Linking taxonomy with other pieces of information: tesserae in the mosaic of bryophyte life

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Introduction

Bryophytes belong to the oldest extant lineages of land plants, having originated from the common ancestors of the embryophytes in probably several successive radiation events starting in the early Palaeozoic (Kenrick & Crane, 1997). Embryophytes appeared on Earth during the Ordovician period about 480 mya (Wickett et al., 2014) and bryophytes were among the earliest embryophyte lineages that colonized terrestrial habitats and assisted transforming the environment on land. Yet, the early history and branching pattern of the first land plant lineages remains obscured for many reasons. These particularly include poor fossil record of bryophytes and sparse taxon sampling for large-scaled analyses of extant taxa. Thus, whereas most of the recent studies agreed on liverworts representing the oldest sister lineage to the rest of embryophytes (Chang & Graham, 2011; Qiu et al., 1998; Gao et al., 2010), mosses, diverging next as a first representative of embryophytes with stomata, and hornworts being the youngest bryophyte lineage, sister to polysporangiophytes (Qiu et al., 2006, 2007; Liu et al., 2014), this idea has recently been challenged by a large phylotranscriptomic analysis (Wickett et al., 2014). The authors suggested several alternative topologies depending on the matrices used and also the evaluation methods; in two of them, either the hornworts or monophyletic bryophytes were recovered as the sister group of the rest of embryophytes. In the light of inconclusive results of phylogenetic reconstruction, it is perhaps better to understand the traditional label 'bryophytes' as a structurally and ecologically defined unit of green, autotrophic land plants with a dominant gametophyte and largely heterotrophic, ephemeral unbranched sporophyte bearing a single sporangium. This group comprises some 15–20 000 species according to the latest estimates in total (Shaw et al., 2011), about one-tenth of all embryophytes.

Constituting only rarely a conspicuous component of the biomass in terrestrial biotopes and being absent from Earth's oceans completely, bryophytes receive even less attention than they would deserve in proportion to their share on Earth biodiversity, owing to the virtual lack of direct positive or negative importance to humans like nutritional value, presence of toxins or destructive abilities, shown by other representatives of green plants. Nevertheless, bryophytes can have important roles in Earth's ecosystems. Boreal peatlands are perhaps the prime example of biotopes where they form a dominating part of the biomass but even on a global scale, the major poikilohydric organisms, bryophytes with lichens, may play an important role in Earth's biogeochemical cycles (Porada et al., 2013, 2014). Northern soil systems, the huge reservoirs of carbon originating to a significant extent from mosses, particularly the

genus *Sphagnum*, may react sensitively to even slight temperature changes. Increased rates of soil decomposition, triggered by global warming, may turn these systems from global carbon sink to carbon source, which could greatly affect the global carbon circulation (O'Neill 2000). It is generally appreciated that, with respect to no effective barriers between their inner cell compartment and the environment, that bryophytes react much more sensitively to the physical and chemical conditions of their sites and their changes, both in the negative and positive senses and their population dynamics can precede the changes exhibited by the populations of dominant vascular plants and organisms connected to them via food webs and other ecological relations.

Bryophytes are also a popular model system with respect to their haploidy and structural simplicity (Shaw & Goffinet, 2000). Milestones in discoveries related to their life cycle and biology, such as the description of alteration of sporophytic and gametophytic generation (Hofmeister, 1851), description of haploid and diploid phase in plants (Strasburger, 1894), discovery of sex-chromosomes in the liverwort *Sphaerocarpos* (Allen, 1919) and heterochromatin in the liverwort *Pellia* (Heitz, 1928), or the first sequenced chloroplast genome (Ohyama et al., 1986) are thus at the same time milestones in plant knowledge in general.

Taxonomy, cryptic diversity, speciation processes in bryophytes

Taxonomic studies of bryophytes, along with the whole bryology as a field of scientific investigation, has a somewhat shorter history than analogous studies in vascular plants. The main reason is the microscopic nature of not only anatomical but even gross morphological characters, which were hidden to human eye prior to the invention and wider use of microscope. Nonetheless, since the beginning of the 19th century, systematics of bryophytes roughly keeps the pace with systematics of vascular plants, despite the always smaller human capacities and material sources. One of the typical characteristics of bryophyte thalli, whether we observe the reduced sporophytic or the generally more elaborate gametophytic phase, is the relatively small number of available morphological and anatomical characters. Together with the general belief in nearly unlimited world-wide dispersal capacities of bryophytes, the 'modern' world-wide taxonomic generic and familial revisions of the 20th century of often arrived at similar conclusions, which resulted in extensive synonymizations of dozens of taxa, with the accepted ones showing broad, often cosmopolitan distribution areas and euryoecious ecology. Indeed, bryophytes were even believed to represent 'unmoving, unchanging sphinxes of the past' (Crum, 1972). The underlying hidden diversity was first evidenced with the advent of molecular studies in studies of thallose liverworts in the

late 1970s, and more often in early 1990s (Szweykowski & Krzakowa, 1979; Dewey, 1989; Odrzykoski & Szweykowski, 1991). Since then, however, the number of documented cases climbs every year (Shaw, 2001), although most studies arrive at discovering of at least subtle morphological, ecological and geographical differentiation among lineages, which allow for re-consideration of earlier synonymized taxa or the description of new ones (e.g., Hedenäs et al., 2014; **Paper 2**, **Paper 3**).

The cryptic and semi-cryptic diversity is however only one of the challenges in current taxonomy of bryophytes, which is perhaps less complicated methodologically. Recent studies of polyploid species complexes, such as those in the genus *Sphagnum* (e.g., Shaw et al., 2008; Shaw et al., 2012), documented a complicated hybridization network that sometimes involves more than two parental taxa (Karlin et al., 2009) and recurrent origins of allopolyploid lineages (Shaw et al., 2015). Such situations, which we also documented in *Tortula muralis* complex (**Paper 7**), have no straightforward taxonomic solution which would allow labelling all specimens, despite the relatively good knowledge of their origin.

Phylogeny & taxonomy of Pottiaceae

The moss family Pottiaceae is considered to be the largest bryophyte family, with more than 1450 species recognized in the last comprehensive monograph (Zander, 1993). Of course, both family and individual species delimitation and recognition may be subject of individual taxonomic views, which might affect the species counts both in Pottiaceae and in families considered for comparison. Taxonomy of Pottiaceae is generally perceived as being notoriously difficult with respect to the generally minute size of plants and the relatively small number of available morphological characters on both gametophyte and sporophyte. These characteristics constitute the basis to identification difficulties referring to individual taxa at or below species rank. Nevertheless, the major challenge of pottiaceous taxonomy of these days might rather dwell in understanding the affinities among species. Many generic concepts in Pottiaceae, which throughout most of the 20th century followed Brotherus (1924–1925) were revolutionized by Zander (1993). He correctly recognized that the earlier sporophyte-focused higher rank taxonomy is often misguided, as the sporophyte in Pottiaceae is very commonly strongly reduced and modifiable by environment. This led him to description of 'reduction series' of sporophytic characters in several larger genera such as *Tortula*, which he understood as containing the earlier generally recognized genera *Desmatodon*, *Pottia* and *Phascum*, or *Weissia* including *Hymenostomum* and *Astomum*. On the other hand, greater focus on gametophytic characters allowed him to segregate *Microbryum* or *Hennediella* from

Pottia, *Syntrichia* from *Tortula*, or *Bryoerythrophyllum*, *Pseudocrossidium*, and *Didymodon* from *Barbula*, although in the latter case the concept was to a large extent adopted from another major treatment of Japanese Pottiaceae (Saito, 1975). Molecular phylogenetic studies, which included broader selections of haplolepidous taxa performed so far (La Farge et al., 2000; Hedderson et al., 2004; Werner et al., 2004; Cox et al., 2010; Stech et al., 2012), confirmed the monophyly of Pottiaceae nearly in sense of Zander (1993), with notable exceptions being the small genera *Timmiella* and *Luisierella* removed to basalmost lineages of Dicranidae (Hedderson et al., 2004; Inoue & Tsubota, 2014), the monogeneric family Hypodontiaceae having been shown to be related with Dicranaceae (Stech & Frey, 2008; Stech et al., 2012) and, on the contrary, similarly not numerous Cinclidotaceae, Ephemeraceae, and Splachnobryaceae being nested within Pottiaceae (Werner et al., 2004; Cox et al., 2010; Stech et al., 2012). While the monophyly of Pottiaceae, pending the above mentioned deviations from older morphology-based concepts, seems to be relatively firmly established, only a few generic concepts have been rigorously tested and the results of the accomplished studies point to large and sometimes surprising rearrangements. One of the first molecular studies in Pottiaceae was devoted to the genus *Tortula* and related genera (Werner et al., 2002). Although this study used a single chloroplast marker (the gene *rps4*) and only 15 of about 200 of accepted species were analysed with a similar number of putatively related genera, it largely confirmed Zander's not yet generally accepted view (Zander, 1993), although it also suggested that smaller genera *Crossidium*, *Pterygoneurum* and *Stegonia* should also probably me merged with *Tortula* or their generic delimitations need to be amended. Also the following study, devoted to another large pottiaceous genus, *Didymodon* (Werner et al., 2005a), confirmed largely the earlier delimitation of the genus by Saito (1975) and Zander (1993). On the contrary, the following study of *Trichostomoideae* (Werner et al., 2005b) showed that species of *Trichostomum* are interspersed among lineages of *Weissia*, and the same applies to *Pleurochaete* with respect to *Tortella*, or *Oxystegus*, *Chionoloma* and *Pseudosymblepharis*. This study thus led to the description of *Pottiopsis* (Ros & Werner, 2007), and helped to reinstate the genus *Oxystegus*, to that time mostly recognized within *Trichostomum* (Köckinger et al., 2010), with the remaining genera still in need to be settled. Following mostly smaller studies with molecular phylogenetic analyses based either on a single nuclear or chloroplast marker or a modest combination of chloroplast, mitochondrial or nuclear ribosomal marker(s) confirmed the nested position of *Pleurochaete* within *Tortella* (Grundmann et al., 2006), partly revised the limits between *Leptodontium* and *Triquetrella* (Hedderson & Zander, 2007), confirmed the monophyly

of *Hennediella* after a slight modification of the concept (Cano et al., 2009), suggested merger of monotypic *Erythrophyllastrum* with *Erythrophyllopsis* (Cano et al., 2010b), confirmed the isolated position of another newly recognized genus, *Indopottia* (Akiyama & Goffinet, 2011) and the affinity of *Plaubelia* with *Hyophila* (Mao et al., 2014), and also helped to establish two new South American genera, *Guerramontesia* and *Andina* (Cano et al., 2010a; Jiménez Fernández et al., 2012). As most of the above mentioned studies except Werner et al. (2002, 2005b) avoided problematically defined larger genera, taxonomic rearrangements based on the molecularly supported affinities were scanty. It is nevertheless obvious that the time for novel generic delimitations will come in near future. Already our first taxonomic study utilizing molecular data (**Paper 3**) suggested that *Hymenostylium*, a moderately large genus of some 20 species, is not monophyletic, and should include the monotypic *Reimersia*, and its affinities with *Molendoa*, *Tuerckheimia* and other, molecularly yet never analysed genera, such as *Quaesticula*, *Hymenostyliella* or *Teniolophora*, should be revised. Truly revolutionary in this context came our paper, revising – yet only partly – the generic delimitation of *Barbula* (**Paper 2**), the putatively largest genus of Pottiaceae, estimated to contain some 200 species (Zander, 2007a). Having used one nuclear (ITS) and two chloroplast markers (*rps4* gene + *rps4–trnS* spacer, *trnM-trnV* spacer) on a possibly representative selection of taxa, we confirmed the earlier suspicion of deep polyphyly of *Barbula* s.l., leaving *Barbula* possibly monotypic (!) in the subfamily Pottioideae, moving most of its species to predominantly palaeotropical genus *Hydrogonium* with unclear affinities within the subfamily Trichostomoideae. This example points to the necessity of revising large and possibly heterogeneous genera, such as *Trichostomum* or *Tortula*.

Studies in *Didymodon* and other taxonomic & phylogenetic studies

My taxonomic studies started with the revision of *Didymodon rigidulus* group in Europe (Kučera, 1999). *Didymodon rigidulus* group was defined as the European taxa included by Zander (1993) into the concept of this species, whether as recognized infraspecific taxa (*D. rigidulus* subsp. *andreaeoides*, and *D. acutus*, *D. icmadophilus* and *D. glaucus* as varieties of *D. rigidulus*), taxonomic synonyms (*D. validus, D. verbanus*), or accepted putatively closely related taxa (*D. mamillosus*). The results of this study, based mostly on microscopic examination of specimens and morphometric analysis could be summarized as follows: (1) *Didymodon rigidulus* subsp. *andreaeoides* is a well-defined species, which has been shown to be a taxonomic synonym of *D. subandreaeoides* (**Paper 4**), (2) *D. mamillosus* is a taxonomic synonym of *D. rigidulus* (Kučera, 2000), (3) *D. glaucus* is a well-defined species with *D. verbanus* being either its taxonomic synonym

or an infraspecific taxon thereof, (4) *D. acutus* and *D. icmadophilus* are well-defined taxa with respect to *D. rigidulus* but gametophytically are often indistinguishable; the sporophytic differences however support at least the varietal status of *D. icmadophilus* within the concept of *D. acutus*, and (5) *D. validus* is a problematic taxon, morphologically intergrading with both *D. acutus* s.l. and *D. rigidulus*, but the occasional presence of axillary gemmae suggests rather its affinity with *D. rigidulus*, and is thus best provisionally recognized as a variety thereof. As morphological and anatomical characters proved insufficient to convincingly support either option under (3) to (5), we started molecular investigation of these and other putatively related taxa from the whole Northern Hemisphere, based on sequence data from two chloroplast regions (*rps4*, *trnM-trnV*) and the nuclear ribosomal ITS. To this date, the results have not been finished and preliminary results were presented only at several conference events (e.g., Kučera, 2010). Principal reason is the extremely complicated nature of both molecular and morphological delimitation of the taxa in the complex. While some of the lineages are well-defined by the molecular markers, and are either nearly invariable (*D. acutus*) or surprisingly variable but still constituting a monophyletic lineage (*D. icmadophilus*), others are well-defined morphologically but hardly forming a distinct lineage with respect to molecular markers (*D. cordatus*, *D. anserinocapitatus*). In other taxa, neither morphological nor molecular definition seems to be straightforward (*D. validus*, *D. constrictus*, *D. tectorum…*) and several morphologically semi-cryptic species need to be described if the morphologically well-defined lineages should not be merged with completely different plants. In any case, none of the morphology-based hypotheses (4–5) mentioned above has support from molecular data.

One at least partly completed chapter from taxonomy of *Didymodon* was the revision of the section *Rufiduli* (**Paper 1**). This paper brought quite a novel insight into the understanding of phylogenetic affinities within *Didymodon*. The only previous molecular study of this large genus (Werner et al., 2005) included only European species and utilized only a single hypervariable marker, nrITS. Both insufficient sampling, which omitted most of the members of sect. *Rufiduli* and the marker, which is too variable to provide reliable data on such a diversified selection of species (which of course hardly could have been anticipated), allowed for only relatively modest conclusions and provided no support for any infrageneric delimitation. The complete sampling in sect. *Rufiduli* and a representative selection of other taxa from all parts of the world allowed much clearer conclusions with respect to infrageneric delimitation in **Paper 1**. Sect. *Rufiduli* was reinstated in the original understanding of Chen (1941), including most of the relatively only recently described Central Asian species with propaguliferous leaf

apices. The newly segregated monotypic genus *Exobryum* (Zander, 2013) was clearly shown to be nested within sect. *Rufiduli*, the genus *Fuscobryum* (l.c.) was removed from the section and transferred as a subsection of sect. *Didymodon*, and the genera *Geheebia*, *Vinealobryum* and *Trichostomopsis* (l.c.) were put into synonymy with traditionally recognized sections *Fallaces*, *Vineales* and *Asteriscium*, respectively. **Paper 1**, however, also pointed to several open problems, including the polyphyly of *D. asperifolius* and *D. gaochienii* in the present concepts, and possible hybridization between *D. hedysariformis* and *D. gaochienii* in the understanding of Russian taxonomists.

Other taxonomic and phylogenetic studies of our group often result from more or less occasional and at first minor stimuli. Our treatment of neglected taxa in European *Hymenostylium* (**Paper 3**) was initiated by the consultation of the generic identity of two putatively undescribed Alpine taxa of gymnostomoid affinities. Similarly, our revision of generic delimitation of *Barbula* (**Paper 2**) started with the discovery of the plant originally identified as *Barbula amplexifolia* in Switzerland, which turned out to be identical with *B. indica* var. *kurilensis*, the putative endemic of the Kuril Islands, which we proved to be the closest relative of the tropical *Hydrogonium consanguineum*. The study of *Hydrogonium* at the world-wide level continues, similarly to studies from *Hymenostylium* and related genera (*Molendoa, Anoectangium, Gymnostomum*). The cooperation with other bryologists however sometimes leads to small-scaled studies of non-pottiaceous mosses, such as *Pohlia* (Köckinger et al., 2005), *Bryum* (Kučera & Holyoak, 2005), *Hymenoloma* (Werner et al., 2013), *Brachythecium* (with H. Köckinger, unpublished), or *Hypnum* s. l. (with M. S. Ignatov, unpublished).

Research of the bryological group at University of South Bohemia

Taxonomy and molecular taxonomy is of course one of the research topics of the bryological team at the Department of Botany. In addition to ongoing taxonomic and molecular-phylogenetic studies in the genera of Pottiaceae mentioned above, further investigation is devoted to speciation processes in the taxonomically difficult *Tortula muralis* aggregate. The latter species complex was studied in the scope of the master thesis and doctoral dissertation of J. Košnar and led to a publication (**Paper 7**), which demonstrated the role of gene flow and polyploidy in the origin of studied lineages, implying difficulties and at times impossibility of simple taxonomic decisions in species groups where speciation patterns are complex and reticulate. An interesting by-product of this study was the first published ITS-based phylogeny of *Tortula* and related taxa, complementing the study of Werner et al. (2002).

Second major topic of the bryological research of our group is the biology of rare mosses, particularly of the threatened rich fen species and the liverworts on decaying wood. Most studies to date were dedicated to *Hamatocaulis vernicosus*, an Annex II species of the European Habitats Directive. The interest in this species has been stimulated with the creation of Natura 2000 network in the Czech Republic, for which *H. vernicosus* was one of the important flagship species of a globally threatened habitat of rich fens. As of 2000, only a handful of recent localities were known but only several years later, in 2005, we were able to start monitoring the basic water chemistry and water level fluctuation at 28 Czech localities and established manipulative experiments to assess the role of management, particularly the mowing, on the dynamics of the patches. The first results were published in 2007 (Štechová & Kučera, 2007) and the more complete outcomes were released five years later (**Paper 8**). We found that the species thrives best at localities with stable and high water level, low herb competition, and relatively high concentration of iron, whereas no relation was found among the concentration of major nutrients and the population demographic characteristics. Pleasing consequence of the focused 'hunt' of *Hamatocaulis vernicosus* was the discovery of numerous additional localities – 54 in total as of 2012 (Štechová et al., 2012), and more than 60 at present. Results of similar studies help the implementation of practical measures in management of fen habitats (Štechová et al., 2014).

The third topic, which we started to develop in last years, are the population genetic studies, focusing likewise on rare bryophyte species. Genetic structure of populations is an informative window into the reproductive system and demographic history. The first of our studies that already has been published investigated the patterns of genetic variation and spatial genetic structure in restricted Central European and large Finnish population of a strictly epixylic liverwort, *Crossocalyx hellerianus* (**Paper 6**). Although some of the results were expected, such as the reduced genetic variation in small Central European populations and much lower levels of gene flow among them, which we attributed to obvious habitat fragmentation, the levels of genetic diversity were still higher than one would expect in putatively only asexually propagating colonies. As high values of linkage disequilibrium indicated the lack of effective recombination among genotypes in the small Czech populations, we hypothesized the major source of the genetic variability in these populations being the somatic mutations. Two other population genetic studies of threatened fen species *Hamatocaulis vernicosus* and *Helodium blandowii* are in the progress now.

Although the three above mentioned topics play a major role in the scientific profile of the bryological team at the Department of Botany, we do not disregard floristic

activities. Whether the possibly thorough inventories in our country or the exploratory study tours abroad, field study of plants in their environment is indispensable both for understanding the ecological demands of individual taxa, but also for the first check of proposed taxonomic concepts or generating meaningful taxonomic hypotheses. For example, the concept of 'mixed stands' of closely related taxa (Frisvoll, 1983) is in fact nothing less than observation of results of a nature's long-time cultivation experiment, unhindered by improper cultivation conditions, which commonly impair our deliberate attempts. Similarly, nothing else than frequent observation of as many taxa of local flora and the effort in estimating the extent of populations and empirical assessment of possible habitat threats can result in informed guesses, which help in creating regional check-lists and particularly red lists. These later serve as a basis for important decisions on focusing the always limited sources to practical conservation measures. We have published the first version of IUCN criteria-based bryophyte red list with a check-list more than a decade ago (Kučera & Váňa, 2003) and recently an updated version with a brief analysis of the Czech bryoflora was issued (**Paper 5**).

Future perspectives

Ongoing taxonomic and phylogenetic studies will focus first of all on resolving the 'Gordian knot' in *Didymodon* of taxa related to *D. icmadophilus*. It obvious that neither the 'European' view, represented by the treatment of Jiménez (2006), who recognizes *D. acutus, D. icmadophilus* and *D. validus* at specific level, nor the 'American' view of Zander (2007b), who accepts these taxa as varieties of *D. rigidulus*, not recognizing *D. validus* for North America at all, have the support from molecular and, in fact, even from morphological data at world-wide scale. It will be obviously necessary to be reconciled with the fact that any simple solution which would result in a dichotomous key to the clearly defined several taxa which could be matched to molecularly delimited lineages is impossible. The reality might rather match the complicated reticulate patterns resolved in some species complexes in *Sphagnum* (Shaw et al., 2008), or our treatment of *Tortula muralis* aggregate (**Paper 7**). There seems to be an inexhaustible supply of unanswered taxonomic questions in Pottiaceae – whether these will be generated from detailed work on disentangling the speciation processes and delimitation of species and emerging species, or understanding of inter-specific affinities. These questions are interesting scientifically but there are even practical consequences of taxonomic conclusions. For example, our taxonomic work, which resulted in synonymization of *Didymodon mamillosus* with *D. rigidulus* (Kučera 1999, 2000), enabled removing *D. mamillosus*, once a putative endemic species of Scotland, from priority species of United

Kingdom's Biodiversity Action Plan, and helped thus focusing these sources to species with real need for protection. On the contrary, several rare species have been resurrected from oblivion or newly described (**Paper 2, Paper 3, Paper 4**), documenting the need for conservation effort to be directed towards these taxa.

Nonetheless, we would also like to continue with our ecology line of research, increasingly supplemented by molecular data. Population genetic studies of two cryptic lineages of *Hamatocaulis vernicosus* and on *Helodium blandowii* were mentioned above, hopefully providing significant new data on the levels of intra- and inter-population variability, which helps us understand their colonization history and the levels of gene flow among the populations separated by habitat fragmentation, analogically to the results retrieved from the study on *Crossocalyx hellerianus* (**Paper 6**). In the sum, the meaningful bryological research topics would hold out for the lifelong scientific career of several teams, while the major concern is rather the human capacities. The biggest challenge for future thus remains the continuity of the bryological team research at the University of South Bohemia.

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Appendix 1: Taxonomy and phylogeny of Pottiaceae (Bryophyta: Dicranidae)

Paper 1: Kučera J. & Ignatov M. S. 2015. Revision of phylogenetic relationships of *Didymodon* sect. *Rufiduli* (Pottiaceae, Musci). – Arctoa 24: 79–97.

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REVISION OF PHYLOGENETIC RELATIONSHIPS OF *DIDYMODON* SECT. *RUFIDULI* (POTTIACEAE, MUSCI)

ПЕРЕСМОТР ФИЛОГЕНЕТИЧЕСКОГО ПОЛОЖЕНИЯ *DIDYMODON* SECT. *RUFIDULI* (POTTIACEAE, MUSCI)

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Abstract

Molecular phylogenetic analysis of the *Didymodon* species, which were assigned to Sect. *Rufiduli* (P.C. Chen) R.H. Zander by different authors showed that most of these species constitute a monophyletic lineage which largely fits the original concept of Chen. *Didymodon asperifolius*, *D. sinuosus*, and surprisingly also *D. revolutus* need to be included in the section, while *D. anserinocapitatus* is more closely related to *D. cordatus* of the sect. *Didymodon*. The genus *Fuscobryum* R.H. Zander (sect. *Rufiduli* in the sense of Zander) represents a well-supported lineage within sect. *Didymodon*, and is therefore combined as a subsection thereof, after *D. norrisii* is removed to sect. *Vineales*. *Didymodon gaochienii* and *D. asperifolius* have been found polyphyletic in present morphological circumscriptions and hybridization between *D. hedysariformis* and the Russian lineage of *D. gaochienii* s.l. has been suggested by incongruence between nuclear and chloroplast data. Revision of types revealed that *D. murrayae* seems to be identical with the type of *D. gaochienii* and at the same time, current understanding of these taxa differs from what is represented by their types, which will probably necessitate description of new taxa following a dedicated study. Additions to known distribution of *Didymodon hedysariformis*, *D. johansenii*, *D. murrayae*, *D. rivicola* and *D. zanderi* are listed.

Резюме

Согласно данным молекулярно-филогенетического анализа виды, относимые разными авторами к секции *Rufiduli* (P.C. Chen) R.H. Zander, образуют монофилетическую группу, соответствующую изначальной концепции секции, предложенной Ченом. *Didymodon asperifolius*, *D. sinuosus*, а также, неожиданно, и *D. revolutus* должны быть включены в эту секцию, в то время как *D. anserinocapitatus* оказался близок к *D. cordatus* из секции *Didymodon*. Род *Fuscobryum* R.H. Zander (Sect. *Rufiduli* в смысле Зандера) представляет собой хорошо поддержанную группу в секции *Didymodon* и рассматривается в ранге подсекции, при этом *D. norrisii* должен быть перемещен в секцию *Vineales*. Показана полифилетичность *Didymodon gaochienii* и *D. asperifolius* в их известных морфологических границах, а также выявлен факт гибридизации между *D. hedysariformis* и представленной в России линией *D. gaochienii* s.l. на основании несоответствия данных ядерной и хлоропластной ДНК. Тип *D. murrayae* оказался идентичен типу *D. gaochienii*, так что виды в их современном понимании, по-видимому, должны быть описаны. Перечислены данные, уточняющие распространение *Didymodon hedysariformis*, *D. johansenii*, *D. murrayae*, *D. rivicola* и *D. zanderi*.

KEYWORDS: *Didymodon* section *Rufiduli*, Potticeae, molecular phylogeny, ITS, *rps*4, *trn*M–*trn*V

INTRODUCTION

Phylogenetic affinities among species of the large genus *Didymodon* based on molecular data have to date been published only in the paper of Werner *et al*. (2005), who studied the relationships among 29 taxa, and tested the monophyly of the genus in the sense of Zander (1993), based on the nuclear ITS sequence data. The authors confirmed the monophyly of *Didymodon* as understood by Zander (l.c.) and clearly refuted the earlier suggested transfer of *D. sinuosus* to *Oxystegus*. Also, they questioned the need for separating the genera *Geheebia* and *Trichostomopsis*. Infrageneric affinities were much less clearly obvious, confirming the monophyly of the only section, *Asteriscium*, and even this was not possible before *D. bistratosus*, a species morphologically close to *D. vinealis*, and *D. paramicola*, earlier segregated into a monotypic genus (*Kingiobryum*) of the family Dicranaceae, was understood as a member of the section. Chloroplast data have been published only for a subset of the taxa employed in the Werner *et al*.'s study. The most comprehensive picture of chloroplast phylogeny to date can be drawn from the paper by Jiménez *et al*. (2012), who

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described a new South American genus, *Andina*, which combined several species earlier recognized within both *Pseudocrossidium* and *Didymodon*. Having analyzed a concatenated matrix of chloroplast *trn*L-F and *trn*G regions, they were able to confirm the monophyly of sections *Fallaces* (with the inclusion of *D. luridus*, as suggested by Werner *et al*., 2005) and of the sect. *Vineales*, with *D. bistratosus* in the sister position to the clade containing both of these sections, and *Andina* + *Gertrudiella* sister to *Didymodon* as a whole.

The results of above named studies need to be supplemented by information from additional species, other geographic regions and multiple genomic compartments before they can be generally accepted. Unfortunately, the selection of taxa for the study of Werner *et al*. (2005), which was intended just as a preliminary investigation into the phylogenetic relationships, included only taxa occurring in Europe and North America and was based on a single hypervariable nuclear marker. Similarly, only European taxa of sections *Fallaces* and *Vineales* and the North American *D. norrisii* were included in the study of Jiménez *et al*. (2012), which might compromise the obtained results. Again, the marker selection included only two regions from one genomic compartment. Zander (2013) published another, rather revolutionary classification scheme of *Didymodon*, based on the re-interpretation of Werner *et al*. (2005), which resulted in splitting the genus into six genera – *Didymodon*, *Trichostomopsis* (re-established), *Geheebia* (amended to include the earlier concept of sect. *Fallaces*), *Vinealobryum* (= sect. *Vineales*), *Fuscobryum*, established for taxa putatively related to *D. nigrescens* (*D. perobtusus*, *D. subandreaeiodes*, *D. norrisii*), and monotypic *Exobryum* with *D. asperifolius*.

One of the species groups in *Didymodon* that has not yet been representatively covered by the above named molecular phylogenetic treatments was the group of taxa with fragile leaf tips, which seems to be particularly well represented in the Central Asian and South Siberian mountains and was taxonomically treated using conventional methods by Otnyukova (2002). Besides the relatively well-known species, *D. johansenii*, she accepted two species previously described from China, *D. anserinocapitatus* and *D. gaochienii* (synonymized later by Sollman (2006) with *D. fragilicuspis*), and described two new species, *D. hedysariformis* from Tuva and *D. murrayae* from Altai, the two neighbouring regions of southernmost part of Siberia, situated along the Mongolian border. Later, another species was described from southern Siberia (Aga-Buryatia of Transbaikalia), *Didymodon zanderi* Afonina & Ignatova (Afonina & Ignatova, 2007), putatively related to *D. hedysariformis*. Phylogenetic affinities of all these species have never been thoroughly discussed, nor studied using molecular approaches. *D. johansenii* and *D. anserinocapitatus* have been considered the only members of section *Didymodon* besides the generitype, *D. rigidulus* by Zander (2013), who merged *D. acutus*, *D. icmadophilus* and *D. validus* into the infraspecific variability of *D. rigidulus*. *Didymodon murrayae* was placed in the section *Vineales* in the same treatment based on swapping that species with *D. sinuosus*, the name under which *D. murrayae* was earlier reported from North America. The phylogenetic position of *D. sinuosus* is nevertheless not clearly established. Although most authors acknowledge morphological and anatomical similarities between that species and the typical representatives of the section *Vineales* (reddish colour, red KOH reaction of lamina walls, absent ventral stereids of the costa), there are also characters not seen among members of *Vineales*, such as the fragile lamina or denticulation of upper leaf margins. Phylogenetic affinities based on nrITS data (Werner *et al.,* 2005) neither support the close relationship of *D. sinuosus* with the section *Vineales*.

