Cyperaceae* Juss.) is the eleventh largest family of the angiosperms and the third largest one of the monocotyledons (Stevens 2012). Plants assignable to *Cyperaceae* have been recognized since Antiquity (Blackstock 2007) and this suggests that at least some members of the family has long been regarded as worthy of people's attention. Although *Cyperaceae* are sometimes considered to be plants of minimum practical importance, particularly in comparison with *Poaceae* Barnhart, more detailed inspection proved that such a view is misguiding. As reviewed by Simpson (2008), there is actually a wide range of historical or current uses of *Cyperaceae* by man. Sedges have provided materials (*Cyperus papyrus* L. is the illustrious example) or food for domestic animals and even for people (e.g., edible rhizome corms of *Eleocharis dulcis* (Burm. f.) Hensch.). Some species are interesting for horticulture as ornamental plants, others are used for consolidation of soils endangered by erosion or for revegetation of extremely infertile sites. In addition, several species of *Cyperaceae* (e.g., *Cyperus rotundus* L. or *C. esculentus* L.) are serious agricultural weeds.

Apart from the purely practical aspects, important role of *Cyperaceae* in plant communities and whole ecosystems is undisputable. Members of the family occur in various habitats from the tropical to the Arctic regions and particularly often they dominate wetlands (Simpson et al. 2003). On the other hand, many species of *Cyperaceae* are competitively inferior, restricted to vulnerable habitats, and thus rare, endangered, and important from the viewpoint of nature conservation. For ecologically oriented research, *Cyperaceae* are very often useful as phytoindicators of site properties (Simpson et al. 2003), because many species of the family possess relatively narrow ecological amplitudes in respect to environmental factors such as soil acidity or water chemistry.

However, *Cyperaceae* are also distinguished by strongly derived morphology, particularly by inconspicuous and reduced generative organs, which make correct determination of species often difficult (Bruhl

1995, Muasya et al. 1998). The ostensible lack of characters for circumscription of taxa and high diversity of morphological forms in *Cyperaceae* are challenging. And perhaps it is not surprising that the endeavour to understand patterns and causes of biological variability in *Cyperaceae* is one of constant directions of plant biosystematic research.

OUTLINE OF THE FAMILY

Cyperaceae are graminoid monocotyledonous herbs with vaginate leaves (very often arranged in three rows as opposed to the two-ranked leaves of superficially similar *Poaceae* and *Juncaceae* Juss.) and usually reduced wind-pollinated flowers; fruits are achenes containing one seed (Ball and Reznicek 2002, Stevens 2012). Given the enormous number of species, almost no morphological character can be emphasized as a perfect synapomorphy of the family. As summarized by Bruhl (1995) and Ball and Reznicek (2002), among-taxon variability exists in life span, growth form, vegetative morphology, and floral characters. For instance, although the stems are very often triquetrous, other shapes (terete, compressed, or multangular) are also present; leaves may be two-ranked or bladeless; inflorescences are either unbranched or branched to various orders; several types of prophylls can be developed; flowers are either bisexual or unisexual; perianths are either present or completely missing; and style is either entire, bifid, trifid, or quadrifid. Common feature of all *Cyperaceae* is the presence of pollen pseudomonads or monads (instead of pollen tetrads typical for *Juncaceae*): only one of the microspores produced by meiosis of a pollen mother cell completes development into a pollen grain, whereas the other three microspores abort (Simpson et al. 2003).

Several phylogenetic studies based on sequences of plastid DNA (Muasya et. al. 1998, Simpson et al. 2007, Muasya et al. 2009) confirmed monophyly of *Cyperaceae* and their sister relationship to *Juncaceae*. The molecular studies also re-examined the infrafamiliar classification of *Cyperaceae* and suggested distinguishing two currently recognized

monophyletic subfamilies, *Mapanioideae* C. B. Clarke and *Cyperoideae* Suess.

Mapanioideae is a small, exclusively tropical and subtropical group with less than 200 species. The subfamily is characterized by specific complex inflorescences. The terminal inflorescence branches bear compact spike-like structures formed by imbricately arranged glumes (bracts). Fertile glumes subtend either bisexual reproductive units consisting of one distal pistil and proximal stamens subtended by scales (bracts) or male reproductive units with aborted gynoecium (Richards et al. 2006, Lunkai et al. 2010).

Cyperoideae are distributed worldwide, include all large genera of *Cyperaceae*, and contain more than 5000 species (Stevens 2012). Although the morphology of mature floral parts is very diverse within such large group, recent studies indicate that early ontogeny of generative structures is common for all members of the subfamily (Richards et al. 2006, Vrijdaghs et al. 2009) and involves formation of primordia of stamens, gynoecium, and perianth bristles (i. e., perfect hermaphroditic flowers; as these are typical for the genus *Scirpus* L. among others, the term "scirpoid ontogenetic pattern" was adopted for this presumably basal developmental scheme). Unisexual or perianth-lacking flowers present in many other cyperoids (particularly in the largest genus *Carex* L.) are then interpreted as derived from the scirpoid pattern (Vrijdaghs et al. 2009).

Total species richness of the family *Cyperaceae* is very high but also very unevenly distributed among genera. More than half of the 98 genera of *Cyperaceae* are small (each containing less than six species), and only in seven genera the number of species exceeds 200 (Muasya et al. 2009, Stevens 2012). These large genera are *Cyperus* L., *Rhynchospora* Vahl, *Scirpus*, *Fimbristylis* Vahl, *Scleria* P. J. Bergius, *Eleocharis* R. Br., and *Carex* (Roalson et al. 2010, Stevens 2012). The latter two will be characterized in more details because their representatives were included in the studies constituting this thesis.

Genus *Eleocharis*

Eleocharis encompasses more than 250 species (Roalson et al. 2010) with superficially uniform morphology. Leaves in *Eleocharis* are bladeless, reduced to membranous sheaths, and the culm is therefore the most important photosynthetic organ; inflorescence is formed by a sole unbranched spike consisting of scale-like bracts (glumes) subtending bisexual flowers with perianth bristles; and the base of a style (stylopodium) persists on a mature achene as a tubercle of various shape and size (Smith et al. 2002).

The most recent infrageneric classification, proposed by González-Elizondo and Peterson (1997), divided *Eleocharis* into four subgenera on the basis of such morphological criteria as relative width of a spike, length and width of internodes of spike axis, number and density of flowers in a spike, presence of flowers at the basal glumes, structure of glumes, shape and ornamentation of achenes, and size and shape of a tubercle. Following brief outlines of the subgenera are compiled from González-Elizondo and Peterson (1997), Smith et al. (2002), and Hinchliff and Roalson (2009).

(1) Subgenus *Scirpidium* (Nees) Kukkonen (c. 12 species), characterized by usually fertile basal glumes and terete achenes, is a relatively small but widespread group, represented on both hemispheres, from tropical to boreal zone. The subgenus includes, among others, a broadly distributed species *Eleocharis acicularis* (L.) R. et Sch. (2) Subgenus *Zinserlingia* T. V. Egorova (c. 8 species) associates the plants with sparse, few-flowered spikes and inconspicuous tubercles (more or less blending with apical parts of achenes). Members of the subgenus occur in temperate and boreal regions of both hemispheres and include, e.g., the Holarctic species *E. quinqueflora* (F. X. Hartmann) O. Schwarz. (3) Subgenus *Eleocharis* R. Br. (c. 160 species) comprises the plants with spikes which are markedly wider than culms and contain numerous, usually membranous fertile glumes. The subgenus has a cosmopolitan distribution and involves, for instance, the taxonomically difficult groups of *E. uniglumis* (Link) Schult. and *E. palustris* (L.) R. et Sch. (4) Finally, the subgenus *Limnochloa* (P. Beauv. et Lestib.) Torr. (c. 30 species) denotes the plants with spikes as wide as culms and dense, numerous, and

tough glumes. The group (encompassing, e.g., edible *E. dulcis*) is predominantly tropical and subtropical. Morphological diversity and species richness of the subgenus *Limnochloa* still has not been fully recognized and evaluated, as evidenced by increasing number of newly described taxa (e.g., Trevisan and Boldrini 2006, Rosen and Hatch 2007, Hinchliff et al. 2010a).

According to the phylogenetic studies based on sequences of internal transcribed spacers (ITS) of the nuclear ribosomal DNA and sequences of chloroplast DNA, the genus *Eleocharis* is paraphyletic and should include also monotypic genera *Websteria* S. H. Wright, *Chillania* Roiv., and *Egleria* L. T. Eiten (Hinchliff et al. 2010b). The subgenera *Zinserlingia* and *Scirpidium* are nearly monophyletic but the subgenus *Eleocharis* is polyphyletic (Roalson and Friar 2000, Roalson et al. 2010). The subgenus *Limnochloa* seems to be a monophyletic group, supposedly basal, sister to the rest of the genus (Roalson et al. 2010).

Genus *Carex*

Carex, comprising about 2000 species, is by far the largest genus of the family *Cyperaceae* and even the fourth largest genus of angiosperms (Stevens 2012). It is distributed almost worldwide, being relatively poorly represented in the tropical lowlands and subtropical deserts, and completely absent only in Antarctica. Most of *Carex* species occur in the temperate, boreal, and arctic zones of the northern hemisphere, with the greatest species diversity in North America and East Asia (Ball 1990). In Europe, 222 *Carex* species are present (Koopman 2011).

Characteristic morphological structure of the genus *Carex* is a completely closed perigynium (utricle), a modified prophyll with connate margins, coating ovary. It is currently accepted that the utricle does not enclose a single female flower (reduced to ovary), but a reduced spikelet, originally bisexual, of which just one female flower and a remnant of axis, termed rachilla, are maintained (Standley 1985). The rachilla in *Carex* almost never exceeds the margin of perigynium (but, e.g., *C. microglochin* Wahlenb. is one of the exceptions with a long rachilla protruding from the perigynium – Reznicek 1990). Closed

perigynium is not exclusive for *Carex* but is present also in closely related genera *Uncinia* Pers. and *Cymophyllus* Mackenzie. *Uncinia* differs from *Carex* in having well-devoloped, long rachilla; *Cymophyllus* (which is a monotypic genus) has short rachilla but differs from *Carex* in vegetative morphology (very broad flat leaves), invariable presence of inflorescence consisting of one androgynous spike only, and entomophily as the exclusive way of pollination (Reznicek 1990). Open perigynia, with incompletely connate or free margins, are present in the genera *Kobresia* Willd. and *Schoenoxiphium* Nees (Reznicek 1990), forming together with *Carex*, *Uncinia*, and *Cymophyllus* the tribe *Caricae* Dumort.

Kükenthal (1909) provided the most comprehensive infrageneric classification of *Carex* based on morphological features, with four distinguished subgenera. (1) Subgenus *Psyllophora* (Degl.) Peterm. (syn. *Primocarex* Kük.) denotes unispicate plants (with inflorescence consisting of one spike only, either bisexual or unisexual). (2) Subgenus *Vignea* (P. Beauv.) Nees involves plants with multiple sessile bisexual spikes lacking cladoprophylls (tubular bracts enclosing the base of lateral inflorescence axes). (3) Subgenus *Carex* L. comprises plants with multiple pedunculate unisexual spikes and cladoprophylls; less often, some species of the subgenus possess bisexual and unisexual (female) spikes within an inflorescence. (4) Members of the subgenus *Vigneastra* Tuck. (syn. *Indocarex* Baill.) have multiple pedunculate lateral inflorescence units (paracladia *sensu* Molina et al. 2012), each consisting of several bisexual spikes with a perigynium-like prophyll at the base; tubular cladoprophylls at the bases of the paracladia are also present.

