



MASARYK UNIVERSITY
FACULTY OF SCIENCE
DEPARTMENT OF BOTANY AND ZOOLOGY

Review of the PhD thesis "Biosystematic studies in the family *Cyperaceae*" submitted by Jan Košnar

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I. General comments

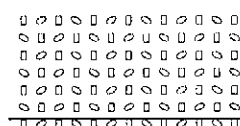
The thesis of Jan Košnar examines the variation in two model taxa of *Cyperaceae*, *Carex nigra* s. lat. and *Eleocharis* subgen. *Limnochloa*. The main topic addresses the relationship between morphological and genetic variability in these species aggregates in Europe and Central America, respectively.

The methods used in the thesis are based particularly on (i) molecular techniques – analyses of sequence polymorphism in ribosomal DNA internal transcribed spacer region (ITS), analyses of polymorphism in microsatellites (simple sequence repeats; SSR) and analyses isozyme polymorphism. (ii) The sets of morphological features were also studied and statistically evaluated in both analyzed groups. In the case of *Carex nigra* s. lat., (iii) sophisticated cultivation experiments were carried out, too. Finally, (iv) vegetation data analyses as proxies of environmental parameters were used for explanation of morphologic variation in the last taxon.

The thesis is well written, clear to read, and nearly free of typographical and other minor errors. It consists of three original papers: two published (in American Journal of Botany and in the journal Flora) plus one paper-manuscript in preparation to be submitted. Theses is completed by a comprehensive general introduction, where objectives, model taxa, analyzed micro-evolutionary processes putatively responsible for detected morphological variation, and summary of results, are presented. The candidate is the first author of all three key parts.

The key results of the thesis are: (i) detection of natural hybridization in *Eleocharis* subgen. *Limnochloa*; (ii) description of complex hybrid zone in this subgenus in Belize; (iii) proof of at least partial instability of traditionally distinguished *Carex nigra*-morphotypes under different cultivation treatments as well as (iv) confirmation of the absence or nearly absence of relationship between morphological and genetic variability at a broad geographical scale in this species.

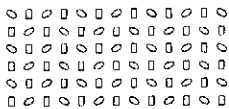
Thesis of Jan Košnar constitutes, in my opinion, a very valuable addition to the study of taxonomy of two difficult complexes in genera *Eleocharis* and *Carex*. The candidate has demonstrated creative capacities and very good skills in molecular and morphometric methods as well as in results presentation. I recommend warmly to accept this thesis for the doctor degree in the field of Botany after its successful defense.



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In Czech: Disertace Jana Košnara představuje podle mého názoru velmi hodnotný přínos k výzkumu taxonomie dvou obtížných komplexů v rodech *Eleocharis* a *Carex*. Kandidát prokázal tvůrčí schopnosti a značné dovednosti jak v metodách molekulárních tak morfometrických, jakožto i při prezentaci výsledků. Vřele proto doporučuji tuto disertační práci k obhajobě a považuji ji za splňující požadavky k udělení doktorské hodnosti v oboru Botanika.

II. Questions for defense/discussion

1. In the introduction the pseudomonad or monad type of microsporogenesis is mentioned as a common feature of *Cyperaceae* "instead of pollen tetrad typical for *Juncaceae*" (p. 4). Such a comparison could suggest the rarity of tetrads pollen formation. --- **In what plant taxa - genera or families – pseudomonad pollen development is actually present? (instead of *Cyperaceae*, of course)**
2. The nuclear genome is probably variable and recombined due to frequent chromosomal fissions and fusions, however, there is one substantial constraint of chromosome rearrangement via crossing-over in holocentric organisms compared to monocentric ones which can reduce the mentioned variability. --- **Which one?**
3. There is really low frequency of polyploidy in the genus *Carex* as mentioned in Introduction (p. 10). --- **Do you have any reason (at least speculative) for this low frequency of whole genome duplication in this species-rich genus?**
4. If there were microsatellite and ITS evidences for hybridization of *Eleocharis cellulosa* and *E. interstincta* with unknown taxon from *Limnochloa*-subgenus, --- **why did you not describe it?**

III. Brief comments/which cannot be answered

5. The subfamily *Mapanioideae* is characterized as "exclusively tropical and subtropical group" (p. 5). --- **Is it really true in the case of small tribe of *Chrysitricheae*?**
6. The term "morphologically highly uniform congeners" (used in the page 27) in relation to hardly distinguishable inter-specific hybrids is a little bit confusing, in my opinion. "Morphologically highly similar congeners" would be less ambiguous, as the hybrids between **morphologically non-uniform but similar** congeners could be much easily overlooked than hybrids between **morphologically uniform but dissimilar** congeners. --- **What do you think?** [in other words: (congeners interspecifically uniform in morphology = morphologically similar congeners) but (congeners intraspecifically uniform in morphology ≠ morphologically similar congeners)]

doc. RNDr. Petr Bureš, Ph.D.