Alternative placement of *D. sinuosus*, *D. murrayae* and potentially the other taxa with fragile leaf apices could be within the section *Rufiduli*. That section was originally described within *Barbula* to account for three Chinese species with mammillose cells and costa ending below apex – *B. rufidula* (=*D. rufidulus*), *B. rivicola* (=*D. rivicola*) and *B. subrivicola* (synonymized later by Saito, 1975 with *Didymodon nigrescens*). The section was largely neglected by recent authors until Zander (1999) revived it for placing the newly described *D. norrisii*, along with the morphologically similar *D. nigrescens*, *D. perobtusus* and *D. subandreaeoides*. He underlined the characters of bulging lamina cells, papillosely crenulate upper leaf margins and dark red – blackish color, red in KOH, whereas Chen (1941) stressed the costa ending well below apex, bulging lamina cells and the leaves twisted in dry state. *Didymodon nigrescens* and *D. perobtusus* were placed by Chen in subsect. *Rigidulae* (roughly equivalent to usual current delimitation of sect. *Didymodon*). However, bulging leaf cells occur also in species that were never compared to species of sect. *Rufiduli* in the sense of either author, such as *D. occidentalis* of the sect. *Vineales* or the South American taxa *Didymodon fuscus* or *D. santessonii*, which are also similar to members of the latter section and were tentatively compared to *D. vinealis* by Jiménez & Cano (2006).

MATERIAL AND METHODS

The sampling included the selection of above named species with fragile leaf apices in multiple accessions covering as much as possible of the distribution area, species of sect. *Rufiduli* in the sense of both Chen (1941) and Zander (1999), as well as several accessions of *D. sinuosus*, *D. asperifolius*, and *D. fuscus*. These were complemented by the representatives of other groups of *Didymodon*, as well as the selection of most probable outgroups, based on the studies of Werner *et al*. (2004), Kučera *et al*. (2013) and unpublished results of our team. Table 1 lists the accessions used in this study. We employed one nuclear (ITS) and two chloroplast markers (*rps*4, *trn*M-*trn*V), which were successful-

ly used in our previous phylogenetic studies in Pottiaceae (Köckinger & Kučera 2011; Kučera *et al*. 2013) and enabled the re-use of earlier results and easier interpretation of new data. Authors of names in the whole text follow the TROPICOS database (www.tropicos.org).

Molecular protocols

Total genomic DNA was extracted using the NaOH method (Werner *et al.*, 2002). The target regions (ITS, *rps*4, *trnM-trnV*) were amplified from diluted crude extracts, and the purified DNA sequenced as specified in our earlier studies (*e.g.*, Köckinger & Kučera, 2011).

Sequence editing, alignment, and phylogenetic analysis

Obtained raw sequences were edited (trimming of primer complements, 18S and 26S rRNA in ITS amplicons, interpretation of ambiguities where possible) in BioEdit v.7.1.7 (Hall, 1999) and Geneious v. 7 (Biomatters Ltd, available from http://www.geneious.com/). Three datasets were built, ITS, chloroplast concatenation (*rps*4 + *trn*M–*trn*V), and ITS + cp concatenation for accessions which were successfully amplified for all regions. The sequences in the above described datasets were aligned using the online interface of MAFFT v7.213 (Katoh & Standley, 2013), employing the Q-INS-i strategy with 20 PAM/ê = 2 scoring matrix, gap opening penalty set to 1.0, and offset value set to 0.0 for ITS sequences (including the ITS part of the concatenated dataset before concatenation) and E-INS-i strategy with the same settings for chloroplast sequences. The resulting alignments were manually inspected for homology problems and manually edited, but these interventions were limited to minimum cases to ensure maximum reproducibility. Indels were scored for chloroplast partitions with SeqState v.1.4 (Müller, 2005) using the simple indel coding method (Simmons & Ochoterena, 2000). Phylogenetic analyses were performed using the Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) criteria on partitioned datasets with partitions assigned to individual DNA regions (ML, BI), and binary indel data. MrBayes v. 3.2.2 (Ronquist *et al.*, 2012) was used for BI, with the gamma model of rate variation across sites sampled across the GTR model space (nst = mixed, rates = gamma) with unlinked parameters for the respective partitions and performed two simultaneous runs with temp set to 0.05 and otherwise default settings for 1 million generations. The convergence between runs in all cases dropped below 0.01. Twenty-five percent of the sampled trees were discarded as burn-in and the rest were used for construction of the majority consensus tree. ML analysis was executed in RaxML using the raxmlGUI interface v 1.3 (Silvestro & Michalak, 2012) using the GTR model of nucleotide substitution with the Γ model of rate heterogeneity. Bootstrap support for the lineages was calculated using the 'thorough bootstrap' option with 500 replicates. MP analysis, with gaps scored as missing data, was executed in TNT ver. 1.1 (Goloboff *et al.*, 2008). Trees were sought using a heuristic search starting by 1000 random addition sequences followed by TBR and keeping 99 trees in each replication. Strict consensus tree was constructed from the most parsimonious trees found and bootstrap support plotted for resolved lineages using 1000 replicates.

Results

Molecular affinities

The chloroplast (cp), ITS, and ITS+cp alignments comprised 1382, 1466, and 2838 nucleotide sites, respectively, with additional 41 characters from the indel scoring of the chloroplast partitions in the latter two datasets. Topology of trees agrees among methods of phylogenetic inference, although with different levels of support, essentially similar for BI and ML, and lower from MP for some of the branches. Hence we present only the topology with branch lengths from the BI and add the support indication from other methods on the respective trees (Figs. 1-3).

The inference from analysed chloroplast regions (Fig. 1) and the ITS (Fig. 2) agrees in most aspects. Most of the species with caducous leaf apices except *D. anserinocapitatus* group into a well-supported clade, which also includes *D. rivicola* and *D. asperifolius*, essentially in agreement with the original delimitation of sect. *Rufiduli* by Chen (1941). Chloroplast data support the inclusion of *D. sinuosus* and *D. revolutus* in the sister position to the rest of the clade, while the ITS data separate these two taxa into a poorly supported position sister to the rest of analysed *Didymodon* taxa. All accessions of *D. anserinocapitatus* are nested within other representatives of sect. *Didymodon*, in a sister position to *D. cordatus*, which itself is closely related to *D. validus* (data not shown but compare also Werner *et al*., 2005). Members of *Fuscobryum* (sect. *Rufiduli* sensu Zander, 1999) except *D. norrisii* form a well-supported clade within the sect. *Didymodon*, and *D. norrisii* appears to be nested within sect. *Vineales*. South Hemisphere taxa with bulging cells, represented by *D. fuscus* and *D. xanthocarpus*, form a moderately supported clade in a sister position to the clade formed by sect. *Asteriscium* and sect. *Didymodon* in the analysis of chloroplast data but one of the *D. fuscus* s.l. accessions appears unsupported sister to sect. *Rufiduli* in the ITS tree (ML analysis nevertheless supports a clade containing this accession in a sister position to *D. fuscus* s.str. + *D. xanthocarpus*). Conflict between chloroplast and ITS data is also seen in the positions of *D. rigidulus*, *D. acutus* and *D. icmadophilus* but the chloroplast information is poorly supported. The concatenation of all regions (Fig. 3) supports the chloroplast-based phylogeny with respect to the position of *D. sinuosus* and *D. revolutus*, as well as retaining *D. fuscus* s.l. and *D. anserinocapitatus* accessions in monophyletic lineages but in case of *D. rigidulus*, *D. acutus* and *D. icmadophilus* the signal from ITS data is stronger.

The species-level view surprisingly shows many of the analysed taxa of sect. *Rufiduli* non-monophyletic. *D. hedysariformis* is monophyletic only after about half of

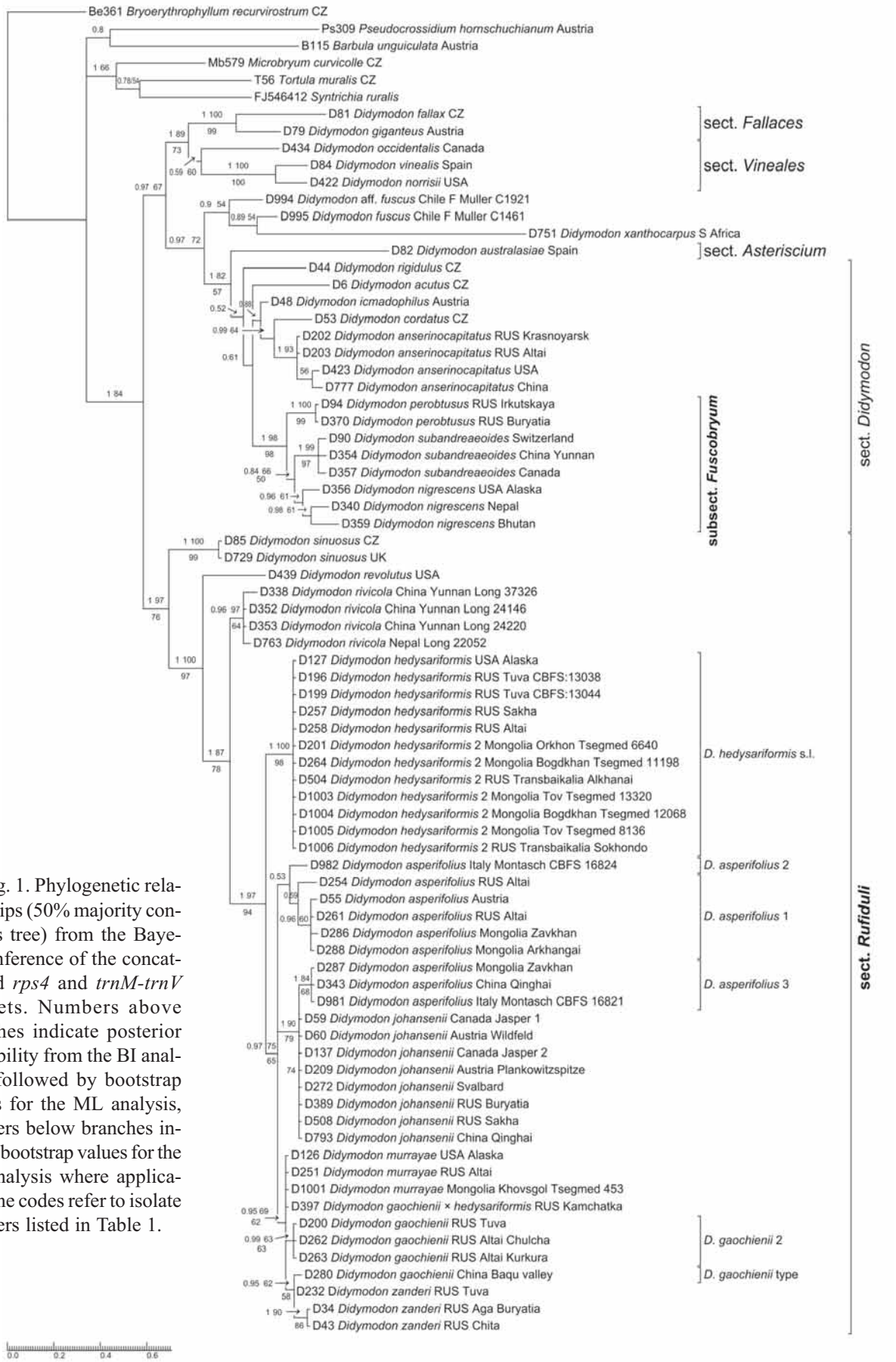


Fig. 1. Phylogenetic relationships (50% majority consensus tree) from the Bayesian inference of the concatenated *rps4* and *trnM-trnV* datasets. Numbers above branches indicate posterior probability from the BI analysis, followed by bootstrap values for the ML analysis, numbers below branches indicate bootstrap values for the MP analysis where applicable. The codes refer to isolate numbers listed in Table 1.

Fig. 2. Phylogenetic relationships as revealed by the Bayesian inference on the ITS dataset. For further explanation see caption to Fig. 1.

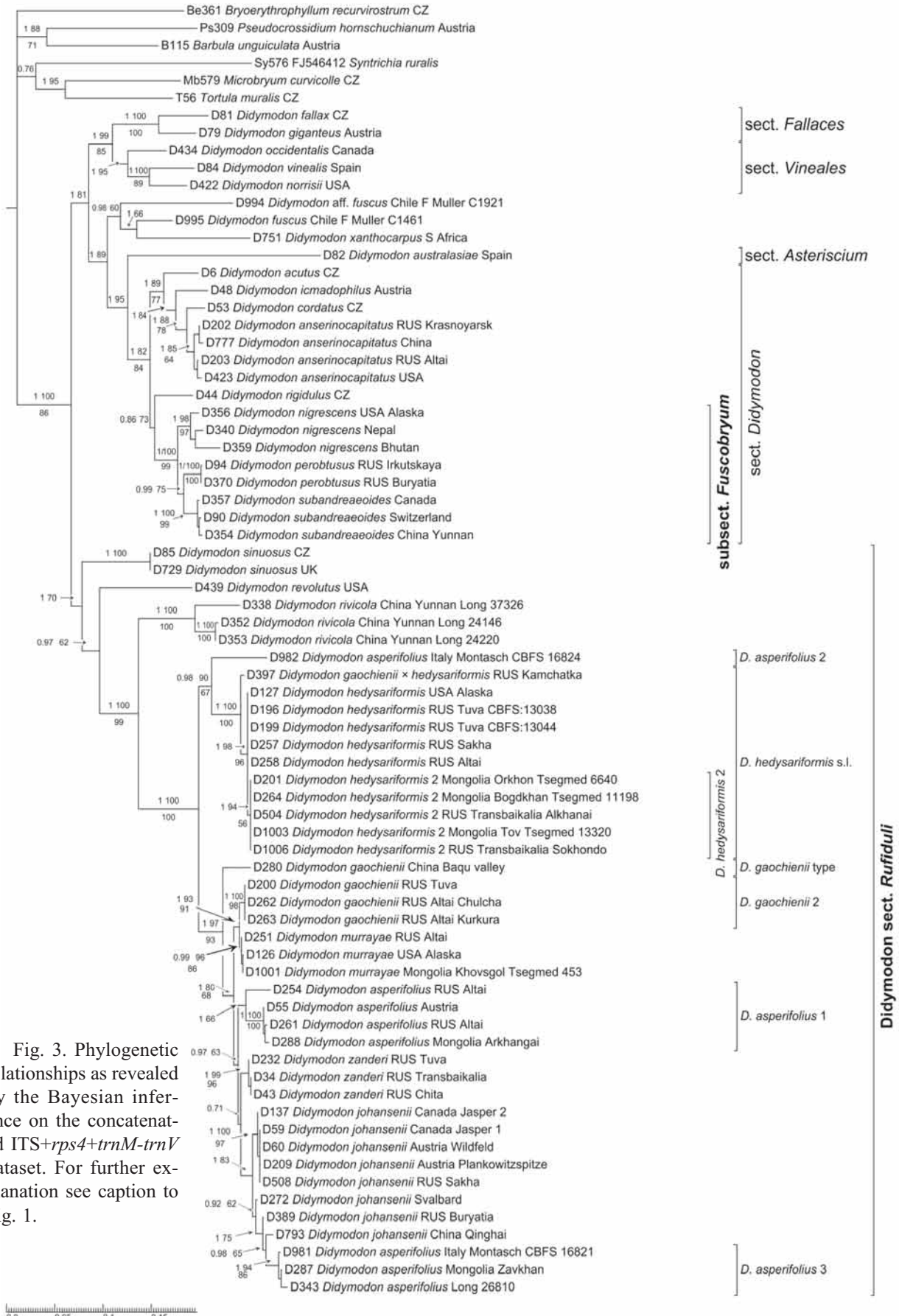


Fig. 3. Phylogenetic relationships as revealed by the Bayesian inference on the concatenated ITS+*rps4*+*trnM-trnV* dataset. For further explanation see caption to Fig. 1.

the analysed accessions with the morphology of *D. gaochienii* sensu Otnyukova (2002) is recognized as '*D. hedysariformis*-2', a taxon with the chloroplast sequence identical to *D. hedysariformis* and ITS sequence differing in one constant ITS1 substitution and two 2-bp deletions from the rest of otherwise rather variable *D. hedysariformis* accessions. Neither the rest of analysed accessions, morphologically corresponding to *Didymodon gaochienii*, is monophyletic. The isotype specimen has a completely unique sequence, different in one *rps4* site and two *trnM-trnV* sites from the Russian accessions of that taxon and being only distantly related to the rest of the section members in its ITS sequence. Interestingly, its chloroplast sequence is identical to *D. murrayae* except for one unique substitution which has not been observed in any other of the analysed accessions. The ITS sequence with many deletions and scarcely alignable regions in ITS1 might however have likely resulted as partial artefact during the cloning procedure. In one of the samples from Kamchatka (isolate 397), the chloroplast sequence is identical to *D. murrayae* (different in only one base of the *trnM-trnV* region from Russian *D. gaochienii*) but the ITS sequence corresponds to *D. hedysariformis*, suggesting the possible hybridization between the two taxa. *Didymodon johansenii* is monophyletic only based on chloroplast data (with their sequences completely uniform), otherwise it necessitates the inclusion of '*D. asperifolius-3*' into one of its ITS clades. *Didymodon asperifolius* in its current morphological understanding is deeply polyphyletic, with individual accessions belonging to at least three lineages, named here provisionally *D. asperifolius 1–3*.

South American taxa with bulging lamina cells, which share the general habit and costa anatomy with typical representatives of sect. *Vineales*, do not seem to be related with this lineage. Rather, they might represent a basal lineage within *Didymodon* together with the South African *D. xanthocarpus*, sister to both sections *Asteriscium* and *Didymodon*. However, this hypothesis needs to be tested with a better representation of South Hemisphere taxa.

Morphological considerations

There is a reasonable level of match between the partly surprising molecular affinities and morphological characters if some of the existing sectional and species-level concepts are reconsidered. The addition of *D. zanderi*, *D. hedysariformis*, *D. gaochienii*, *D. murrayae*, *D. johansenii*, and *D. sinuosus* into the Chen's concept of sect. *Rufiduli* necessitates no significant morphological amendments except for accounting for the variability in costa excurrency, which is percurrent to excurrent in some of these taxa. On the other hand, in addition to the rufous colour and bilaterally bulging to mammillose lamina cells, all these taxa share characters that have not been mentioned by Chen, such as the fragile and disintegrating lamina in the upper part of the leaf and disintegrating and caducous tip of the costa in taxa with its excurrent part. Also, in contrast to other representatives of *Didymodon*, the members of sect. *Rufiduli* in the amended sense share a disproportionally narrow costa with reduced anatomical differentiation. This character is useful *e.g.* in the differentiation of *D. anserinocapitatus* (sect. *Didymodon*) from the superficially similar *D. johansenii*, in addition to the colour differences. Both *D. anserinocapitatus* and *D. johansenii* were recognized as species closely related to *D. rigidulus*, and hence considered to represent two of few taxa recognized within the sect. *Didymodon* by Zander (2013). While *D. anserinocapitatus* indeed belongs within sect. *Didymodon*, as evidenced by the well-differentiated costa anatomy and absence of rufous colour of plants, *D. johansenii* only shares the obviously convergent character of swollen breaking excurrent parts of costa (Figs. 32-33), but otherwise matches well the delimitation of sect. *Rufiduli*, with rufous colour of plants, reduced costa anatomy and bulging lamina cells. The addition of *D. asperifolius* in sect. *Rufiduli* might look more surprising, as even Chen (1941) recognized this taxon within the sect. *Fallaces* (named there incorrectly *Barbula* subsect. *Reflexae* Mönk.), followed by most other authors (Zander, 1993; Jiménez *et al*., 2005 but not Saito, 1975), until Zander (2013) established a genus of its own, *Exobryum*, to account for the unusual combination of morphological characters found in this species. However, *D. asperifolius* shares the rufous colour and proportionally weak costa with reduced anatomy, as well as the sometimes fragile upper leaf lamina and sometimes bulging leaf cells with the other members of the section, hence only the robust habit, patent to squarrose leaves in wet state, porose basal cells, and mostly absent central strand of the stem are the alien characters of the species, shared with some species of sect. *Fallaces*. Much more problematic is the inclusion of *Didymodon revolutus*. This seems to be an extremely specialized species of the genus with characters hardly attributable to any of the generally recognized sections, such as the broadly obtuse leaves with revolute margins up to the apex and costa ending below apex, with bifurcations (spurs) in its terminal part. The unusual combination of characters led Cardot (1909) to the establishment of a new monotypic genus, *Husnotiella* at the time of the description and since then, its affinities have never been thoroughly discussed, neither by Williams (1913), who synonymized *Husnotiella* with *Didymodon*, nor by Zander (2013), who combined the species into *Trichostomopsis* (=*Didymodon* sect. *Asteriscium*). Although *D. revolutus* shares the somewhat reduced costa anatomy with other members of sect. *Rufiduli* as recognized here, it is also strikingly different in the absence of rufous colour, non-fragile leaf lamina and hardly bulging lamina cells except for the ventral epidermal cells of the costa. Hence, its inclusion in sect. *Rufiduli* is only tentative at the moment and should be tested more thoroughly in the future, although one should bear in mind that analogical surprising affinities of highly specialized taxa are not so exceptional (compare, *e.g.*, the affinity of *Hydrogrim-*

mia mollis with members of *Grimmia* subgenus *Orthogrimmia*; Streiff 2006; Hernández-Maqueda *et al.*, 2008, or the affinity of *Ephemerum* with *Pottiaceae* trib. *Trichostomeae*; Werner *et al.*, 2004; Cox *et al*., 2010; Goffinet *et al.*, 2011; Kučera *et al.*, 2013).

One of the serious potential flaws of this study is the absence of *Didymodon rufidulus* in our molecular analysis and the lectotypification of the sect. *Rufiduli* with this species at the same time. Chen (1941) has not typified his newly established *Barbula* sect. *Rufidulae* and after *B. subrivicola* has been synonymized with *Didymodon nigrescens*, the choice of the lectotype is only possible with either *D. rufidulus* or *D. rivicola*. The latter species would unequivocally match the original description of the section, has been analysed molecularly and clearly belongs to the lineage recognized here as sect. *Rufiduli,* but the name and the first position in Chen's listing the species favours *D. rufidulus* as the first choice candidate for the lectotypification. The first author was able to study the isotype of *D. rufidulus* from herbarium JE and the isotype of *Didymodon handelii* from E, synonymized with *D. rufidulus* by Chen (1941). Although the isotype of *D. rufidulus* only includes one stem fragment of the plant, it matches well the Chen's illustration and the original description and in fact resembles the recently described *D. zanderi* in both shape of narrowly lanceolate leaves with costa ending below apex and occasionally fragile upper part of the lamina, and bilaterally bulging leaf cells (hardly papillose in the type of *D. rufidulus* but slightly papillose in the type of *D. handelii*). The short straight peristome teeth are also very similar in both taxa, which together provide strong arguments for inclusion of *D. rufidulus* into the lineage of molecularly barcoded taxa containing *D. rivicola*. The type of *D. handelii* looks similar to the type of *D. rufidulus* but it is smaller in stature, its leaves are more similar in shape to *D. fallax* and the upper cells are not conspicuously bulging, hence the identity with *D. rufidulus* is in our opinion not certain.

Molecular data have shown that the morphological characteristics related to the shape of the segments of the fragile costa or upper lamina, which were used to the delimitation of *D. hedysariformis* from *D. gaochienii* (Otnyukova, 1998, 2002) are not in agreement with the molecular data, and probably are to be considered homoplasic. Unfortunately it is not possible to solve this discrepancy between morphological and molecular data by the synonymization of the two taxa, as the *D. hedysariformis* clade, which includes the Mongolian *D. gaochienii*-like accessions appears sister to all other taxa of the sect. *Rufiduli*, including the morphologically very different *D. zanderi*, *D. johansenii* and *D. asperifolius*, and the synonymization all these taxa would bring little sense to the practical taxonomy of the group. It is also not quite impossible that the deep polyphyly of the three lineages within *D. gaochienii*, seen in the three studied gene regions from two genomic compartments arises from the conflict between gene trees and species trees due to the deep coalescence / incomplete lineage sorting and might not reflect the situation of the whole genome. It is interesting to mention that similar deep polyphyly was seen in the analysed accessions of *Streblotrichum convolutum* in the study by Kučera *et al*. (2013). Another important fact was found following the detailed comparison of types of *D. gaochienii* and *D. murrayae*. The characteristically toothed acute apex of *D. murrayae* was found on plants of the studied isotype of *D. gaochienii* (Fig. 8), which, together with the above mentioned molecular data constitutes a solid argument for synonymization of the two taxa, although the molecular affinities should be studied on more accessions from the Tibetan area to account for uncertainties which result from the incompletely preserved DNA in the type of *D. gaochienii*. Anyway, the highly probable identity of the two types necessitates the formal description of *D. gaochienii* sensu Otnyukova (2002), here named *D. gaochienii* 2, which nevertheless should not be accomplished prior to the examination of the type of *D. fragilicuspis*, regarded identical to *D. gaochienii* by Sollman (2006).

The polyphyly of *D. asperifolius* in present circumscription is only superficially similar to the situation of *D. gaochienii*. Upon the morphological examination of the specimens, assigned to the three revealed lineages within the contemporary concept of the species, we were able to find differences, which might later prove sufficient for the description of new taxa corresponding to the molecularly barcoded lineages. These characters include the presence of stem central strand, character of papillosity of lamina cells, the stature of the plants and subtle differences in the leaf shape. It may be noted that Jiménez *et al*. (2005), in agreement with Saito (1975) have not observed the central strand in the stem of studied specimens, while the other authors did (Zander, 1979; Kučera, 2000). Nevertheless, given the large collection numbers of *D. asperifolius* worldwide and the existence of several older types that have been put into synonymy with *D. asperifolius*, we prefer to perform a more thorough revision before attempting at describing new taxa within the complex.

Molecular support for Zander's delimitation of the genus *Fuscobryum* (recognized as a subsection of *Didymodon* sect. *Didymodon* here, see below) only requires the removal of *Didymodon norrisii*, which seems to be closely related to *D. vinealis*, in agreement with earlier results of Jiménez *et al.* (2012), based on different chloroplast genes. Moreover, as already pointed out by Zander (1999), *D. norrisii* differs from the members of sect. *Rufiduli* in the stout costa and pluripapillose lamina cells, both characteristic of sect. *Vineales*. The differences of subsect. *Fuscobryum* from sect. *Rufiduli* include deep brown to blackish, rather than rusty brownish colour and non-fragile lamina. An interesting autapomorphy of the section might include the flattened, spirally twisted seta,

as seen in *D. nigrescens*, the type species of the subsection; in other species of that group the sporophyte is unfortunately not known.

Taxonomic synopsis of the taxa

In the following synopsis, we list the taxa accepted and excluded from *Didymodon* sect. *Rufiduli*. We refer to existing sources for synonymy, descriptions and distribution data and only list additional information if applicable.

Didymodon Sect. ***Rufiduli*** '*Rufidulus*' (P.C. Chen) R.H. Zander, Bull. Buffalo Soc. Nat. Sci. 32: 162. 1993. Lectotype: *Didymodon rufidulus* (Müll. Hal.) Broth., ***here designated***.

Barbula Sect. *Rufidulae* '*Rufidula*' P.C. Chen, Hedwigia 80: 210. 1941.

Exobryum R.H. Zander, Framew. Post-Phylogenet. Syst. p. 96. 2013. (14 Sep 2013), ***syn. nov.*** Type: *Exobryum asperifolium* (Mitt.) R.H. Zander (= *Didymodon asperifolius* (Mitt.) H.A. Crum, Steere & L.E. Anderson).

Husnotiella Cardot, Rev. Bryol. 36: 71, ***syn. nov.*** Type: *Husnotiella revoluta* Cardot (=*Didymodon revolutus* (Cardot) R.S. Williams).

Characteristics of the section include rusty red coloration, dark green in less exposed parts of plants, tendency towards development of fragile upper part of leaf lamina and/or excurrent part of costa, serving for vegetative propagation, bilaterally bulging lamina cells with often only single papillae, and relatively weak costa with few guide cells in one row and ventral stereids absent. Sporophyte production is rare; the peristome (when known) is reduced, of short, straight, irregularly divided filiform teeth.

Accepted species

Didymodon asperifolius (Mitt.) H.A. Crum, Steere & L.E. Anderson, Bryologist 67: 163. 1964.

Barbula asperifolia Mitt., J. Proc. Linn. Soc., Bot., Suppl. 1: 34. 1859, basionym.

Exobryum asperifolium (Mitt.) R.H. Zander, Framew. Post-Phylogenet. Syst. p. 96. 2013. (14 Sep 2013); *Didymodon rufus* var. *gorodkovii* Abramova & I.I. Abramov, *Didymodon gorodkovii* (Abramova & I.I. Abramov) Schljakov, *Didymodon asperifolius* var. *gorodkovii* (Abramova & I.I. Abramov) Afonina, Problemy Briologii v SSSR p. 13. 1989.

For additional synonymy, see Jiménez *et al*. (2005).

Description and distribution summarized in Jiménez *et al*. (2005).

It is probable that at least one new taxon will be described from within the current circumscription of the species. Nevertheless, the type, according to existing descriptions, seems to agree with representatives of the first lineage (*asperifolius 1*) and these at least overwhelmingly show the typical characters reported for the species, such as the large stature, completely absent stem central strand and papillose upper lamina cells.

Didymodon gaochienii B.C. Tan & Y. Jia, J. Hattori Bot. Lab. 82: 309. f. 12–19. 1997. Figs. 4-8, 17-22(-26)

(?=*Didymodon fragilicuspis* Broth., Ann. Bryol. 1: 31. 1928)

(?=*Didymodon murrayae* Otnyukova, Arctoa 11: 345. f. 6. 2002)

The description and illustration of Tan & Jia (1997) and Otnyukova (2002) do not fully correspond to our examination. Morphologically, the type of *D. gaochienii* (Figs. 4-8) matches the type of *D. murrayae* (Figs. 9-13), whereas the other examined plants of these species show subtle morphological differences, as well as molecular differences, which are nevertheless much smaller than the position in phylogenetic trees suggests. Broader sampling, particularly in the Chinese part of the distribution area, is necessary to resolve the question. Moreover, *D. gaochienii* sensu Otnyukova (2002), which probably should be described as a taxon of its own, falls within two molecularly defined lineages and we have not found characters which would allow assigning the specimens to them. Whether deep coalescence / incomplete lineage sorting is responsible for the polyphyletic nature of '*D. gaochienii*' lineages, or indeed more species should be recognized with morphological characteristics that we were not able to elaborate, needs to be addressed in future studies. Moreover, hybridization probably occurs between *D. gaochienii 2* (*D. gaochienii* sensu Otnyukova) and *D. hedysariformis*. Adding to the complexity of problems, Sollman (2006) synonymized *D. gaochienii* with the older *D. fragilicuspis* Broth., described from Kashmir (Brotherus, 1928), which would also mean a significant range extension for the species (known distribution until that study included the eastern part of Tibetan plateau, Southern Siberia and Mongolia but see below). Unfortunately, we were not able to check the type material (the loan request to herbarium H was not answered) and Sollman provides no details on the Brotherus's type ("The type collections of *D. f.* and *D. g.* were carefully compared and were found to match well"). In conclusion, the application of the name *Didymodon gaochienii* (or *D. fragilicuspis*) remains problematic and cannot be matched to molecularly resolved lineages at present. The only guaranteed specimen, which can be unequivocally assigned to *D. gaochienii* s. str. is the type specimen, and very probably, the type of *D. murrayae* from Russia, Altai belongs here as well.