The morphologically defined subgenera of *Carex*, as well as the other supraspecific taxa of the tribe *Cariceae*, were subjected to several revisions using a molecular phylogenetic approach. These studies, based on sequences of nuclear and chloroplast DNA, revealed that some of the morphologically circumscribed supraspecific taxa within *Caricae* represent natural, monophyletic lineages, whereas others do not. The tribe *Cariceae* as a whole appears to be a monophyletic group (Muasya et al. 2009) and so does the genus *Uncinia* (Starr and Ford 2009, Waterway et al. 2009). The unispicate sedges (*Psyllophora*), on the other hand, are

clearly polyphyletic and occur in several different clades across *Cariceae* (Starr and Ford 2009, Waterway et al. 2009). Some of *Psyllophora* species were found to be more related to *Schoenoxiphium*, whereas others to *Uncinia*, *Cymophyllus*, and *Kobresia* (which also appears to be a polyphyletic group) (Starr and Ford 2009, Waterway et al. 2009). Thus the genus *Carex* in the traditional morphological delimitation is paraphyletic. None of the Kükenthal's subgenera of *Carex* are monophyletic. The subgenus *Vignea* is polyphyletic but removal of a small number of morphologically distinct tristigmatic species (e.g., *Carex baldensis* L. and *C. curvula* All.) and inclusion of some *Psyllophora* species (e.g., *Carex dioica* L.) would make it monophyletic (Starr and Ford 2009). The subgenus *Vigneastra* is clearly polyphyletic, but the clade consisting of the subgenera *Carex* and *Vigneastra* and some *Psyllophora* species appears to be monophyletic (Starr and Ford 2009).

The subgenera of *Carex* are further divided into numerous sections circumscribed on the basis of morphological characters. Molecular phylogenetic studies ascertained some of the sections to be monophyletic whereas many others to be artificial groups (Starr and Ford 2009). Section *Phacocystis* Dumort. is one of the largest in the genus and comprises about 90 species (Dragon and Barrington 2009), *Carex nigra* (L.) Reich. being one of them. The section appears to be non-monophyletic (Roalson et al. 2001, Hendrichs et al. 2004) but is quite distinctive morphologically from the remainder of the subgenus *Carex* (particularly by the combination of unbranched unisexual spikes, reduced sheaths of inflorescence bracts, dorsiventraly compressed utricles, and bifid styles) and is therefore sometimes treated in a separate subgenus *Kreczetoviczia* Egor. (Egorova 1999). Although a frequent subject of systematic research (e.g., Faulkner 1972, 1973, Standley 1985, Volkova et al. 2008, Jiménez-Mejías et al. 2011), the section *Phacocystis* can still be considered as taxonomically critical and delimitation of some species within the section is controversial.

POSSIBLE SOURCES OF MORPHOLOGICAL DIVERSITY

Variability of genome and chromosomes

Cyperaceae possess several unusual cytogenetic features. In particular, the chromosomes are holocentric, i.e., microtubules of the mitotic spindle can be attached to any part of a chromosome (as opposed to usual monocentric chromosomes in which the microtubules can be attached to a relatively restricted region of centromere only). Even the broken parts of the holocentric chromosomes retain their kinetic activity and are not lost during cell divisions. As a result, the nuclear genome of *Cyperaceae* is very variable and prone to frequent structural reorganizations by fissions and fusion of the holocentric chromosomes (Faulkner 1972).

Studies of the karyotype evolution in holocentric plants, conducted mainly in the model genera *Carex* and *Eleocharis*, represent a dynamic part of current plant biosystematics. It is generally accepted that the genome in the genus *Carex* evolves particularly by fissions (agmatoploidy) and fusions (symploidy) of chromosomes (Faulkner 1972, Hipp et al. 2009, Lipnerová et al. 2012). Unlike in most angiosperms, multiplication of whole chromosome set (polyploidy) is a relatively rare phenomenon in *Carex* (Hipp et al. 2009), and particularly in the subgenus *Vignea* (Lipnerová et al. 2012). The chromosomal fissions in *Carex* are probably accompanied by losts of repetitive DNA, whereas the proliferation of repetitive DNA is connected with the fusions; however, in some sections of the subgenus *Carex*, including section *Phacocystis*, increase in chromosome number (fissions) does not reduce DNA content (Lipnerová et al. 2012). Anyway, it still has not been clarified whether the chromosomal variability is a cause or rather a consequence of species diversity in *Cyperaceae* (Hipp et al. 2009). Interesting in this respect may be the notion of Faulkner (1972), who found in *Carex nigra* no correlation between morphological variability (which was high) and chromosome number variability (which was relatively low in comparison with other closely related species).

In contrast to other *Cyperaceae*, numerous taxa of *Eleocharis* are polyploids (Bureš 1998, Yano et al. 2004). However, polyploidization in *Eleocharis* is probably an evolutionary novelty of phylogenetically younger clades, since the putatively basal groups (*Limnochloa* and *Zinserlingia*) seem to display the pattern common to other *Cyperaceae*, i.e., small genome sizes associated with high chromosome numbers (Zedek et al. 2010). Proliferation of long terminal repeat (LTR) retrotransposons was shown to be responsible for increased chromosome size of phylogenetically younger lineages of *Eleocharis* and it was hypothesized that the activity of the retrotransposons may create genetic variation necessary for adaptive radiation (Zedek et al. 2010).

Natural hybridization

Natural hybridization is a spontaneous crossing of individuals belonging to populations differing in at least one hereditary trait (Arnold 1999). This definition involves crosses among infraspecific taxa as well but, as species has generally been taken for basic taxonomic and evolutionary unit, particular attention has always been paid to interspecific crosses. Since the early generations of hybrid offspring are usually less viable or less fertile than their parents (Arnold et al. 1999), hybridization was sometimes regarded as an anomal trespassing of limits of well established species and was thought to have minimum evolutionary significance. Existence of successful (viable and fertile) interspecific hybrids was then regarded as a proof of unsuitable delimitation of hybridizing species and merger of such species was usually proposed (e.g., Schmid 1983).

Apparent biological obstacles to hybridization of well-established species indeed exist, which is actually necessarry for the species to remain distinct. The obstacles to hybridization reflect the fact that the extant species possess combinations of traits (co-adapted gene complexes *sensu* Hufford and Mazer 2003) that are advantageous under natural selection. Alterations in the co-adapted gene complexes are often disadvatageous, and thus the species evolved various isolating mechanisms impeding among-species gene flow and decreasing frequency of unwarranted

recombinations. These mechanisms include phenological differences among sympatric species, dominance of conspecific pollen in pollen competition (Arnold 1999 and references therein), sterility of F_1 hybrid progeny, and decreased survival of hybrids in parental habitats (outbreeding depression).

However, substantial body of evidence suggests that hybridization is one of the most important mechanisms of diversification and speciation in vascular plants. As stressed by Arnold (1999), the barriers to successful hybridization are buffered by frequent possibilities to hybridization events. Thus even though the probability of successful interspecific crosses is often very low, the absolute number of successful hybridization events can be high. Although nearly all hybrid genotypes in a progeny may be less fit than parents, some of new recombinant variants may be, in contrary, more fit (display hybrid vigour). Positive selection of such variants will then tend to maintenance of newly established co-adapted gene complexes, to reproductive isolation, and thus formation of well defined new species of hybridogeneous origin.

Speciation by hybridization questions fundamental cladistic scheme recognizing entirely monophyletic species originating from unique events of divergence within ancestral populations (Arnold 1999). Not only the hybridogeneous species arise from recombination, instead of divergence, of ancestral lineages, but these species can also be of recurrent (polytopic, multiple) origin (e.g. Soltis and Soltis 1991).

Many well documented natural hybrids in *Cyperaceae* belong to the large genus *Carex* (e.g., Cayouette and Catling 1992, Ford and Ball 1993, Waterway 1990, Waterway 1994) but hybrids are known also from other genera, such as *Cyperus* (Carter and Bryson 1991), *Schoenus* L. (Scotti et al. 2002), *Scirpus* (MacKay et al. 2010), *Schoenoplectus* (Rchb.) Palla (De Greef and Triest 1999, Fay et al. 2003), and *Eleocharis* (Lewis and Johns 1961, Strandhede 1965, Bureš 1998). Some of *Carex* species are apparently of hybridogeneous origin (Faulkner 1972, Volkova et al. 2008, Korpelainen et al. 2010).

Hybridization in *Cyperaceae* is usually first detected from morphological intermediacy, in well documented cases then corroborated

by findigs of reduced fertility, irregular chromosome pairing, and additivity of parent-specific molecular markers in the hybrids. Given the low number of conspicous differences among many taxa of *Cyperaceae* and the fact that morphological intermediacy is not very reliable indication of interspecific crossing (Rieseberg and Ellstrand 1993), the real extent of hybridization in the family may be largely unrecognized.

Phenotypic plasticity

Phenotypic plasticity is the ability of a genotype to produce more than one distinct phenotype in response to environmental conditions (Pfennig et al. 2010). Thus the plastic character can display variations that are not genetically based, and the extent of morphological variability in such case is wider than the extent of genetic variability. The plasticity itself, however, seems to be a genetically conditioned trait (de Jong 2005). Plastic genotypes have wider ecological niche and therefore plasticity is a generally advantageous trait unless there are either physiological or environmental constraints that make it impossible or too demanding (de Jong 2005, Valladares et al. 2007).

The plasticity may delay or prevent evolutionary change since it allows genotype to produce a phenotype that is not eliminated after natural selection (de Jong 2005). Nevertheless, although sometimes regarded as an alternative solution to genetically fixed adaptation, the plasticity does not completely exclude genetic differentiation among populations of a species (de Jong 2005). Widening of species ecological niche due to plasticity actually can expose populations to such new environments where selective pressures may strictly favour particular genotypes only (Bennigton et al. 2012). In addition, genotypes are not subjected to selective forces directly but through selection of phenotypes. West-Eberhard (2003) deduced that extrinsically induced, selectively non-neutral, and non-hereditary (i.e., plastic) phenotypic change at the level of individual may have represented a necessary condition for changes of allele frequencies and genetic divergence at the level of populations.

In *Cyperaceae*, response to environment may be solely plastic (Smythe and Hutchinson 1989) or may involve combination of plastic and

genetically based variations (Stenström et al. 2002). There is some indication that plasticity may be particularly important in the competitively weak species forming small populations in spatially and temporally unstable habitats. Under such circumstances, genetic drift depauperates population genetic variability and the genotypes capable of wider range of response to largely unpredictable and fluctuating environmental conditions gain greater probability of survival than the specialized, less plastic genotypes (Schmid 1984).

Ecotypic differentiation

Ecotypes are the groups of populations belonging to one species but adapted to different habitats within the distribution area of the species. In contrast to the cases of phenotypic plasticity, the differences among ecotypes are hereditary and genetically based.

The probability of structuring of a species population into ecotypes (subpopulations) increases with the strength of selection pressure. Distinct ecotypes are thus often composed of individuals highly adapted to a particular habitat and are to be found in extreme environment, such as serpentine rocks (Sambatti and Rice 2006), high altitudes (e.g., Geburek et al. 2008), or Arctic habitats (e.g., Bennigton et al. 2012). In less extreme environments, less severe selection against alternative phenotypes results in largely or completely free gene-flow within and among conspecific populations and genetic differentiation of subpopulations can be prevented. Moreover, phenotypic plasticity is usually more advantageous than a genetic fixation of a phenotype in more productive (less extreme) habitats (Bennigton et al. 2012).

Species populations in one type of environment can be selected for a different ecological strategy than those (of the same species) in the other environment, which may result in considerable morphological diversification. Sambatti and Rice (2006) showed that plants of serpentine ecotype of *Helianthus exilis* A. Gray were selected for drought stress tolerance, whereas the riparian ecotypes for competitive ability. Thus the serpentine ecotype was distinguished out by small height, reduced leaf

area, and rich root system, whereas the plants of the riparian ecotype were higher, possessed broader leaves, and shallow root system.

Ecotypic differentiation, if followed by partial or complete reproductive isolation and subsequent accumulation of further differences among the ecotypes, may stand at the beginning of speciation (Via 2009). Similarly to the speciation by hybridization, the species arising from the ecotypic differentiation need not to be monophyletic and can have multiple origin, since the environmental stimuli causing the initial divergence of an ancestral population can occur repeatedly and polytopically (Levin 2001).

