



March 28th 2014

Evaluation of Ph. D. thesis: *Taxonomic study of the Eurasian taxa of Tortula muralis (Pottiaceae, Musci) complex* by Jiří Košnar.

With approximately 1500 species, the moss family Pottiaceae comprises approximately 10% of the bryophyte diversity. The Systematics and taxonomy of the family are extremely complex at all levels. This is also true for the genus *Tortula* Hedw. within the Pottiaceae, which has suffered great changes in the last decades. There are still many unsolved questions regarding this genus. These concern on the one hand the inclusion or exclusion of many species, traditionally considered as belonging to other genera like *Phascum* Hedw., *Pottia* Ehrh. ex Fűrnr., *Crossidium* Jur. or *Pterygoneurum* Jur., and on the other hand the delimitation of species belonging to this genus.

The thesis presented here tries to clarify one of the important taxonomic problems of the second type. *Tortula muralis* Hedw. is a frequent, widely distributed species. Its morphology is highly variable and several closely related species have been described in the scientific literature. In his Ph.D. thesis, Jiří Košnar treats these problems using a wide variety of techniques spanning from classical morphological work with detailed statistical analyses over flow cytometry to assess the ploidy level of his samples to DNA sequence data. The first part of his thesis consists of a general introduction, which over 16 pages (literature excluded) gives an excellent general overview over the state of the investigation before his thesis. In the following he presents three papers published in journals with impact factor, two of them with a factor of almost 3.0.

In his first paper he presents the results of a morphometrical study of four European taxa of the *Tortula muralis* complex. Cultivation experiments were used in order to test the genetic nature of morphological variation. In this work Jiří Košnar uses complex statistical analyses in order to study the morphological variability present in this species complex. Furthermore, he shows that the variation in ploidy level frequently observed in this species complex is only partly related with morphology.



Jiří Košnar's second paper treats a more specific problem. He shows that *Barbula montenegrina* Breidl. Szyszyl. is actually a synonym of *Tortula muralis* subsp. *obtusifolia* (Schwägr.) Culm. This work is largely based on the morphological data obtained in the first contribution, which allowed a detailed comparison of the type of *Barbula montenegrina*.

In his third contribution, Jiří Košnar uses nrITS sequences in order to define the *Tortula muralis* complex and to study the relationships within the complex. But contrasting with most studies, he not only uses one sequence per sample but shows the intragenomic sequence variation existing in many samples of this complex and the genus *Tortula* in general. His data show an extensive gene flow connecting several of the traditionally recognized species. There are only two well defined groups within this complex: *Tortula muralis*, a morphologically very broad species and the much more uniform *T. lingulata* Lindb.. As before with the morphological study of his first contribution, the candidate relates the ploidy level and in this case he finds is no phylogenetic signal.

Taken together, in his thesis the candidate shows almost perfectly how taxonomic work can and should be done today. The integration of morphological data, sequence data and flow cytometry is the way to go whenever possible. We did some work with *Tortula muralis* in the past and what Jiří Košnar has done is almost exactly what I would have tried given the opportunity to keep on working on the *Tortula muralis* species complex. I only would have liked to have some data on chloroplast sequences. But I understand that often time and the available money limit the possible choices and under these circumstances the use of nrITS is surely the best choice as these sequences are normally more informative than the most variable chloroplast DNA regions.

As a conclusion, I highly recommend that this thesis should be defended.

Dr. Olaf Werner



CHARLES UNIVERSITY IN PRAGUE

FACULTY OF SCIENCE

DEPARTMENT OF BOTANY

Mailing address: Benátská 2, CZ-128 01 Praha 2, Czech Republic

Phone: +420 221 951 646

Fax: +420 221 951 645

To whom it may concern

Review of the Ph.D. thesis of Jiří Košnar "Taxonomic study of the Eurasian taxa of *Tortula muralis* (*Pottiaceae*, *Musci*) complex"

The Ph.D. thesis of Jiří Košnar represents a significant contribution to our understanding of morphological, karyological and genetic variation of the taxonomically challenging group of mosses. The core consists of three papers published in WoS-indexed journals (*Preslia*, *Journal of Bryology and Taxon*); the candidate is the first author in all contributions. The papers included in the thesis cover many topics of contemporary biosystematics research (assessment of phenotypic variation using multivariate statistical methods, insights into ploidy differentiation with the aid of DNA flow cytometry, evaluation of gene flow and evolutionary relationships using different molecular markers, typification work, etc.) and demonstrate the candidate's knowledge of various contemporary methodological approaches. A combination of different complementary methods allowed gaining holistic insights into the patterns and processes shaping the evolutionary history of the group under investigation. In general, the addressed questions are meaningful and challenging, conclusions clear and well-founded, and the results are interpreted in the light of the most recent studies.