Didymodon hedysariformis Otnyukova, Arctoa 7: 207. f. 1–36. 1998. Figs. 29-31

[+14-16 for *D. hedysarimosmis* 2]

For description and illustration, see Otnyukova (1998, 2002). Reported characters only apply to the lineage described here as *hedysariformis 1*, which has been using molecular data confirmed to occur in Russian Altai, Tyva (Otnyukova, 2002), Yakutia (Ivanova *et al.*, 2005), Kamchatka (Czernyadjeva, 2012), and North American Alaska (which is a new record for America). The occurrence in Mongolia, reported by Tsegmed (2001), is nevertheless probable.

Other records: Afonina (2007): Transbaikal Territory; Bezgodov *et al.* (2013): Amurskaya Province.

New record: U.S.A., Alaska: Talkeetna Quad. Denali State Park, Lower Troublesome Creek state recreation site, George Parks Hwy, 62°37'N, 150°14'W, on bark of roadside mature *Populus balsamifera*, 7.7.1991 *A.R. Perry* 7670 (NMW).

Didymodon johansenii (R.S. Williams) H.A. Crum, Canad. Field-Naturalist 83: 157. 1969. Figs. 32-34

Barbula johansenii R.S. Williams, Rep. Canad. Arctic Exped. 1913–1918, 4(E): 4. f. 1–12. 1921.

For description and illustration, see Otnyukova (2002).

Previous records: Otnyukova (2002): Chukotka, Altai, Khakassia; Ivanova *et al*. (2005): Yakutia; Fedosov *et al.* (2011): Taimyr; Bardunov (2000) and Fedosov (2008): Irkutsk Province; Afonina (2009): Buryatia; Jiménez (2006): Tajikistan; Redfearn *et al*. (1996): China (Qinghai); Sollman (2008, 2010): Bhutan, Pakistan; Zander (2007): NW North America.

Didymodon murrayae Otnyukova, Arctoa 11: 345. f. 6. 2002. Figs. 9-13,27-28

For description and illustration, see Otnyukova (2002). Toothed apex of excurrent part of the costa in juvenile leaves has been found to be the best diagnostic character of *D. murrayae*. As mentioned above, the type seems to be identical with the type of *D. gaochienii*. The differences in the invariable sequences of Siberian *D. murrayae* and the type of *D. gaochienii* might well be found to be not important but should their differentiation be confirmed, *D. murrayae* would stay a species of its own, pending the amendment of morphological characteristics with respect to *D. gaochienii*.

Previous records: Asia: Altai (Russia, Altai Rep.), North America: Alaska, British Columbia (Zander 2007).

New country record: Mongolia: Khövsgöl Province (Aimag), Renchinlkhümbe Sum, Mt Khar-Murugu-Uul, stony fields, on rocks, 21.6.2006 *Ts. Tsegmed* 453 (CBFS).

Didymodon revolutus (Cardot) R.S. Williams, Bryologist 16: 25. 1913.

Basionym: *Husnotiella revoluta* Cardot, Rev. Bryol. 36: 71. 1909.

Trichostomopsis revoluta (Cardot) R.H. Zander, Framew. Post-Phylogenet. Syst. p. 93. 2013. (14 Sep 2013).

For additional synonymy, description and illustration see Allen (2002), Jiménez *et al.* (2005), or Zander (2007). The reasons for transferring the species to *Trichostomopsis* have not been specified by Zander (2013) but Allen (2002) lists similarities between *D. australasiae* and *D. revolutus*, which include the bulging ventral epidermal cells of the costa and the slightly developed stem hyalodermis. On the other hand, thickened non-hyaline basal leaf cells and costa guide cells in one row in *D. revolutus* contradict the affinity with *Didymodon* sect. *Asteriscium* on morphological reasons.

Didymodon rivicola (Broth.) R.H. Zander, Ann. Bot. Fenn. 20: 222. 1983. Figs. 40, 49-50

Barbula rivicola Broth., Symb. Sin. 4: 41. 1929.

For description and illustration see Chen (1941) or Li *et al.* (2001). The species is quite similar to *D. zanderi*, from which it differs in broader leaves and shorter apices, and more pronouncedly mammillosely bulging lamina cells. The leaves show also less pronounced tendency for disintegration of the upper lamina.

The species was believed to be endemic to China, where it is quite broadly distributed with the centre of distribution in Yunnan (Li *et al*., 2001). Miehe (1991) however also published a record from Central Nepal. Below, we list new regional occurrences for India (Jammu and Kashmir, Uttarakhand) and Nepal.

INDIA: Jammu and Kashmir: Gangabal, W end of the larger lake, ca. 3640 m, damp rock crevice in a rock bluff descending almost to the water, 12.8.1989 *C.C. Townsend 89/469* (E); Uttarakhand, Garhwal Himal: between Dhanolti and Mussoorie, 30°26'N, 78°13'E, on half-shaded rock in a cultured land 2360 m, *M. Lüth 6686* (herb. Lüth, dupl. CBFS).

NEPAL: Rasuwa distr., N bank of Langthang Khola between Lama Hotel and Ghora Tabela, 28°10'N, 85°27'E, 2610 m, on boulder, 24.4.1992, *Long 22052* (E).

Didymodon rufidulus (Müll. Hal.) Broth., Nat. Pflanzenfam. I(3): 405. 1902. Figs. 38, 42-44, 47

Basionym: *Barbula rufidula* Müll. Hal., Nuovo Giorn. Bot. Ital., n.s. 3: 102. 1896.

?= *Didymodon handelii* Broth.

According to Chen (1941), followed by other authors, additional synonyms include *Trichostomum sulphuripes* Müll. Hal. and *T. nodiflorum* Müll. Hal. (not seen).

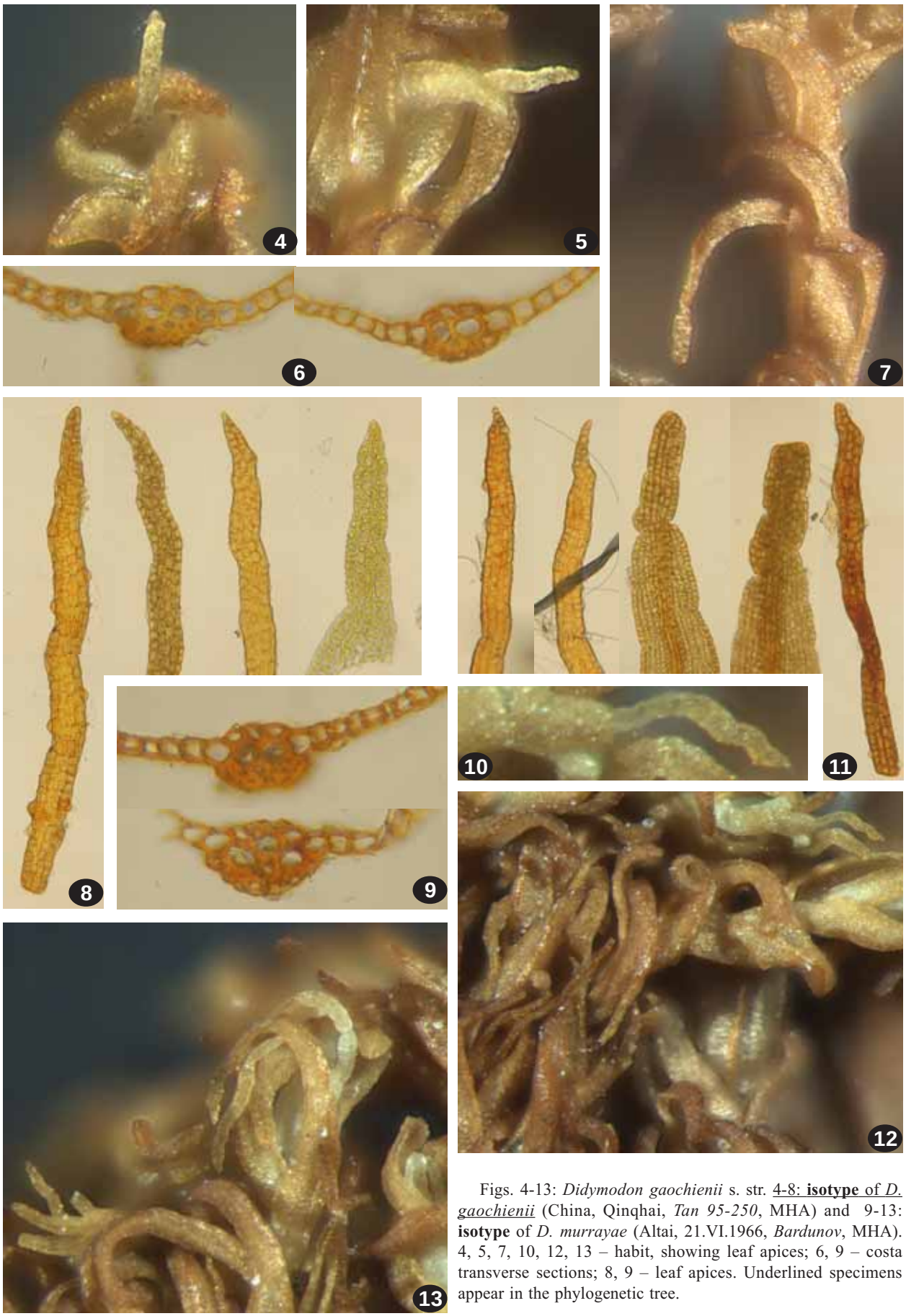
For description and illustration see Chen (1941) or Li *et al*. (2001). It seems that the taxon has not been generally well understood. For instance, none of the (anyway few) specimens housed in herbarium E with generally large collections of Sino-Himalayan bryophytes matches the type, except perhaps the isotype of *Didymodon handelii*. The other specimens belonged either to *D. icmadophilus* or to *D. asperifolius* s.l. The species seems to be morphologically transitional between *D. zanderi* and *D. rivicola*, as illustrated in Figs. 38-50.

Didymodon sinuosus (Mitt.) Delogne, Bull. Soc. Roy. Bot. Belgique 12: 423. 1873.

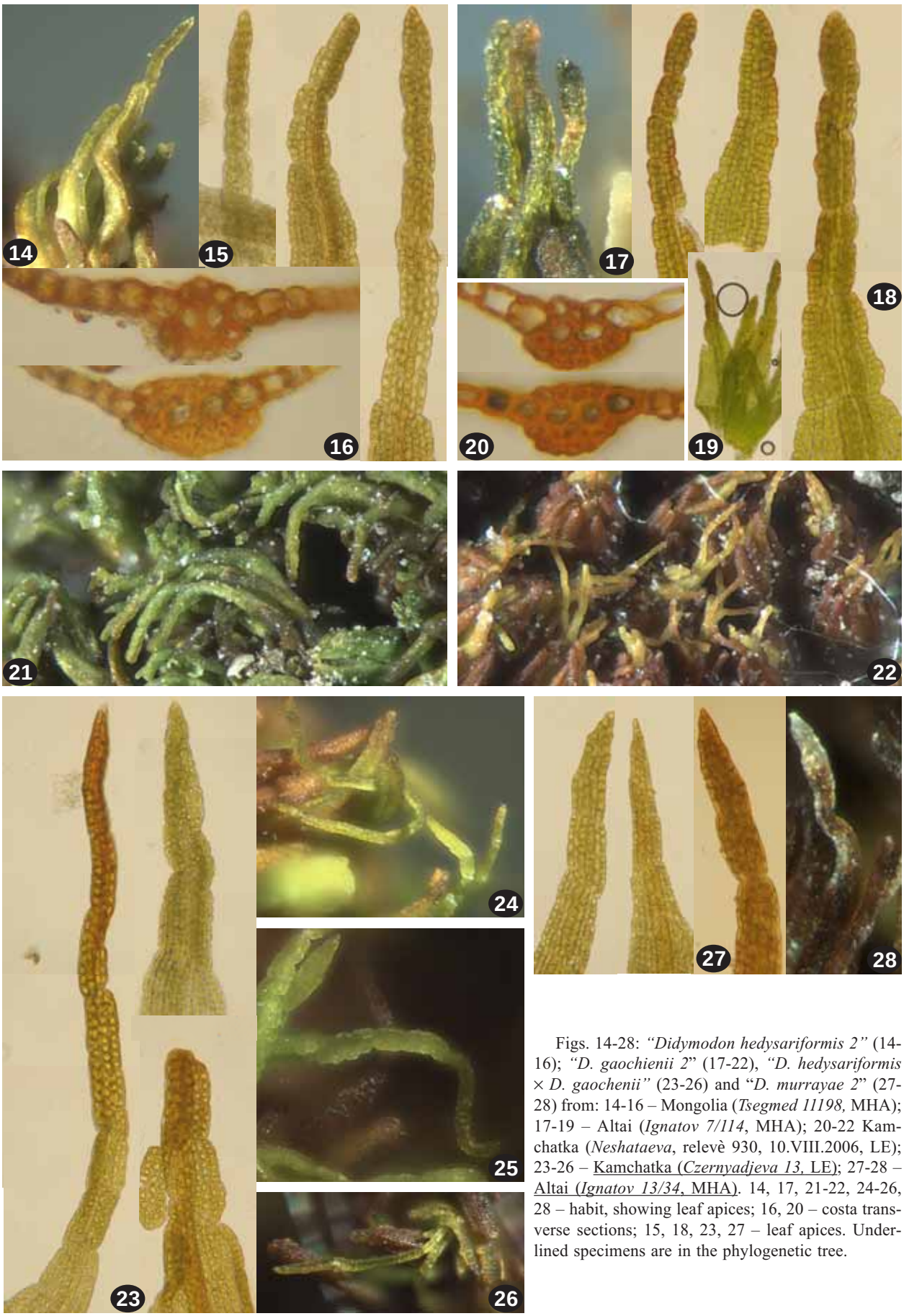
Basionym: *Tortula sinuosa* Mitt., J. Bot. 5: 327. 1867.

For additional synonymy, description and illustration see Jiménez (2006).

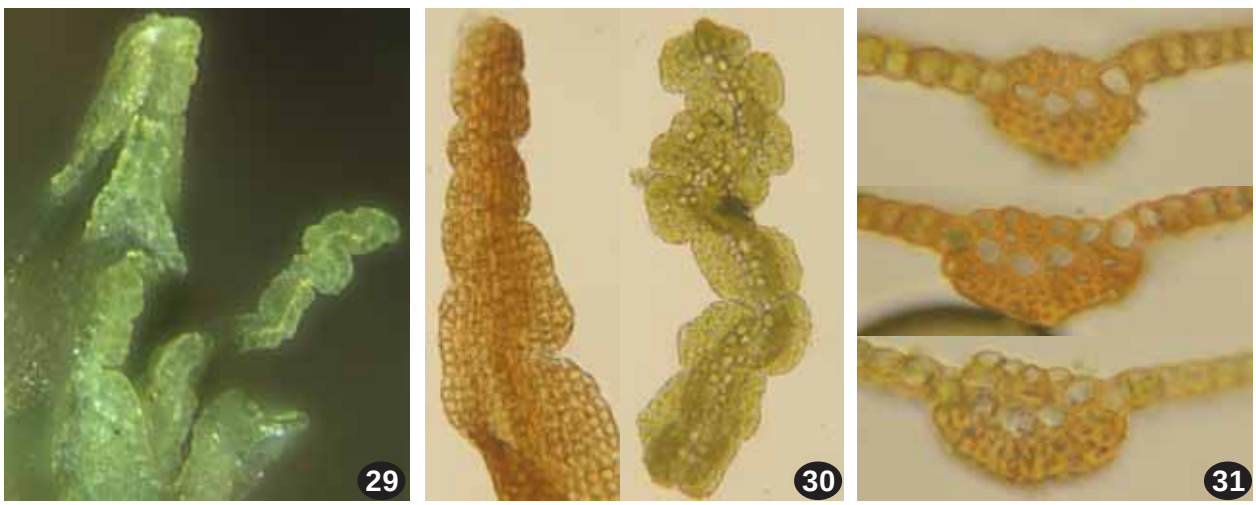
The species matches well the morphology of sect. *Rufiduli* except for the relatively stout costa with commonly present two rows of guide cells, typical of species of sect. *Vineales* and denticulate leaf apices and costa in younger leaves, which are unique for this species (the character of denticulation is different in *D. erosodenticulatus*). Jiménez (2006) also reports the occurrence of multicellular gemmae developed on the upper ventral part of the costa seen in a sample from Azerbaijan, but this character has never been observed in any other specimen.



Figs. 4-13: *Didymodon gaochienii* s. str. 4-8: **isotype** of *D. gaochienii* (China, Qinqhai, *Tan 95-250*, MHA) and 9-13: **isotype** of *D. murrayae* (Altai, 21.VI.1966, *Bardunov*, MHA). 4, 5, 7, 10, 12, 13 – habit, showing leaf apices; 6, 9 – costa transverse sections; 8, 9 – leaf apices. Underlined specimens appear in the phylogenetic tree.



Figs. 14-28: *"Didymodon hedysariformis 2"* (14-16); *"D. gaochienii 2"* (17-22), *"D. hedysariformis × D. gaochenii"* (23-26) and "*D. murrayae 2*" (27-28) from: 14-16 – Mongolia (*Tsegmed 11198,* MHA); 17-19 – Altai (*Ignatov 7/114*, MHA); 20-22 Kamchatka (*Neshataeva*, relevè 930, 10.VIII.2006, LE); 23-26 – Kamchatka (*Czernyadjeva 13,* LE); 27-28 – Altai (*Ignatov 13/34*, MHA). 14, 17, 21-22, 24-26, 28 – habit, showing leaf apices; 16, 20 – costa transverse sections; 15, 18, 23, 27 – leaf apices. Underlined specimens are in the phylogenetic tree.



Figs. 29-31: *Didymodon hedysariformis* from isotype: Tuva (13.VII.1996, *Otnyukova*, MHA), 29 – habit, showing leaf apices; 30 – leaf apices; 31 – costa transverse sections. Underlined specimen is in phylogenetic tree.

Previous records: Europe, Middle East, Caucasus. In Russia was reported from Gelendzhik, Caucasus (Abramova & Abramov, 1962). Some additional collections were made along Black Sea coast, from Sochi area (Ignatov & Ignatova, 1.VIII.2002, MHA) to Utrish (*e.g.* Ignatov & Ignatova #05-178, MHA).

Didymodon zanderi Afonina & Ignatova, Arctoa 16: 135. f. 1–3. 2007. Figs. 39, 45-46, 48

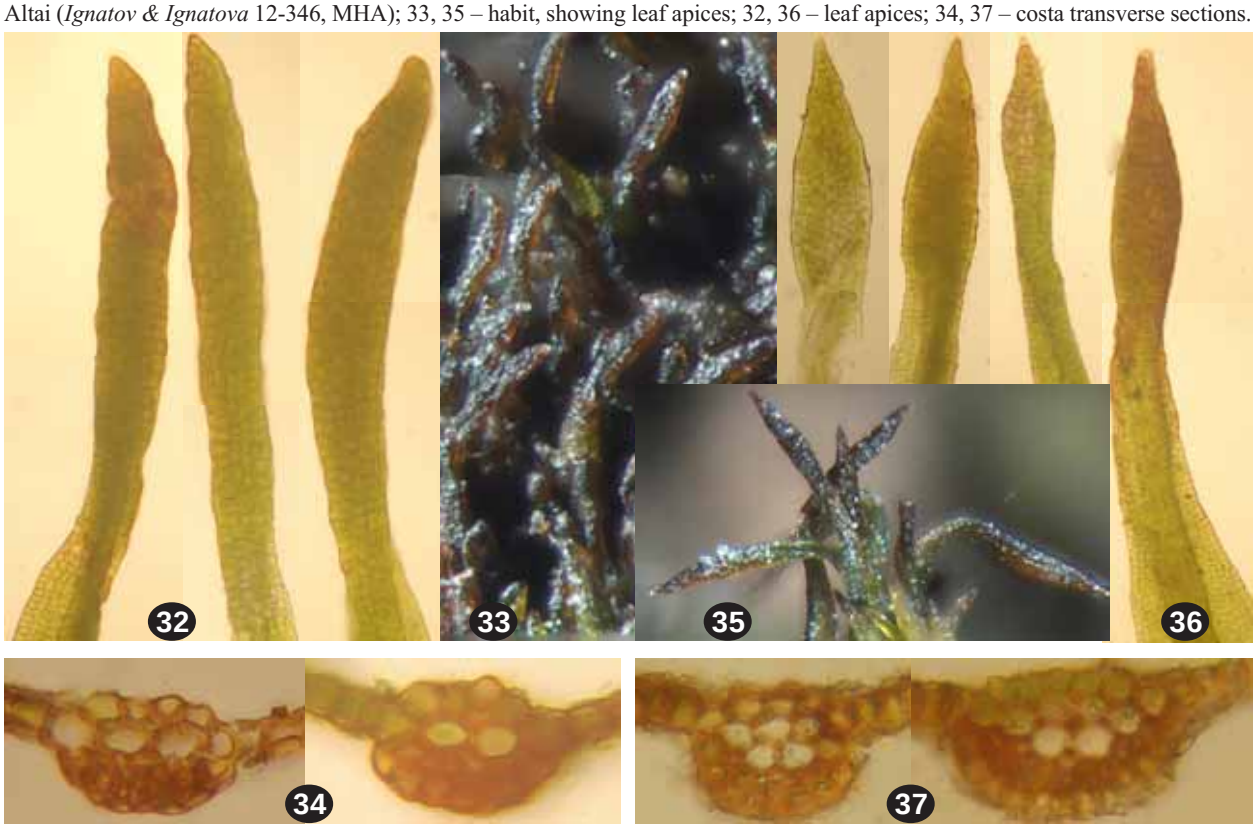
For description and illustration, see Afonina & Ignatova (2007). Relationship to *D. hedysariformis* has already been suggested by the authors of the description and in fact, this species is morphologically closest to *D. rufidulus*, as argued above.

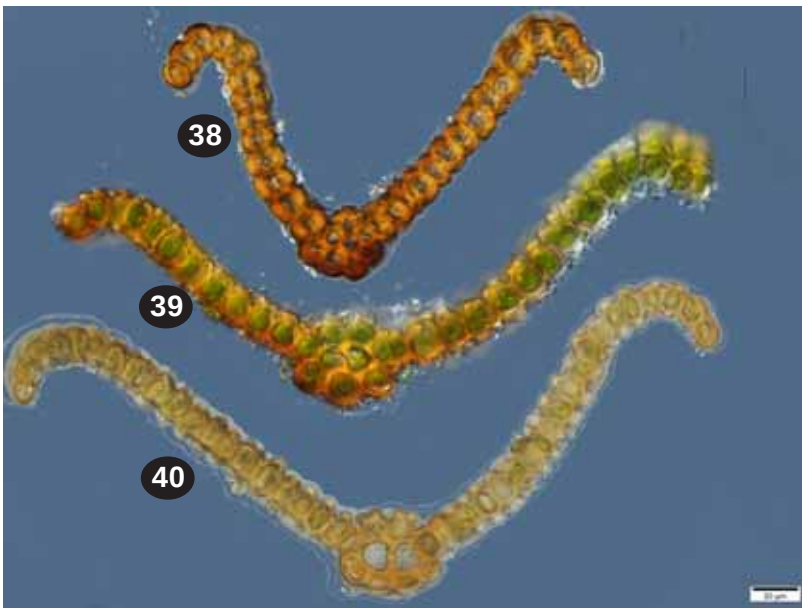
In addition to distribution in original description (Trasnbaikalia, Buryatia, Yakutia, Taimyr, Altai, Kamchatka, Primorsky Territory), the species was found in Khabarovsk Territory (Ignatova *et al*., 2013), Mongolia (Tsegmed, 2010) and Inner Mongolia Province of China (Bai *et al*., 2008).

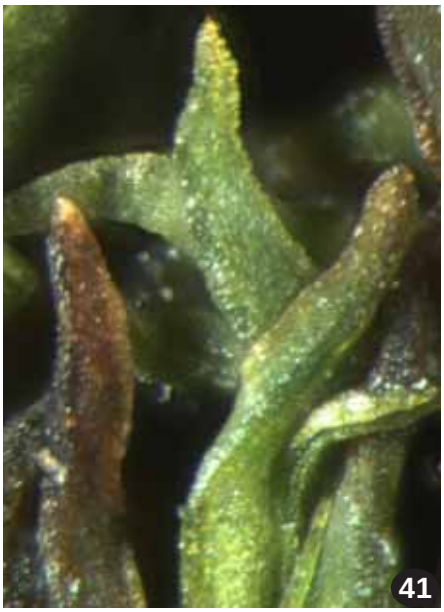
Didymodon sect. **Didymodon subsect. Fuscobryum** (R.H. Zander) J. Kučera, ***comb. nova***

Basionym: *Fuscobryum* R.H. Zander, Framew. Post-Phylogenet. Syst. p. 98. 2013. (14 Sep 2013).

Figs. 32-37: *Didymodon johansenii* (32-34) and *D. anserinocapitatus* (35-37). 32-34 – Yakutia (*Ignatov 11-4049*, MHA), 35-37 – Altai (*Ignatov & Ignatova* 12-346, MHA); 33, 35 – habit, showing leaf apices; 32, 36 – leaf apices; 34, 37 – costa transverse sections.







Figs. 38-50. *Didymodon rufidulus,* **isotype,** JE (38, 42-44, 47); *D. zanderi*, **holotype,** Transbaikalia, *Afonina 3406*, MHA (41) and *Afonina 3405*, CBFS (39, 45-46, 48); *D. rivicola,* China, Long 24146, E (40, 49-50). 38-40 – costa transverse sections; 44, 46, 50 – leaves; 41, 47-49 – habit, showing leaf apices; 42, 44, 45 – leaf apices. Underlined specimens are in phylogenetic trees.

Type: *Didymodon nigrescens* (Mitt.) K. Saito, J. Hattori Bot. Lab. 39: 510. 1975.

Characteristics of the subsection include dark brown to blackish coloration, upper part of leaf lamina not fragile, costa hardly excurrent, and vegetative propagation occasional by means of axillary gemmae. Lamina cells bilaterally bulging or not, commonly conspicuously thick-walled, with multiple/branched papillae. Costa in well-developed plants with a single row of guide cells and a weak band of ventral and a larger group of dorsal stereids. Sporophyte production known only in *D. nigrescens*; the seta is flattened and twisted dextrosely.

ACCEPTED SPECIES

Didymodon nigrescens (Mitt.) K. Saito, J. Hattori Bot. Lab. 39: 510. 1975. *Barbula nigrescens* Mitt., J. Proc. Linn. Soc., Bot., Suppl. 1: 36. 1859.

Fuscobryum nigrescens (Mitt.) R.H. Zander, Framew. Post-Phylogenet. Syst. p. 99. 2013. (14 Sep 2013).

For description and illustration see Chen (1941), Allen (2002), Li *et al*. (2001) and Zander (2007).

Didymodon subandreaeoides (Kindb.) R.H. Zander

Fuscobryum subandreaeoides (Kindb.) R.H. Zander, Framew. Post-Phylogenet. Syst. p. 99. 2013. (14 Sep 2013).

For additional synonymy, description and illustration see Kučera & Köckinger (2000) and Jiménez (2006).

Didymodon perobtusus Broth., Rev. Bryol., n.s., 2: 1. 1928.

Barbula perobtusa (Broth.) P.C. Chen, Hedwigia 80: 194. 28 f. 1–5. 1941.

Fuscobryum perobtusum (Broth.) R.H. Zander, Framew. Post-Phylogenet. Syst. p. 99. 2013. (14 Sep 2013).

For description and illustration see Chen (1941) or Zander (2007).

SPECIES EXCLUDED FROM SECTIONS *RUFIDULI* AND *DIDYMODON* SUBSECT. *FUSCOBRYUM*

Didymodon anserinocapitatus (X.J. Li) R.H. Zander, Bull. Buffalo Soc. Nat. Sci. 32: 162. 1993 (*Barbula anserinocapitata* X.J. Li, Acta Bot. Yunnan. 3: 103. f. 2: 1–9. 1981.) Figs. 35-37

For description and illustration see Otnyukova (2002) or Jiménez (2006). The species has convergent shape of swollen excurrent part of the costa (Figs. 35-36) serving vegetative propagation to *D. johansenii* (Figs. 32-33) but in fact is closely related to *D. cordatus / validus / tectorum* group of taxa, which belong to *Didymodon* Hedw. sect. *Didymodon* subsect. *Didymodon*. Morphological evidence for the relationship with the above named taxa includes the relatively strong costa with several layers of dorsal stereids, two rows of guide cells at least sometimes seen in all of the above named taxa and green to dark green colour of plants without reddish tones. In contrast to species of sect. *Vineales* which can have convergently identical anatomy of the costa, the red KOH reaction of cell walls and multiple branched papillae are not present.

Didymodon norrisii R.H. Zander, Bryologist 102: 112. f. 1–11. 1999. (*Fuscobryum norrisii* (R.H. Zander) R.H. Zander, Framew. Post-Phylogenet. Syst. p. 99. 2013. (14 Sep 2013).

For description and illustration see Zander (1999). This species shares the general look with *D. nigrescens*, owing to the typically dark brown colour and quite similar leaf shape. However, the anatomy of the costa is typical for other species of *Didymodon* sect. *Vineales* with two rows of guide cells and absent ventral stereids. The papillosity is less developed than in most species of the section but similar to, *e.g*., *D. brachyphyllus*, and still more developed than in *D. nicholsonii*. Bulging lamina cells approach these of *D. occidentalis* R.H. Zander, another rather similar species of sect. *Vineales*.

KEY TO THE TREATED TAXA
(*DIDYMODON* SECT. *RUFIDULI*, SUBSECT. *FUSCOBRYUM*, *D. ANSERINOCAPITATUS* AND *D. NORRISII*)

1. Plants green, with imbricate, not contorted, ovate-lingulate rounded leaves, margin revolute up to the apex ..... *D. revolutus*
— Plants rufous to dark brown or blackish at least in exposed parts, margin recurved not up to the apex 2
2. Leaves mostly ovate to broadly ovate-lanceolate with ± rounded apex 3
— Leaves mostly longer, from the ovate or oblong base long-lanceolate; if ovate, apex hardly rounded 4
3. Vegetative propagation by regularly formed deciduous flagelliform innovations with reduced, cochleariform leaves, axillary gemmae absent ..... *D. subandreaeoides*
— Occasional vegetative propagation by means of mostly unicellular axillary gemmae, flagelliform innovations with reduced leaves absent ..... *D. perobtusus*
4. Specialized vegetative propagation by means of swollen excurrent parts of costa 5
— Specialized vegetative propagation by means of irregularly disintegrating upper lamina or disintegrating apices formed mostly by costa, but the costa not swollen 6
5. Plants green to dark green, costa strong, with two layers of guide cells and well developed dorsal stereids in multiple rows, upper lamina cells around 8 µm ..... *D. anserinocapitatus*
— Plants typically rufous, costa weak, with a single layer of guide cells and weak dorsal stereid band, upper lamina cells mostly over 10 µm ..... *D. johansenii*
6. At least young leaf apices with mostly regularly toothed margins ..... *D. sinuosus*
— Leaf apices with margins entire or with few irregular teeth in the apical caducous part of the leaf ... 7
7. Leaves mostly patent to squarrose when wet, from ovate base gradually tapering to apex; plants typically robust, with long and porose basal cells and stem central strand absent ..... *D. asperifolius* s.l.