Genetic differentiation due to disruptive selection is easier to be experimentally revealed in the organisms producing many generations over a short time period. Conversely, in the long-lived perennials, such as many *Cyperaceae*, genetic differentiation may require substantially longer time to be established or detected (Bennington et al. 2012). Nonetheless, cases of ecotypic differentiation in *Cyperaceae* were reliably proven and involve, for instance, populations of *Carex aquatilis* Wahlenb. (Chapin and Chapin 1981) or *Eriophorum vaginatu*m L. (Bennington et al. 2012) from high latitudes. These populations (from sites with relatively most severe conditions) were in comparison to the southern ones less plastic but better adapted to survival in their habitats.

AIMS OF THE THESIS

The thesis attempted to contribute to understanding mechanisms that generate phenotypic (morphological) diversity in some taxonomically challenging members of the family *Cyperaceae*. The study groups showed conspicous levels of morphological variability. In all partial studies of the thesis, the common aim was to answer the following questions: Is observed morphological variability underlaid by hereditary genetic differences that could stand at the begining of new evolutionary lineages? Or does it represent rather responses of morphologically plastic genotypes

to environmental conditions, i.e., reversible non-hereditary variations not indicating evolutionary change?

The first part of the thesis deals with *Eleocharis* subgenus *Limnochloa*, a group with extraordinarily diversified vegetative morphology (stem architecture). Field observations revealed existence of *Limnochloa* morphotypes with stem architecture not corresponding to any of currently known species. Searching for the origin of such morphotypes, more or less morphologically intermediary among the known species, led into the study of natural interspecific hybridization, a phenomenon sometimes suggested but so far not evidenced to occur in *Eleocharis* subgenus *Limnochloa* (paper I).

The remaing two parts of the thesis are devoted to a widespread member of the problematic section *Phacocystis*: *Carex nigra*, a highly polymorphic species with unresolved taxonomy. Variability of growth forms of *C. nigra*, from loose rhizomatous to dense caespitose, brought about description of several taxa, either on infraspecific or even specific level. Adequacy of such taxonomic treatments was examined by testing the role of morphological plasticity and ecotypic differentiation among contrasting growth forms (paper II), by evaluating the reliability of the morphological characters used for the circumscription of the traditionally distinguished taxa and by assessing genetic differentiation of these taxa (paper III).

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I.

Natural hybridization in tropical spikerushes of *Eleocharis* subgenus *Limnochloa* (*Cyperaceae*): evidence from morphology and DNA markers

American Journal of Botany 97, 1229–1240

Natural hybridization in tropical spikerushes of *Eleocharis* subgenus *Limnochloa* (*Cyperaceae*): evidence from morphology and DNA markers

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ABSTRACT

- *Premise of the study:* Natural hybridization represents an important force driving plant evolution and affecting community structure and functioning. Hybridization may be overlooked, however, among morphologically highly uniform congeners. An excellent example of such a group is *Eleocharis* subgenus *Limnochloa*, which has no reliably proven hybrids. Does this reflect biological barriers to interspecific crosses or difficulties in detecting the hybrids? We tested the hypothesis that hybridization occurs among sympatric *Eleocharis cellulosa*, *E. interstincta* and *E. mutata* in northern Belize, Central America.

- *Methods:* Morphometric study (407 plants) was followed by examination of inter-simple sequence repeat (ISSR) polymorphism (44 plants) and ITS sequence variation (33 plants).
- *Key Results:* Two putatively hybrid morphotypes were discerned – *E. cellulosa*-resembling and *E. interstincta*-resembling. DNA markers of *E. cellulosa* and *E. interstincta* displayed additive constitution in plants from one *E. cellulosa*-resembling population only. The other putatively hybrid populations contained ISSR and ITS markers of the species they resembled morphologically, several unique ISSR markers, and ITS sequences of an undescribed South American *Limnochloa* entity. DNA markers of *E. mutata* were absent in the putative hybrids.
- *Conclusions:* Simultaneous use of various types of molecular markers can overcome many pitfalls of investigations concerning hybridization among closely related and morphologically similar species. Northern Belize represents a hybrid zone of *E. cellulosa* and *E. interstincta.* A third participant in the hybridization events occurring in this zone is an unknown *Limnochloa* lineage but is not *E. mutata*. Interspecific hybridization may play a significant role in the diversification of *Eleocharis*.

Key words: Belize; *Cyperaceae*; DNA markers; *Eleocharis*; hybridization; ISSR; ITS; *Limnochloa*; molecular cloning; morphometrics.

Publikace vyšla tiskem v časopise American Journal of Botany.

II.

Environmental control of clonal growth in *Carex nigra*: What can be masked under the name *Carex nigra* subsp. *juncella* in the Czech Republic?

Flora 207, 294–302

Environmental control of clonal growth in *Carex nigra*: what can be masked under the name *Carex nigra* subsp. *juncella* in the Czech Republic?

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ABSTRACT

Carex nigra plants forming elevated dense tussocks are often named *C. nigra* subsp. *juncella*, as opposed to rhizomatous *C. nigra* subsp. *nigra*. It is uncertain, however, whether the cespitose growth form is a hereditary trait useful for definition of the distinct taxon or a site modification of little taxonomic value. We used vegetation analyses (phytosociological relevés) to reveal main patterns in ecological demands of the cespitose *C. nigra* plants in the Czech Republic, and three cultivation experiments to assess changes in clonal growth of *C. nigra* under various environmental conditions. In the field the cespitose *C. nigra* plants were typically found in abandoned wet meadows near open water, whereas the rhizomatous morphotypes frequently occurred also in regularly mown wet meadows and in peat bogs. The cespitose growth form disappeared in the cultivations, and the rhizome system responded plastically to immediate environmental stimuli. Number of rhizome branches and mean rhizome length decreased after defoliation of aboveground parts and denudation of belowground parts, whereas increased due to inundation. In the population from the cold site in high altitude (Modrava, Šumava Mts.), however, the originally cespitose plants repeatedly produced shorter and less numerous rhizome branches than the rhizomatous plants cultivated in the same conditions. This suggests ecotypic (genetic) differentiation in some populations of *C. nigra*, driven by environmental selection for more

compact growth form in climatically severe sites. The cespitose *C. nigra* plants thus arise polytopically, by different mechanisms. The growth form itself therefore cannot serve as the character reliably delimiting *C. nigra* subsp. *juncella* as the distinct taxon.

Keywords: cespitose morphotype, cultivation experiments, ecotype, plasticity, polymorphic taxa, rhizomatous sedge

III.

Morphological and DNA variability of *Carex nigra* in Czechia and northern Europe provides no support for taxonomic splitting

Manuscript

Morphological and DNA variability of *Carex nigra* in Czechia and northern Europe provides no support for taxonomic splitting

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ABSTRACT

Morphological and genetic variability of *Carex nigra* from low and medium altitudes (c. 10–1100 m) of central (Czechia) and northern Europe (Sweden, Norway, Finland, and Russia) was studied to evaluate the degree of differentiation among four taxa traditionally recognized in this geographic and altitudinal range: *C. nigra* subsp. *nigra*, *C. n.* subsp. *recta*, *C. n.* subsp. *tornata*, and *C. n.* subsp. *juncella*. Morphometric study involving 268 plants was accompanied by the analysis of ISSR polymorphism in 103 samples. Both the methods failed to find any discontinuities among the taxa. The described taxa were widely or completely overlapping, referred to only a part of overall morphological variability of *C. nigra*, and did not form any genetically distinct groups. We particularly conclude that the recognition of densely cespitose narrow-leaved plants as a distinct taxon (*C. nigra* subsp. *juncella* or *C. juncella*) should be avoided even in the northern Europe. We did not find any support for distinguishing any taxa even at a varietal level.

Keywords: *Cyperaceae*, ecotype, ISSR, morphometric analysis, morphological plasticity, species, variety

INTRODUCTION

In the genus *Carex* L., morphology of rhizome system and a resulting growth form represent important taxonomic characters (Kukkonen and Toivonen 1988). Some *Carex* species possess a genetically fixed production of long extravaginal rhizome branches (creeping rhizomes) and thus achieve loose growth form (e. g., *Carex dioca* L., *C. disticha* Huds., *C. acutiformis* Ehrh.), whereas others almost or completely lack the creeping rhizomes and grow in dense clumps or elevated tussocks (e. g., *Carex davalliana* Sm., *C. appropinquata* Schum., *C. elata* All.). On the other hand, a widespread wetland sedge *Carex nigra* (L.) Reich. can serve as an example of a species whose rhizome system is markedly variable. It displays a continuous range of growth forms found in the genus, from the loosely rhizomatous to the compact dense tussocks.

Variability of the growth forms in *C. nigra* attracted a taxonomic attention. The nominate subsp. *nigra* is represented by the plants of lower growth with frequent long rhizomes (named *C. nigra* subsp. *nigra*). Several other taxa (for simplification we use the rank of subspecies for them throughout the text) were described with respect to variation in the production of creeping rhizomes, plant height, leaf width, and the length and density of female spikes.

C. nigra subsp. *tornata* (Fr.) Lemke was described from Sweden as a rigid, densely cespitose plant with broad leaf blades and crowded female spikes (Fries 1842). *C. nigra* subsp. *juncella* (Fr.) Lemke was originally described from Sweden as an elongate and gracile plant with narrow leaf blades and slightly distant female spikes (Fries 1842). Later authors emphasized additional character for the delimitation of *C. nigra* subsp. *juncella*, namely the densely cespitose growth form without any creeping rhizomes (Fries 1853, Sylvén 1963, Hess et al. 1967, Egorova 1976, Dostál 1989, Malyshev 1990, Egorova 1999). *C. nigra* subsp. *recta* (Fleischer) Rothm. was described from Germany as a tall plant with creeping rhizomes, narrow leaf blades, and distant female spikes (Fleischer 1832). Later interpretations of this name admitted variability in

clonal growth, from loosely rhizomatous to cespitose (Sylvén 1963, Dostál 1989).

Apart from the mentioned subspecies, referring to the plants from low and medium altitudes of Europe, additional taxa were reported from high mountain ranges of Central and Southern Europe. Accepted in some recent compendia (e.g. Chater 1980, Koopman 2011) are the dwarf, longely rhizomatous, narrow-leaved plants named *C. nigra* subsp. *alpina* (Gaudin) Lemke, described from Switzerland (Gaudin 1830), and dwarf, tufted, broader-leaved plants called *C. nigra* subsp. *intricata* (Tineo) Mayre et Weiller, described from Sicily (Gussone 1843). However, as our study was focused on the tall, often densely cespitose morphotypes from the lower and medium altitudes, the taxa from high altitudes, exhibiting rather opposite morphology, were not examined.

There is a general consensus that *C. n.* subsp. *recta* and *C. n.* subsp. *tornata* refer to infraspecific variability within *C. nigra*. These two taxa were never distinguished at the rank higher than subspecies (Sylvén 1963, Schulze-Motel 1980, Klimko 1981, Dostál 1989), and particularly in the taxonomic syntheses from larger geographical areas they are not distinguished at all (Chater 1980, Egorova 1999, Koopman 2011). On the contrary, the status of *C. n.* subsp. *juncella* is more controversial. The most frequent (and the most conservative) approach is to regard this morphotype as a genetically-based modification of *C. nigra*, deserving an infraspecific taxonomic rank (cf. Chater 1980, Koopman 2011). However, some authors (e.g. Sylvén 1967, Egorova 1999, Fischer et al. 2005) consider the morphological differences between *C. nigra* subsp. *juncella* and the typical rhizomatous *C. nigra* to be so profound that they treat the former taxon even as a distinct species. Opinions about the distribution of *C. nigra* subsp. *juncella* are fundamentally different as well. While some authors (Dostál 1989, Fischer et al. 2005, Bernátová 2005) report occurrence of this taxon from the Central Europe, others consider it to be restricted to northern Europe and west Siberia (Sylvén 1963, Egorova 1999, Hultén and Fries 1986). It was hypothesized that the tussocky growth form of the plants from northern Europe is based genetically, whereas in the plants from lower latitudes the same growth

form arises as a non-hereditary morphological response to environmental conditions (Jermy 1957, Egorova 1999).