The **general introduction** sets nicely the scene for subsequent chapters and provides the readers with the overview of the study systems, microevolutionary processes and aims of the thesis. The text is far from a boring reading, is well-structured and well-balanced.

Questions:

- p. 11: the author claims that the role of cryptospeciation varies among geographic regions. Can this information be elaborated in more detail?
- p. 14: the frequency of polyploidy in mosses is a hotly debated topic, with estimates ranging from ~ 20% to ~ 80%. The candidate seems to prefer the upper end of this range – which pieces of evidence support his opinion?

Technical comments and typos

- recent data indicate higher proportions of polyploids in angiosperms than those published in 1990s (p. 14)
- similarly to bryophytes, allopolyploidy has generally been assumed to be the major mode of genome duplication in angiosperms (p. 15)

Paper 1 deals with morphological and cytological variation of four European taxa of the *Tortula muralis* agg. The results challenged some previous taxonomic concepts and allowed to draw more robust taxonomic treatment. I appreciate that the stability of selected characters was assessed using a cultivation experiment.

Questions:

- a crucial prerequisite of discriminant analysis is an accurate assignment of all investigated samples to a particular taxon (i.e., correct delimitation of groups subjected to discrimination). I believe that the most correct approach is to include type specimens of all taxa. Because the analyzed samples were determined on the basis of their morphology (which may introduce a subjective bias), I would like to see evidence that the analyzed taxonomic groups were correctly circumscribed (i.e., were homogeneous and corresponded to a particular named taxon).
- the number of analyzed individuals in some groups was quite small (occasionally smaller than was the number of measured characters) – can this fact bias results of discriminant analysis?
- can maternal effect be an issue in cultivation experiment? Was the duration of cultivation (5-7 months) sufficient to separate the genetic variation vs. environmental plasticity? Cultivation conditions should be provided (optimally, plants should be cultivated under different conditions).
- were some morphological differences observed between haploid and diploid accessions of the same ploidy-variable taxon (to elucidate the effect of genome duplication on phenotype)?

Comments:

- p. 38: all fluorochromes are solid – final concentration should therefore be expressed as $\mu\text{g/ml}$ (not $\mu\text{l/ml}$). It should be indicated whether the genome size value of *Glycine* corresponds to 1C or 2C.
- p. 39: I would appreciate more details about the adopted protocol for chromosome counts
- p. 40: close to the *T. lingulata* cluster (not *T. obtusifolia* cluster)
- p. 42: same number of decimal points should be presented for both mean and SD. In fact, two decimal points would be sufficient because higher resolution can hardly be achieved in flow cytometric analyses.
- it would be valuable if a representative FCM histogram is presented (actually, the standard / sample ratio for diploid accessions is too small – beyond recommended values)
- p. 47: ... incidence OF common...

In **Paper 2**, the taxonomic status of an enigmatic species described from Montenegro is elucidated. The taxonomic conclusion is well substantiated and I have no special questions. It would be nice to know ploidy level of the type material but I understand that obtaining fresh material from *locus classicus* might be challenging.

Paper 3 addresses the question of gene flow between different taxa and the origin of polyploids using molecular (ITS) markers. Both major findings (the incidence of intragenomic ITS variation and support for autopolyploid origin of some lineages) are truly novel and valuable, and I believe that the paper will be appreciated by a wide scientific community.

Questions:

- there are dramatic discrepancies between taxonomic conclusions based on “conventional” morphological approach (Paper 1) and genetic relationships inferred from molecular sequence data. In addition, several recognized taxa are ploidy heterogeneous and many intermediate morphotypes occur. Would not be better to adopt some different taxonomic concept (i.e., is it meaningful to recognize all species, subspecies and varieties within the complex)?

- have the candidate considered the assessment of morphological variation in individual evolutionary lineages (i.e., to search for lineage-specific morphological characters, which can possibly turned out to be taxonomically informative)?

Comments:

- *obtusifolia* – as subspecies on p. 89 while full species on p. 90
- correct formula for DAPI is 4',6 ... (not 4,6)

In summary, I do consider that the thesis not only fulfils all criteria necessary for obtaining the Ph.D. degree but the work of J. Košnar appears to me as a paradigmatic example how a modern Ph.D. thesis should be made.

Final evaluation: I warmly recommend the thesis for the award of the degree of Doctor of Philosophy (Ph.D).

Signed in Prague on April 6, 2014

Doc. RNDr. Jan Suda, Ph.D.