— Leaves spreading, never squarrose when wet, from oblong-ovate base more abruptly narrowed to long-lanceolate apical part; basal cells never porose and at least weak stem central strand always present . 8

8. Leaf apices acute, gradually tapering, only occasionally fragile and disintegrating into variously large lamina parts, not containing the costa 9
— Leaf apices narrow and nearly lingulate, conspicuously fragile and mostly broken, disintegrating into segments containing costa and adjacent parts of lamina 13

9. Plants dark chestnut brown to blackish 10
— Plants dark green or rufous 11

10. Leaf cells typically with extremely thickened cell walls, costa weak, hardly ventrally prominent, with a single layer of guide cells, leaf apex not cucullate ..... *D. nigrescens*
— Leaf cells with moderately thickened cell walls, costa stout, ventrally prominent, with two layers of guide cells and ventral stereids absent, leaf apex cucullate ..... *D. norrisii*

11. Leaves broadly lanceolate or ovate-lanceolate, gradually tapering to acute apex ..... *D. rivicola*
— Leaves lanceolate with long apex; if broadly lanceolate, than apiculate or blunt 12

12. Plants mostly dark green, leaf apex narrowly acuminate to apiculate, somewhat cucullate, in cross-section hollow, leaf cells bulging and papillose .... *D. zanderi*
— Plants mostly rufous, leaf apex gradually acuminate, not cucullate, keeled, leaf cells bulging, hardly papillose ..... *D. rufidulus*

13. Terminal part of the caducous leaf tip acute, slightly irregularly toothed, solid for (15–)20–30 cells, which falls off as one fragment, composed mostly of the excurrent costa; below near transition to lamina notched and separates into fragments of usually 4-8(-12) cells long 14
— Terminal part of the caducous leaf tip blunt, composed of the costa lined with narrow lamina border, not toothed, notched and easily broken into fragments 4–8(–12) cells long 15

14. Terminal part of the caducous leaf tip composed of thin-walled cells, some of them conspicuously bulging ..... *D. gaochienii* s.str.
— Terminal part of the caducous leaf tip composed of moderately thick-walled cells, without bulging cells ..... *D. murrayae* 2

15. Leaf apex formed by irregularly notched fragments in a flexuose line, leaf cells around 8 µm .... *D. hedysariformis* s.str.
— Leaf apex formed by relatively regularly notched fragments in a ± straight line, leaf cells mostly 10–14 µm ..... *D. gaochienii* 2 incl. the *D. hedysariformis-2* lineage

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Table 1. Label data and accession numbers of studied specimens. New accessions are boldfaced.

Species	Isolate	Provenance	Collector_No	Herbarium	ITS	rps4	trnM-trnV
Barbula unguiculata	B115	Austria, Carinthia, Heiligenblut	Kučera 12829	CBFS	HM147804	HM147777	JQ890366
Bryoerythrophyllum recurvirostrum	Be361	Czech Republic, Sumperk, Mestske skaly	Kučera 12925	CBFS	JQ890527	JQ890468	JQ890407
Didymodon acutus	D6	Czech Republic, Breclav, Sedlec	Kučera 12684	CBFS	**KP307477**	**KP307551**	**KP307667**
D. anserinocapitatus	D202	Russia, Krasnoyarsk	Otnyukova	CBFS:13039	**KP307480**	**KP307545**	**KP307640**
D. anserinocapitatus	D203	Russia, Altai Republic, Malyi Yaloman	Ignatov & Ignatova 25/155	CBFS:13045	**KP307485**	**KP307558**	**KP307664**
D. anserinocapitatus	D423	U.S.A., Colorado, Vrain Canyon	Weber & Wittmann B-114031	DUKE	**KP307497**	**KP307544**	**KP307636**
D. anserinocapitatus	D777	China, Yunnan, Diqing, Deqin	Long 23918	E	**KP307466**	**KP307582**	**KP307616**
D. asperifolius	D55	Austria, Carinthia, Mt Gr. Hafner	Kučera 12575	CBFS	**KP307455**	JQ890472	**KP307600**
D. asperifolius	D254	Russia, Altai Republic, Kobiguayuk Cr	Ignatov 0/113	CBFS:13302	**KP307494**	**KP307597**	**KP307665**
D. asperifolius	D261	Russia, Altai Republic, Mt Tabozhok	Ignatov 31/281	CBFS:13303	**KP307492**	**KP307596**	**KP307659**
D. asperifolius	D286	Mongolia, Zavkhan, Tsagaan Gol	F-Muller	DR:039336	–	**KP307595**	**KP307605**
D. asperifolius	D288	Mongolia, Arkhangai, Ogtojn Am	F-Muller	DR:039402	**KP307502**	**KP307553**	**KP307631**
D. asperifolius	D788	India, Sikkim, Goichang	Long 26560	E	**KP307489**	–	–
D. asperifolius 2	D982	Italy, Friuli, Mt Montasch	Kučera 16824	CBFS	**KP307457**	**KP307588**	**KP307608**
D. asperifolius 3	D287	Mongolia, Zavkhan, Tsagaan Gol	F-Muller	DR:039368			
				clone 2:	**KP307516**		
				clone 3:	**KP307522**		
				clone 4:	**KP307499**	**KP307587**	**KP307622**
D. asperifolius 3	D343	China, Qinghai, Huashixia	Long 26810	E	**KP307514**	**KP307540**	**KP307660**
D. asperifolius 3	D981	Italy, Friuli, Mt Montasch	Kučera 16821	CBFS	**KP307510**	**KP307590**	**KP307637**
D. australasiae	D82	Spain, Granada, Trevelez	Kučera 5425	CBFS	**KP307472**	**KP307571**	**KP307651**
D. cordatus	D53	Czech Republic, Breclav, Dolni Vestonice	Kučera 12702	CBFS	**KP307460**	**KP307564**	**KP307668**
D. fallax	D81	Czech Republic, Breclav, Klentnice	Kucera 2023	CBFS	**KP307504**	**KP307552**	**KP307663**
D. aff. fuscus	D994	Chile, Reg. XI, Puyuhapi	F-Muller C1921	CBFS:16866	**KP307476**	**KP307546**	**KP307615**
D. fuscus	D995	Chile, Reg. VII, Altos de Lircay	F-Muller C1461	CBFS:16865	**KP307467**	**KP307537**	**KP307601**
D. gaochienii	D280	China, Qinghai, Baqu valley	Tan 95-250	MHA (isotype)	**KP307474**	**KP307538**	**KP307658**
D. gaochienii 2	D200	Russia, Tuva, Lake Kadysh	Otnyukova	CBFS:13040	**KP307461**	**KP307591**	**KP307641**
D. gaochienii 2	D262	Russia, Altai Republic, Chulcha River	Ignatov 9/42	CBFS:13318	**KP307488**	**KP307532**	**KP307649**
D. gaochienii 2	D263	Russia, Altai Republic, Kurkura Range	Ignatov 8/329	CBFS:13319	**KP307482**	**KP307592**	**KP307623**
D. gaochienii 3	D397	Russia, Kamchatka, Pravyi Kikhchik	Chernyadyeva 13	CBFS:13724	**KP307506**	**KP307541**	**KP307620**
D. giganteus	D79	Austria, Salzburg, Mt Waldhorn	Kučera 12897	CBFS	**KP307468**	**KP307548**	**KP307669**
D. hedysariformis	D127	U.S.A., Alaska, Denali	Perry 7670	CBFS:12916	**KP307525**	**KP307569**	**KP307629**
D. hedysariformis	D196	Russia, Tuva, Toora-Khem River	Otnyukova	CBFS:13038	**KP307465**	**KP307555**	**KP307618**
D. hedysariformis	D199	Russia, Tuva, Toora-Khem	Otnyukova	CBFS:13044	**KP307464**	**KP307557**	**KP307628**
D. hedysariformis	D255	Russia, Tuva, Azas River	Otnyukova	CBFS:13304	**KP307462**	–	–
D. hedysariformis	D257	Russia, Sakha, Ezhantsy	Ignatov 00-67	CBFS:13305	**KP307478**	**KP307550**	**KP307624**
D. hedysariformis	D258	Russia, Altai Republic, Ust-Sema	Ignatov 24/53	CBFS:13306	**KP307486**	**KP307574**	**KP307632**
D. hedysariformis 2	D201	Mongolia, Orkhon River basin	Tsegmed 6640	CBFS:13041	**KP307518**	**KP307556**	**KP307655**
D. hedysariformis 2	D264	Mongolia, Ulan Bator, Bogdkhan Uul	Tsegmed 11198	CBFS:13317	**KP307529**	**KP307581**	**KP307635**
D. hedysariformis 2	D504	Russia, Transbaikalia, Alkhanai	Afonina 07507	CBFS:14104	**KP307495**	**KP307580**	**KP307666**
D. hedysariformis 2	D1003	Mongolia, Tov, Khustain Ridge	Tsegmed 13320	CBFS:14930	**KP307528**	**KP307572**	**KP307612**
D. hedysariformis 2	D1004	Mongolia, Ulan Bator, Bogdkhan Uul	Tsegmed 12068	CBFS:14941	–	**KP307578**	**KP307610**
D. hedysariformis 2	D1005	Mongolia, Tov, Hentei Ridge	Tsegmed 8136	CBFS:14942	–	**KP307560**	**KP307634**
D. hedysariformis 2	D1006	Russia, Transbaikalia, Sokhondo, Enda	Czernyadjeva 47-11	CBFS:15096	**KP307458**	**KP307561**	**KP307633**
D. icmadophilus	D7/D48	Austria, Styria, Mt Hochwildstelle	Kučera 12490	CBFS	**KP307475**	**KP307598**	**KP307604**
D. johansenii	D59	Canada, Alberta, Jasper, Devona cabin	Cleavitt	CBFS:4472	**KP307470**	**KP307542**	**KP307662**
D. johansenii	D60	Austria, Styria, Mt Wildfeld	Kučera 7204	CBFS	**KP307517**	**KP307593**	**KP307602**
D. johansenii	D137	Canada, Alberta, Jasper, Snake Indian River	N. Cleavitt	CBFS:4473			
				clone 1:	**KP307487**		
				clone 2:	**KP307493**	**KP307583**	**KP307603**

D. johansenii	D209	Austria, Salzburg, Mt Plankowitzspitze	Köckinger 97-631	CBFS:13254	**KP307471**	**KP307577**	**KP307614**
D. johansenii	D272	Norway, Svalbard, Petuniabukta	Kosnar	CBFS:13322	**KP307526**	**KP307579**	**KP307653**
D. johansenii	D389	Russia, Buryatia, Sorok River	Afonina 02408	CBFS:13718	**KP307456**	**KP307573**	**KP307626**
D. johansenii	D508	Russia Sakha, Suntar Khayata ridge	Ivanova & Krivoshapkin	CBFS:14105	**KP307481**	**KP307530**	**KP307645**
D. johansenii	D793	China, Qinghai, Jungun Naichong	Long 26962	E	**KP307515**	**KP307594**	**KP307657**
D. murrayae	D126	U.S.A., Alaska, Liberty Falls	Perry 7912	CBFS:12917	**KP307503**	**KP307563**	**KP307650**
D. murrayae	D251	Russia, Altai Republic, Kayru Creek	Ignatov	CBFS:13300	**KP307513**	**KP307576**	**KP307613**
D. murrayae	D1001	Mongolia, Khovsgol, Khar-Murugu-Uul	Tsegmed 453	CBFS:14920	**KP307521**	**KP307567**	**KP307639**
D. nigrescens	D340	Nepal, Langtang valley	Long 30589	E	**KP307498**	**KP307543**	**KP307611**
D. nigrescens	D356	U.S.A., Alaska, Izembek NWR	Schofield 109554	NY	**KP307512**	**KP307554**	**KP307656**
D. nigrescens	D359	Bhutan, Bumthang Road	Andreas	NY	**KP307505**	**KP307562**	**KP307648**
D. norrisii	D422	U.S.A., California, Upper Chico Canyon	Shevock 27907	DUKE	**KP307509**	**KP307585**	**KP307617**
D. occidentalis	D434	Canada, British Columbia, Botaniae Mt	McIntosh 7521	DUKE	**KP307524**	**KP307533**	**KP307599**
D. perobtusus	D94	Russia, Irkutskaya, Lake Baykal	Pujmanova	CBFS:12920	**KP307523**	**KP307539**	**KP307609**
D. perobtusus	D370	Russia, Buryatia, River Sorok	Afonina 02408	CBFS:13691	**KP307490**	**KP307549**	**KP307654**
D. revolutus	D420/439	U.S.A., Oklahoma, Hinton	Merrill 13249	DUKE	**KP307501**	JQ890471	**KP307646**
D. rigidulus	D44	Czech Republic, C. Budejovice	Kučera 1815	CBFS	**KP307473**	**KP307589**	**KP307647**
D. rivicola	D338	China, Yunnan, Gaoligong Shan	Long & Shevock 37326	E	**KP307491**	**KP307566**	**KP307607**
D. rivicola	D351	China, Yunnan, Diqing, Litiping Plateau	Long 24534	E	**KP307507**	–	–
D. rivicola	D352	China, Yunnan, Diqing, Deqin	Long 24146	E clone 1:	**KP307479**		
				clone 2:	**KP307520**	**KP307565**	**KP307661**
D. rivicola	D353	China, Yunnan, Diqing, Benzilan	Long 24220	E	**KP307500**	**KP307575**	**KP307652**
D. rivicola	D763	Nepal, Langtang Khola	Long 22052	E	–	**KP307568**	**KP307619**
D. sinuosus	D85	Czech Republic, Breclav, Pohansko	Kucera 12059	CBFS	JQ890529	JQ890476	JQ890410
D. sinuosus	D729	United Kingdom, Scotland, Allt Mor	Hodgetts 8230	CBFS:16366	**KP307508**	**KP307536**	**KP307627**
D. subandreaeoides	D90	Switzerland, Schwyz, Mt Rigi	Kučera 7389	CBFS	**KP307483**	**KP307570**	**KP307630**
D. subandreaeoides	D354	China, Yunnan, Wo Tu Di	Long 19030	E	**KP307519**	**KP307547**	**KP307642**
D. subandreaeoides	D357	Canada, NWT, Virginia Falls	Steere 76-603	NY	**KP307484**	**KP307531**	**KP307644**
D. vinealis	D84	Spain, Malaga, Ronda Mts	Kučera 5567	CBFS	**KP307469**	JQ890475	**KP307606**
D. xanthocarpus	D751	South Africa, Cape, Mt Synott	Magill & Schelpe 4030	E	**KP307459**	**KP307534**	**KP307638**
D. zanderi	D34	Russia, Transbaikalia, Alkhanay, Ubzholgos	Afonina 3405	CBFS:12909	**KP307527**	**KP307535**	**KP307621**
D. zanderi	D43	Russia, Chita, Kyra	Afonina 11706	CBFS:12907	**KP307463**	**KP307559**	**KP307643**
D. zanderi	D232	Russia, Tuva, Lake Kadysh	Otnyukova	CBFS:13273	**KP307496**	**KP307586**	**KP307625**
Microbryum curvicolle	Mb579	Czech Republic, Breclav, Pouzdrany	Kosnar 358	CBFS:15119	JX679969	JX679986	JX679936
Syntrichia ruralis		Canada, Alberta, Bow River		UC/JEPS	–	FJ546412	FJ546412
Syntrichia ruralis	Sy576	Czech Republic, Vyskov, Kojatky	Kosnar 1035	CBFS:15126	clone 1	–	–
Tortula muralis	T56	Czech Republic, Tachov, Studanka	Kosnar 771	CBFS	JN544795	JN581679	JQ890421

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SYSTEMATICS AND PHYLOGENY

Partial generic revision of *Barbula* (Musci: Pottiaceae): Re-establishment of *Hydrogonium* and *Streblotrichum*, and the new genus *Gymnobarbula*

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Abstract Large genera, that were defined using a restricted suite of morphological characters, are particularly prone to be polyphyletic. We analysed a representative selection of species traditionally assigned to the genus *Barbula*, believed to represent the largest genus of the moss family Pottiaceae, but which recently was suggested to be polyphyletic. Special attention was paid to species traditionally assigned to *Barbula* sect. *Hydrogonium* and sect. *Convolutae*, in which phylogenetic relationships are likely to be incongruent with morphological traits, which could have evolved in adaptation to hydric and otherwise extreme habitats. Our phylogenetic analysis was based on nrITS and chloroplast *rps4* and *trnM-trnV* sequence data and resolved only the type of the genus, *B. unguiculata*, plus *B. orizabensis*, in subfamily Pottioideae, while most of the species occurring in the Northern Hemisphere are part of Trichostomoideae and need to be recognized within the re-established and partly re-defined genera *Hydrogonium* and *Streblotrichum*. The phylogenetically and morphologically divergent *B. bicolor* needs to be removed from *Streblotrichum* to a newly described genus, *Gymnobarbula*. Numerous taxonomic changes and nomenclatural novelties, resulting from the molecular, morphological and nomenclatural studies are proposed for taxa of *Hydrogonium*, particularly within the *H. consanguineum* clade. Lectotypes are selected for *Tortula angustifolia* Hook. & Grev. (≡ *Hydrogonium angustifolium* (Hook. & Grev.) Jan Kučera, comb. nov.), *Tortula consanguinea* Thwaites & Mitt. (≡ *Hydrogonium consanguineum* (Thwaites & Mitt.) Hilp.) and *Tortula flavescens* Hook. & Grev. (= *Hydrogonium consanguineum* (Thwaites & Mitt.) Hilp.).

Keywords *Barbula*; *Gymnobarbula* gen. nov.; *Hydrogonium*; ITS; nomenclature; polyphyly; Pottiaceae; *rps4*; *Streblotrichum*; taxonomy; *trnM-trnV*

Supplementary Material The alignment is available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

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INTRODUCTION

The genus *Barbula* Hedw. has been considered to represent the largest genus of the moss family Pottiaceae Schimp., with Zander (2007) estimating *Barbula* to contain some 200 species. The current taxonomic concept of *Barbula* dates back to Saito (1975), who emphasized gametophytic characters (e.g., leaf shape and anatomy and characters of axillary hairs). This allowed him to exclude the species of *Didymodon* Hedw. with twisted peristome teeth, and those of *Bryoerythrophyllum* P.C. Chen from the earlier concepts of *Barbula*, while including the species of *Hydrogonium* (Müll. Hal.) A. Jaeger. Saito recognized three subgenera—*B.* subg. *Barbula* with sect. *Barbula* and sect. *Hydrogonium* (Müll. Hal.) K. Saito, *B.* subg. *Streblotrichum* (P. Beauv.) K. Saito, and the newly established *B.* subg. *Odontophyllon* K. Saito. His concept was slightly extended by Zander (1993) from a global perspective. Zander classified the genus only down to section level, merging *B.* subg. *Odontophyllon* with *B.* sect. *Convolutae* (= *B.* subg. *Streblotrichum*), while retaining *B.* sect. *Hydrogonium*, and further recognizing several mostly monotypic and partly obscure sections not occurring in Japan, such as the principally Central American *B.* sect. *Hyophiladelphus* Müll. Hal., or the Central to South African *B.* sect. *Bulbibarbula* Müll. Hal. This delimitation of *Barbula* has never been challenged in later treatments and hence it has been widely accepted, except by Li & al. (2001), who retained *Hydrogonium* distinct from *Barbula*. Even Zander (1993), however, acknowledged the difficult delimitation of *Barbula* with respect to, e.g., *Trichostomum* Bruch and *Hyophila* Brid., and envisaged the future splitting of the genus into segregate genera.

Phylogenetic inferences from *rps4* data resolved *Barbula* as a polyphyletic entity (Werner & al., 2004). Its generitype, *Barbula unguiculata* Hedw., appeared in subfamily Pottioideae (Limpr.) Broth., while the other two analyzed species, *B. bolleana* (Müll. Hal.) Broth. and *B. indica* (Hook.) Spreng., appeared in a clade in subfamily Trichostomoideae (Schimp.) Limpr. The close relationship between the latter two species was perhaps unexpected, as *B. bolleana* has been regarded by recent authors (Frahm & al., 1996; Zander, 2007) to be taxonomically

identical with *B. ehrenbergii* (Lorentz) M. Fleisch., the type of *B.* sect. *Hydrogonium*, while *B. indica* (Hook.) Spreng. represents a group of taxa which had been recognized by some pre-Saitoan authors as the genus *Semibarbula* Herzog ex Hilp. (e.g., Hilpert, 1933; Gangulee, 1972). *Semibarbula* has mostly been synonymized with *Barbula* sect. *Barbula* in the following treatments (Saito, 1975; Li & al., 2001). Later, Cox & al. (2010) were able to confirm the isolated position of *B. agraria* Hedw., elevated to the rank of genus (*Hyophiladelphus* (Müll. Hal.) R.H. Zander) by Zander (1995). The isolated position of some European members of *B.* sect. *Convolutae* Bruch & Schimp. could be inferred from a study by Köckinger & Kučera (2011), which essentially studied the members of tribe Pleuroweisieae (Limpr.) P.C. Chen.

It is obvious that building a robust backbone phylogeny of *Barbula* sensu Zander (1993) is a voluminous task which can be accomplished only by the combination of molecular phylogenetic analyses with the careful re-consideration of morphological and anatomical characters. The sampling for such a study will not only have to include the representatives of the genus, as understood by earlier authors, from its entire range, but also species currently assigned to the genera *Trichostomum* or *Hyophila*, which contain species morphologically similar to *Barbula*. Similarly, the relatively recently segregated genus *Pseudocrossidium* R.S. Williams should be included, because its delimitation has not been tested using molecular markers. One way of dealing with *Barbula* is the successive revision of relatively well-defined groups that will be removed from the wastebasket assemblage of the original *Barbula*, keeping in mind that the morphological delimitation must not always reflect phylogenetic relationships. Based on the molecular studies discussed above, we identified two such groups, *B.* sect. *Hydrogonium* and sect. *Convolutae*.

Barbula sect. *Hydrogonium* was first recognized as a "Gruppe", i.e., an unranked infrageneric group, within the genus *Trichostomum* by C. Müller (Müller 1876: 297) to accommodate three similar hydrophytic taxa, *B. bolleana* and *B. ehrenbergii* (now regarded as conspecific), and *B. meidensis* Cufod. (≡ *Trichostomum fontanum* Müll. Hal.), a little known Somali species that may, according to the protologue, be identical with the preceding two species. Fleischer (1904) broadened the concept of *Hydrogonium* (at subgeneric level within *Barbula*) to accommodate the hygrophilous Indo-Malayan s.l. *Barbula* species with broadly lanceolate to lingulate leaves with smooth to little papillose, relatively wide lamina cells (*B. javanica* Dozy & Molk., *B. inflexa* (Duby) Müll. Hal., *B. pseudoehrenbergii* M. Fleisch., *B. tjibodensis* M. Fleisch.). Later Hilpert (1933) included in *Hydrogonium* the less hygrophilous species with densely papillose lamina cells, such as *H. consanguineum* (Thwaites & Mitt.) Hilp., and drew attention to axillary gemmae of a type different from, e.g., *Streblotrichum*. Hilpert's delimitation of *Hydrogonium* was accepted and applied to East Asian species by Chen (1941), who also formalized the delimitation of two groups within *Hydrogonium*, i.e., *H.* sect. *Hydrogonium* ['*Euhydrogonium*'], comprising the traditionally recognized hygrophilous taxa, and the newly distinguished *H.* sect. *Barbuliella* P.C. Chen comprising species with small papillose cells and sharply acute or apiculate leaves. Finally, Zander (1993) extended sect. *Hydrogonium* to include the genus *Semibarbula*, a view that was later (Werner & al., 2004) corroborated by their molecular relationships. Zander (2007) later changed his mind and transferred the North American species of *Semibarbula* and *H.* sect. *Barbuliella* to *H.* sect. *Convolutae*. Most of the taxa assigned historically to *Hydrogonium* have their distribution centre in the Indo-Malayan region or occur exclusively in this area.

Barbula sect. *Convolutae*, typified by *B. convoluta* Hedw., has been traditionally recognized to include *Barbula* s.l. species with strongly differentiated convolute perichaetial leaves, mostly yellow seta and an annulus of strongly differentiated, vesiculose cells (Limpricht, 1890; Zander, 1993). This definition agrees well only with the characters of the primarily European species *Streblotrichum convolutum*, *S. commutatum* (Jur.) Hilp., and *S. enderesii* (Garov.) Loeske, while other taxa like *S. bicolor* (Bruch & Schimp.) Loeske or *S. croceum* (Brid.) Loeske share only some of these characters. Only a few taxa occurring exclusively outside Europe have historically been assigned to this group of mosses. Although Brotherus (1902: 410–411) listed 30 species worldwide, most of them were later transferred to other genera or synonymized.

Our aims were (1) to identify phylogenetically defined supra-specific units within the supposedly polyphyletic genus *Barbula* and to compare the phylogenetic signal from chloroplast and nrITS datasets, (2) to identify morphological and anatomical characters which match these phylogenetially defined units and permit their formal description or referral to earlier described taxa, and (3) to develop a taxonomic and nomenclatural synopsis of taxa referred to *Barbula* sect. *Convolutae* and sect. *Hydrogonium* occurring in the Northern Hemisphere, focused on the revision of specific limits within the *Barbula indica* complex where these limits are uncertain considering the contrasting treatments of Sollman (2004b), Zander (2007) and Ignatova & Ignatov (2009).

■ MATERIALS AND METHODS

Herbarium material and sampling for the molecular analysis. — Sampling of the material followed the main goals specified above. We sampled representative species of sect. *Convolutae* and sect. *Hydrogonium*, with a focus on exemplars from their putative centres of origin in SW Asia and the Holarctic, respectively. More detailed sampling was necessary in the *B. indica* complex. Types of the sections as well as taxa which differ in morphological characters which might prove to be taxonomically important were included in the analysis. This selection was complemented by representatives of other Pottiaceae, based on Zander (1993), Werner & al. (2004, 2005b), Cox & al. (2010) and Köckinger & Kučera (2011). The taxa sampled for this study are listed in Appendix 1.

The molecular study was complemented by the study of herbarium material and the most relevant types to ensure the correct application of names. These studies particularly concentrated on European and American collections named *Barbula*

indica (BP, DUKE, Z, priv. herb. G. Amann) and *B. indica* var. *kurilensis* (MHA). Following the treatment of Sollman (2004b), who synonymized many taxa of the *B. indica* complex under *B. tenuirostris* Brid., the type material of *B. tenuirostris* and their taxonomically and nomenclaturally most relevant synonyms, *B. flavicans* D.G. Long and *B. consanguinea* were obtained from BM, E and NY, and additional recent material from Southeast Asia of these taxa and of *B. javanica* was obtained from E. Later findings prompted us for morphological study of the types of *B. subcomosa* Broth. and *B. majuscula* Müll. Hal., which were obtained from BM. The plants studied are listed in Appendix 1. We have also extensively utilized the results of our previous morphological studies of *B. amplexifolia* and *B. gregaria* (Köckinger & Kučera, 2011), as well as unpublished morphological studies of European species of *Barbula* by the first author.

Molecular protocols. — Total genomic DNA was extracted using the NaOH method (Werner & al., 2002). Three regions were selected for amplification: the chloroplast loci *rps4* with the flanking *rps4-trnS* spacer (hereafter denoted as *rps4*) and *trnM-trnV*, and the nuclear ITS region. Chloroplast *rps4* is the region best represented for Pottiaceae in GenBank, followed by nuclear ITS, which was used in the treatments of Trichostomoideae by Werner & al. (2005b) and Köckinger & Kučera (2011). The variability of *trnM-trnV* has been shown to be useful in the study of Werner & al. (2009). Amplification and sequencing reactions followed the protocols described in Köckinger & Kučera (2011), and primers and amplification of *trnM-trnV* followed Werner & al. (2009). When data obtained from the direct ITS sequencing indicated a mixed template and more than two polymorphic positions within one sequence were detected, cloning was performed following the procedure described by Košnar & al. (2012).

Sequence editing, alignment and phylogenetic analysis. — The sequences were edited in BioEdit v.7 (Hall, 1999). The partial sequences of the *trnS* gene were trimmed from the *rps4* amplicons, as were the invariable 5′ and 3′ ends of ITS amplicons which belong to the 18S and 26S rRNA genes. The sequences were aligned using the online version of MAFFT v.6 (Katoh & Toh, 2008) using the Q-INS-i strategy with 200PAM / κ = 2 scoring matrix, gap opening penalty set to 1.0, and the offset value set to 0.0. The resulting alignments were manually inspected for homology problems and edited but these interventions were limited to very obvious cases to ensure maximum reproducibility. For purposes of phylogenetic analyses, three data matrices were produced: ITS, *rps4*, and a concatenated matrix of *rps4* and *trnM-trnV*. Information from indels was included in the phylogenetic analyses of the chloroplast datasets by coding them into the data matrix with SeqState v.1.4 (Müller, 2005) using the simple indel coding method (Simmons & Ochoterena, 2000).

Selection of outgroup taxa was based on earlier studies by Werner & al. (2004) and Cox & al. (2010). This selection could not be fully identical among datasets because *trnM-trnV* could not be amplified in *Pseudephemerum* and *Pleuridium*, and ITS sequences of *Blindia*, *Fissidens* and *Scopelophila* were not alignable with the rest of the dataset. Phylogenetic analyses were performed using the maximum parsimony (MP) criterion in PAUP* v.4b10 (Swofford, 2002) and Bayesian inference using MrBayes v.3.2.1 (Ronquist & al., 2012). The MP analysis was run using a heuristic search with the following settings: tree bisection-reconnection (TBR) branch swapping, random additions with 1000 replicates, hold = 1, multrees = yes, steepest = yes, collapse = yes. The 'maxtrees' limit was not restricted in analyses of concatenated *rps4* + *trnM-trnV* data, but was set to 100,000 trees in the analysis of *rps4* and to 50,000 in the analysis of ITS. A bootstrap analysis was performed with 1000 replicates using the heuristic search strategy as described, except for the following options: the 'maxtrees' limit was set to 10,000 and 10 replicates of random additions were used for analysis of concatenated *rps4* + *trnM-trnV*; the 'maxtrees' limit was set to 1000 and simple additions were used for the analysis of ITS and *rps4* matrices. For Bayesian inference, we have not partitioned DNA data from the concatenated chloroplast matrix, as the phylogenetic signal from separate genes and spacers was weak (compare support for clades in the separate *rps4* analysis and the analysis of the concatenated chloroplast dataset). We used a gamma model of rate variation across sites sampled across the GTR model space (lset nst = mixed rates = gamma). The analyses in MrBayes were performed using two simultaneous runs each with four separate chains, sampling one tree every 100 generations and running until the average standard deviation of split frequencies between runs dropped below 0.01. The temperature of a hot chain was set empirically to 0.05. Following the inspection of log likelihood values we found no reason to change the default setting of burn-in of the first 25% of sampled trees, and the remaining trees were used for construction of a 50% majority consensus tree. The trees were edited using TreeGraph v.2 (Stöver & Müller, 2010). Alternative topological hypotheses were evaluated using Bayesian inference. The datasets were re-analysed using the same settings as described above, except that models were constrained to monophyly/polyphyly of particular groups. The marginal model likelihoods of constrained trees were estimated using the harmonic mean of the likelihood values of the MCMC samples (Ronquist & al., 2012). Differences in log likelihoods >3 log units were considered as significant (Kass & Raftery, 1995).