On the other hand, several experimental works showed that the tussocky *C. nigra* plants differed from the typical rhizomatous *C. nigra* neither in karyotype (Faulkner 1972 and 1973), sequences of non-coding regions of chloroplast DNA, nor in amplified fragment length polymorphism (AFLP) of nuclear DNA (Jimenez-Mejías et al. 2012). In addition, the morphological character mostly used for delimitation of *C. nigra* subsp. *juncella*, i.e. tussocks without creeping rhizomes, was revealed to be taxonomically unreliable due to its high plasticity and frequent dependence on environmental conditions rather than on genotype (Košnar et al. 2012).

The unresolved status of *C. nigra* subsp. *juncella* and, more generally, the obscure delimitation of all the taxa described among cespitose and tall rhizomatous morphotypes of *C. nigra*, apparently figures from (1) the lack of critical evaluation of the morphological characters of these taxa and (2) a missing synthesis of information obtainable from morphological characters (presumably strongly influenced by environmental factors) and markers reflecting entirely genetic constitution of plants (i.e. selectively neutral molecular markers). As the non-morphological markers used so far did not provide any support for taxonomic splitting of *C. nigra*, the morphological characters remain the only ones for taxa delimitation, although the extent of their plasticity (i.e. taxonomically confusing variability) may be substantial. We therefore attempted to compare morphometric data with the highly variable, selectively neutral nuclear DNA markers (inter-simple sequence repeat polymorphism, ISSR; Zietkiewicz et al. 1994) to answer the following questions: (1) Do the patterns of the morphological and molecular variation in *C. nigra* correspond to each other? (2) How strong is morphological and genetic differentiation among the morphotypes of *C. nigra* that are traditionally classified to the subsp. *nigra*, subsp. *recta*, subsp. *tornata*, and subsp. *juncella*?

MATERIAL AND METHODS

Plant material

Plants for the morphometric study were collected during the vegetation seasons 2003–2008 at 55 localities (Fig. 1 and Appendix 1) in Czechia (37 localities), Sweden (3), Norway (10), Finland (2), and Russia (3). The aim was to represent both regions with reported continuous distribution of *C. nigra* subsp. *juncella* (northern Europe, according to Hultén and Fries 1986) and the regions where absence of "true" *C. nigra* subsp. *juncella* is presumed (central Europe). Number of sampled plants per population varied between 1 and 16 in order to represent each morphotype at a locality by at least one sample. In total 268 plants were collected for the morphometric study. Each sampled plant was immediately pressed and dried as a herbarium specimen (deposited in CBFS), and tentatively determined as one of the four studied taxa (*C. nigra* subsp. *nigra*, *C. n.* subsp. *recta*, *C. n.* subsp. *tornata*, and *C. n.* subsp. *juncella*) or as morphological transitions among these taxa, according to original descriptions and their subsequent widely adopted interpretations (Table 1).

Since the original descriptions and subsequent interpretations of the studied taxa usually did not provide detailed, clear, and unambiguous values of distinguishing characters, following morphological criteria were used for evaluation of the characters for purposes of the tentative determination. (1) Herbarium specimens that contained neither ascending nor horizontally growing extravaginal rhizome branches and were collected from the plants growing in conspicuous elevated tussocks were labeled as "densely cespitose plants without creeping rhizomes". If the herbarium specimen of the tussock-forming plant contained at least one horizontally growing or ascending extravaginal rhizome branch, the plant was labeled as "densely cespitose with creeping rhizomes". The plants of loose growth form and with frequent or long extravaginal rhizome branches were scored as "rhizomatous". (2) "Narrow leaves" were scored if the modus of the leaf blade width (from five measurements in the lowest thirds of the leaf blades) was lower than or equal to 1 mm and the

maximum leaf blade width was lower than 2 mm. “Broad leaves” were scored if the modus of leaf blade width was equal to or higher than 1 mm and the minimum leaf blade width was higher than 1 mm. The leaves not meeting the conditions of being either narrow or broad were labeled as “moderately wide”. (3) A plant was labeled as “high” if the lenght of the longest flowering stem was equal to or higher than 30 cm. Plants with the longest flowering stems shorter than 30 cm were scored as “low”. (4) “Crowded spikes” were scored on a plant if the mean value of ratio of the length of a female spike and of the nearest subsequent internode was equal to or higher than 1.5 and if the minimum value of the ratio was equal to or higher than 1. “Distant spikes” were scored if the mean value of the ratio was equal to or lower than 0.9 and if the maximum value of the ratio was equal to or lower than 1. Spikes not fulfilling conditions of being either crowded or distant were labeled as “moderately distant”. (5) Female spikes were scored as “almost as long as male spikes” if the ratio of the average length of a female spike and of a male spike was equal to or higher than 0.9. If the value of the ratio was lower than 0.9, the female spikes were scored as “shorter than male spikes”.

Of the 268 specimens collected for the morphometric study, 103 samples from 36 populations (18 Czech, 3 Swedish, 10 Norwegian, 2 Finnish, and 3 Russian) were chosen for the study of ISSR polymorphism. These samples covered whole geographic range of the sampling for the morphometric analysis and included all morphotypes distinguished by the tentative determination.

Morphometric analysis

In total, 43 morphological characters (Table 2), including all those used in literature for delimitation of *C. nigra* subsp. *recta*, *C. n.* subsp. *tornata*, and *C. n.* subsp. *juncella*, were observed or measured at 40× magnification using a stereomicroscope. Twenty-five characters were quantitative variables (numbers, lengths, or widths), eleven characters were inferred as various ratios of the quantitative variables, and seven characters were categorial variables. All measurements and observations of inflorescence parts were carried out on the longest flowering stems.

DNA isolation

Total genomic DNA was extracted using the Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany) according to the manufacturer's instructions with minor modifications. Approximately 4–7 mg of air-dried leaf tissue (excised from herbarium specimens) was ground by shaking with 3-mm tungsten carbide beads in a mixer mill MM400 (Retsch, Haan, Germany), and 100 µg of RNase A (Promega, Madison, Wisconsin, USA) was added to the extract. Elution was carried out with 75 µl of elution buffer, and DNA eluates were stored at -20 ºC.

ISSR analysis

Thirteen primers were initially tested for their ability to provide variable and reproducible PCR products. Three primers were selected as suitable after optimization: $(GA)_8YT$, $(GA)_7RC$, and $(ATG)_6$. PCRs were performed in a reaction mixture containing 0.8 µl of genomic DNA (diluted 1:10 in sterile water), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.6 µM of a primer (Invitrogen, Carlsbad, California, USA), 0.75 U Taq polymerase (Top-Bio, Praha, Czech Republic) in the manufacturer's reaction buffer, and sterile water to make up a final volume of 15 µl. Amplifications were performed in a Biometra T3000 thermocycler (Biometra, Göttingen, Germany) with an initial denaturation of 3 min at 94 °C; followed by 38 cycles of 1 min at 94 °C, 1 min at 65 to 61 °C (see next sentence), and 2 min at 72 °C; and a final extension of 10 min at 72 °C. The annealing temperature was decreased by 1 °C in the first five cycles until the primer-specific temperature was reached [58, 60, and 56 °C for $(GA)_8YT$, $(GA)_7RC$, and $(ATG)_6$, respectively]. This temperature was then used for the remaining 33 cycles. PCR product aliquots of 6 µl were mixed with loading buffer and separated by electrophoresis running for 8–10 h at 80–120 V on 1.3% (w/v) agarose gels with Tris-borate-EDTA (TBE) buffer. Gels were stained in 1×GelRed (Biotium, Hayward, California, USA) staining solution (TBE buffer, pH 8.0) for 25–45 min, and band patterns were visualized with a UV transilluminator. The size (molecular mass) of PCR products (bands)

was estimated with 100-bp ladder size standard (New England Biolabs, Ipswitch, Massachusetts, USA).

Each band was assumed to be the gene product of a dominant allele at a separate genetic locus. At least two PCR amplifications were performed for each sample, and only clear and reproducible bands were considered for data analysis. Bands were manually scored as present (1) or absent (0) at each locus. From the samples with identical ISSR phenotypes (putative clones), only one randomly selected sample was maintained in the dataset, whereas the remaining were removed prior to statistical analyses.

Data analysis

A principal components analysis (PCA) was used to find the main gradients in the variability of the morphological characters and to assess the morphological differentiation among the studied plants. The values of characters were standardized, and the levels of the qualitative characters were coded as binary dummy variables. Computations and construction of ordination plots were carried out with the programs CANOCO for Windows 4.5 and CanoDraw for Windows 4.0 (ter Braak and Šmilauer 2002).

A classification tree (CART) was used to find which additional morphological characters are the most efficient for distinguishing the tentatively determined taxa. To infer the classification criteria, only the samples tentatively determined as almost or fully corresponding to one of the four taxa could have been included, i.e. the samples tentatively not assignable to any taxon were omitted. Computation procedure was carried out in the library *rpart* of the software package R 2.9.0 (R Development Core Team 2009). The default values of CART parameters were maintained, with exceptions of *minsplit* (set to 2) and *minbucket* (set to 1). The final tree, selected after cross-validation, was the one that displayed the highest complexity parameter together with the lowest value of the relative error of predictions.

A principal coordinates analysis (PCoA) was employed to find the main trends in the genetic (ISSR) variability and to visualize the correlation of genetic and morphological variability. The distance matrix

for PCoA was constructed from pair-wise genetic distances calculated from standard Jaccard's coefficients using FAMD1.2 (Schlüter and Harris, 2006). Other computations were carried out in CANOCO for Windows 4.5 and CanoDraw for Windows 4.0 (ter Braak and Šmilauer, 2002).

For further investigations of the structure of genetic variability, two different methods of Bayesian analysis implemented in the program BAPS 5.4 (Corrander et al. 2006, 2008) were used. A population mixture analysis was performed to group the samples (individuals) into the clusters which were maximally genetically divergent from each other. The highest number of the clusters (*K*) was set to 2–87, and the analysis was replicated five times. In addition, several various *a priori* specified (hypothetical) genetic structures were compared. The hypotheses were given equal prior probabilities. Their posterior probabilities were evaluated according to the calculated logarithmic marginal likelihood (logML) values.

RESULTS

Morphometric analysis

Of the 268 tentatively determined plants, only 14% fully corresponded to the descriptions (Table 1) of any of the four subspecies (25 to *C. n.* subsp. *nigra*, 3 to *C. n.* subsp. *recta*, 1 to *C. n.* subsp. *tornata*, and 9 to *C. n.* subsp. *juncella*). Most (59%) of the plants almost corresponded to some taxa (106 plants to *C. n.* subsp. *nigra*, 8 to *C. n.* subsp. *recta*, 16 to *C. n.* subsp. *tornata*, and 29 to *C. n.* subsp. *juncella*) when the criteria for determination were allowed to deviate from the descriptions in one character. Morphology of the remaining 71 samples (27%) did not enable unequivocal classification, and these samples were thus considered as transitions among the subspecies. Almost all studied populations were composed of several different morphotypes (Appendix 1). The plants morphologically fully corresponding to *C. n.*

subsp. *juncella* were represented both in north-European and Czech populations (Appendix 1).

The PCA (Fig. 2 and Fig. 3) revealed no distinct groups, besides the several outliers representing individual plants (from different populations) with unusually long inflorescences or uppermost female spikes. The samples belonging to various tentatively determined taxa were randomly intermingled and widely overlapping.

The cross-validated CART contained merely one branch and thus did not enable to distinguish between any taxa. This indicated that no additional morphological characters were correlated to the criteria for tentative determination. In other words, no additional morphological characters useful for circumscription of the taxa were found.

ISSR analysis

PCR amplifications using the primers $(GA)_8YT$, $(GA)_7RC$, and $(ATG)_6$ yielded 34 scorable loci (markers, bands). Each of the markers was polymorphic across the sample set. The size of the bands ranged from 290 bp to 1100 bp. Seventy-five ISSR profiles were unique in the dataset (i.e., present in one sample only), eight profiles were represented by two samples, and four profiles by three samples. The plants with identical ISSR profiles (putative clones) were found exclusively within populations (not among populations). The samples from the same clone were tentatively determined as identical morphotype in four cases only, whereas in the remaining eight cases samples from the same clone were assigned to different morphotypes.