Opponent's review of the Doctoral Thesis

Thesis title: **Biosystematic studies in the family *Cyperaceae***

Author: **Jan Košnar**

The submitted thesis covers selected aspects of the *Cyperaceae* family systematics, with the focus on some representatives of *Eleocharis* and *Carex* genera. It contains an Introduction and a Special part, consisting of two published research articles (journal IF=2,664 and 1,639) and one article in press.

The Chapter 1, Introduction, is given to make the thesis self-contained. It shortly reviews morphological characteristics of the family of *Cyperaceae* and presents recent phylogenetic studies, which significantly influenced its classification. *Eleocharis* and *Carex* genera are presented in the same ways. Following is the discussion over possible sources of the morphological diversity of the *Cyperaceae* family. Chapter Introduction concludes with defining the objectives of the thesis.

In the Special part selected taxonomic questions are addressed. The groups of interest are the tropical plants of *Eleocharis* subgenus *Limnochloa* and plants of the group of *Carex nigra*. In both cases, the aim is to explain the significant morphotype diversity and its impact on taxonomy of the studied groups. The results are based on morphometric and molecular data analyses. The author explains the origin of ambiguous *Eleocharis* subgenus *Limnochloa* morphotypes from northern Belize by interspecific hybridization between representatives of *Eleocharis* and *Limnochloa*. On the contrary, the overwhelming range of morphotypes of the *Carex nigra* is explained by morphological plasticity of the rhizome system rather than genetically determined traits of these conspicuous morphological forms. Based on this outcome, the author sees no justification for taxonomic evaluation of cespitose and non-cespitose morphotypes of *Carex nigra* at any level.

The questions for a discussion:

1) The author often uses terms morphological diversity and morphological variability, especially in unpublished parts of the thesis (Introduction or General conclusions). For example in the paragraph Aims of the thesis he says: The thesis attempted to contribute to understanding mechanisms that generate phenotypic (morphological) **diversity** in some taxonomically challenging members of the family *Cyperaceae*. In the next sentence, however, says: the common aim was to answer the following questions: Is observed morphological **variability** underlain by hereditary genetic differences that could stand at the beginning of new evolutionary lineages? My question is: observed variability or diversity? Could author explain differences of using of terms morphological diversity and morphological variability?

2) The author performed a detailed morphometric analysis of four infraspecific taxa of *Carex nigra*. I recommend to show a comprehensive table of measured values of observed characters in Paper III (manuscript). In particular, taxa *C. nigra* subsp. *tornata* and *C. nigra* subsp. *recta* are mentioned in botanical literature only sparsely, and the values of their morphological characters are not much known. In the Paper II, the author states that the nordic tufted morphotypes never form creeping rhizomes (p. 81). In Paper III he observed this character more profoundly. Did he reach the same findings (from the text this finding is not clear)? If so, then the plants from Central and Northern Europe are different at least in the presence or absence of creeping rhizome. However, in Paper III the author states that he found no significant morphological differences (p. 109).

3) In the Paper III, the author evaluates the infraspecific taxonomy of the cespitose and non-cespitose morphotypes of *Carex nigra*. He accepted the name *C. nigra* subsp. *juncella* (Fr.) Lemke ex Rothm. Feddes Repert. 67: 4 (1963). Could he explain why did he not accept the name *C. nigra* subsp. *juncea* (Fr.) Soó Feddes Repert. 83: 148 (1972)? I suppose that the taxon under consideration was originally described by Elias Fries in 1842 (Novit. Fl. Suec. Mant. 3: 154), as *C. vulgaris* subsp. *juncea*. In 1843, the same author changed the epithet „*juncea*“ to „*juncella*“ (Bot. Not. 1843:105). In response to a treatment of *C. nigra* given in Fries work (1843) and to Fries reference to Novit. Fl. Suec. Mant. 3, 1842: 153, it is clear that the name *C. vulgaris* subsp. *juncella* should be treated as the later synonym of *C. vulgaris* subsp. *juncea*. Does the author of presented thesis consider *C. vulgaris* subsp. *juncea* and *C. vulgaris* subsp. *juncella* as different taxa with names based on different Type specimens? If not, *C. nigra* subsp. *juncella* (Fr.) Lemke ex Rothm. is illegitimate name.