■ RESULTS

Data matrices and phylogenetic reconstruction. — Data characteristics of the sequences are summarized in Table 1. The strict consensus trees obtained from MP had similar topologies as the 50% consensus Bayesian trees, differing only in poorly supported internal branches. Therefore, only the Bayesian trees (Figs. 1–3) are shown here with bootstrap support from the MP analyses shown where applicable. The topologies of trees inferred from each individual region as well as from the combined chloroplast data confirmed the polyphyly of *Barbula* sensu Zander (1993). While the type of the genus, *Barbula unguiculata*, plus *B. orizabensis* are resolved in Pottioideae, the remaining *Barbula* s.l. species appear among members of subfamily Trichostomoideae. A possible exception

Table 1. Characteristics of data matrices. Characters are listed for each of the regions in the *rps4* + *trnM-trnV* matrix.

	rps4	*rps4* + *trnM-trnV*	ITS
Number of sequences	100	87	114
Number of characters	696	679 / 945	1911
Variable characters	332	269 / 439	1116
Parsimony-informative characters	196	157 / 250	826

is the position of *Streblotrichum*, which is ambiguous: sister to Trichostomoideae+Pottioideae (*rps4* dataset, Fig. 1), basal within Trichostomoideae (concatenated chloroplast dataset, Fig. 2) or even polyphyletic (ITS dataset, Fig. 3), with low support for any of these placements. Monophyletic *Barbula* containing *B. unguiculata* and *B. orizabensis* received poor support only in the Bayesian inference of ITS data (PP = 0.94, Fig. 3). All tests using constrained trees rejected monophyly of *Barbula* s.str. with any other clade of other *Barbula* s.l. accessions (see Table 2).

While the chloroplast datasets render members of *Streblotrichum* (except for *B. bicolor*, see below) monophyletic with high support, the ITS analysis surprisingly suggests their polyphyly. While *B. enderesii* and one of the accessions of *B. convoluta* appear in a clade containing *Hyophila involuta*, *B. bicolor* and the *Leptodontium*+*Triquetrella* clade, which is largely congruent with the concatenated chloroplast dataset, the rest of accessions of *B. convoluta* with *B. commutata* appears in Pottioideae in a poorly supported clade containing *Didymodon* and *Syntrichia*.

Barbula bicolor has an uncertain position in Trichostomoideae. Chloroplast data marginally support its affinity to *Hyophila involuta* rather than to *Streblotrichum*, while ITS data failed to resolve its position with statistical support. The analysis of constrained trees strongly supported the non-monophyly of *Streblotrichum*+*B. bicolor* based on the *rps4* dataset and marginally did so in the analysis of the concatenated chloroplast dataset, while the ITS data marginally supported the possible monophyly of *Streblotrichum*+*B. bicolor* (Table 2). Nevertheless, the appearance of the *Leptodontium*+*Triquetrella* clade in this poorly supported clade again points to homology problems in ITS, and morphologically this grouping of taxa has no support at all.

The relationships of *Hyophiladelphus* could be analyzed only in the *rps4* dataset, which was slightly extended compared to the last study of Cox & al. (2010). *Hyophiladelphus* appears closely related with *Hyophila* Brid., *Gymnostomiella* M. Fleisch., *Splachnobryum* Müll. Hal. and possibly *Indopottia* A.E.D. Daniels & al., and a close relationship with *B. bicolor* was rejected in the test of monophyly (Table 2).

All analyses resolved a strongly supported clade within Trichostomoideae, which contained the traditionally recognized species of sect. *Hydrogonium* (*B. bolleana*—the type of the section, *B. pseudoehrenbergii*, *B. javanica*) together with taxa which were recognized only by some authors as members of *Hydrogonium* (*B. amplexifolia*, *B. subcomosa*, *B. indica*), and with taxa that have never been attributed to this group (*B. crocea*, *B. convoluta* var. *gallinula*). All the tests using constrained trees rejected monophyly of this clade with any other clade of *Barbula* s.l. (Table 2). The molecular analysis of samples originally identified as *B. indica* (incl. var. *kurilensis* and var.

Table 2. Comparison of constrained trees using marginal model likelihoods.

Group constrained to monophyly	*rps4*	*rps4* + *trnM-trnV*	ITS
Barbula s.l.	–	PPP	PPP
Barbula s.str. (*B. orizabensis*+*B. unguiculata*)	–	PP	n.s.
Barbula s.str.+*Gymnobarbula*	–	PPP	PPP
Barbula s.str.+*Streblotrichum*	–	PPP	PPP
B. orizabensis+*Streblotrichum*	–	PPP	–
B. unguiculata+*Streblotrichum*	–	P	–
Gymnobarbula+*Hydrogonium*	–	PP	PP
Gymnobarbula+*Hydrogonium*+*Streblotrichum*	–	PPP	PP
Gymnobarbula+*Streblotrichum*	PP	P	M
Hydrogonium+*Streblotrichum*	–	PPP	PP
Gymnobarbula+*Hyophiladelphus*	PP	–	–
Gymnobarbula+*Hydrogonium*+*Hyophiladelphus*	PPP	–	–
Hydrogonium+*Hyophiladelphus*	PPP	–	–

M, tree constrained to monophyly is significantly better, difference in marginal likelihoods >3 log units;
P, tree constrained to polyphyly is significantly better, difference in marginal likelihoods >3 log units;
PP, tree constrained to polyphyly is significantly better, difference in marginal likelihoods >5 log units;
PPP, tree constrained to polyphyly is significantly better, difference in marginal likelihoods >30 log units;
n.s., no significant difference in marginal likelihoods of constrained trees. In ITS analyses, only the clade comprising accessions of *B. enderesii* and *B. convoluta* JQ890491 was considered as *Streblotrichum*.

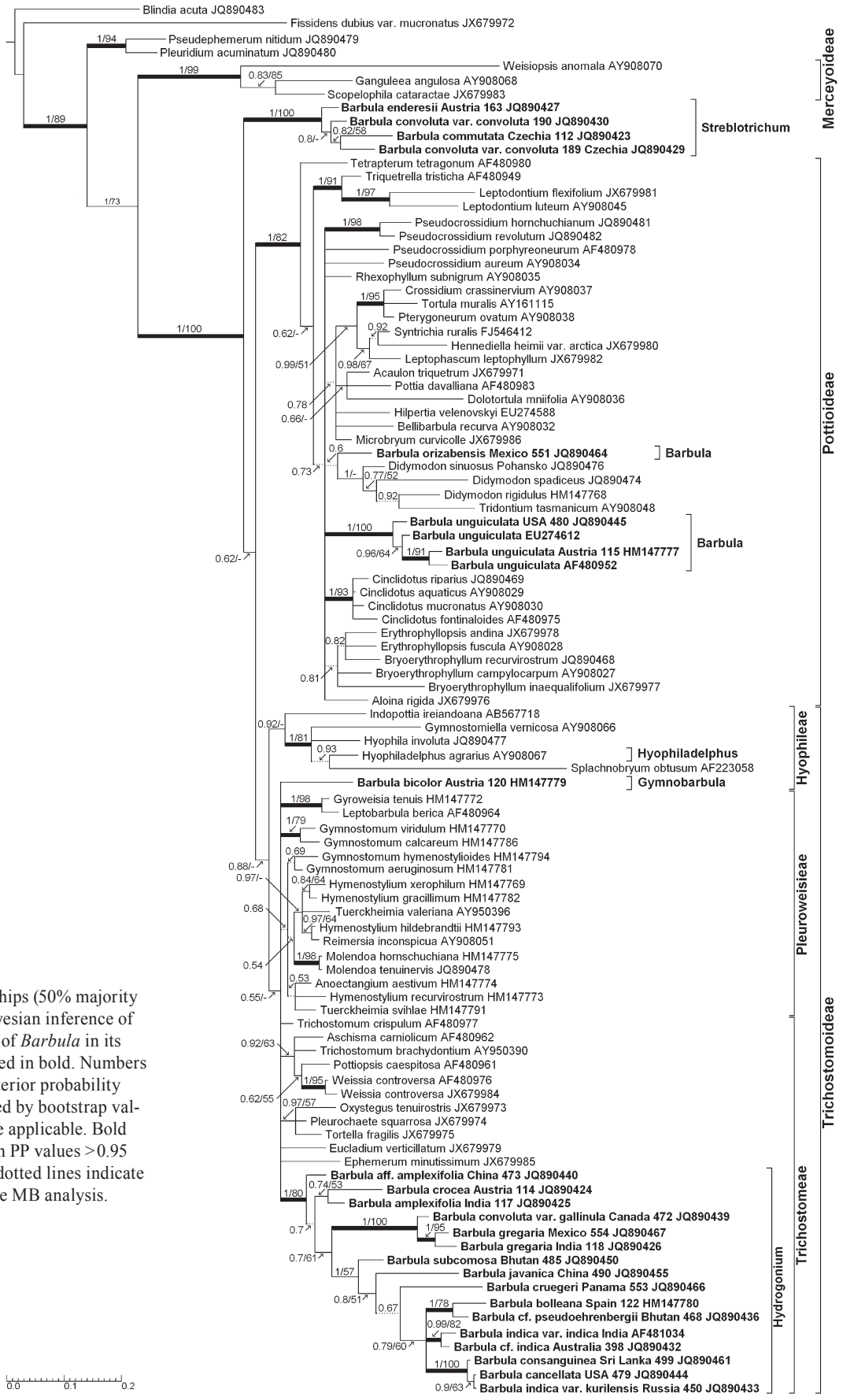

Fig. 1. Phylogenetic relationships (50% majority consensus tree) from the Bayesian inference of the *rps4* dataset. Accessions of *Barbula* in its earlier circumscription printed in bold. Numbers above branches indicate posterior probability from the BI analysis, followed by bootstrap values of the MP analysis where applicable. Bold branches indicate clades with PP values >0.95 and bootstrap values >0.75, dotted lines indicate branches resolved only by the MB analysis.

Fig. 2. Phylogenetic relationships as revealed by the analysis of the concatenated *rps4* and *trnM-trnV* datasets. For further explanation see Fig. 1.

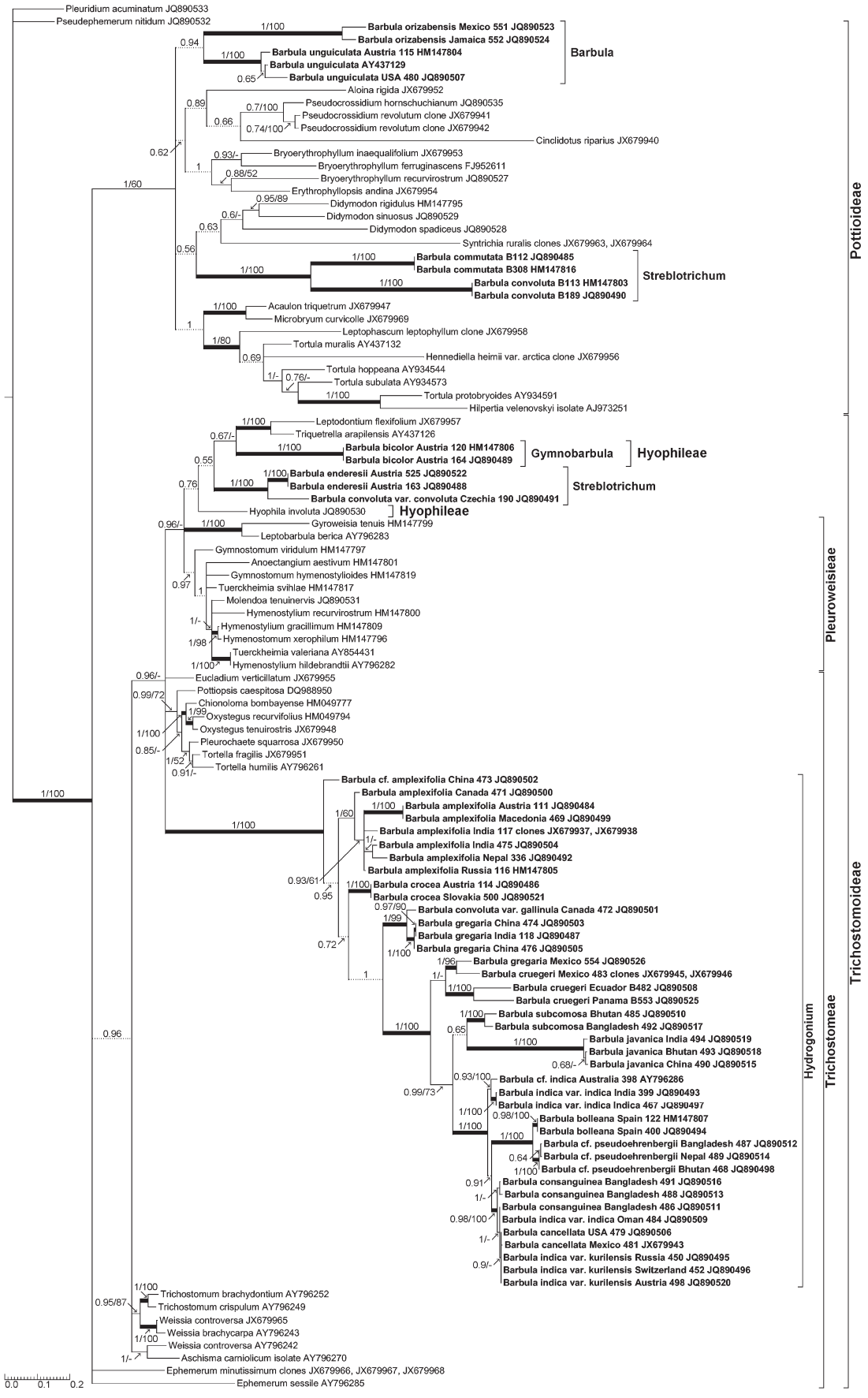

Fig. 3. Phylogenetic relationships based on ITS. For further explanation see Fig. 1.

gregaria), *B. tenuirostris*, *B. consanguinea* and *B. convoluta* var. *gallinula* revealed that the morphological characters used in Floras and other treatments for the differentiation of these taxa do not reflect molecular relationships. The morphological re-consideration of the samples nevertheless showed that it is possible to find characters which are in good agreement with the pattern detected by the molecular study. These characters will be described and discussed in the taxonomic synopsis and were used for the final identification of taxa. The individual clades within *Hydrogonium* will be described in more detail in the following text.

***Barbula amplexifolia* clade. —** *Barbula amplexifolia* accessions are highly variable. Identical ITS, *rps4* and *trnM-trnV* haplotypes were obtained only from European samples, while all Asian and North American accessions are unique. One accession (*Long 18818*) is so distant from other accessions that it does not appear in the *B. amplexifolia* clade in any of the analyses. It probably belongs to a different species, although it still matches the described morphological variability of *B. amplexifolia*. *Barbula crocea*, never before assigned to sect. *Hydrogonium*, appears nested within the *B. amplexifolia* clade in the analysis of the combined chloroplast dataset, yet its position in the ITS analysis suggests a poorly supported sister relationship to the rest of *Hydrogonium* excluding *B. amplexifolia*.

***Barbula gregaria* and *B. cruegeri*. —** All inferences agree in their confirmation of no close relationship of specimens identified as *B. gregaria* and their putative synonyms (*B. indica* var. *gregaria*, *B. cruegeri*, *B. horrinervis*) to *B. amplexifolia* or *B. indica* s.str. Surprisingly, these inferences also resolve *B. gregaria* and *B. cruegeri* s.str. as separate entities that do not constitute a monophyletic taxon. American *B. cruegeri* can be morphologically defined by the characters listed below in the taxonomic synopsis, although the differentiation is not always easy. Putatively endemic North American *B. convoluta* var. *gallinula* is resolved as sister to the remaining *B. gregaria* accessions. It differs in two unique transitions in the coding region of *rps4* and one substitution and two indels in the *rps4-trnS* spacer; ITS is somewhat more divergent, differing in several larger indels, but anyway *B. convoluta* var. *gallinula* forms a well-supported monophyletic group with the rest of *B. gregaria* except for the above-named specimen *Eckel 188986*.

***Barbula subcomosa*. —** Two accessions of plants originally identified as *B. consanguinea* appeared in a well-supported clade separate from a clade containing other accessions of that taxon. That clade appears in a poorly supported lineage as sister to *B. javanica* (ITS dataset), resp. sister to a poorly supported clade of *B. javanica* + *B. bolleana* + *B.* cf. *pseudoehrenbergii* + *B. indicav* + *B. consanguinea* s.l. (cp dataset). Subsequent morphological reconsideration and study of the type of *B. subcomosa* (see Synopsis) indicated the highly probable identity of this lineage with *B. subcomosa*, that has been to date regarded as synonymous with *B. consanguinea*.

***Barbula bolleana* clade. —** This clade, sister to *B. indica* + *B. consanguinea* s.l., includes *B. bolleana* and another taxon, which consists of several accessions originally named *B. consanguinea, B. javanica* or *B. tenuirostris*, which are morphologically uniform and different from all other taxa included. This taxon may be identical to *B. pseudoehrenbergii* (see Taxonomic synopsis for details).

***Barbula indica*+*B. consanguinea* clade. —** All Indian and the Omani sample of *B. indica* share identical sequences in the chloroplast regions, and the *rps4* sequences are also identical with the morphologically unusual Mauritanian samples deposited in GenBank, studied earlier by Werner & al. (2003). One sample of morphologically divergent *B.* cf. *indica* from Australia (*Streimann 39344*) differs by three substitutions in *rps4*, and similarly its ITS shows multiple substitutions and indels compared to the otherwise nearly invariable *B. indica*. The ITS and chloroplast inferences differ mainly in the position of the Omani sample (*Rothfels 2763*), identified as *B. indica*, which in the ITS dataset is more closely related to the accessions of *B. consanguinea*. The possibility of a hybrid origin of *Rothfels 2763* between *B. indica* and *B. consanguinea* would have some support from its intermediate morphology. The tropical accessions of *B. consanguinea* from SW Asia are identical in their chloroplast sequences but slightly diverge in their ITS, hence the pattern seen in the ITS tree, in which *B. consanguinea* sample *Long 28197* clusters with North American samples of *B. cancellata* and European and Asian samples of *B. indica* var. *kurilensis*. The type of *B. indica* var. *kurilensis* is identical with the European plants named earlier *B. indica* (re-interpreted as *B. consanguinea* by Köckinger & al., 2012), and differs only by one substitution in the *trnM-trnV* spacer from North/Central American *B. cancellata*, and in four, respectively three substitutions in their chloroplast loci from *B. consanguinea* s.str. The phylogenetic signal of ITS is similar to the signal of the chloroplast regions in this clade. The three accessions of *B. indica* var. *kurilensis* are identical except for one microsatellite repetition in one of the samples. The two samples of the North American *B. cancellata* diverge from each other in three microsatellite motifs, and both differ from *B. indica* var. *kurilensis* in two homopolymer segments. The two *B. consanguinea* s.str. accessions show greater genetic divergence particularly in their ITS2 region.

■ DISCUSSION

Our results confirm that all earlier delimitations of *Barbula* not only do not meet the criterion of monophyly with respect to the analyzed sequence data, but show that *Barbula* is so clearly polyphyletic that several genera need to be re-established or newly recognized. *Barbula*, with more than 200 species accepted by Zander (1993, 2007) the largest genus of Pottiaceae, must be substantially restricted. In addition to monotypic *Hyophiladelphus*, which has already been removed from *Barbula* by Zander (1995), the species attributed to sections *Convolutae* and *Hydrogonium* as circumscribed by Zander (1993) must further be removed from the genus. The new delimitation of the genus *Barbula* within Pottioideae requires further investigation, because *Barbula* s.str. was not representatively sampled in this study. Especially Southern

Hemisphere taxa seem to be of special importance for the future delimitation of *Barbula* s.str. Also the exact placement of *B. orizabensis* will be interesting, because a sister-group relationship of *B. unguiculata* and *B. orizabensis* was suggested only by the ITS data (without bootstrap support; constraining the chloroplast data to monophyly was significantly worse than the tree found). Morphology nevertheless strongly supports that the latter two taxa are congeneric as recognized by earlier authors (Thériot, 1931; Zander, 1979). Shared characters of the two species include the relatively long stems with even foliage, lowermost leaves nearly identical to the uppermost ones, leaf costa excurrent in stout mucro, lingulate leaf apex, strongly recurved leaf margins, multiple simple conical papillae on lamina cells in the transition zone between the upper pluripapillose cells with c-shaped or composite papillae and smooth basal cells, dorsal superficial cells of the costa pluripapillose with simple conical and evenly distributed papillae, perichaetial leaves little differentiated, seta orange-reddish to reddish brown, long-cylindric capsule with long, sinistrorsely twisted peristome, spores to 15 µm, and axillary gemmae (subglobose, brownish, pluricellular and spontaneously developed in *B. orizabensis*, but unicellular, and known only from cultivation in *B. unguiculata*; Zander, 1979).

Barbula sect. *Convolutae* needs to be recognized as a separate genus, i.e., *Streblotrichum*, although in a delimitation which partly differs from historical understanding (after *B. bicolor*, *B. crocea*, *B. hiroshii*, *B. convoluta* var. *gallinula* are removed, see Synopsis). This delimitation of *Streblotrichum* has strong support in our molecular analyses (see Results) and represents a morphologically clear-cut entity (see Synopsis) although the diversification of ITS sequences makes the genus biphyletic. The apparent non-monophyly might well reflect the problems of homology within ITS due to the low level of sequence similarity rather than really challenging the monophyly of *Streblotrichum* (similar to the case of the *Leptodontium* + *Triquetrella* clade, which also belongs to Pottioideae according to chloroplast data), but needs to be addressed in future studies. ITS sequences of both *Streblotrichum* clades are probably functional nrDNA molecules. Both sequence types have a conserved 5.8S gene, and no differences in free energy of RNA secondary structure nor in CG content were observed (data not shown). Nevertheless, the intrafamilial position of *Streblotrichum* could not yet been ascertained due to low support for any of the placements described above.

Barbula bicolor always constituted a morphologically odd element in the earlier delimitation of *Streblotrichum*, as already acknowledged by Brotherus (1902: 410), and it does not even fit the broad global description of *Barbula* s.l. by Zander (1993: 146) considering its large spores and absent peristome. As the molecular relationships based on the analysis of chloroplast regions do not support the monophyly of *Streblotrichum* s.str. + *B. bicolor*, and the results of the ITS analysis are weakly supported, the species is best accommodated in a genus of its own that is newly described below as *Gymnobarbula*.

The re-established genus *Hydrogonium* becomes one of the phylogenetically best-supported genera of Pottiaceae (Figs. 1–3). Historically, the acceptance and delimitation of *Hydrogonium* in the treatments that followed Chen (1941) varied substantially, with the majority of opinions tending towards sectional or no infrageneric rank within *Barbula*, with the notable exception of Li & al. (2001), who accepted the genus. Interestingly, they attributed *B. subcomosa* and the little known *B. dixoniana* (P.C. Chen) Redf. & B.C. Tan to *Barbula*, although they basically adopted Chen's delimitation of *Hydrogonium*, judging from the descriptions and key characters provided. Similarly, it is not obvious why Saito (1975), along with earlier treatments, classified *B. subcomosa* within sect. *Hydrogonium* but *B. amplexifolia* (as *B. coreensis*) and *B. gregaria* (as *B. horrinervis* K. Saito) were left in sect. *Barbula*. Our molecular results are consistent with Zander's perhaps surprising view (Zander, 1993) that the hygrophilous taxa, such as *B. bolleana*, are species with a morphology derived from xerophilous species, such as *B. indica*, and hence that their unusual morphology may reflect adaptations to a special habitat. Earlier delimitations of *Hydrogonium* always strived to look for morphological characters that were such derived adaptations, a view that was unintentionally but strongly supported by the name of the genus itself. One of the obvious differences of our treatment from Zander's delimitation is the transfer of subg. *Odontophyllon* from the synonymy of *Streblotrichum* to the synonymy of *Hydrogonium*. The type of *Odontophyllon*, *Barbula hiroshii*, described by Saito (Saito, 1975: 499) is morphologically very similar to *B. crocea*, which was traditionally accommodated in *Streblotrichum* (Limpricht, 1890; Loeske, 1909) despite the fact that it substantially differs from other members of the genus in its perichaetial leaves (much less differentiated), colour of seta (red versus yellow in *Streblotrichum*), or anatomy of annulus (non-revoluble in *B. crocea*). These characters, which are common to *B. crocea* and *B. hiroshii*, are clearly all diagnostic characters of *Hydrogonium* and the two species are thus combined in this genus in the Synopsis below. Starting with Hilpert (1933), earlier authors correctly pointed out unclear morphological limits between *Hydrogonium* and *Hyophila*, which differ only in the absence of a peristome in members of the latter genus, while sharing leaf shape, hygric habitat, ventrally bulging leaf cells in some species and densely papillose in others of both genera, and production of axillary gemmae. Unfortunately, molecular data have been acquired only for *Hyophila involuta*, which is not even the type of the genus. *Hyophila involuta* was resolved in a group of its own within Trichostomoideae that can be identified with tribe Hyophileae, but in a delimitation that differs both from Chen's original (Chen, 1941) and Zander's later concepts (Zander, 1993).

The unexpected grouping of *Hydrogonium* taxa called for several reconsiderations at specific and infraspecific levels. The first of these is the confirmation of the status of *B. gregaria* as distinct from *B. amplexifolia* and *B. indica*, which was advocated on morphological grounds by Köckinger & Kučera (2007). Further, *B. cruegeri* and *B. subcomosa* should be distinguished from *B. gregaria* and *B. consanguinea*, respectively. Although the morphological differentiation is not always easy and will probably be refined by future revisionary studies (see below in Synopsis), the molecular relationships of *B. cruegeri*

and *B. subcomosa* support the recognition of these taxa at the specific level. The nested position of the *B. gregaria* specimen *Eckel 188986* in the nrITS dataset (within *B. cruegeri*) points towards possible hybridization or shared ancestral polymorphism of the two taxa. On the other hand, the morphological similarity of *B. convoluta* var. *gallinula* to *B. gregaria* and its sister relationship to all (cp dataset) or all but one (ITS dataset) *B. gregaria* accessions favour its recognition at the infraspecific level within *B. gregaria*, which itself is proposed to be combined under *Hydrogonium* below. Similarly, *B. indica* var. *kurilensis* and *B. cancellata*, which seem to be molecularly uniform but slightly distinct, yet closely related to the more variable *B. consanguinea* s.str., might be best recognized as infraspecific taxa of *B. consanguinea*. In all three latter cases (*B. convoluta* var. *gallinula*, *B. indica* var. *kurilensis*, *B. cancellata*), we formally propose the status of variety as most appropriate below.

The extent of polyphyly in *Barbula* sensu Zander (1993) has no known parallel in Pottiaceae and in fact has very few parallels among other bryophytes and embryophytes. Even in the largest angiosperm genera, which proved to be polyphyletic following recent molecular studies, such as *Astragalus* L. (Wojciechowski, 2005), *Euphorbia* L. (Horn & al., 2012), or *Senecio* L. (Pelser & al., 2007), and which outnumber the estimated species count in ex-*Barbula* by one order, the species removed from the core genus remained interspersed among taxa of the same tribe or subtribe. Generally speaking, polyphyly is perhaps largely confined to taxa with specialized morphology and a reduced number of easily observable characters. This may be less common in higher plants but commonly has been documented, e.g., in algae and lichens (Gaya & al., 2008; Draisma & al., 2010). In mosses polyphyly has best been documented for several lineages of pleurocarpous mosses at both generic and familial levels (Gardiner & al., 2005; Ignatov & al., 2007; Olsson & al., 2009). The situation in liverworts is generally less well known but a polyphyletic origin was demonstrated in only a few of the recently analyzed large genera, e.g., *Jungermannia* (Hentschel & al., 2007) or *Lophozia* (De Roo & al., 2007). The polyphyly in hypnalean moss lineages could be expected considering their rapid radiation concommitant with the evolution of angiosperm-dominated tropical forests in the Tertiary (Shaw & al., 2003; Pedersen & Newton, 2007). In case of the above-named liverwort genera, the broad circumscriptions were based on the lack of unequivocally differentiating morphological characters of the groups after their revision on a world-wide level (Váňa, 1973; Schuster, 2002). Pottiaceae have been monographed relatively recently and thoroughly (Zander, 1993) with great emphasis placed on the formalized cladistic phylogenetic analysis of morphological and anatomical characters. While some of the large genera seem to have withstood the test of phylogenetic relationships using DNA sequence data (*Didymodon*, Werner & al., 2005a), and most others show some level of paraphyly but do not include accessions now found in other subfamilies (*Tortula* Hedw., Werner & al., 2002; Košnar & al., 2012; *Weissia* Hedw. and *Tortella* (Lindb.) Limpr., Werner & al., 2005b), the level of polyphyly in the modern definition of *Barbula* is unique. It implies that homoplastic morphological and anatomical characters cannot be easily recognized without the help of molecular data.

■ TAXONOMIC AND NOMENCLATURAL SYNOPSIS

The synopsis applies to the taxa occurring in the Holarctic, Indomalayan, and northern part of Neotropical ecozones.

Gymnobarbula Jan Kučera, **gen. nov.** – Type: *G. bicolor*.

Closely resembling *Streblotrichum* P. Beauv. but differing in the rudimentary peristome, reddish seta, persistent annulus, large spores (>20 µm), absence of rhizoidal gemmae, rusty brown coloured cells of the leaf base, and the little differentiated anatomy of the weak, flat leaf costa, consisting only of a row of guide cells and 1–2 rows of dorsal stereids in the lower part of leaves.

Lindberg (1863: 386) was the first who used the name *Gymnobarbula*, unfortunately without description and rank designation, i.e., having created a nomen nudum, to accommodate this morphologically odd species of *Barbula*. He noted its similarity to "*B. convolutae*", i.e., *Streblotrichum*, and particularly to *B. crocea*. The name *Gymnobarbula* also appeared in a different context as a generic nomen nudum in C. Müller's *Genera Muscorum Frondosorum* (Müller, 1901: 456), and two specific nomina nuda, ascribed to Schimper, were listed in that genus in the same publication (*G. weddellii*, *G. subulirostris*). However, there is no other known mention of these taxa in the literature.