The PCoA detected no apparent structure in the variability of the ISSR markers. The samples were almost evenly dispersed throughout the ordination space, without any correlation to the tentative morphological determination (Fig. 4).

The mixture analysis of population structure found that the most probable partitioning of the genetic variability was into four clusters, however, without any relation to either tentative morphological determination or geographic origin of the samples. For instance, the samples from the north-European plants accurately corresponding to *C. n.*

subsp. *juncella* were grouped in the cluster containing Czech rhizomatous plants as well (Appendix 1).

Of the eight *a priori* specified hypotheses describing the genetic structure in *C. nigra*, the one corresponding to absence of any partitioning was the most plausible. The hypotheses corresponding to the genetic differentiation of the plants fully or almost corresponding to *C. nigra* subsp. *juncella* were evaluated as somewhat more probable than those assuming each taxon or population to be a distinct genetic group (Table 2).

DISCUSSION

Utilization of multivariate morphometric methods in the complexes of closely related but distinct species repeatedly proved to be a powerful tool for thorough evaluation of overall morphological variability and for precise circumscription of existing taxa. Namely in *Carex*, multivariate morphometrics recently revealed a range of overlooked but well-defined species (Naczi et al. 1998, Saarela and Ford 2001, Ford et al. 2008). Although the phenotypic plasticity may obscure morphological circumscription of closely related species, some genetic discontinuities must be present among them (Hedrén 2003). Such discontinuities reflect either accumulation of genetic differences among independently evolving allopatric populations (Mayr 1963) or shift in habitat preferences and adaptations in sympatric populations (Diehl et Bush 1989). On the other hand, a lack of any differentiation even when formalized morphological and molecular data are combined serves as a strong evidence for conspecific nature of the studied group (Foggi et al. 2005). The results obtained for *C. nigra* convincingly demonstrate that all the studied morphotypes belong to the single species. The recognition of *C. juncella* as a species distinct from *C. nigra* is no more sustainable because of the absence of any remarkable partition in the morphological and ISSR variability. Our results are in full agreement with the previously reported karyological uniformity (Faulkner 1972), absence of reproductive

isolation (Faulkner 1973), and absence of structure in variability of chloroplast DNA and AFLPs (Jimenez-Mejías et al. 2012) between the rhizomatous and the cespitose plants of *C. nigra*.

Moreover, even the rank of variety seems to be too high for evaluation of the variation patterns in *C. nigra*. In fact, varieties in the genus *Carex* often refer to relatively well demarcated groups. For instance, Standley (1985) reported varieties in *C. aquatilis* that clearly differed in several independent morphological characters. Hedrén (1998) demonstrated the trend in clinal variability of *C. oederii,* with the nominate variety and *C. oederii* var. *bergrothii* standing on the opposite parts of the continuum.

Nonetheless, according to Hedrén (2003), the morphological differentiation among varieties in the genus *Carex* may be very subtle (merely one character) and incomplete, resulting in many transitional individuals. Moreover, the infraspecific taxa in plants may be of repeated polytopic origin (Levin 2001), and thus the individuals belonging to the same variety need not to be closely genetically similar ("parallel evolution" sensu Schlutter and Nagel 1995). The only genetic similarity among the individuals from the same variety can be represented by a genetically determined trait which passed through a process of natural selection to give the individuals an advantage under particular, often very local, environmental conditions. The varieties then correspond to ecotypes (Turesson 1922, 1925) and assignment of an individual to a variety provides biologically meaningful information about the hereditary trait with an adaptive importance. However, the existence of a genetic basis of any trait does not implicitly mean control by the same genes in all individuals, as parallel evolution of traits based on different genetic pathways was proven in some cases (e.g., Fenster and Barrett 1994, Andersson 1995).

The status of the ecotypic variety is sometimes proposed for the tussock-forming plants of *C. nigra* since it is sometimes claimed that the compact growth form is the hereditary (genetically fixed) character (Faulkner 1973) and seems to be of an adaptive value in harsh climatic conditions. The group of samples from the tussock-forming plants more

or less fitting the description of *C. n.* subsp. *juncella* showed somewhat higher genetic coherence than the remaining tentatively determined subspecies (Table 3), which may be interpreted as an indication of shift in genetic constitution due to ecotypic differentiation. However, delineation of an ecotypic variety based on the growth form would bring a risk of inclusion of many "inappropriate" individuals in the variety, since many tussock-forming plants are actually non-hereditary site modifications (Košnar et al. 2012) and, as demonstrated in our morphometric analysis, there is no way to distinguish them morphologically from other plants. Thus it could not be assured that the diagnostic character of the cespitose variety was a hereditary trait that have resulted from selection. Instead, the plasticity of clonal growth may be the prevailing adaptive trait which enables *C. nigra* to adjust a phenotype to a particular environment prior to a selection. Phenotypic plasticity is probably an important source of variability not only in growth form but also in other morphological characters used for delimitation of infraspecific taxa in *C. nigra*, as indicated by the lack of correlation between morphological and genetic variability and even by findings of genetically identical plants displaying different morphotypes.

The morphological characteristics of *C. n.* subsp. *juncella*, *C. n.* subsp. *recta*, and *C. n.* subsp. *tornata* apparently fit to a small fraction of individuals only. The pattern of morphological and genetic variability of *C. nigra* in the studied part of central and northern Europe corresponds to a large population of outcrossing sexual species with extensive gene flow that prevents differentiation of local subpopulations. We suggest that in such a system distinguishing of any infraspecific taxa is impossible and useless.

ACKNOWLEDGEMENTS

We thank Ester Ekrtová, Libor Ekrt, Milan Soukup, and Filip Kolář for help with collection of samples and Jitka Laburdová and Jiřina Laburdová for comments on the first drafts of the manuscript. The study

was supported by Mattoni Awards for Studies of Biodiversity and Conservation Biology 2004 and 2005 and grant MSM6007665801 from the Ministry of Education, Youth and Sports of the Czech Republic.

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Table 1. Morphological characters of the studied taxa of *Carex nigra*, compiled from literature with particular emphasis on original descriptions. Details about classification of character states are given in text.

	subsp. *nigra*	subsp. *recta*	subsp. *juncella*	subsp. *tornata*
Growth form	Rhizomatous plants	Rhizomatous plants	Densely cespitose plants, without creeping rhizomes	Densely cespitose plants, with or without creeping rhizomes
Leaf width	Narrow or moderately wide	Narrow	Narrow	Broad
Plant height	Low	High	High	High
Distances among spikes	Spikes crowded or moderately distant	Spikes distant	Spikes distant or moderately distant	Spikes crowded
Relative length of female spikes	Shorter than male spikes or almost as long as male spikes	Almost as long as male spikes	Shorter than male spikes or almost as long as male spikes	Shorter than male spikes or almost as long as male spikes

Table 2. Characters studied in the morphometric analysis.

Character	Description
1-3	Clonal growth form: 1 - densely cespitose without creeping rhizomes, 2 - densely cespitose with creeping rhizomes, 3 - loose with creeping rhizomes
4	Yellow coloration of root hairs present
5	Red coloration in basal leaf sheaths present
6	Length of the longest flowering stem (including inflorescence) [mm]
7	Ratio of the longest flowering stem length to the longest leaf length
8	Width of leaf in the lowest third of its length [mm]; calculated as average from measurements of five randomly selected leaves on a plant
9	Inflorescence length [mm] on the longest flowering stem
10	Ratio of the lowermost bract legth to inflorescence length
11	Length of the female portion of inflorescence [mm]
12	Length of the male portion of inflorescence [mm]
13	Ratio of the lengths of the female portion and the male portion of inflorescence
14	Multiple male spikes present
15	Number of female spikes
16	Number of spikes
17	Number of spikes per 1 cm of the length of inflorescence axis (not including the axis of the uppermost male spike)
18	Ratio of lengths of a female spike and of the subsequent internode of inflorescence; average calculated from measurements of all female spikes and the respective internodes in an inflorescence
19	Length of the lowermost female spike [mm]
20	Width of the lowermost female spike [mm]
21	Ratio of the length and the width of the lowermost female spike
22	Ratio of the distance from the base of the widest part of the lowermost female spike to the length of the lowermost female spike
23	Length of the uppermost female spike [mm]
24	Width of the uppermost female spike [mm]
25	Ratio of the length and the width of the uppermost female spike
26	Ratio of the distance from the base of the widest part of the uppermost female spike to the length of the uppermost female spike
27	Legth of the uppermost male spike [mm]
28	Width of the uppermost male spike [mm]
29	Length of the lowermost male spike [mm]
30	Width of the lowermost male spike [mm]
31	Length of the stalk of the lowermost female spike [mm]
32	Length of the stalk of the uppermost male spike [mm]
33	Ratio of average female spike length and average male spike length; averages calculated from measurements of all spikes in an inflorescence

34	Distance between the female and the male portion of the inflorescence [mm]
35	Number of utricles in 1 cm of the length of the central part of the lowermost female spike
36	Number of utricles in 5 mm from the base of the lowermost female spike
37	Width of a glume [mm]; calculated as average from measurements of five glumes from the middle of a randomly selected female spike on the longest flowering stem
38	Length of a glume [mm]; calculated as average from measurements of five glumes from the middle of a randomly selected female spike on the longest flowering stem
39	Glume apex acute; observed on five glumes from the middle of a randomly selected female spike on the longest flowering stem, scored if at least three glumes were acute
40	Length of a utricle [mm]; calculated as average from measurements of five utricles from the middle of a randomly selected female spike on the longest flowering stem
41	Width of a utricle [mm]; calculated as average from measurements of five utricles from the middle of a randomly selected female spike on the longest flowering stem
42	Ratio of the distance from the apex of the widest part of the utricle to the length of the utricle; calculated as average from measurements of five utricles from the middle of a randomly selected female spike on the longest flowering stem
43	Ratio of glume length to utricle length

Table 3. Bayesian analysis of population genetic structure – comparison of the hypotheses. The probability of a hypothesis (*H1–H8*) decreases with logarithmic marginal likelihood (logML) value. *H1:* Each population is genetically distinct group. *H2:* The plants from northern Europe tentatively determined as fully corresponding to *C. n.* subsp. *juncella* differ from the others. *H3:* The plants from northern Europe tentatively determined as almost or fully corresponding to *C. n.* subsp. *juncella* differ from the others. *H4:* The plants tentatively determined as fully corresponding to *C. n.* subsp. *juncella* differ from the others. *H5:* The plants tentatively determined as fully or almost corresponding to *C. n.* subsp. *juncella* differ from the others. *H6:* The four described taxa are genetically distinct and include also the plants that almost but not fully correspond to the descriptions of the taxa. Each morphotype not assignable to one of the taxa is a genetically distinct group. *H7:* Each tentatively distinguished morphotype is genetically distinct group. *H8:* All morphotypes and all populations are members of a single, not structured genetic group.