4) The protolog of the name *C. vulgaris* subsp. *juncea* Fr. and *C. vulgaris* subsp. *juncella* Fr. includes no notices about the absence or presence of the creeping rhizomes. Also Soó (1972) and Rothmaler (1963) did not mention this character, when published new combinations. Theodor Fries 1857 (Bot. Not. 1857: 207 1857, this citation is absent in the List of literature cited, Paper III) was the first who mentioned densely cespitose growth in description of *C. juncella* (Fr.) Th. Fr. Did the author see the type specimen of *C. vulgaris* subsp. *juncea*? Without typification of every taxa under consideration, it is still not clear, which names corresponds to which morphotypes, I suppose. The difficulties connected with unambiguous determination of the plants included in the analysis may be partly consequences of this nomenclatory noise.

5) *Carex nigra* subsp. *tornata* was described from Sweden. Fries in his protolog (1842:154) mentioned region in the vicinity of Uppsala. However, no plants from this region were analysed. Did the author study any herbarium material from Sweden or was he looking for corresponding plants on the field around Uppsala? It surprises me that only one sample was determined as subsp. *tornata*, and it was even collected from the Czech Republic. How often did the author notice the subsp. *tornata* in Czech Republic?

This thesis convincingly documented that Jan Košnar acquired necessary theoretical knowledge and that he can build up working hypotheses and test them. Thus, author of this thesis showed his capability for independent scientific work. Summarizing, the thesis presents findings significantly contributing not only to explanation of taxonomic complexity within studied groups, but also to our general knowledge of evolutionary mechanisms acting in plant organisms. Overall quality of the thesis meets high standards, and I therefore recommend it to be admitted for defence.

Průhonice 19. 11. 2013

RNDr. Jitka Štěpánková, CSc.
Institute of Botany, CAS
Průhonice



The Committee for PhD studies in Botany

Faculty of Science, University of South Bohemia

Madrid, 6th November 2013

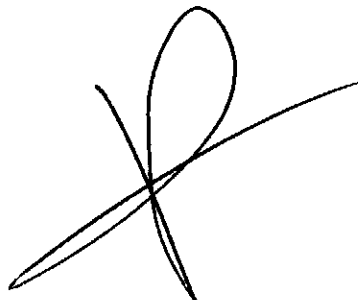
I, Pedro Jiménez-Mejías, being designated reviewer of the PhD thesis of Jan Košnar, presents the following inform about the PhD thesis Project.

This Thesis Project about the microevolutionary mechanism involved in the morphological diversity of Cyperaceae, presents hypothesis that are relevant for the current scientific knowledge, and that are accurately answered. The used techniques are at the vanguard of the molecular systematics. The discussion of the results conforms important innovations on taxonomy, systematics, plant hybridization, and ecology.

The first chapter provides the first reliable insights of hybridization within *Eleocharis* subgen. *Limnochloa*. The author reveals hybridization as the mechanism subjacent behind the observed morphological diversity. A previously unnoticed taxonomic entity is also detected.

The second and third chapters contributes to the understanding of the morphological variation of *C. nigra*, a species of unstable taxonomy that have longely confused the taxonomists. Different growth forms have been considered as subspecies or variaties, and even as different species (*C. juncella*). The author combines molecular analysis, morphometrics, and cultivation experiments to reveal the polytopic origin of the tussock-forming morphotypes from rhizome-creeping forms. It is demonstrated that the growth form is genetically mediated only in some instances, whereas in other cases it is due to environmental factors. The extreme morphology are shown to be part of a clinal variation, which prevents the distinction of any taxonomical partition within North and Central European populations of *C. nigra*. All these results together conform the first solid epistemological basis to solve this taxonomic problem, simply relegating all these forms as part of the variation of *C. nigra*.

As expert in Cyperaceae systematics in general, specialized in microevolution of *Carex*, I consider that this PhD Project is an excellent and impressive piece of work. And I propose that it is suitable to be defended in the University of South Bohemia.



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