According to our present knowledge the genus is monotypic and its description is thus identical with the description of *Gymnobarbula bicolor* (see e.g., Bruch & Schimper, 1846: 76–77; Limpricht, 1890: 626–627).

Gymnobarbula bicolor (Bruch & Schimp.) Jan Kučera, **comb. nov.** ≡ *Gymnostomum bicolor* Bruch & Schimp. in Bruch & al., Bryol. Europ. 1: 76, pl. 29 (fasc. 33–36. Mon. 4. pl. 1). 1846 – Type: In terra calcarea m. Radstädter Tauern Alp. Salisburgiae (*Funk*) et in Alpibus Julicis (*Sendtner*).

This is a relatively rare species, only known from the European Alps (Switzerland, Italy, Austria, Germany). The virtually absent peristome, unusually large spores, and the rusty brown cells of the leaf base are characters which are probably unknown in any other member of *Barbula* s.l., at least among the well-known taxa. In addition to the diagnostic characters of *Gymnobarbula*, *Barbula crocea* differs by the presence of axillary gemmae, much larger size, longer yellowish basal leaf cells, and hardly differentiated perichaetial leaves. The species of *Streblotrichum* differ in their yellow seta, well-developed twisted peristome, separating annulus and much smaller spores (to ca. 12 µm). In contrast to most members of tribe Hyophileae, to which *Gymnobarbula* might belong phylogenetically, the leaf cells of *Gymnobarbula* are not unilaterally and ventrally bulging, and are covered by dense, multiple, massive warty papillae reminding of *Anoectangium* Schwägr. or *Molendoa* Lindb.

Streblotrichum P. Beauv. in Mag. Encycl. 5: 317. 1804 ≡ *Barbula* subg. *Streblotrichum* (P. Beauv.) K. Saito in J. Hattori Bot. Lab. 39: 499. 1975 ≡ *Barbula* sect. *Streblotrichum*

(P. Beauv.) Limpr., Laubm. Deutschl. 1: 626. 1888 – Type (designated by Saito, 1975: 499): *S. convolutum* (Hedw.) P. Beauv., Prodr. Aethéogam.: 89. 1805 ≡ *Barbula convoluta* Hedw., Sp. Musc. Frond.: 120. 1801 ≡ *Bryum convolutum* (Hedw.) Dicks. ex With., Syst. Arr. Brit. Pl., ed. 4, 3: 799. 1801 ≡ *Tortula convoluta* (Hedw.) P. Gaertn. & al., Oekon. Fl. Wetterau 3(2): 92. 1802.

= *Barbula* sect. *Convolutae* (De Not.) Bruch & Schimp. in Bruch & al., Bryol. Europ. 2: 91 (fasc. 13–15. Mon. 29). 1842 ≡ *Tortula* sect. *Convolutae* De Not. in Mem. Reale Accad. Sci. Torino 40: 287. 1838.

Streblotrichum (on generic, subgeneric or sectional rank) has traditionally been recognized to include *Barbula* s.l. species with strongly differentiated convolute perichaetial leaves, and an annulus of differentiated, vesiculose cells, which agrees well with the characters of *S. convolutum*, *S. commutatum*, *S. enderesii* and *S. bicolor* (Bruch & Schimp.) Loeske. After *S. bicolor* is moved to *Gymnobarbula*, as discussed above, the first three taxa are moreover characterized by the yellow seta, revoluble annulus, and the formation of brown, spherical, rhizoidal gemmae, and these characters can be added to the revised delimitation of *Streblotrichum*. *Barbula crocea* was also traditionally assigned to *Streblotrichum*, but its molecular relationships, as well as the less markedly differentiated perichaetial leaves, red seta, non-revoluble annulus, absence of rhizoidal gemmae and presence of axillary gemmae support its inclusion in *Hydrogonium*. The same applies to *B. convoluta* var. *gallinula* R.H. Zander (see below). Whether *B. convoluta* var. *eustegia* (Cardot & Thér.) R.H. Zander, which is also reported to have perichaetial leaves less markedly differentiated, is to be retained in or to be removed from *Streblotrichum* needs to be ascertained. Its automatic combination to *Streblotrichum* is to be avoided, having in mind the case of *B. convoluta* var. *gallinula*. Whether *Barbula calyculosa* (Mitt.) A. Jaeger, type of *Barbula* sect. *Leptopogon* (Mitt.) Lindb., and regarded as synonymous with *Streblotrichum* by Zander (1993), belongs here, also needs to be ascertained. Among the austral taxa, *B. calycina* Schwägr. and *B. microcalycina* Magill have been reported to have markedly convolute, differentiated perichaetial leaves (Magill, 1981), but other diacritical characters as identified by the analysis of the northern taxa are lacking except for the yellow seta in the latter species, and hence molecular data are necessary to resolve their affinities. As judged from illustrations, the putatively endemic Chinese *Streblotrichum propaguliferum* X.J. Li & M.X. Zhang seems to belong to *Dichodontium* Schimp., as it has no diagnostic characters of *Streblotrichum*. The genus *Streblotrichum* was synonymized with *Barbula* sect. *Convolutae* (1842) at the sectional level (Zander 1993). This is correct (*Barbula* sect. *Streblotrichum* is a later combination) except for the author citation, which should read (De Not.) Bruch & Schimp., as the basionym of the epithet is *Tortula* sect. *Convolutae* De Not. (1838), not vice versa, as stated in Index Muscorum.

Accepted species studied: *Streblotrichum convolutum* (Hedw.) P. Beauv., *S. commutatum* (Jur.) Hilp., *S. enderesii* (Garov.) Loeske.

Hydrogonium (Müll. Hal.) A. Jaeger in Ber. Thätigk. St. Gallischen Naturwiss. Ges. 1877–78: 405 (Ad. 2: 669). 1880 ≡ *Trichostomum* [unranked] *Hydrogonium* Müll. Hal. in Linnaea 40: 297. 1876 ≡ *Barbula* subg. *Hydrogonium* (Müll. Hal.) M. Fleisch., Musci Buitenzorg 1: 352. 1904 ≡ *B.* sect. *Hydrogonium* (Müll. Hal.) K. Saito in J. Hattori Bot. Lab. 39: 492. 1975 ≡ *Didymodon* subg. *Hydrogonium* (Müll. Hal.) Kindb., Eur. N. Amer. Bryin. 2: 273. 1897 – Type (designated by Saito, 1975: 492): *H. ehrenbergii* (Lorentz) A. Jaeger in Ber. Thätigk. St. Gallischen Naturwiss. Ges. 1877–78: 405 ≡ *Trichostomum ehrenbergii* Lorentz in Abh. Königl. Akad. Wiss. Berlin 1867: 25, t. 4 f. 1–6, t. 5 f. 7–19. 1868 = *Hydrogonium bolleanum* (Müll. Hal.) A. Jaeger fide Frahm & al. in Trop. Bryol. 12: 123–154. 1996.

= *Semibarbula* Herzog ex Hilp. in Beih. Bot. Centralbl., Abt. 2, 50(2): 626. 1933 – Type: *S. indica* (Hook.) Herzog ex Hilp. in Beih. Bot. Centralbl., Abt. 2, 50(2): 626. 1933.

= *Barbula* subg. *Odontophyllon* K. Saito in J. Hattori Bot. Lab. 39: 499. 1975 ('*Odontophylla*'), **syn. nov.** – Type: *B. hiroshii* K. Saito in J. Hattori Bot. Lab. 39: 499. 1975.

Hydrogonium is distinguished from *Barbula* primarily by the nearly constant presence of axillary gemmae even in natural conditions, which differ in shape from the gemmae of *Barbula* in being mostly markedly elongate, seriate, ellipsoid, clavate to fusiform or corniculate, green, light brownish-green to reddish brown, as opposed to spherical, unicellular to pluricellular, irregularly subsphaerical non-seriate brownish gemmae with protuberant cells of *Barbula* and no axillary gemmae in other segregate genera (except for the rhizoidal gemmae of *Streblotrichum*). The indistinct ornamentation of the cell surface together with the loose areolation of relatively large cells and the generally flaccid habit of plants holds true only for a minor part of derived *Hydrogonium* species, which occur in markedly wet habitats, and the non-hygrophytic species such as *H. orientale* may show remarkable phenotypic plasticity, acquiring the "typical" *Hydrogonium* characters when growing in humid places, as excellently demonstrated by Werner & al. (2003). Perichaetial leaves of *Hydrogonium* are slightly differentiated, mostly smaller than the vegetative leaves, and subsheathing. The peristome is typically well-developed, composed of 32 long, sinistrorsely twisted filiform prongs, but shows a progressive reduction via shorter anastomosing teeth, as typically developed in the North American '*Barbula cancellata*' (see below), to the short, more or less erect, fugacious teeth of *H. orientale*. We revised in detail the taxa related or believed to be related to *Hydrogonium orientale* and *H. consaguineum*, which form the larger part of *Hydrogonium* taxa. Several tropical taxa, known from the very limited number of historical observations, need to be addressed in the future, including taxa that have earlier not been assigned to *Hydrogonium*, such as *Barbula pachyloma* Broth., *B. calodictyon* Broth., *B. sumatrana* Baumg. & Dixon or *B. robbinsii* Bartr. However, we believe that we have addressed a significant proportion of the existing diversity of the genus.

Species addressed in this treatment, for which nomenclatural changes are proposed or taxonomic understanding was re-considered:

1. ***Hydrogonium angustifolium*** (Hook. & Grev.) Jan Kučera, **comb. nov.** ≡ *Tortula angustifolia* Hook. & Grev. in Edinburgh J. Sci. 1: 298, t. 12. 1824 ≡ *Barbula angustifolia* (Hook. & Grev.) Müll. Hal., Syn. Musc. Frond. 1: 603. 1849, non Brid. 1826 ≡ *B. tenuirostris* Brid., Bryol. Univ. 1: 826. 1827 – **Lectotype (designated here):** Nepal, *Wallich* (E! [E00049216]).

Typification notes. – The herbarium material of *Tortula angustifolia* found in BM and E is extremely sparse. In BM, there are two envelopes bearing this name. One of them (BM000867496) has the seal "Herbarium Hookerianum 1867" and was annotated by Wilson (initial W.) as "*Tortula angustifolia* Hook. [from Harvey's own specimen], closely allied to *T. flavescens*. Nepal, Wallich". It contains two fragments of one plant with seta but without capsule. The other type specimen at BM contains a heavy paper sheet with four miniature glued capsules. The upper two capsules (BM000867497) are annotated by Wilson in a similar manner as BM000867496 as "*Tortula angustifolia* Hook. Nepal. Wallich [from Harvey's own specimen]", and are accompanied by sketches of the plant habit, several leaves, capsule and the peristome, re-drawn after Hooker. The capsules include fragments of one plant. The lower two capsules (BM000867498) contain two and one shoot, respectively, of the type collection of *Tortula flavescens*, and both types were compared and annotated by Wilson. It appears that Wilson realized that the two types are very similar and planned to unite them under the name *Tortula crenulata* (reference to a manuscript from June 1857). His annotation under *Tortula angustifolia* reads: "differs from *T. flavescens*—see below—in the more opaque texture of leaves, which are more lax when dry; differs also in areolation, somewhat bordered with larger cellules, crenate, nerve more pellucid … [illegible]". The Greville herbarium at E contains two specimens annotated as type specimens. One (E00049216) includes a small heavy paper sheet with four glued plants (one of them with sporophyte, although deoperculated and with lost peristome) annotated as "*Tort. angustifolia* H. & Gr. Nepal. Dr. Wallich"; this seems to be a part of the original collection. The other specimen (E00049217) contains two glued tufts of *Hydrogonium javanicum* (!) but is annotated by Wilson (June 1850) as "*Tortula tenuirostris* Hook. & Greville/*Tortula angustifolia* Hook. & Greville". The original Wallich collection from Nepal was obviously separated into several duplicates but the time of origin of the duplicates is unknown. Wilson's annotations on the authenticity of the duplicates housed at BM referring to W.H. Harvey are not quite relevant with respect to the authenticity of Hooker's material, as Harvey (b. 1811) must have acquired the material from Hooker not earlier than after 1834, when they met in Glasgow (Long, 1995). Hence, Greville's duplicate from his own herbarium (E00049216) housed now at E is more suitable as type and is selected as lectotype here; moreover the specimen contains more material. The status of the two specimens from BM as isolectotypes would be inappropriate, as they hardly originated as duplicates of the Greville specimen.

With respect to the taxonomic identity of *Hydrogonium angustifolium*, the type specimens include large-leaved plants (up to 2.8 mm long), flaccid and crisped when dry, which are superficially very similar to the types of *Tortula flavescens* Hook. & Grev. (= *Hydrogonium consanguineum*, see below). However, no gemmae were seen among the leaves and, more importantly, the cross-section of leaves shows distinctly bistratose to polystratose margins, which are nevertheless not distinct under the microscope under incident light, so that this character could easily have been overlooked. Among similar species, bi- or polystratose leaf margins have otherwise only been described for the New Guinean *Barbula pachyloma* Broth. (cf. Norris & Koponen, 1989; Eddy, 1990). The eventual identity of the two taxa needs to be studied. Anyway, the name *Hydrogonium angustifolium* (≡ *Barbula tenuirostris*) is thus not applicable to any taxon of the *H. orientale* complex and the synonymy proposed by Sollman (2004a, b) has to be suspended until more material can be studied, ideally using molecular tools; our attribution to *Hydrogonium* is based solely on its morphological similarity to other species of the genus from the region and may even prove erroneous in the future. *Hydrogonium angustifolium* s.str. has not been known from other than the type collections until the treatment of Sollman (2004a, 2004b), although already Chen (1941), who could not study the original material, speculated about its identity with *H. consanguineum*. Anyway, our revision of the material at E and BM has not revealed any additional specimens of *H. angustifolium*.

2. ***Hydrogonium consanguineum*** (Thwaites & Mitt.) Hilp. in Beih. Bot. Centralbl., Abt. 2, 50(2): 626. 1933 ≡ *Tortula consanguinea* Thwaites & Mitt. in J. Linn. Soc., Bot. 13: 300. 1873 (prior to Oct. 9) ≡ *Barbula consanguinea* (Thwaites & Mitt.) A. Jaeger in Ber. Thätigk. St. Gallischen Naturwiss. Ges. 1877–78: 409. 1880 – **Lectotype (designated here):** "Ins. Ceylon, ad terram, Dr. Thwaites." (BM! [BM001006686]).

Two varieties are recognized here:

2a. ***Hydrogonium consanguineum*** var. ***consanguineum***

= *Barbula flavicans* D.G. Long in J. Bryol. 18: 356. 1994 ≡ *Tortula flavescens* Hook. & Grev. in Edinburgh J. Sci. 1: 297, pl. 12. 1824, non (Dicks. ex With.) P. Beauv. 1805 – **Lectotype (designated here):** "On a clayey soil. Nepaul; Dr. *Wallich*" (E! [E00108463]; isolectotypes: E! [E00208466, E00208467, E00246543, E00108465], BM! [BM000671526, BM000671529, BM001031296, BM000671527, BM000867498]).

• *Typification notes for Tortula consanguinea Thwaites & Mitt.* – JK was able to study the material from both NY and BM, and the additional isotype from E. The reason for all earlier confusion was the fact that the type material, consisting of many duplicates of the original collection (*Thwaites 67* from Ceylon), contains a mixture of species. The two major elements of the mixture are *H. consanguineum* and *H. javanicum*, two relatively closely related and macroscopically very similar species. While the duplicates from BM contain both species either in pure tufts or mixed in quantitatively comparable proportions, the isotype at NY (NY371655) contains only *H. javanicum*

(two other packets glued on the sheet with this isotype do not have any writing on them and contain a mixture of *Bryum* sp. and *Hydrogonium* cf. *pseudoehrenbergii*, elements unknown from any other duplicate of *Thwaites 67*, and hence probably not part of the original collection), and the isotype at E contains only *H. consanguineum*. The sheet with the isotype of *H. consanguineum* from NY contains a label "Holotype of *Tortula consanguinea* Thw. & Mitt. ≡ *Hydrogonium consanguineum* (Thw. & Mitt.) Hilp.", and this confused earlier authors, particularly Sollman (2000b). This designation obviously cannot be attributed to Mitten (Hilpert's combination dates to 1933), and must not be followed. In case of heterogeneous type material, it is important to identify which taxon was intended for the description by the author(s). The description (Mitten, 1873) unfortunately does not specifically mention the most important diacritical characters between *H. consanguineum* and *H. javanicum* as we understand them, but mentions the percurrent, dorsally scabrous costa, a condition which can rarely occur in *H. javanicum* (the nerve in this species mostly ends below the apex and the back of the costa is lowly papillose) but is typical for *H. consanguineum*, particularly under lower magnification and in optically inferior devices that scientists used at that time. It thus seems safe to typify the name *H. consanguineum* with the element from Thwaites' herbarium at BM that does not belong to *H. javanicum*, and this is best accomplished by the homogeneous material of BM001006686. Sayre (1977) moreover identified the major part of Thwaites' herbarium to be housed at BM. Consequently, duplicates of the original collection that contain only the admixed species *H. javanicum* (specimens BM000867492 and NY371655) are excluded from consideration as type material.

• *Typification notes for Barbula flavicans D.G. Long.* – At least five duplicates of Wallich's original collection of *Tortula flavescens* are housed at E (E00108463 containing a coloured, unsigned sketch; E00108464 annotated by Wilson in 1852; E00208466 and E00208467 [Menzies Herbarium]; and E00246543 [Arnott Herbarium]; another possible duplicate [E00108465 annotated as *Tortula flavescens* Nepaul—probably by Wallich himself] was bequested to E from Herb. Wight). At BM, four possible duplicates of the original Wallich collection are housed—two of them bearing the "Herbarium Hookerianum 1867" stamp and number "H.1653" (BM671526, 671529), another specimen (BM1031296) is annotated as "H.1653 dupl.", and specimen BM671527 was labelled by "Arnott 18252, with another, differently written property designation "Hb. Benth."—perhaps bequeathed to Bentham from Arnott. The lectotype was chosen from the Greville material at E (E00108463), which is annotated as "Nepal: Dr. Wallich to Hook." The rest of the type material, which looks convincingly like duplicates of the original collection, is designated here as isolectotypes.

Hydrogonium consanguineum was described from Sri Lanka (Ceylon) by Mitten (1873) and has been consistently in use for the taxon's occurrences in Sri Lanka, Singapore and Java (Fleischer, 1904), Vietnam and the Philippines (Chen, 1941), and generally Malesia (Eddy, 1990), while in the Himalayas (Nepal, Bhutan) the taxon was known as *Barbula flavicans*, described from Nepal (Gangulee, 1972; Long, 1994). The occurrences in Japan, Taiwan and China (Chen, 1941; Saito, 1975; Li & al., 2001) have been named *B. subcomosa*, *Hydrogonium sordidum* or otherwise, although the synonymy is either dubious or wrong (see below). The above-described problems with the heterogeneous type material led to the synonymy with *H. javanicum*, first proposed by Saito (1975: 495–496), and followed by most later authors, including Zander (1993) and Sollman (2000b), who studied the same type material at NY. A different opinion was recently only expressed by Eddy (1990), who studied other parts of type material from BM (see above) that included *H. consanguineum* as understood by earlier authors, e.g., Fleischer (1904), i.e., differing from *H. javanicum* in the densely papillose upper lamina cells, not bulging ventrally, and strongly papillose dorsal surface of the leaf costa. The species was first described under the name *Tortula flavescens* in 1824, but unfortunately this name was already in use at the time of description, and even the later common usage of the replacement name *Barbula fuscescens* of 1849 was invalid, and hence *Barbula flavicans* was newly proposed for the taxon by Long (1994), who at that time did not know about the identity with *H. consanguineum*. According to Art. 11.4 of *ICN*, *Tortula consanguinea* Thwaites & Mitt. and combinations based on this basionym have nomenclatural priority over *Barbula flavicans*, first validly used in 1994. Sollman (2000b) contributed to the nomenclatural confusion, having agreed with Saito on the interpretation of the type of *H. consanguineum* as being identical with *H. javanicum* but at the same time having kept the earlier usage of *H. consanguineum* (spelled as *Barbula consanguinea* sensu Eddy). Such a treatment would have required a previous conservation of the name. The confusion has grown even bigger after Sollman (2004a) realized that '*B. consanguinea* sensu Eddy' is taxonomically identical with *Barbula flavicans* but unfortunately did not realize the consequences of Art. 11.4 of *ICN*, and incorrectly synonymized the taxa under *B. flavicans*. Later he nevertheless probably realized his error, having published another (Sollman, 2004b), nearly identical article in the same volume of the journal (without any explanation of the previous nomenclatural somersaults), where the taxa, including '*B. consanguinea* sensu Eddy' were synonymized under the valid and legitimate name *B. tenuirostris*, which was correct from the nomenclatural point of view but contradicts our taxonomic findings described below in the paragraph on *Hydrogonium angustifolium*. Among the taxa, proposed as synonyms of *H. consanguineum* by Sollman (2004b), no taxon except for *H. angustifolium* endangers its priority. However the types of *Barbula gracilenta* Mitt. (1859) and *B. gangetica* Müll. Hal. (1872), not considered by Sollman and accepted as good species by Li & al. (2001), need to be checked, although the descriptions and illustrations do not raise the suspicion of obvious identity.

2b. ***Hydrogonium consanguineum*** var. ***cancellatum*** (Müll. Hal.) Jan Kučera, **comb. & stat. nov.** ≡ *Barbula cancellata* Müll. Hal. in Flora 56: 483. 1 Nov 1873 − Type: "Texas, Dallas Co., *J. Boll* cum *Aongstroemia varia* associatam legit 1870".

North and Central American plants of *H. consanguineum* s.l. (a name never used in North America) were traditionally named *B. cruegeri*, with the type from Trinidad (Steere, 1938), *B. pringlei* Cardot and *B. hypselostegia* Cardot, with types from Mexico (Bartram, 1949), and *B. cancellata*, with a type from Texas (Crum & al., 1973). Zander (1979) synonymized all of these and some other types (including *B. gregaria* treated below) under *Barbula indica*, within which he later (Zander, 2007) recognized two varieties: var. *indica* with small gemmae and recurved margins, and var. *gregaria* with large gemmae and plane margins. Zander (1979) cited the observation of Crum that the taxon with small gemmae occurring predominantly in North America north of Mexico matches the type of *Barbula cancellata*. It can be inferred that this taxon has later (Zander, 2007) been identified as *B. indica* var. *indica*. According to the descriptions, *B. pringlei* and *B. hypselostegia* are likely identical with *B. cancellata*, although the types have not been examined by us.

Plants morphologically matching *B. cancellata* were shown above to be nested within *H. consanguineum*. In addition to the molecular differences described above, *B. cancellata* differs from *H. consanguineum* s.str. in the irregularly anastomosing rami of the peristome teeth, relatively broad, ovate-cuspidate leaves with a broadly cuspidate to lingulate leaf apex, reminding of the leaf shape of *B. gregaria*, and the dorsal superficial cells of the costa being mostly shortly rectangular to subquadrate, commonly chlorophyllose, and densely papillose with papillae not markedly associated to the cell ends. Nevertheless, the morphological differences are not always clear-cut and the molecular divergence is low, which seems to be most adequately evaluated by distinguishing *B. cancellata* at the varietal level within *H. consanguineum*. The taxonomic synonymy (at the species level) of *Tortula consanguinea* with *B. cancellata* called for investigation of the dates of publication of both taxa, because both species names were published in 1873. *Barbula cancellata* was published in *Flora* (Müller, 1873), issued in clearly dated fascicles; this article was the first in No. 31 in November 1873. Dating of the description of *T. consanguinea* in Vol. 13 (1873) of the *Journal of the Linnean Society, Botany*, is more complicated, as the precise dates of publication of individual issues have not been printed. Fortunately, starting with the next volume of the journal (Vol. 14, 1875), the journal introduced the practice of printing the publication dates after completion of the volume, so that we know that No. 1 of Vol. 14 was published on 9 October 1873, which is one month earlier than the publication date for *B. cancellata*. This constitutes a good argument for considering the name *Tortula consanguinea* as having the priority over *Barbula cancellata*.

Hydrogonium consanguineum var. *cancellatum* is distributed mainly in the southwestern United States and the neighbouring part of Mexico (see Appendix 1 for the studied specimens), but the exact distribution needs to be elucidated as the taxon was not consistently distinguished from related taxa. According to Sollman (2000b), which escaped later attention, *B. consanguinea* occurs in Florida (*Allen 7541*, MO; *Drouet* & *Nielsen s.n.*, L) and Hawaii (*Hoe 3347*, L), which may well be based on specimens of *H. consanguineum* var. *cancellatum*.

2c. ***Hydrogonium consanguineum*** var. ***kurilense*** (Ignatova & Ignatov) Jan Kučera, **comb. nov.** ≡ *Barbula indica* var. *kurilensis* Ignatova & Ignatov in Arctoa 18: 138. 2010 (‘2009’) − Holotype: “Russia … Kunashir Island … Ignatov #06–1884” (MHA; isotype MHA!).

The type of *Barbula indica* var. *kurilensis*, which has been the only specimen of the taxon known (Ignatova & Ignatov, 2009), was collected on Kunashir Island of the Kurils, today belonging to Russia. Although it was carefully compared to the type of *B. indica*, the possible identity with related Japanese taxa, particularly *B. subcomosa* in the sense of Saito (1975) was not considered, despite the geographical proximity of Japanese occurrences. Molecular affinities clearly nest *B. indica* var. *kurilensis* within *H. consanguineum* s.str., along with the very slightly different *H. consanguineum* var. *cancellatum*. Interestingly, *B. indica* var. *kurilensis* does not seem to be morphologically differentiated from *H. consanguineum* s.str., i.e., *B. subcomosa* sensu Saito, while the differences from var. *cancellatum* can be equated to those between var. *cancellatum* and var. *consanguineum*. All European material (see Appendix 1) is morphologically identical with the Asian type, as are the DNA sequences of recent specimens, despite the considerable geographical distance that is not known to be bridged by any other occurrence in between. The ecology of the Far Eastern and European plants is also virtually identical (cf. Köckinger & al., 2012). The unknown sporophytes might differ from var. *consanguineum* in a similar way to those of var. *cancellatum*.

3. ***Hydrogonium croceum*** (Brid.) Jan Kučera, **comb. nov.** ≡ *Tortula crocea* Brid., Muscol. Recent. Suppl. 1: 257. 1806 ≡ *Barbula crocea* (Brid.) F. Weber & D. Mohr, Bot. Taschenbuch: 481. 1807 ≡ *Streblotrichum croceum* (Brid.) Loeske in Hedwigia 49: 30. 1909 – Type: In monte Meissner Cattorum [Hoher Meißner near Kassel] Junio 1805 legi … Ex Heluetiâ etiam …

= *Barbula paludosa* F. Weber & D. Mohr, Bot. Taschenbuch: 482. 1807, nom. illeg. (*ICN* Art. 52.2) – Type: *Schleicher*, Cent. 3 No. 22.

Morphological reasons for inclusion of this species in *Hydrogonium* were discussed above. The taxon is only known from Europe.

4. ***Hydrogonium cruegeri*** (Sond. ex Müll. Hal.) Jan Kučera, **comb. nov.** ≡ *Barbula cruegeri* Sond. ex Müll. Hal., Syn. Musc. Frond. 1: 618. 1849 − Type: Insula Trinitatis Antillarum, ad La Ventille, in terra argillosa, *Crüger* legit Aug. 2, 1846, in muris et rupibus calcareis formam confertiorem Nov. 28.

As discussed above under *H. consanguineum* var. *cancellatum*, *Barbula cruegeri* has earlier been believed to represent, together with *B. gregaria*, the taxon of the *Barbula indica* complex from Central to tropical South America with large gemmae. It can be deduced that Zander (2007) synonymized *B. cruegeri* with *B. indica* var. *gregaria*, although the synonymy has never been officially published except for the more inclusive synonymy of *B. indica* and *B. cruegeri*. Indeed, *Hydrogonium cruegeri* is morphologically very similar to *H. gregarium*, and

we realized the differences between the two only after some of the South/Central American plants were resolved in a clade separate from *H. gregarium* in the phylogenetic analysis. *Hydrogonium cruegeri* differs morphologically from *H. gregarium* in its stronger costa, which is more prominent dorsally and more highly papillose (commonly the costa remains even if the surrounding lamina erodes), by the leaf cells on both sides being ampullaceous-mammillose with extremely high papillae (this character has been observed in some specimens of *H. gregarium* as well), and by the leaf margins being mostly narrowly recurved in the proximal 1/3–2/3. The perichaetial leaves are less differentiated and not markedly sheathing basally. Although we have not yet seen the type of *B. cruegeri*, the characters listed above seem to be visible in the type material present at BM (BM000872606–7, scanned for plants.jstor.org), which is the basis of our belief in the identity of this type. We have to admit the possibility of a certain amount of gene flow between *H. cruegeri* and *H. gregarium*, as discussed above.

At present, *H. cruegeri* is believed to be a (sub)tropically distributed taxon in Central America and the northeastern part of South America (see Appendix 1), but more detailed revision work is needed to confirm this hypothesis.

5. ***Hydrogonium gregarium*** (Mitt.) Jan Kučera, **comb. nov.** ≡ *Tortula gregaria* Mitt. in J. Proc. Linn. Soc., Bot., Suppl. 1: 29. 1859 ≡ *Barbula gregaria* (Mitt.) A. Jaeger in Ber. Thätigk. St. Gallischen Naturwiss. Ges. 1871–72: 424. 1873 ≡ *Barbula indica* var. *gregaria* (Mitt.) R.H. Zander in Cryptog. Bryol. Lichénol. 2: 6. 1981 − Syntypes: In Nepaliae orient. reg. temp., J.D. Hooker (no. 166); In Tibetiae reg. temp. T. Thomson (No. 126).

Two varieties are recognized here:

5a. ***H. gregarium*** var. ***gregarium***

= *Barbula horrinervis* K. Saito in J. Hattori Bot. Lab. 39: 486. 1975 − Holotype: Japan, Nippara, *Saito 4936* (TNS).

This is a broadly distributed and in many regions probably quite common taxon from India, Nepal, Bhutan, China and Japan but extends along the Pacific coast of North America far south (see Appendix 1). Saito's *Barbula horrinervis* (Saito, 1975) is clearly identical with *H. gregarium*, and was distinguished by overemphasizing the importance of leaf shape. Interestingly, while Zander (1979) observed the morphological transitions between *B. indica* and *B. gregaria*, Sollman (2000a) stated that "this is not correct" and that rather *B. gregaria* is "identical with, or very near *Barbula amplexifolia*". Indeed, according to Sollman's identification of SW Asian material of *H. gregarium* and *H. amplexifolium* at E, he did not distinguish between the two taxa, although JK could not find a single specimen that would show intermediate characters between the two species (for a more detailed discussion see Köckinger & Kučera, 2007). Li & al. (2001) also did not recognize *H. gregarium* for China, but its synonym *B. horrinervis* is listed in the synonymy of *B. indica*. In our opinion, the differentiation of *H. gregarium* from *H. amplexifolium* is quite straightforward and the problems may only emerge in the differentiation of *H. gregarium* from *H. cruegeri*, as discussed above under the latter taxon.