Hypothesis	Prior probability	LogML	Posterior probability
H8	0.125	-1303.187	1
H2	0.125	-1317.5257	0
H4	0.125	-1331.2431	0
H3	0.125	-1331.3237	0
H5	0.125	-1341.71	0
H6	0.125	-1511.3282	0
H7	0.125	-1578.0931	0
H1	0.125	-1714.5589	0

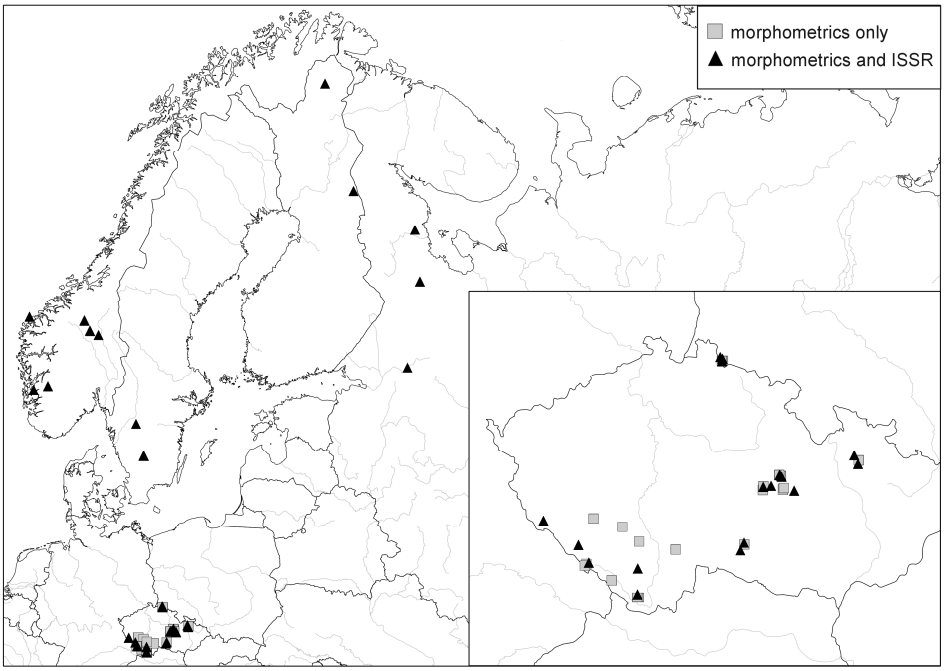

Fig. 1. Populations of *Carex nigra* sampled for morphometric and ISSR study.

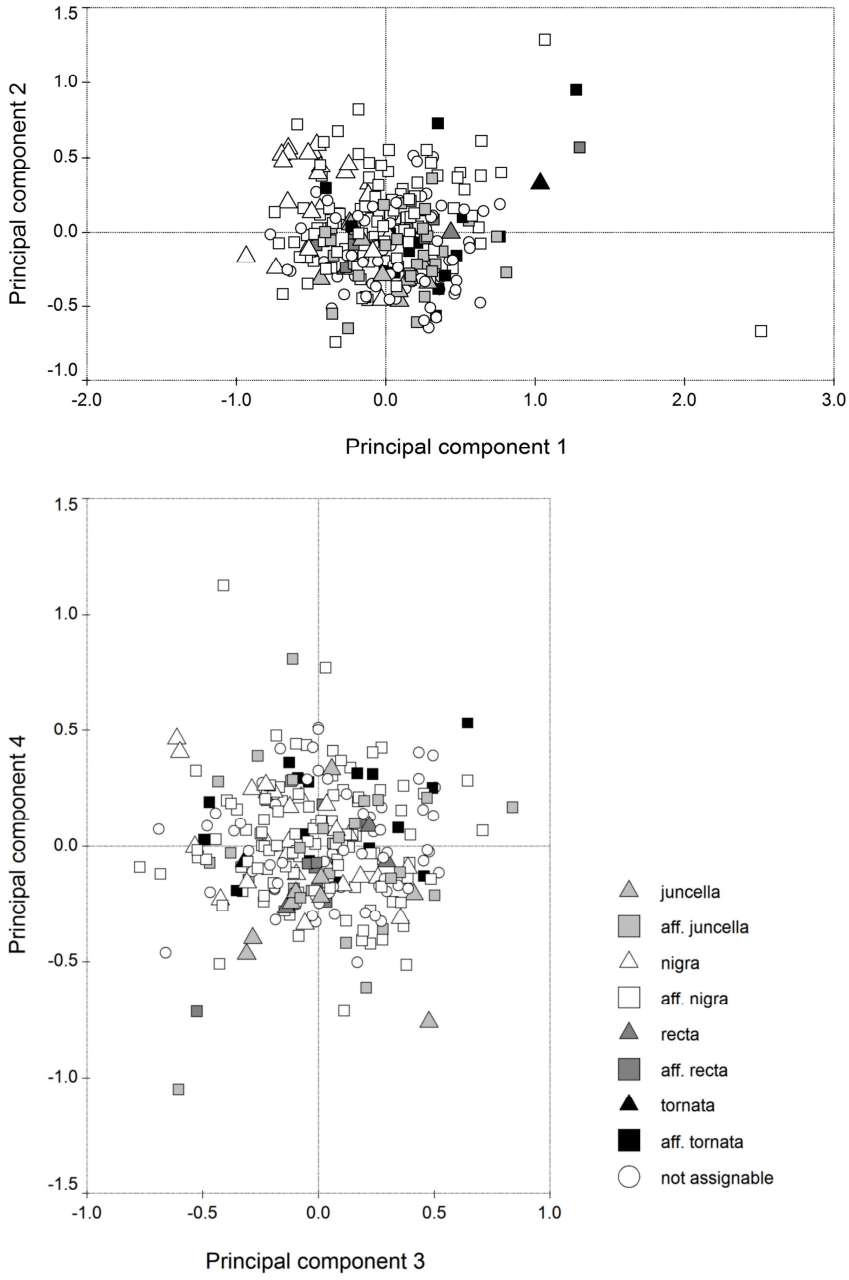

Fig. 2. Principal components analysis (PCA) of morphological variability: ordination of the plant samples. The first, the second, the third, and the fourth principal components explained 16.8%, 9.8%, 8.3%, and 7%, respectively, of the total variability in the 39 morphological characters studied (Table 1).

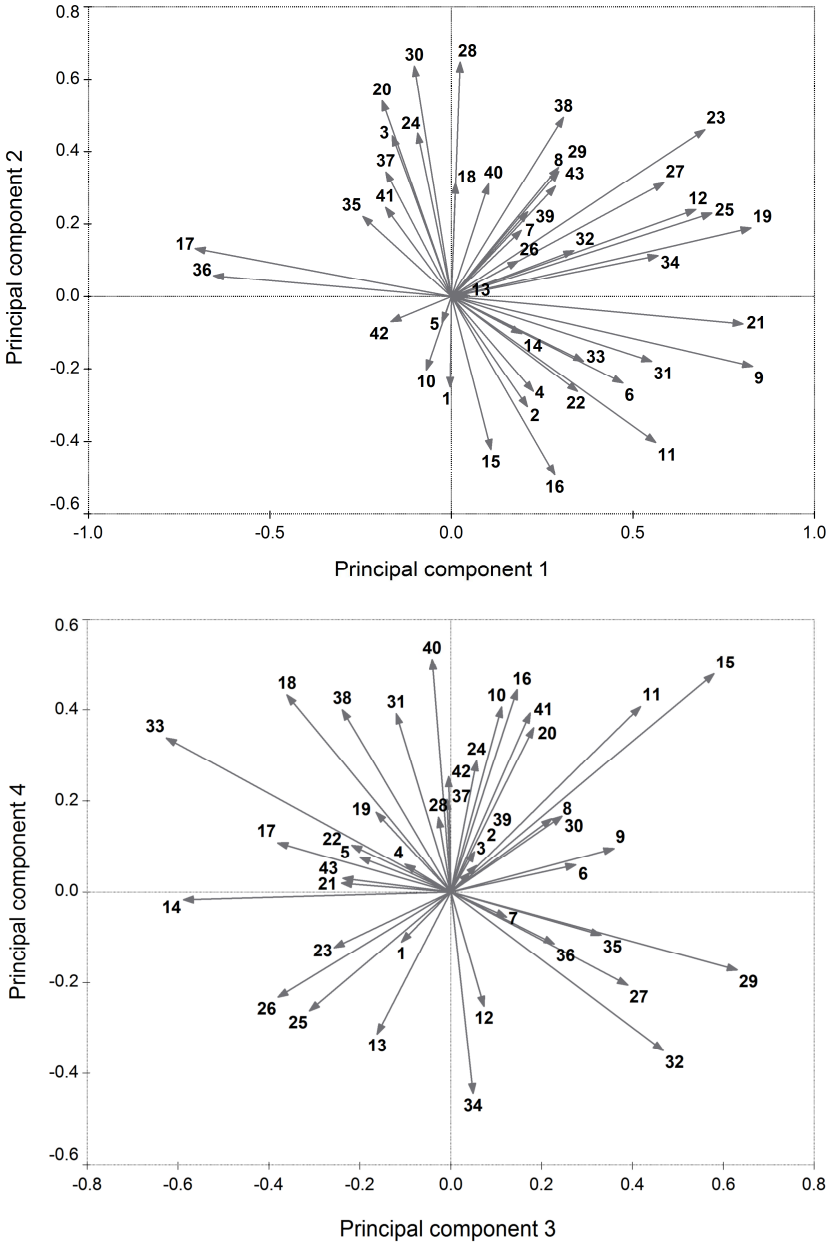

Fig. 3. Principal components analysis (PCA) of morphological variability: vectors of morphological characters (Table 1). The first, the second, the third, and the fourth principal components explained 16.8%, 9.8%, 8.3%, and 7%, respectively, of the total variability in the 39 morphological characters studied.

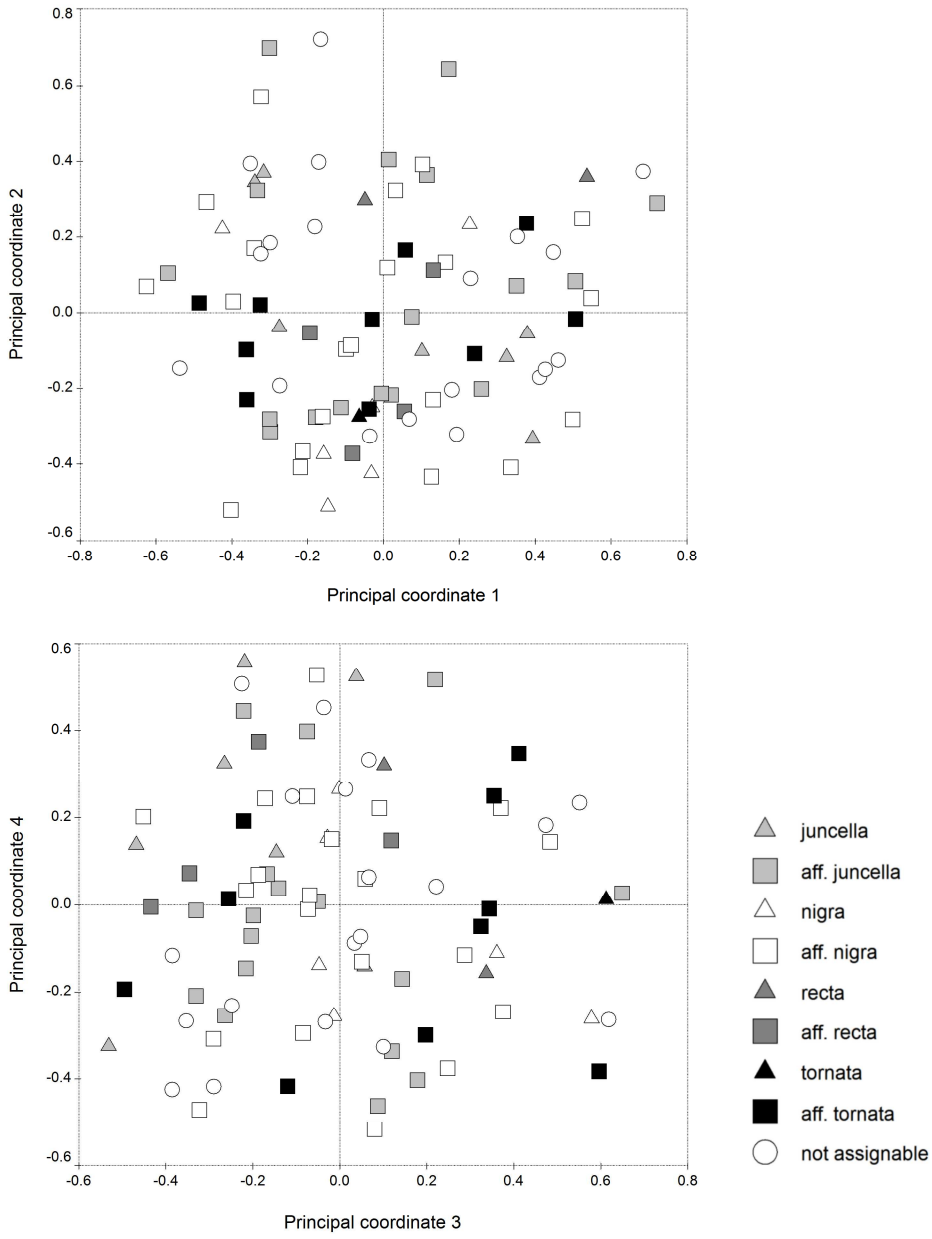

Fig. 4. Principal coordinates analysis (PCoA) of genetic (ISSR) variability: ordination of the plant samples. The first, the second, the third, and the fourth principal coordinates explained 10%, 8.4%, 8.2%, and 7.4%, respectively, of the total variability in 34 ISSR markers.