5b. ***Hydrogonium gregarium*** var. ***gallinulum*** (R.H. Zander) Jan Kučera, **comb. nov.** ≡ *Barbula convoluta* var. *gallinula* R.H. Zander in Phytologia 44: 195, f. 15–19. 1979 − Holotype: Canada, Northwest Territories, Nahanni Natl. Park, Virginia Falls, *Scotter 22433* (NY).

Morphologically, Zander (1979) differentiates this taxon by the presence of simple papillae on the abaxial surface of the costa as opposed to the prorulae of *H. gregarium*, and by larger leaf cells (9–12 vs. 7–10 µm). The ornamentation of the costa is very variable in *H. gregarium* but we admit that the leaf cells in var. *gallinulum* are extremely large and out of the range observed in other specimens of *H. gregarium*. Moreover, the constantly obtuse leaves with a weak costa that never reaches the apex is also unusual. Hence, the taxon might at the moment most conveniently be considered a variety of *H. gregarium*, as proposed above.

6. ***Hydrogonium hiroshii*** (K. Saito) Jan Kučera, **comb. nov.** ≡ *Barbula hiroshii* K. Saito in J. Hattori Bot. Lab. 39: 499, f. 48: 12–22. 1975 − Holotype: Japan [Honshu], Tokyo, Okutama, Nippara, Ogawi-dani, *Saito 10379* (TNS).

The putatively endemic Japanese *Barbula hiroshii* was described by Saito (1975) as the closest relative of *H. croceum*, and a new subgenus, *Odontophyllon*['-*phyllae*'], was established for it, based on the toothed leaf margin, large grape-shaped gemmae, and differentiated hyalodermis as diacritical characters. However, toothed margins are rather typically present in many *Hydrogonium* species, though never as strongly developed. This combination is being proposed purely for morphological reasons; we have not yet studied any specimens.

7. ***Hydrogonium majusculum*** (Müll. Hal.) P.C. Chen in Hedwigia 80: 242, t. 46 f. 6–7. 1941 ≡ *Barbula majuscula* Müll. Hal. in Nuovo Giorn. Bot. Ital., n.s., 5: 182. 1898 – Type: China interior, prov. Schen-si sept., in alveo fluminis Laoy-huo prope Shan-gen-ze, Martio 1897 (isotype: *Giraldi s.n.*, BM!).

The taxon was regarded to represent a good species by Li & al. (2001), while it was synonymized with *H. consanguineum* by Sollman (2004a, b). The studied isotype of *Barbula majuscula* is indeed similar to *H. consanguineum* in its general habit and leaf and costa shape, but differs in substantially larger upper lamina cells (10–15 µm). The gemmae were not observed in the type specimen at BM, although their presence was noted on the revision label by Sollman from 1999. Hence we regard the identity of *H. majusculum* with *H. consanguineum* unwarranted at the moment, but before additional material is studied, a final taxonomic conclusion cannot be drawn.

8. ***Hydrogonium orientale*** (F. Weber) Jan Kučera, **comb. nov.** ≡ *Trichostomum orientale* F. Weber in Arch. Syst. Naturgesch. 1(1): 129, t. 4 f. 6. 1804 ≡ *Semibarbula orientalis* (F. Weber) Wijk & Margad. in Taxon 8: 75. 1959 − Type: Ex India orientali misit *Rottler.*

= *Barbula indica* (Hook.) Spreng., Nomencl. Bot. 2: 72. 1824 ≡ *Tortula indica* Hook., Musci Exot. 2: 135. 1819 ≡ *Semibarbula indica* (Hook.) Herzog ex Hilp. in Beih. Bot.

Centralbl., Abt. 2, 50(2): 626. 1933 – Type: In India orientali. *Röttler*. In muris Horti Botanici Calcuttæ. *Gul. Wallich, M.D.*

This is the closest relative of *H. consanguineum*, and as discussed above, a certain amount of gene flow between the two cannot be ruled out. However, the pattern of known morphological and molecular variability still allows recognizing both taxa at species level.

9. ***Hydrogonium subcomosum*** (Broth.) P.C. Chen in Hedwigia 80: 236. 1941 ≡ *Barbula subcomosa* Broth. in Hedwigia 38: 211. 1899 – Holotype: Kanagawa, *Wichura 1400* (H-BR; isotypes: BM!)

The two studied isotypes superficially match *H. consanguineum* except for being slightly more robust (plants to ca. 4 cm high, leaves to about 2.5 mm long). Importantly, no gemmae were observed in the two duplicates present at BM, despite Sollman's (2004a) explicit reference to this character. However, his revision labels were not present in the herbarium sheet from BM. The gemmae were also not mentioned in the protologue, which is important, as we cannot automatically assume that they were neglected as was commonly the case with older authors. The reason for our belief is that the next species treated in Brotherus (1899) was the equally newly described *Hyophila propagulifera* Broth. with gemmae similar to *H. consanguineum*. Also Chen (1941) did not mention the gemmae despite his careful observation of this character. We can also confirm his observation that the leaf apex of *H. subcomosum* is gradually tapered and the costa is not excurrent, as opposed to the more abruptly narrowed, broader apex in *H. consanguineum* with a mucronate excurrent costa, although this character does not seem to be sufficiently constant in additional material studied of the two taxa. The foliage of *H. subcomosum* is less dense, exposing the stem between the leaves. An identical condition was observed in recent collections of '*Barbula consanguinea*' from Bangladesh and Bhutan, which also differed in their molecular affinities as described above. Hence we refer to these plants as *H. subcomosum*. It needs to be underlined, however, that Saito's (1975) description refers to both *H. subcomosum* and *H. consanguineum*, as the axillary gemmae were explicitly mentioned and illustrated; whether both var. *consanguineum* and var. *kurilense* occur in Japan and if they differ in their regional distribution, needs to be ascertained.

10. ***Hydrogonium* sp.** In the course of the revision of *Hydrogonium consanguineum*, we encountered plants similar in morphology to *H. consanguineum* and *H. orientale* that produced nearly identical axillary gemmae but had broader leaves with a broadly cuspidate apex, much more pellucid, less papillose and bigger cells (9–12 µm), markedly bulging on both sides in cross-section. This taxon is very closely related to *H. bolleanum* as discussed above. According to the descriptions of Fleischer (1904), Chen (1941) and Eddy (1990), the taxon might be identical with *H. pseudoehrenbergii* (M. Fleisch.) P.C. Chen but until the type has been studied, this identity is not certain.

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Appendix 1. Newly acquired sequences with GenBank accession numbers.

Species, country, locality, collector, collector number, herbarium code, isolate number, GenBank accession numbers in the order *rps4*, *trnM-trnV*, ITS. * denotes species for which taxonomic changes (tranfer to the genera *Streblotrichum*, *Gymnobarbula* or *Hydrogonium*) are proposed.

Acaulon triquetrum (Spruce) Müll. Hal.: Czechia, Pouzdřany, *Košnar 356*, CBFS, 558, JX679971, JX679921, JX679947. ***Aloina rigida*** (Hedw.) Limpr.: Czechia, Pavlov, *Košnar 954,* CBFS, 565, JX679976, JX679926, JX679952. ***Anoectangium aestivum*** (Hedw.) Mitt.: Austria, Heiligenblut, *Kučera 12848*, CBFS, 104, HM147774, JX679910, HM147801. ***Barbula amplexifolia*** (Mitt.) A. Jaeger*: Austria, Seidlwinkltal, *Kučera 12792*, CBFS, 111, JQ890422, JQ890363, JQ890484; Russia, Sakha, Selyakh, *Ignatov 00-36*, MHA, 116, HM147778, JQ890367, HM147805; India, Nainital, *Hallingbäck s.n.*, CBFS, 117, JQ890425, JQ890368, JX679937(clone2), JX679938 (clone3); Nepal, Phulchowki, *Townsend 92/89*, E, 336, JQ890431, –, JQ890492; Macedonia, Popova Šapka, *Kučera 13775*, CBFS, 469, JQ890437, JQ890378, JQ890499; Canada, NWT, Virginia Falls, *Steere 76-605*, MO, 471, JQ890438, JQ890379, JQ890500; India, Sikkim, *Long 26378*, E, 475, JQ890442, JQ890381, JQ890504; ***Barbula*** aff. ***amplexifolia*** (Mitt.) A. Jaeger*: China, Yunnan, Gang Ho Ba, *Long 18818*, E, 473, JQ890440, JQ890380, JQ890502. ***Barbula bicolor*** (Bruch & Schimp.) Lindb.*: Austria, Seidlwinkltal, *Košnar 1540*, CBFS, 120, HM147779, JQ890370, HM147806; Mt. Bielschitza, *Köckinger 14262*, CBFS, 164, 170, JQ890428, JQ890372, JQ890489; ***Barbula bolleana*** (Müll. Hal.) Broth.*: Spain, Motril, *Vadam s.n.*,CBFS, 122, HM147780, JQ890371, HM147807; Spain, Bullas, *Kučera 13670*, CBFS, 400, JX679970, JQ890374, JQ890494. ***Barbula cancellata*** Müll. Hal.*: U.S.A., Berkeley Co. (NC), *B.Shaw 8846*, DUKE, 479, JQ890444, JQ890383, JQ890506; Mexico, Tabasco, S. Fé, *Zamudio 1181*, DUKE, 481, JQ890446, JQ890384, JX679943. ***Barbula commutata*** Jur.*, Czechia, Rabí, *Kučera 12658*, CBFS, 112, JQ890423, JQ890364, JQ890485; ***Barbula convoluta*** Hedw. var. ***convoluta****: Czechia, Hus, *Kučera 3882*, CBFS, 113, HM147776, –, HM147803; Czechia, Vilémovice, *Kučera 13021*, CBFS, 189, JQ890429, JQ890401, JQ890490; Czechia, Suchý žleb, *Kučera 13023*, 190, JQ890430, JQ890402, JQ890491; ***Barbula convoluta*** var. ***gallinula*** R.H. Zander*: Canada, NWT, Virginia Falls, *Steere 76-605*, MO, 472, JQ890439, JX679916, JQ890501. ***Barbula consanguinea*** (Thwaites & Mitt.) A. Jaeger*: Bangladesh, Chittagong, *Long 28072*, E, 486, JQ890451, JQ890389, JQ890511; Bangladesh, Alikadam, *Long 28197*, E, 488, JQ890453, JQ890391, JQ890513; Bangladesh, Kaptai, *Long 28117*, E, 491, JQ890456, JQ890394, JQ890516; Sri Lanka, Ella, *Townsend 73/1093*, E, 499, JQ890461, JQ890398, –. ***Barbula crocea*** (Brid.) F. Weber & D. Mohr*: Austria, Rotgülden, *Kučera 12556*, CBFS, 114, JQ890424, JQ890365, JQ890486; Slovakia, Motyčky, *Kučera 1087*, CBFS, 500, JQ890462, JQ890399, JQ890521; ***Barbula cruegeri*** Müll. Hal.*: Ecuador, Pichincha, *Arts 19/003*, DUKE, 482, JQ890447, JQ890385, JQ890508(direct read), JX679944 (clone1); Mexico, Chiapas, *Eggers & Frahm 23*, DUKE, 483, JQ890448, JQ890386, JX679945(clone2), JX679946(clone3); Panama, Cerro Jefe, *Allen 9020*, DUKE, 553, JQ890466, JQ890405, JQ890525. ***Barbula enderesii*** Garov.*: Austria, Pfarreben, *Köckinger 14261*, CBFS, 163, JQ890427, JX679911, JQ890488; Austria, Lechnergraben, *Köckinger 14911*, CBFS, 525, JQ890463, JQ890400, JQ890522. ***Barbula gregaria*** (Mitt.) A. Jaeger*: India, Nainital, *Hallingbäck s.n.*, CBFS, 118, JQ890426, JQ890369, JQ890487; China, Yunnan, Bapo, *Long 33624*, E, 474, JQ890441, JX679917, JQ890503; Yunnan, Bawan Cun, *Long 32244*, E, 476, JQ890443, JQ890382, JQ890505; Mexico, Guerrero, Puentecilla, *Eckel 188986*, DUKE, 554, JQ890467, JQ890406, JQ890526. ***Barbula indica*** (Hook.) Spreng. var. ***indica****: Australia, Wombarella Gap, *Streimann 39344*, NY, 398, JQ890432, –, AY796286; India, Naini Tal, *Arts 08/05*, MUB, 399, AF481034, JQ890373, JQ890493; India, Lucknow, *Long 30794*, E, 467, JQ890435, JQ890377, JQ890497; Oman, Wadi Tiwi, *Rothfels 2763*, DUKE, 484, JQ890449, JQ890387, JQ890509. ***Barbula indica*** var. ***kurilensis*** Ignatova & Ignatov*: Russia, Mt Ruruy, *Ignatov 06-1884*, MHA, 450, JQ890433, JQ890375, JQ890495; Switzerland, Rottenschwil, *Hofmann 183139*, Z, 452, JQ890434, JQ890376, JQ890496; Austria, Hard, *Amann s.n.*, CBFS, 498, JQ890460, JX679919, JQ890520. ***Barbula javanica*** Dozy & Molk.*: China, Yunnan, Kunming, *Long 24613*, E, 490, JQ890455, JQ890393, JQ890515; Bhutan, Gaylegphug, *Long 8159*, E, 493, JQ890458, JQ890396, JQ890518; India, Sikkim, *Long 22418*, E, 494, JQ890459, JQ890397, JQ890519. ***Barbula orizabensis*** Müll. Hal. Mexico, Tzitzio, *Delgadillo 5010*, 551, JQ890464, JQ890403, JQ890523; Jamaica, Cedar Valley, *Crosby 3370*, DUKE, 552, JQ890465, JQ890404, JQ890524 I. ***Barbula*** sp., cf. ***pseudoehrenbergii*** M. Fleisch.*: Bhutan, Phuntsholing, *Long 10352*, E, 468, JQ890436, JX679915, JQ890498; Bangladesh, Manichari, *Long 28169*, E, 487, JQ890452, JQ890390, JQ890512; Nepal, Chitwan Lodge, *Townsend 92/318*, E, 489, JQ890454, JQ890392, JQ890514. ***Barbula subcomosa*** Broth.*: Bhutan, Phuentsholing, *Long 7725*, E, 485, JQ890450, JQ890388, JQ890510; Bangladesh, Teknaf, *Long 28215*, E, 492, JQ890457, JQ890395, JQ890517. ***Barbula unguiculata*** Hedw. Austria, Heiligenblut, *Kučera 12829*, CBFS, 115, HM147777, JQ890366, HM147804; USA, Davidson Co. (SC), *AJ Shaw 5692*, DUKE, 480, JQ890445, JX679918, JQ890507; ***Blindia acuta*** (Hedw.) Bruch & Schimp.: Portugal, Peneda, *Kučera 10525*, CBFS, 121, JQ890483, JQ890416, JX679939; ***Bryoerythrophyllum inaequalifolium*** (Taylor) R.H. Zander: Russia: Sokhondo, Agutsa River, *Czernyadyeva 36-10*, CBFS:15095, 567, JX679977, JX679927, JX679953. ***Bryoerythrophyllum recurvirostrum*** (Hedw.) P.C. Chen: Czechia, Šumperk, *Kučera 12925*, CBFS, 361, JQ890468, JQ890407, JQ890527. ***Cinclidotus riparius*** (Host ex Brid.) Arn.: Czechia, Sokolohrady, *Košnar s.n.*:CBFS, 197, JQ890469, JX679912, JX679940. ***Didymodon rigidulus*** Hedw.: Czechia, Hříběcí Boudy, *Kučera 12905*, CBFS, 15, HM147768, JQ890408, HM147795. ***Didymodon spadiceus*** (Mitt.) Limpr.: Czechia, Ostravice, *Plášek s.n.*, CBFS:12722, 78, JQ890474, JQ890409, JQ890528. ***Didymodon sinuosus*** (Mitt.) Delogne: Czechia, Pohansko, *Kučera 12059*, CBFS, 85, JQ890476, JQ890410, JQ890529. ***Ephemerum minutissimum*** Lindb.: Czechia, Velká nad Veličkou, *Košnar 692,* CBFS, 578, JX679985, JX679935, JX679966(clone1), JX679967(clone2), JX679968(clone3). ***Erythrophyllopsis andina*** (Sull.) R.H. Zander: Ecuador: Mt Chimborazo, *Soldán s.n.*, CBFS:7418, 568, JX679978, JX679928, JX679954. ***Eucladium verticillatum*** (Hedw.) Bruch & Schimp.: Czechia, Tetín, *Kučera 14692*, CBFS, 570, JX679979, JX679929, JX679955. ***Fissidens dubius*** var. ***mucronatus*** (Breidl. ex Limpr.) Kartt., Hedenäs & L. Söderstr.: Czechia, Velká nad Veličkou, *Košnar 696,* CBFS, 559, JX679972, JX679922, JX679949. ***Gymnostomum hymenostylioides*** (Broth. & Dixon) R.H. Zander: India, Nainital, *Long 30847*, CBFS:13299, HM147794, JQ890411, HM147819. ***Gymnostomum viridulum*** Brid.: Czechia, Lukov, *Hradílek s.n.*, CBFS:12914, 99, HM147770, JQ890412, HM147797. ***Gyroweisia tenuis*** (Schrad. ex Hedw.) Schimp.: France, Mont-Dore, *Kučera 10748*, CBFS, 102, HM147772, JX679908, HM147799. ***Hennediella heimii*** var. ***arctica*** (Lindb.) R.H. Zander: Norway, Svalbard, Petuniabukta, *Košnar 1932,* CBFS, 571, JX679980, JX679930, JX679956(clone1). ***Hymenostylium xerophilum*** Köckinger & Jan Kučera: Austria, Reiting, *Köckinger 05-954*, CBFS:12913, 62, HM147769, JQ890415, HM147796. ***Hymenostylium gracillimum*** (Nees & Hornsch.) Köckinger & Jan Kučera: Austria, Tiboldgraben, *Köckinger 14264*, CBFS:12972, 165, HM147782, JQ890413, HM147809. ***Hymenostylium recurvirostrum*** (Hedw.) Dixon: Austria, Seidlwinkltal, *Kučera 12780*, CBFS, 103, HM147773, JX679909, HM147800. ***Hyophila involuta*** (Hook.) A. Jaeger: Costa Rica, Barra Honda, *T.Hauer s.n.*, CBFS:14557, 495, JQ890477, JQ890414, JQ890530; ***Leptodontium flexifolium*** (Dicks.) Hampe: Russia: Duldurga, Elo-Rakhanai, *Afonina s.n.*, CBFS:14332, 572, JX679981, JX679931, JX679957. ***Leptophascum leptophyllum*** (Müll. Hal.) J. Guerra & Cano: Spain: Los Pulpites, *Kučera 13661*, CBFS, 573, JX679982, JX679932, JX679958(clone1). ***Microbryum curvicolle*** (Hedw.) R.H. Zander: Czechia, Pouzdřany, *Košnar 358,* CBFS, 579, JX679986, JX679936, JX679969. ***Molendoa tenuinervis*** Limpr. Mongolia, Mt. Ikh-Bogd, *Ignatov 01-789*, MHA, CBFS:12954, 134, JQ890478, JQ890417, JQ890531. ***Oxystegus tenuirostris*** (Hook. & Taylor) A.J.E. Sm.: Czechia, Třísov-Dívčí Kámen, *Košnar 431,* CBFS, 561, JX679973, JX679923, JX679948. ***Pleuridium acuminatum*** Lindb.: Czechia, Mokrsko, *Kučera 13738*, CBFS, 557, JQ890480, –, JQ890533. ***Pleurochaete squarrosa*** (Brid.) Lindb.: Czechia, Tmaň-Kotýz, *Košnar 1266,* CBFS, 562, JX679974, JX679924, JX679950. ***Pseudephemerum nitidum*** (Hedw.) Loeske: Czechia, Pořešín, *Kučera 13593*, CBFS,

Appendix 1. Continued.

556, JQ890479, –, JQ890532. ***Pseudocrossidium hornschuchianum*** (Schultz) R.H. Zander: Austria, Plankowitzspitze, *Kučera 12610*, CBFS, 309, JQ890481, JQ890420, JQ890535. ***Pseudocrossidium revolutum*** (Brid.) R.H. Zander: UK, Kindrogan, *Kučera 10091*, CBFS, 310, JQ890482, JX679913, JQ890534(direct), JX679941(clone1), JX679942(clone2). ***Scopelophila cataractae*** (Mitt.) Broth.: U.S.A: Silver Hill Mine, Davidson Co., NC, *B. Shaw s.n.*, CBFS:15042, 575, JX679983, JX679933, JX679959-JX679962 (clones1–4). ***Syntrichia ruralis*** (Hedw.) F. Weber & D. Mohr: Czechia, Kojátky, *Košnar 1035,* CBFS, 576, –, –, JX679963–4 (clones 1–2). ***Tortella fragilis*** (Hook. & Wilson) Limpr.: Switzerland, Mt. Sidelhorn, *Košnar 954,* CBFS, 564, JX679975, JX679925, JX679951. ***Tortula muralis*** Hedw. Czechia, Studánka, *Košnar 771*, CBFS, T56, –, JQ890421, –. ***Trichostomum crispulum*** Bruch: Spain, Bullas, *Ros & Werner s/n*, MUB, OW1507, –, JQ890418, –. ***Tuerckheimia svihlae*** (E.B. Bartram) R.H. Zander: U.S.A: Marianna Caverns, FL, *Cash & Rapp M193*, DUKE, 312, HM147791, JX679914, HM147817. ***Weissia controversa*** Hedw.: Czechia, Hrubá Vrbka, *Košnar 1253,* CBFS, 577, JX679984, JX679934, JX679965; New Zealand, *J. Beever 99-94*, MUB, OW2100, –, JQ890419, –.

Additional specimens studied (for list of *Barbula amplexifolia* specimens see Köckinger & Kučera, 2007): ***Barbula arcuata*** Griff.*: India: Darjeeling, *R.S. Chopra & Singh 39*, BM. — Nepal: Mardi Khola, *Stainton & al. 7193a*, BM (cf. *B. gangetica* Müll. Hal.). ***Barbula bolleana*** (Müll. Hal.) Broth.*: Spain: Caravaca de la Cruz, *Kučera 13685*, CBFS. — Switzerland: Rümikon, *E. Steiger s.n.*, Z. ***Barbula cancellata*** Müll. Hal.*: (all specimens from DUKE) U.S.A.: Alabama: *Bowers 12234, 15227*, *Anderson 26721, 27769*; Forida: *Anderson 14310, 24671*, *Peck 8*, *Small 7831*, *Rapp 136*, *Purcell 300MF49*, *Ris & al. 6155*, *Schornherst 20*. — Mexico, S. Luis Potosí, *Frye 2143*, DUKE. ***Barbula consanguinea*** (Thwaites & Mitt.) A. Jaeger*: India: Uttarakhand, Mussoorie, *Duthie s.n.*, BM; Mohand Pass, *Duthie s.n.*, BM; Doiwala, *Brotherus s.n.*, E; Maharashtra, Poona [Pune], *Sedgwick s.n.*, BM; Odisha, Jeypore, *Walker 552, 564, 568*, BM; Karnataka, Shiggaon, *Dixon 3487*, BM; Kerala, Kumily, *Foreau s.n.*, BM; W. Bengal, Calcutta [Kolkata], *Gangulee s.n.*, BM. — Philippines: Luzon, Mt. S. Isidro, *Fénix s.n.* Nov. 1917, E. ***Barbula indica*** var. ***kurilensis*** Ignatova & Ignatov*: Switzerland: Aargau, Rottenschwil, Reuss, *H. Hofmann* 170396, *N. Müller* 171213, Bad Ragaz, Rhein, *N. Müller* 171894, Emmen, Reuss, *F. Zemp* 183290. — Hungary: Budapest, *Boros* 8.1925, BP 107073. — Croatia: Kotoriba-Alsódomború [D. Dubrava], *Boros* 14.8.1943, BP 179185. ***Barbula gregaria*** (Mitt.) A. Jaeger*: Mexico, Chiapas, *Hermann 26399*, DUKE; Puebla, Xicotepec de Juarez, *Delgadillo 1220*, L. Bhutan, Pemagatshel, *Long 8561*, E; Chendebi, *Long 8737*, E; Chapcha, *Long 8824*, E. — India, Himachalpradesh, Mount Jako, *Gollan s.n.* 26.6.1906, PC; Beas, *Lillie 1372*, L; West Bengal, Tiger Hill, *Long 22375*, E; Ghum, *Long 23036*, E; Uttaranchal, Rajpur, *Bowen s.n.*, L; Mussoorie, *Maas Geesteranus 14730-1*, *de Haas B6, B-34, B-44, B-54A*, L; Mallital, *Long 30799*, E; Sikkim, Bop, *Long 26378*, E; Samiti Lake, *Long 22791*, E; Bitu, *Long 26337*, E; Tista valley, *Long 26365*, E. — China, Yunnan: Stone Forest, *Touw 23504C*, L; Zhongdian, *Long 18720*, E; Gang Ho Ba, *Long 18842*, E; Lijiang, *Long 18886*, E; Qionhzu Temple, *Long 23540*, E; Bawan, *Long 32161*, E; Shabadi, *Long 32573*, E; Nankang, *Long 32652*, E; Bingzhongluo, *Long 33522*, E; Liuku, *Long 34272*, E; Qinlangdang, *Long 36260*, E; Nu Jiang, *Long & Shevock 37061*, E. Sichuan, Jinfu Shan, *Wu 21156*, MO; Taiwan: Li-shan, Nantou, *Chuang & Schofield 869A*, L. — Indonesia, Sumatra, Prapat on Toba Lake, *Staal S-4*, L; Lombok, Rinjani, *Elbert 1285F*, L; — Japan, Ōita,. Mt. Sobo, *Iwatsuki* 7 Aug. 1962, L. — Thailand, Payap, Doi Chiengdao, *Touw 9361*, L. ***Barbula javanica*** Dozy & Molk.*: China: Yunnan, Weixi, *Long 24513*, E. — India: Maharashtra, Mahabaleshwar, *Townsend 73/416, 445, 473*, E; Sikkim, Raniphul, *Long 26302*, E. — Indonesia: Java, Sindanglaya, *M. Fleischer, Musci Archipelagi Indici, Ser. III. 1900. No. 124*, E; Tjipannas, *M. Fleischer. Musci Archipelagi Indici, Ser. V. 1902. No. 214*, E. — Nepal: Godawari, *Long 17597–8*, E. — Sri Lanka: Peradeniya, *M. Fleischer, Musci Archipelagi Indici, Ser. X. 1908. No. 460*, E; Rapava, *illegible coll.* No. 2083, BM. ***Barbula subcomosa*** Broth.*: Japan: Kanagawa, *Wichura* 1400 (isotype of *Barbula subcomosa* Broth., 2 duplicates, BM); Etchū, Yabuda(?), *Dixon(?)* 19 Aug. 1915. — Bangladesh, Rangamati, *Long 28158*, E.

Paper 3: Köckinger H. & Kučera J. 2011. *Hymenostylium xerophilum*, sp. nov., and *H. gracillimum*, comb. nov., two neglected European mosses and their molecular affinities. – Journal of Bryology 33: 195–209.

Hymenostylium xerophilum, *sp. nov.*, and *H. gracillimum*, *comb. nov.*, two neglected European mosses and their molecular affinities

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Hymenostylium xerophilum is described as a new species from the European Alps. Molecular *rps*4 and ITS data support its recognition and elucidate its affinities to other species of the tribe Pleuroweisieae. It is closely related to *H. gracillimum*, *comb. nov.*, which is based on the old and neglected *Gymnostomum gracillimum*, which replaces the recent name *G. boreale*. Both species share non-coloured to pale yellowish-brown rhizoids, stem central strand and indistinct sclerodermis, keeled leaves, and concave laminae in abaxial view. They differ from each other in leaf shape and several essentially quantitative characters. Sporophytes have never been found in *H. xerophilum*, but they are known from several localities in *H. gracillimum*. The former colonizes rather dry, sunny to half-shaded calcareous rocks, whereas the latter needs moist and shaded rock habitats and shows a preference for subneutral slate. At present, *H. xerophilum* is known only from the Alps (Austria, and a single site in Germany), where it is rather widespread in calcareous regions. *H. gracillimum* seems to be a distinctly rarer plant, to date known only from eight Austrian sites and one locality in Russian Karelia. Other published records under the name *G. boreale* have been wrongly attributed to this species. Lectotypes are designated for *G. gracillimum* and *Gyroweisia acutifolia*. A key to *Hymenostylium* and the genera of Pleuroweisieae in Europe is presented.

Thicker rhizoids of both species are covered with a thick, non-coloured protective layer and filled with oil-droplets and leucoplasts. They represent a subterranean secondary protonema, which plays an important role in the survival and propagation of these mosses, vital especially in the case of the non-sporulating *H. xerophilum*.

Keywords: *Gymnostomum*, Pleuroweisieae, Pottiaceae, Taxonomy, Phylogeny, ITS, *rps*4

Introduction

Gymnostomum and *Hymenostylium* are moderately large genera of Pottiaceae (each comprising about 20 species) that were traditionally recognized as closely related, in many earlier treatments even synonymous. Although Zander (1993) suggested placement of the two genera in different tribes of the subfamily Merceyoideae Broth. (Barbuleae Herz. and Leptodontieae Herz., respectively) based on a cladistic analysis of morphological characters, this view found no support in later phylogenetic analyses based on chloroplast and nuclear sequences (Werner *et al.*, 2004, 2005). The latter treatments seem to favour the traditional older concept of placing the two genera juxtaposed in the tribe Pleuroweisieae (Limpr.) P.C.Chen *sensu* Saito (1975) within a broadly defined subfamily Trichostomoideae (Schimp.) Limpr. Species of the two genera show both great reduction and morphological plasticity in gametophytic and sporophytic characters, causing difficulties in taxonomy. This has led to extremely wide and artificial species concepts, e.g. in Zander (1977) or Norris & Koponen (1989). A critical worldwide revision is still lacking; only in recent years has more attention been paid to the '*Gymnostomum calcareum* complex' in the Mediterranean area (Whitehouse & Crundwell, 1991; Cano *et al.*, 1994; Sérgio, 2006).

Since 1994, the first author, HK, has been aware of a problematical, non-sporulating moss growing on dry calcareous rocks. It was originally attributed to *Hymenostylium recurvirostrum*, representing an unusual xeromorphic expression. In subsequent years, the number of collections from different areas and

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altitudes of the Austrian Alps increased considerably and allowed a thorough and critical reconsideration of the status of this plant. It became apparent that this moss has not been described. Based on the generic characters given in Zander (1993), especially those of the stem cross-section, the species was tentatively placed in *Gymnostomum*. The frequent occurrence of true mixed stands with *G. aeruginosum* and more rarely with *H. recurvirostrum* was highly useful for learning about its variation, allowing a secure differentiation. The existence of this plant was previously mentioned, based on determinations by HK, under '*Gymnostomum* sp.' in Schlüsslmayr (2005) and Meinunger & Schröder (2007).