Appendix 1. Sampled populations of *Carex nigra*: code of population (number of samples for the morphometric/ISSR analyses), municipality, localization, coordinates (WGS 84), altitude, date of collection, name of collector (if not mentioned, the collector was Jan Košnar) [tentatively determined morphotypes in a population (number of samples for the morphometric/ISSR analyses) – genotypes found in the samples of a morphotype studied for ISSR, with capital letter denoting a cluster identified in the population mixture analysis and subscript denoting an ISSR profile (if a genotype was found in more than one sample, the number of the samples is mentioned)].

Czechia:

BAL (15/8), Modřec, abandoned fen meadow in a wold of a brook, c. 1.2 km W from the summit of Baldský vrch hill (692 m), 49.6797°N, 16.3286°E, 620 m, 28 June 2004 [aff. subsp. *juncella* (3/2) – D_1, D_2; aff. subsp. *nigra* (6/2) – C_3, D_5; aff. subsp. *tornata* (3/2) – C_4, D_5; not assignable (3/2) – D_1, D_6].

BOB (9/0), Slavkov, edge of the peat bog Bobovec, 48.7164°N, 14.1844°E, 780 m, 12 June 2004 [aff. subsp. *nigra* (8/0); not assignable (1/0)].

BOR (10/2), Bor u Skutče, edge of an abandoned fen meadow "Na Tintěrkách", c. 500 m SSW from the centre of village, 49.8189°N, 16.1239°E, 477 m, 20 June 2004 [aff. subsp. *nigra* (3/0); subsp. *tornata* (1/1) – A_8; aff. subsp. *tornata* (1/1) – D_7; not assignable (5/0)].

BUD (5/3), Budislav, peaty sites within a spruce plantation, c. 1 km W from the centre of the village, c. 150 WNW from the northernmost edge of the pond Nový rybník, 49.8044°N, 16.155°E, 510 m, 2 July 2004 [subsp. *juncella* (1/1) – A_{11}; aff. subsp. *nigra* (1/0); aff. subsp. *recta* (1/1) – A_9; aff. subsp. *tornata* (1/1) – C_{10}; not assignable (1/0)].

CHL (6/1), Chlumětín, abandoned oligotrophic meadow in a wold of a brook, c. 500 m SE from the village, 49.7231°N, 16.0092°E, 650 m, 28 July 2005 [aff. subsp. *juncella* (2/1) – D_{12}; aff. subsp. *nigra* (3/0); not assignable (1/0)].

HLA (1/1), Hladov, abandoned fen meadow at the pond, c. 1.7 km from the centre of the village, 49.2122°N, 15.6356°E, 12 June 2004, 610 m, E. Ekrtová, L. Ekrt [aff. subsp. *juncella* (1/1) – C_{13}].

KAR (8/3), Bor u Skutče, sandstone valley Karálky, bank of a brook in a spruce forest, c. 500 m SE from the centre of the village, 49.8186°N, 16.1314°E, 440 m, 27 June 2004 [subsp. *nigra* (7/3) – A_{14} (3); aff. subsp. *nigra* (1/0)].

KEP (12/6), Hartmanice, abandoned fen meadow c. 900 m SSW from the settlement Keply and c. 1 km NE from the summit of the hill Hadí vrch (1021.7 m), 49.1917°N, 13.3497°E, 960 m, 10 August 2005 [aff. subsp. *juncella* (3/1) – C_{15}; aff. subsp. *nigra* (4/1) – C_{16}; aff. subsp. *tornata* (2/2) – B_{17}, B_{18}; not assignable (3/2) – A_{19}, C_{20}].

KOR (16/7), Lhenice, wet meadow at the Koubovský rybník pond, c. 2 km SE from the church in the town, 48.98°N, 14.1689°E, 535 m, 18 May 2004 [aff. subsp. *nigra* (9/2) – A_{21}, B_{22}, B_{23}; subsp. *recta* (1/1) – B_{25}; aff. subsp. *recta* (1/1) – B_{25}; aff. subsp. *tornata* (2/1) – A_{24}; not assignable (3/1) – B_{25}].

KOZ (8/0), Bor u Skutče, wet meadow below a former sandstone quarry, c. 750 m SW from the centre of the village, c. 450 m SSE from the summit "Na Kozinci" (485.4 m), 49.8189°N, 16.1203°E, 470 m, 26 June 2004 [subsp. *nigra* (2/0); aff. subsp. *nigra* (5/0); not assignable (1/0)].

KPR (8/0), Plánička, sloped wet meadow, c. 2 km ESE from the crossroad at the south-western edge of the village, 48.7169°N, 14.1603°E, 750 m, 12 June 2004 [aff. subsp. *nigra* (7/0); not assignable (1/0)].

LAS (2/0), Jizerka, ditch along the path Lasičí cesta, c. 935 m SSW from the summit of the mountain Jelení stráň (1018 m), 50.8292°N, 15.3453°E, 915 m, 6 August 2005 [subsp. *nigra* (1/0); aff. subsp. *nigra* (1/0)].

LSR (10/6), Pila, abandoned fen meadow c. 440 m SE from the railway station, 49.4125°N, 12.8658°E, 460 m, 26 May 2005 [aff. subsp. *juncella* (4/2) – A_{27} (2); subsp. *nigra* (1/1) – B_{26}; aff. subsp. *nigra* (4/2) – B_{26} (2); not assignable (1/1) – A_{27}].

LUC (3/0), Telecí, abandoned wet meadow between a forest and a field, c. 1.4 km SE from the summit of Lucký vrch hill (739 m), 49.7047°N, 16.1853°E, 640 m, 20 July 2004 [aff. subsp. *tornata* (1/0); not assignable (2/0)].

MIL (1/0), Radomyšl, abandoned meadows c. 980 m NNE from the railway station Rojice, at the west bank of the pond Milava, 49.3564°N, 13.9558°E, 460 m, July 1987, M. Štech [aff. subsp. *nigra* (1/0)].

MJL (6/0), Jizerka, edge of the peat bog Malá Jizerská louka, 50.8281°N, 15.3311°E, 863 m, 6 August 2005 [aff. subsp. *nigra* (3/0); aff. subsp. *recta* (1/0); not assignable (2/0)].

MOD (16/6), Modrava, abandoned sloped wet meadow, c. 1500 m NNW from the centre of the village, 49.0328°N, 13.4928°E, 1015 m, 26 July 2005 [subsp. *juncella* (2/2) – A_{30}, B_{31}; aff. subsp. *juncella* (2/1) – A_{28}; aff. subsp. *nigra* (6/1) – A_{29}, C_{32}; not assignable (6/3) – C_{32}, C_{33}, C_{34}].

MYT (7/2), Černá v Pošumaví, abandoned wet meadow below the road between Muckov and Hořice na Šumavě, c. 600 m S from the settlement Mýtina, 48.7469°N, 14.165°E, 805 m, 12 June 2004 [aff. subsp. *nigra* (1/0); aff. subsp. *recta* (2/1) – A_{35}; aff. subsp. *tornata* (1/1) – A_{36}; not assignable (3/0)].

NVC (12/6), Studnice (at Hlinsko), drier edges of the peaty heath "Na Velkém Černém" (sites of former manual peat-mining), c. 500 m SSW from the chapel in the village, 49.7139°N, 15.9008°E, 613 m, 18 August 2005 [subsp. *juncella* (3/2) – A_{40}, C_{41}; aff. subsp. *nigra* (4/3) – A_{37}, D_{38}, D_{39}; subsp. *recta* (1/0); not assignable (4/2) – B_{42}, D_{43}].

OPZ (2/0), Opatov, fen meadow c. 3.4 km SSW from the church in the village, 49.1967°N, 15.64°E, 650 m, 12 June 2004, E. Ekrtová, L. Ekrt [not assignable (2/0)].

OTP (1/1), Bohuslavice, wet meadow in the wold of the brook Otvrňský potok, c. 950 m SE from the centre of the village, 49.1439°N, 15.5853°E, 540 m, 9 June 2004, E. Ekrtová, L. Ekrt [aff. subsp. *juncella* (1/1) – D_{44}].

PMS (1/0), Modrava, edge of the forest road in the peat bog Přední Mlynářská slať, c. 2.5 km WSW from the centre of the village Modrava, 49.0219°N, 13.4594°E, 1060 m, 11 September 2004, E. Ekrtová, L. Ekrt [aff. subsp. *juncella* (1/0)].

POL (8/0), Polánka (at Nepomuk), fen meadow with willow shrubs, in a wold of a brook c. 500 m S from the chapel in the village, 49.4311°N, 13.5575°E, 550 m, 3 August 2005 [aff. subsp. *juncella* (1/0); subsp. *nigra* (1/0); aff. subsp. *nigra* (2/0); not assignable (4/0)].

PST (7/0), Rýmařov, fen meadow in a wold of the brook Pstruží potok, c. 2 km W from the castle in the town, 49.95°N, 17.2194°E, 680 m, 13 August 2005 [aff. subsp. *nigra* (3/0); aff. subsp. *recta* (1/0); not assignable (3/0)].

ROP (1/0), Modrava, peaty sites along the brook Roklanský potok, c. 5 km SW from the centre of the village Modrava, 49.0069°N, 13.4369°E, 1075 m, 11 September 2004, E. Ekrtová, L. Ekrt [aff. subsp. *nigra* (1/0)].

RUD (3/0), Veselí nad Lužnicí, north-eastern edge of the peat bog Ruda, c. 2 km SE from the church in the village Horusice, 49.1519°N, 14.6919°E, 395 m, 20 May 2004 [subsp. *nigra* (1/0); aff. subsp. *nigra* (1/0); not assignable (1/0)].

RYL (6/2), Jizerka, the peat bog Rybí loučky, 50.8469°N, 15.3386°E, 850 m, 6 August 2005 [subsp. *nigra* (3/0); aff. subsp. *nigra* (3/2) – B_{45}, B_{45}].

SAF (7/3), Jizerka, sloped oligotrophic meadow above the wold of the brook Safírový potok, 50.825°N, 15.3325°E, 860 m, 6 August 2005 [subsp. *nigra* (5/2) – A_{46}, B_{47}; aff. subsp. *nigra* (1/0); not assignable (1/1) – B_{47}].

SKA (5/2), Horní Město, fen meadow c. 1.3 km WNW from the church in the village Skály, 49.9181°N, 17.21°E, 700 m, 13 August 2005 [aff. subsp. *nigra* (3/2) – A_{48} (2); not assignable (2/0)].

SKP (1/0), Skály (at Protivín), wet oligotrophic meadow (*Violion caninae*) E from the pond Skalský rybník, c. 760 m S from the fort Klokočín, 49.2239°N, 14.1872°E, 374 m, 20 May 2003, L. Soukup [not assignable (1/0)].

SKR (7/3), Sobotín, open sites within a complex of bog spruce forests, c. 530 m SSE from the roadhouse Skřítek, 49.9947°N, 17.1578°E, 850 m, 13 August 2005 [aff. subsp. *nigra* (6/2) – A_{49}, C_{50}; aff. subsp. *recta* (1/1) – C_{50}].

STL (7/0), Stožec, abandoned wet meadows in a wold of a brook, c. 1 km W from the rock formation "Stožecká skála", 48.8742°N, 13.8083°E, 805 m, 14 July 2004 [subsp. *juncella* (1/0); aff. subsp. *juncella* (1/0); aff. subsp. *tornata* (2/0); not assignable (3/0)].

SUK (2/0), Vojnův Městec, sloped fen meadow with scattered willow shrubs, c. 1500 m NE from the church in the town, 49.6861°N, 15.8956°E, 665 m, 18 August 2005 [aff. subsp. *nigra* (2/0)].

TEL (6/0), Telecí, abandoned miry meadow within spruce plantation, c. 1.5 km SSW from the church in the village, 49.6892°N, 16.1722°E, 674 m, 20 July 2004 [aff. subsp. *nigra* (1); aff. subsp. *tornata* (1/0); not assignable (4/0)].

VJL (4/2), Smědava, peaty wold of the river Jizera, 50.8614°N, 15.3075°E, 840 m, 6 August 2005 [aff. subsp. *nigra* (2); subsp. *recta* (1/1) – D_{52}; aff. subsp. *recta* (1/1) – C_{51}].

VOL (4/0), Budislav, peaty bank of the brook in the sandstone valley Voletín, c. 1 km NW from the settlement Borek, 49.8103°N, 16.1458°E, 500 m, 10 July 2004 [aff. subsp. *nigra* (3/0); not assignable (1/0)].

ZAL (3/0), Studnice (at Hlinsko), bank of a brook in the fen meadow, c. 1 km NNE from the village Zalíbené, 49.7211°N, 15.9042°E, 614 m, 18 August 2005 [aff. subsp. *juncella* (1/0); not assignable (2/0)].

Sweden:

KAV (3/3), Hillerstorp, pine forest in the peat bog Store Moose, north-west bank of the lake Kävsjön, c. 1.5 km ESE from the traffic circle at the NE

edge of the town, 57.3153°N, 13.935°E, 170 m, 12 July 2008 [aff. subsp. *juncella* (1/1) – B_{53}; subsp. *nigra* (1/1) – D_{54}; not assignable (1/1) – B_{55}].

STM (1/1), Hillerstorp, peat bog Store Moose, east bank of the lake Kävsjön, c. 4.5 km ESE from the traffic circle at the NE edge of the town, 57.3067°N, 13.9813°E, 170 m, 12 July 2008, M. Štech [not assignable (1/1) – D_{56}].

VAN (1/1), Sandtorp, wet meadow c. 2.3 km NNE from the village, 58.5636°N, 13.3942°E, 147 m, 13 July 2008 [aff. subsp. *juncella* (1/1) – A_{57}].

Norway:

ATN (1/1), Stor-Elvdal, stony bank of the river Atna, c. 11 km from the railway station in the town Atna and c. 8.5 km E from the town Mogrenda, 61.7878°N, 10.6619°E, 420 m, 14 July 2008, F. Kolář [aff. subsp. *nigra* (1/1) – B_{58}].

BAK (2/2), Kvinnherad, mountain range Folgefonna, wet acidophilous grassland at a road verge, c. 4 km NNE from the town Utåker, c. 350 m NNE from the easternmost edge of the pond Bakkastølsvatnet, 59.8236°N, 5.9158°E, 225 m, 20 July 2008 [subsp. *nigra* (1/1) – D_{59}; not assignable (1/1) – B_{60}].

GAV (3/3), Oppdal, mountain range Dovrefjell, sloped peat bog above the NE bank of the lake Gåvålivatnet, c. 7 km NE from the village Hjerkinn, 62.2731°N, 9.6278°E, 940 m, 15 July 2008 [aff. subsp. *nigra* (2/1) – B_{61}, D_{62}; not assignable (1/1) – D_{63}].

KON (4/4), Oppdal, mountain range Dovrefjell, a brook flowing to a peat bog, c. 8 km NE from the town Hjerkinn, c. 2 km SSE from the Kongsvoll Alpine Garden, 62.2833°N, 9.6303°E, 1046 m, 15 July 2008 [aff. subsp. *nigra* (3/3) – A_{64} (2), B_{65}; not assignable (1/1) – A_{66}].

ROB (1/1), Folldal, mountain range Rondane, bank of a brook at the road c. 12 km NW from the town Atnbruna, 61.9211°N, 10.0561°E, 710 m, 14 July 2008 [aff. subsp. *juncella* (1/1) – D_{67}].

ROG (3/3), Folldal, mountain range Rondane, acidophilous grassland on shallow stony soil at the road c. 14 km NW from the town Atnbruna, 61.9361°N, 10.0322°E, 710 m, 14 July 2008 [subsp. *nigra* (1/1) – A_{69}; aff. subsp. *nigra* (1/1) – D_{68}; not assignable (1/1) – B_{70}].

RUB (1/1), Herøy, island Runde, brook in a wet meadow, c. 2 km NW from the town Runde, 62.4053°N, 5.6194°E, 25 m, 16 July 2008 [aff. subsp. *tornata* (1/1) – A_{71}].

RUC (1/1), Herøy, island Runde, edge of a coastal cliff, c. 2.7 km NW from the town Runde, 62.4103°N, 5.6122°E, 108 m, 16 July 2008 [aff. subsp. *juncella* (1/1) – A_{72}].

VLB (1/1), Odda, mountain range Hardangervidda, bank of a brook under a basiphilous fen c. 14 km NE from the town Røldal and c. 350 m SW from the NE edge of the lake Valldalsvatnet, 59.9472°N, 6.9636°E, 780 m, 21 July 2008 [subsp. *nigra* (1/1) – A_{73}].

VLF (3/3), Odda, mountain range Hardangervidda, sloped basiphilous fen c. 14.5 km NE from the town Røldal, c. 220 m subsp. nigra from the NE edge of the lake Valldalsvatnet, 59.9519°N, 6.9647°E, 750 m, 21 July 2008 [aff. subsp. *juncella* (1/1) – A_{74}; not assignable (2/2) – B_{75}, B_{76}].

Finland:

KMN (2/2), Inari, peat bog at the road c. 23 km NNW from the town Kaamanen, 69.3214°N, 27.2164°E, 220 m, 25 July 2004, Jiří Košnar [subsp. *juncella* (2/2) – B_{77}, B_{78}].

OUL (2/2), Kuusamo, miry sites subsp. nigra from the river Oulankajoki, c. 11 km NNE from the village Käylä, at the path c. 500 m NNW from the tourist centre Luontokeskus, 66.3767°N, 29.2961°E, 200 m, 27 July 2004, L. Ekrt, M. Štech [aff. subsp. *juncella* (2/2) – B_{79}, B_{80}].

Russia:

SVR (1/1), Kovkenitsy, peat bog at the bank of the river Svir, c. 600 m NE from the bridge in the village Kovkenitsy, 60.6508°N, 33.2425°E, 10 m, 17 July 2004, Jiří Košnar [not assignable (1/1) – D_{81}].

TAB (7/5), Segezha, bank of the pool in the village Taboyporog, 63.5836°N, 34.1519°E, 90 m, 18 July 2004, Jiří Košnar [aff. subsp. *juncella* (1/1) – A_{82}; aff. subsp. *nigra* (4/2) – A_{83}, D_{84}; aff. subsp. *tornata* (1/1) – D_{85}; not assignable (1/1) – B_{86}].

VIK (1/1), Kem, bank of the river Viksh, at the bridge (road between Sankt Petersburg and Murmansk) c. 47 NW from the town Kem, 65.2128°N, 33.7886°E, 70 m, 19 July 2004, Jiří Košnar [aff. subsp. juncella (1/1) – B_{87}].

General conclusions

General conclusions

Main stream of current biosystematic research in *Cyperaceae* is particularly focused on karyotype evolution, investigations of species genetic structure, and phylogenetic reconstructions inferred from molecular markers. Although the presented thesis did not explicitly follow this research scheme, it did not fail to reveal new findings of importance for improving our understanding the mechanisms causing morphological diversity and taxonomic complexity within the family.

Natural hybridization was for the first time documented, by jointed evidence from morphological and molecular markers, in *Eleocharis* subgenus *Limnochloa* (paper I). It was found that the structure of hybrid zone in the area of sympatry of several *Limnochloa* species can be more complex than one would intuitively infer from morphological characters. One of the parental lineages was, at the time of the study, regarded as a supposedly new undescribed species. For further taxonomic research, which is very active in the predominantly tropical subgenus *Limnochloa* and entails an increasing number of new species delineated usually at the base of morphological characters only, the potential role of hybridization in formation of new morphotypes should be taken into account.

Morphological plasticity of rhizome system was found to be an important source of the variability of *Carex nigra*. Whether the plants of the species achieve loose rhizomatous or dense cespitose growth form can be in many cases determined purely environmentally. Several environmental factors responsible for variability of rhizome system of *C. nigra* were identified, and it is plausible that these factors may play role in others rhizomatous graminoids as well. On the other hand, at least subtle genetic (ecotypic) differentiation of the cespitose morphotypes of *C. nigra* exists in some populations. These findings represent one of the few experimentally-based proofs that in *C. nigra* the mode of clonal growth cannot be regarded as a reliable taxonomic character (paper II).

Critical revision of the taxa considered either as infraspecific variants of *C. nigra* or as a species closely related to *C. nigra* revealed inadequacy of the narrow taxonomic concept (paper III). This finding implies that if

any taxonomically relevant genetic structure exists in broadly distributed sexual species, such as *C. nigra*, it may occur at much greater geographical scales than has been traditionally expected.

Curriculum vitae

Curriculum vitae

PERSONAL DATA

Jan Košnar,
born 28 February 1982, Polička, Czech Republic,
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EDUCATION

2000–2003: Faculty of Biological Sciences, University of South Bohemia in České Budějovice, bachelor study in biology, Bc. Thesis – Srovnávací studie lučních ostřic [Comparative study of meadow sedges].

2003–2006: Faculty of Biological Sciences, University of South Bohemia in České Budějovice, master study in botany, Mgr. Thesis – Morfologické a ekologické aspekty problematiky trsnatých morfotypů *Carex nigra* (L.) Reich. v České republice [Morphological and ecological studies on the tussock-forming types of *Carex nigra* in the Czech Republic].

Since 2006: Faculty of Biological Sciences, University of South Bohemia in České Budějovice, doctoral study in botany, PhD. Thesis – Biosystematic studies in the family *Cyperaceae*.

EMPLOYMENT

2008–2010: Faculty of Science, University of South Bohemia in České Budějovice, part-time job; *position:* skilled employee in research projects in systematic botany; *responsibilities:* collecting of plant material in the field, cultivation experiments, DNA analyses, and data analyses.

2010: Administration of the Protected Landscape Area Broumovsko, Police nad Metují, part-time job; *position*: employee for updating of

habitat mapping; *responsibilities*: mapping and documenting of vegetation.

2011: Nature Conservation Agency of the Czech Republic, Pardubice, full-time job; *position*: botanist (with focus on the system NATURA 2000); *responsibilities*: mapping, monitoring, and documenting of vegetation and plant species, co-ordination of habitat mapping in the Pardubice region (East Bohemia), evaluation of impacts of development projects on plant species and communities, and designing of nature conservation management.

Since 2012: Administration of the Protected Landscape Area Žďárské vrchy, Žďár nad Sázavou, full-time job; *position*: botanist; *responsibilities*: mapping and monitoring of plant species and communities, designing, organizing, and pursuing of nature conservation management, evaluation of impacts of development projects on plant species and communities, and administration in nature conservation.

TEACHING

2006–2009: practicals of the course Botany of higher plants (Faculty of Science, University of South Bohemia in České Budějovice).

2004–2009: field botanical excursions (Faculty of Science, University of South Bohemia in České Budějovice)

PUBLICATIONS

Journals with impact factor

Košnar J., Košnar J., Herbstová M., Macek P., Rejmánková E., Štech M., 2010. Natural hybridization in tropical spikerushes of *Eleocharis* subgenus *Limnochloa* (*Cyperaceae*): evidence from morphology and DNA markers. American Journal of Botany 97, 1229–1240.

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Biosystematic studies in the family *Cyperaceae*
Ph.D. Thesis Series, 2013, No. 7

Printed in the Czech Republic by Vlastimil Johanus
Edition of 20 copies

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