Molecular analyses, carried out since 2007 by the second author, JK, offered a good opportunity to independently assess the affinities of this plant. Sequences from both chloroplast (*rps*4) and nuclear (ITS) genomes demonstrated a closer relationship to *Hymenostylium* than to *Gymnostomum s.str.*; hence, we describe the species within the former genus, as *H. xerophilum*. Sequencing of another specimen with dwarf sporophytes, erroneously believed by HK to represent *Gyroweisia acutifolia* Philib., demonstrated a close relationship to the new species. A morphological re-examination of further material revealed that this 'sister species' had been collected by HK in the Alps on several previous occasions, although mostly without sporophytes. In contrast to the other plant, literature and type studies yielded three valid names attributed to it, the earliest being *G. gracillimum* Nees & Hornsch.

Material and Methods

Fieldwork and herbarium studies

Plant material has accumulated gradually during the last 15 years of bryological fieldwork by HK, enriched by a few collections of G Schlüsslmayr and L Meinunger. Focused excursions by HK with special emphasis on the search for mixed stands with related species were carried out between 2007 and 2009. The Austrian herbaria GJO, GZU, and KL were checked for the possible presence of the two treated species. Type material and other specimens were borrowed from AUT, BM, DUKE, E, MHA, MUB, MW, PR, S, and W. Nomenclature of European mosses follows Hill *et al.* (2006).

Sampling and selection of analysed DNA regions

The selection of taxa for molecular analysis followed two main goals: (1) testing the morphological concepts of *H. xerophilum* and *H. gracillimum* by means of identifying taxon-specific molecular markers and their variability; and (2) ascertaining their generic placement, which could not be unambiguously addressed with morphological data alone.

In order to gain phylogenetically relevant information from multiple sources, and at the same time keep the budget relatively low, we decided to analyse two widely sampled regions that have been shown to provide relevant phylogenetic information in haplolepidous mosses, the chloroplast gene *rps*4 with the adjacent spacer towards the *trn*S gene, and the hypervariable nuclear spacers ITS1 and ITS2, including the 5.8S rRNA gene (ITS). This choice enabled us to compare our results with preceding works by Werner *et al.* (2004, 2005), Grundmann *et al.* (2006), and Ros & Werner (2007).

Due to the low degree of morphological variability, putative absence of sporophytes, and relatively narrow span of known ecology and distribution, only two specimens of *H. xerophilum* were analysed, whereas the greater variability of the sporulating *H. gracillimum* called for wider molecular sampling (four analysed samples of seven recent collections and the paratype of *Gymnostomum boreale*). For the analysis of wider relationships, we needed to acquire a representative selection of species from the tribe Pleuroweisieae (genera *Molendoa*, *Hymenostylium*, *Reimersia* Chen, *Tuerckheimia* Broth., *Gymnostomum*, *Gyroweisia*, and *Anoectangium*), which was undersampled in comparison with the tribe Trichostomeae Dixon (genera *Trichostomum*, *Weissia*, and *Tortella* s.l.) in the previous study by Werner *et al.* (2005). We also tried to include several taxa morphologically similar to *H. xerophilum*, for which a close relationship to Pleuroweisieae seemed unlikely but *a priori* was unknown (*Barbula convoluta*, *B. bicolor*, and *B. amplexifolia* (Mitt.) A.Jaeger). Due to the availability of material, the selection was strongly biased towards taxa occurring in Europe, supplemented however by important Asian material from the collections of M Ignatov (Moscow) and D G Long (Edinburgh), which together represents most of the existing diversity in the studied group. The list of sequences used appears in Table 1; the analyses were supplemented with sequences retrieved from GenBank, selected using the BLAST search tool and omitting several incomplete sequences of misnamed taxa (notably AY908053 '*Molendoa sendtneriana*' and AF478273 '*Barbula convoluta*').

Molecular protocols

Total genomic DNA was extracted using the NaOH method (Werner *et al.*, 2002). Crude extracts were diluted $\times 10$ (amplification of *rps*4) or $\times 100$ (amplification of ITS) with 100 mM Tris — 1 mM EDTA (pH 8.3). Polymerase chain reactions (25 μl final volume) were performed with 1 μl DNA solution in a Biometra T3000 Cycler using Plain PP MasterMix kit (TOP-BIO JSC, Brno, Czech Republic) with the primers m-18-S and m-25-R (7.5 pmol each) described by Spagnuolo *et al.* (1999) for ITS, and the primers rps5 (Nadot *et al.*, 1994) and trnas

Table 1 Taxon sampling for molecular dataset

Species	Provenance	Voucher	*rps*4	ITS	Reference
Hymenostylium xerophilum	Austria: Reiting	Köckinger 05-954 (CBFS 12913)	**HM147769**	**HM147796**	
H. xerophilum	Austria: Krastal	Köckinger 14243 (CBFS 12934)	**HM147771**	**HM147798**	
H. gracillimum	Austria: Tiboldgraben	Köckinger 14264 (CBFS 12972)	**HM147782**	**HM147809**	
H. gracillimum	Austria: Virgental	Köckinger 14267 (CBFS 12981)	**HM147784**	**HM147811**	
H. gracillimum	Austria: Maltatal	Köckinger 14268 (CBFS 12982)	**HM147785**	…	
H. gracillimum	Austria: Weissensee	Köckinger 14650 (CBFS 13477)	**HM147792**	**HM147818**	
H. gracillimum (paratype of *Gymnostomum boreale*)	Russia, Karelia, Kulmakkapuro	A. Hülphers coll. 1938 (S: B147722)	**HM147789**	**HM147815**	
H. hildebrandtii	Morocco: Anti Atlas	R.M. Ros (MUB 13690)	**HM147793**	AY796282	Werner *et al.*, 2005
H. recurvirostrum var. *recurvirostrum*	Austria: Seidlwinkltal	Kučera 12780 (CBFS)	**HM147773**	**HM147800**	
H. recurvirostrum var. *insigne*	Austria: Haselschlucht	G. Schlüsslmayr s.n., coll. 1998 (CBFS 12971)	**HM147783**	**HM147810**	
H. recurvirostrum	Spain, Albacete	MUB 5394	AF480967	…	Werner *et al.*, 2004
Reimersia inconspicua	Taiwan: Hua-Lien	Shevock 15069 (MO)	AY908051	…	GenBank
Tuerckheimia valeriana	Costa Rica	Holz & Schaefer (GOET: Bryotheca Goettingensis Fasc. 9, No. 38)	AY950396	AY854431	Grundmann *et al.*, 2006
T. svihlae	USA, FL: Marianna Caverns	Cash & Rapp s.n. coll. 1984 (DUKE)	**HM147791**	**HM147817**	
Molendoa hornschuchiana	Austria: Seidlwinkltal	Kučera 12790 (CBFS)	**HM147775**	**HM147802**	
M. sendtneriana	Russia, Yakutia: Lenskie Stolby	Ignatov 00-258 (MHA)	**HM147787**	**HM147813**	
Gymnostomum aeruginosum	Czechia: Sněžka	Kučera 3612 (CBFS)	**HM147781**	**HM147808**	
G. aeruginosum	Russia: Taimyr, Medvezhya River	Fedosov 05-228 (MW)	**HM147788**	**HM147814**	
G. hymenostylioides	India, Uttaranchal: Nainital	Long 30847 (E)	**HM147794**	**HM147819**	
G. calcareum	Switzerland: Muzzano	Kučera 6508 (CBFS)	HM147786	HM147812	
G. calcareum	Spain, Cádiz: Algeciras	MUB 10136	…	AY796279	Werner *et al.*, 2005
G. calcareum (sub *Anoectangium aestivum*)	Morocco: Rif	Cano s.n., MUB 10482	AF480963	…	Werner *et al.*, 2004
G. calcareum (sub *A. aestivum*)	Morocco: Rif	Cano & Ros s.n., MUB 13792	…	AY796280	Werner *et al.*, 2005
G. viridulum	Czechia: Lukov	Hradílek s.n., coll. 2007 (CBFS: 12914)	**HM147770**	**HM147797**	
Anoectangium aestivum	Austria: Heiligenblut	Kučera 12848 (CBFS)	**HM147774**	**HM147801**	
Anoectangium angustifolium	Canary Islands: La Palma	Köckinger s. n. (CBFS 12967)	**HQ651843**	**HQ651842**	
Gyroweisia tenuis	France: Mont Dore	Kučera 10748 (CBFS)	**HM147772**	**HM147799**	
Gyroweisia tenuis	Northern Hemisphere	Long 16061 (DUKE)	AY908062	…	Shaw *et al.*, 2005
Leptobarbula berica	Spain, Alicante	MUB 4219	AF480964	…	Werner *et al.*, 2004
Leptobarbula berica	Spain, Menorca	Cano 1014 (MUB 13995)	…	AY796283	Werner *et al.*, 2005
Eucladium verticillatum	Slovenia: Triglav reserve	BM824490	AY950356	AY854392	Grundmann *et al.*, 2006
Weissia controversa	Spain: Sevilla	MUB 11704	AF480976		Werner *et al.*, 2004
Weissia controversa	China, Guizhou Province	Tan 91–659 (NY)		AY796242	Werner *et al.*, 2005
Aschisma carniolicum	Spain, Huelva	Cano et al. s.n., MUB 7932	AF480962	AY796270	Werner *et al.*, 2005
Trichostomum brachydontium	Spain, Cádiz	MUB 12650		AY796252	Werner *et al.*, 2005
Trichostomum brachydontium	Germany: Northrhine-Westphalia	BM824497	AY950390		Grundmann *et al.*, 2006
Trichostomum crispulum	Spain, Murcia	MUB 9709	AF480977		Werner *et al.*, 2004
Trichostomum crispulum	Spain, Murcia	MUB 14239		AY796249	Werner *et al.*, 2005
Oxystegus tenuirostris	Germany: Northrhine-Westphalia: Plettenberg	BM824487	AY950393	AY854428	Grundmann *et al.*, 2006
Triquetrella arapilensis	Spain, Ciudad Real	Fuertes s.n. (MUB 6465)	AF480984	AY437126	Werner *et al.*, 2004, 2005

Table 1. Continued

Species	Provenance	Voucher	*rps*4	ITS	Reference
Leptodontium flexifolium	Spain, Orense	MUB 2563	AF480973		Werner *et al.*, 2004
Leptodontium gemmascens	UK: Somerset	Hedderson 12996 (TAH)		AM497951	Hedderson & Zander, 2007
Hyophila involuta	India, Uttar Pradesh	Arts s.n. (MUB 12222)	…	AY796284	Werner *et al.*, 2005
Hyophila involuta	Belize	F.J. Rumsey (BM824492)	AY950357	…	Grundmann *et al.*, 2006
Barbula bicolor	Austria: Seidlwinkltal	Košnar s.n. (CBFS: 12960)	**HM147779**	**HM147806**	
Barbula amplexifolia	Russia, Yakutia: Selyakh Creek	Ignatov 00-36 (MHA)	**HM147778**	**HM147805**	
Barbula bolleana	Spain: Motril	Vadam s.n. (CBFS: 10740)	**HM147780**	**HM147807**	
Barbula unguiculata	Austria: Heiligenblut	Kučera 12829 (CBFS)	**HM147777**	**HM147804**	
Barbula convoluta	Czechia: Hus	Kučera 3882 (CBFS)	**HM147776**	**HM147803**	
Barbula commutata	Spain: Montserrat	Kučera 5323 (CBFS)	**HM147790**	**HM147816**	
Tortula muralis	Chile	MUB 13820	AY161115	…	Werner & Guerra, 2004
Tortula muralis	Yugoslavia, Serbia	Sabovljević s.n. (MUB 13827)	…	AY437132	Werner *et al.*, 2005
Didymodon rigidulus	Czechia: Hřiběcí Boudy	Kučera 12510 (CBFS)	**HM147768**	**HM147795**	
Scopelophila cataractae	Spain, Sevilla	MUB 11941	AF480974	…	Werner *et al.*, 2004
Cynodontium polycarpon	Czechia: Krkonoše, Kotel	Kučera 8176 (CBFS)	**HQ651844**		

Note: Newly acquired sequences are printed in bold.

(Buck *et al.*, 2000) for the *rps*4 region. The amplification cycle for ITS started with 3-minute denaturation at 95°C, followed by 35 cycles of 1 minute at 95°C, 1 minute at 52°C, and 1 minute at 72°C, and a final extension step of 10 minutes at 72°C. The amplification cycle for *rps*4 differed in denaturation at 94°C, 35 cycles including only 30-second steps at 94°C, 30 seconds at 50°C and 1 minute at 72°C, with an elongation step of 5 minutes at 72°C. Successful amplifications, visualized using GelRed dye (Biotium Inc., Hayward, CA, USA), were cleaned with the JETQUICK PCR Purification Spin Kit (GENOMED GmbH, Bad Oeynhausen, Germany). Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) at the Genomic Centre of the University of South Bohemia and the Biological Centre of the Academy of Sciences.

Sequence editing, alignment, and phylogenetic analysis

The sequences were manually inspected in SequenceScanner (Applied Biosystems) and edited in BioEdit ver. 7 (Hall, 1999). The partial sequences of the *trn*S gene were trimmed from the *rps*4 sequences, similarly the invariable 5′- and 3′-ends of 'ITS' sequences which belong in fact to the 18S rRNA and 26S rRNA genes. The alignments were constructed using the online version of MAFFT version 6 (Katoh *et al.*, 2009) with the Q-INS-i option and the resulting alignments manually edited. Selection of taxa outside of the Trichostomoideae was based on the earlier studies of Werner *et al.* (2004, 2005).

Phylogenetic analyses were performed using the maximum likelihood (ML) criterion in the phyML 3.0 program (Guindon & Gascuel, 2003) using the default settings except for the nucleotide substitution model which was selected according to the results from jModeltest 0.1.1 (Posada, 2008), with the proportion of invariable sites estimated and optimized equilibrium frequencies, and Bayesian inference using the programme MrBayes ver. 3.1.2 (Huelsenbeck & Ronquist, 2001). In case of *rps*4, phylogenetic information from indel events was included in the phylogenetic analyses by coding indel events into a separate data matrix with SeqState (Müller, 2005) using the simple indel coding method (Simmons & Ochoterena, 2000). The analyses in MrBayes were performed using two simultaneous runs each with four separate Markov Chain Monte Carlo chains, sampling one tree every 100 generations and running until the average standard deviation of split frequencies between runs was <0.01. The first 25% of the sampled trees, representing the burn-in, was discarded. Majority rule 50% consensus trees

were prepared using TreeGraph 2 (Stöver & Müller, 2010).

Results

Molecular results

The acquired partial *rps*4 sequences together with the *rps*4–*trn*S spacer comprise 642 bases, of which the last 54 belong to the spacer. All five *H. gracillimum* accessions, including that of the paratype of *Gymnostomum boreale*, are identical, while the two *H. xerophilum* sequences differ from each other in one unique substitution (pos. 86), which is not shared by any other sequenced member of the Pottiaceae. Disregarding this, *H. gracillimum* and *H. xerophilum* differ in three substitutions, of which two are in the non-coding spacer and the third is a synonymous substitution in the *rps*4 gene (pos. 291). The nearest related taxa according to *rps*4, i.e. *Hymenostylium hildebrandtii* (Müll.Hal.) R.H.Zander and *Reimersia inconspicua* (Griff.) Chen (see below), differ from *H. gracillimum* by two and by three substitutions respectively in the coding region, one of these being non-synonymous, while *H. xerophilum* differs by an additional base in the coding region and by one or two further substitutions in the non-coding spacer.

The ITS1-5.8S rRNA-ITS2 sequence of both species is 709 bp long (ITS1, 231 bp; 5.8SrRNA, 159 bp; and ITS2, 319 bp). The three accessions of *H. gracillimum* from Austria are identical. The two accessions of *H. xerophilum* differ by one substitution in ITS1 while the two taxa mutually differ by one substitution in ITS1 and two substitutions in ITS2. The sequence of *H. gracillimum* from Karelia is unique in sharing two ITS2 substitutions with *H. xerophilum* and one ITS1 substitution with the Austrian *H. gracillimum*, in addition to having two unique substitutions in ITS2. The differences from the nearest related taxa according to ITS (see below) — *Hymenostylium hildebrandtii*, *H. recurvirostrum*, *Molendoa hornschuchiana*, *M. sendtneriana*, and *Tuerckheimia valeriana* (E.B.Bartram) R.H.Zander — are rather extensive in both the ITS1 and ITS2 spacers and include dozens of substitutions and many indel events from 1 to 17 bp long.

The 50% majority rule consensus trees resulting from the Bayesian analysis are shown in Figures 1 and 2. The topology of the ML tree is nearly identical and is not shown. A moderately supported clade in both datasets defines the subfamily Trichostomoideae as consisting of the traditionally recognized taxa (*Tortella* s.l. and *Trichostomum* s.l.), the tribe Pleuroweisieae in the sense of Saito (1975), and several taxa of the tribe Barbuleae in the same sense, namely the *Hyophila*/*Hyophiladelphus*/*Gymnostomiella* and the *Barbula amplexifolia*/*bolleana* clades. The *Leptodontium*/*Triquetrella* clade appears in markedly different positions in the *rps*4 tree (basal within the Pottioideae clade) and the ITS results (basal within the Trichostomoideae clade). Within Trichostomoideae, the tribe Trichostomeae as represented by *Tortella* s.l., *Trichostomum*, *Weissia*, *Aschisma*, *Eucladium*, and the *Barbula amplexifolia*/*bolleana* clade, is well supported in both datasets. The tribe Pleuroweisieae in the sense of Saito (1975), to the exclusion of *Eucladium*, is supported in the ITS dataset but lacks support in the *rps*4 dataset owing to the ambiguity in topology of the *Gyroweisia*/*Leptobarbula*, *Gymnostomum calcareum* s.l., and Trichostomeae clades. Nevertheless, the rest of Pleuroweisieae forms a well-supported clade in both datasets. The two closely related taxa *Hymenostylium gracillimum* and *H. xerophilum* together form a strongly supported clade within the core Pleuroweisieae, but their position within the Pleuroweisieae is ambiguous with respect to the difference between the ITS and *rps*4 data. While *Hymenostylium hildebrandtii*, *Reimersia*, *Tuerckheimia valeriana*, and *Molendoa* species appear to be among the nearest related taxa, forming together a monophyletic group according to both datasets, the position of *H. recurvirostrum* varies, appearing in that monophyletic group according to ITS data but in the sister clade with *Anoectangium* and *Tuerckheimia svihlae* (E.B.Bartram) R.H.Zander according to *rps*4. Nevertheless, *Gymnostomum* — particularly the *G. calcareum* group — is significantly more distant from the *H. gracillimum*/*xerophilum* clade.

Taxonomic treatment

Hymenostylium xerophilum* Köckinger & J.Kučera, *sp. nov. (Figure 3.1–3.10)

Diagnosis. *Caulis cum filo centrali, sine sclerodermide. Rhizoidea plerumque non colorata. Folia carinata, 2–4(5)plo longiora quam latiora, in statu sicco contorta, apex late triangularis, costa percurrens et crassa usque ad apicem, lamina aspectu dorsali concava ad plana.*

Holotype. Austria, Styria, Eisenerzer Alpen, Reiting (mt.), Kaisertal NW of Seiz, *ca* 980 m; S-facing and half-shaded rock wall of limestone, shallow crevices in an inclined rock surface, associated with *Gymnostomum aeruginosum*, *Trichostomum crispulum*, *etc*., 31. 10. 2005, *leg*. H. Köckinger, No. 05-954, E (Isotypes in GZU, KL, CBFS).

Description. *Plants* in dense turfs to flat cushions, living part green to dark-brown, not or moderately glossy, old parts bleached, light brownish. *Stems* up to 20 mm high (but mostly <10 mm), weakly branched, smooth, usually not coloured, with short-rectangular superficial cells; transverse section circular to bluntly triangular, with distinct central strand (about 20 μm wide), weak to absent sclerodermis and missing hyalodermis; a weak greyish tomentum

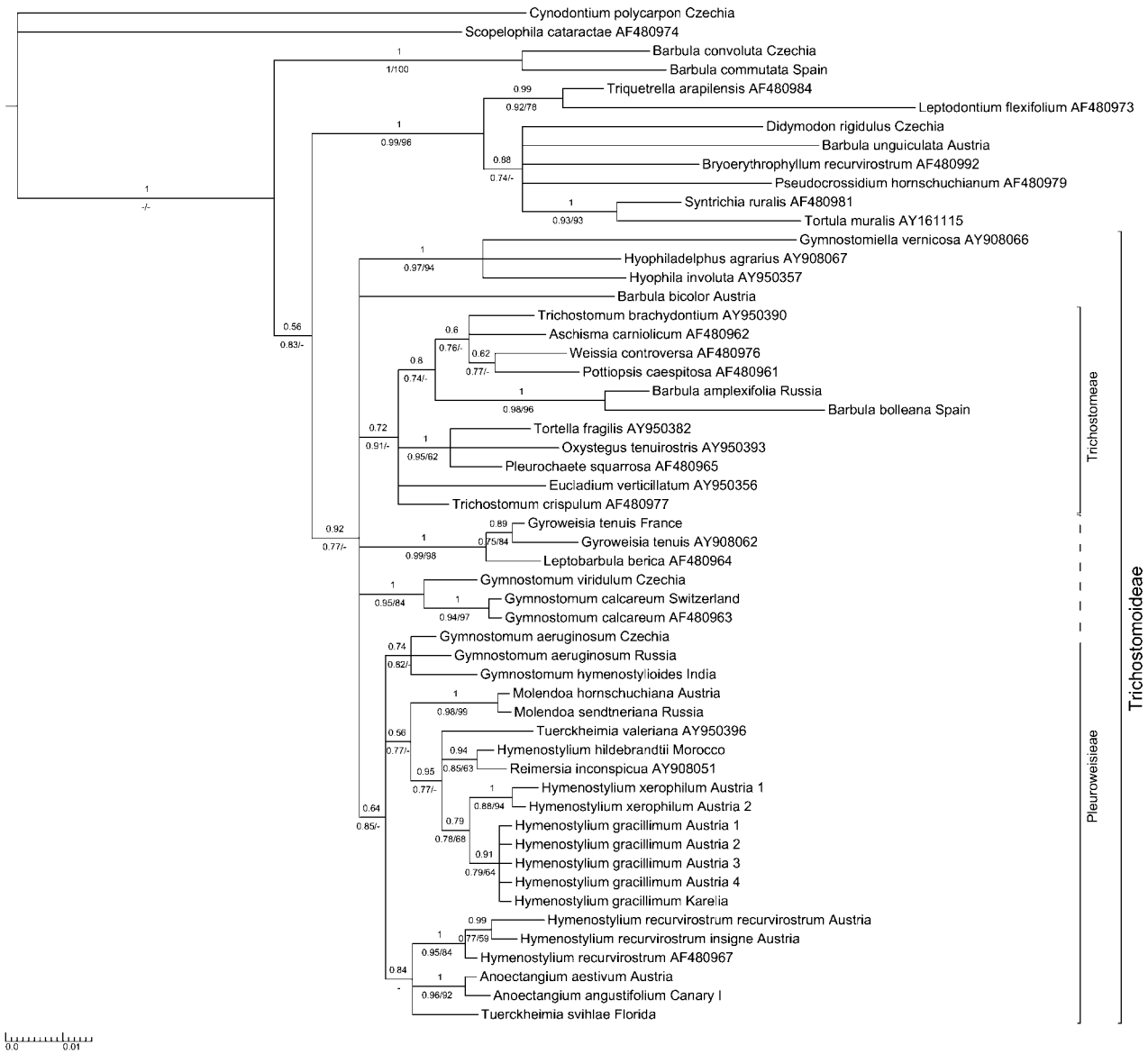

Figure 1 Bayesian inference 50% majority rule consensus tree inferred from chloroplast *rps*4 data, using an unconstrained GTR+I+G model, with PP values indicated above and support values from ML analysis (SH-like aLRT/bootstrap %) indicated below the branches.

sometimes present; subterranean rhizoids usually non-coloured, pale yellowish-brown when old, rarely purple when exposed, thicker rhizoids covered with a thick non-coloured integument, rhizoidal tubers absent. Axillary hairs of about eight elongated cells, non-coloured throughout. *Leaves* weakly to distinctly contorted (in both directions, usually mixed) when dry, erecto-patent (rarely homomallous) when moist; distinctly keeled, short-lanceolate or short-lingulate with a more or less broadly triangular apex, (0.5–) 0.6–1.3 mm long, 0.2–0.3 mm wide, leaf length/width ratio about 2–4(5) : 1, base often somewhat narrowed to insertion, non-sheathing and weakly differentiated, not decurrent along stem. *Margin* not recurved, usually entire, rarely remotely and minutely weakly toothed or notched distally, unistratose throughout. *Costa* (sub)percurrent to shortly excurrent as a rather blunt mucro, (30–)40–70(–80) μm wide near base, hardly narrowed and weakened towards the apex; adaxial superficial cells distally mostly short-rectangular and papillose, proximally elongate and less papillose, sometimes elongate throughout, abaxial superficial cells usually elongate throughout, variably papillose to smooth; transverse section distally almost circular, at base semi-circular, with two stereid bands, the abaxial with 2–3 rows of stereid cells, the adaxial weaker, with 1–2 rows (sometimes only 1–3 stereid cells or all substereid), guide cells 2–6, epidermis at both sides distinct. *Lamina* in dorsal (abaxial) view concave to plane in mid-leaf, unistratose, areolation well discernible, pellucid; basal cells short-rectangular, rather thin-walled, hyaline and non-papillose, distal cells not bulging, mostly quadrate (or rectangular, rarely irregular), thin- to thick-walled, with moderate hyaline corner thickenings, walls not porose, often arranged in rather

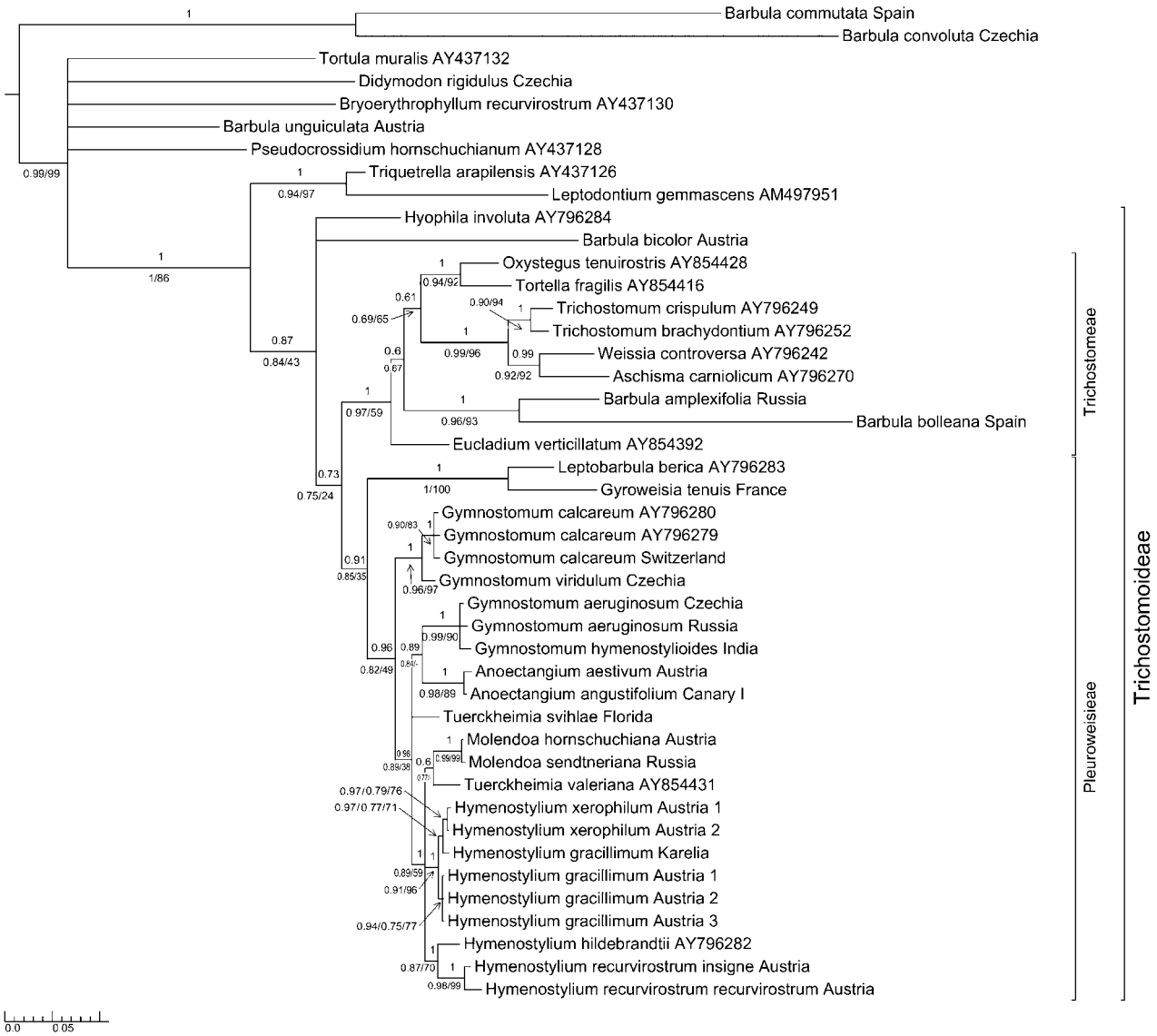

Figure 2 Bayesian inference 50% majority rule consensus tree inferred from nuclear ITS data, using an unconstrained GTR+G model, with PP values indicated above and support values from ML analysis (SH-like aLRT/bootstrap %) indicated below the branches.

distinct horizontal and vertical rows, 8–14(–16) μm wide, hardly larger and never elongate along costa, in mid-leaf about 10–20 cell rows between costa and margin; finely papillose (sometimes nearly smooth), papillae densely set, low, (2)3–6 per cell but hardly correlated with areolation.

Sexual condition dioicous; perichaetia terminal, perichaetial leaves somewhat longer than cauline leaves, base slightly sheathing; perigonia not known. *Sporophytes* not known.

Ecology. *Hymenostylium xerophilum* prefers drier habitats compared with other species of the genus. At high altitudes, the colonized rocks are mostly fully exposed to sunlight whereas it favours half-shaded situations below 1000 m, predominantly growing in shallow depressions of sloping (seldomly vertical) rock walls, more rarely in indistinct and superficial crevices. It has never been found in N-facing or deeply shaded rock habitats. The species shows a preference for dolomite; more rarely it has also been found on limestone, marble, and calcareous schist. Its ecological amplitude is distinctly wider at dolomitic sites and there it also tolerates periodically irrigated (but often dry) or soil- and detritus-covered rock ledges; twice it was even detected as small-leaved, dark-brown plants on dolomitic gravel away from rocks. The most characteristic associate by far is *Trichostomum crispulum*, followed by *Ditrichum flexicaule*, *Tortella densa*, *Gymnostomum aeruginosum*, *Didymodon rigidulus*, and *Hypnum vaucheri*. In subalpine and alpine environments, *Hymenostylium recurvirostrum* tolerates drier habitats than usual and there it sometimes forms mixed stands with *H. xerophilum*.

Sporophytes are not known; hence there must be some mode of vegetative reproduction, although we could not observe any common mode such as fragile leaves and stems, axillary or protonemal gemmae, or rhizoidal tubers. A simple culture experiment demonstrated that the species is able to reproduce successfully

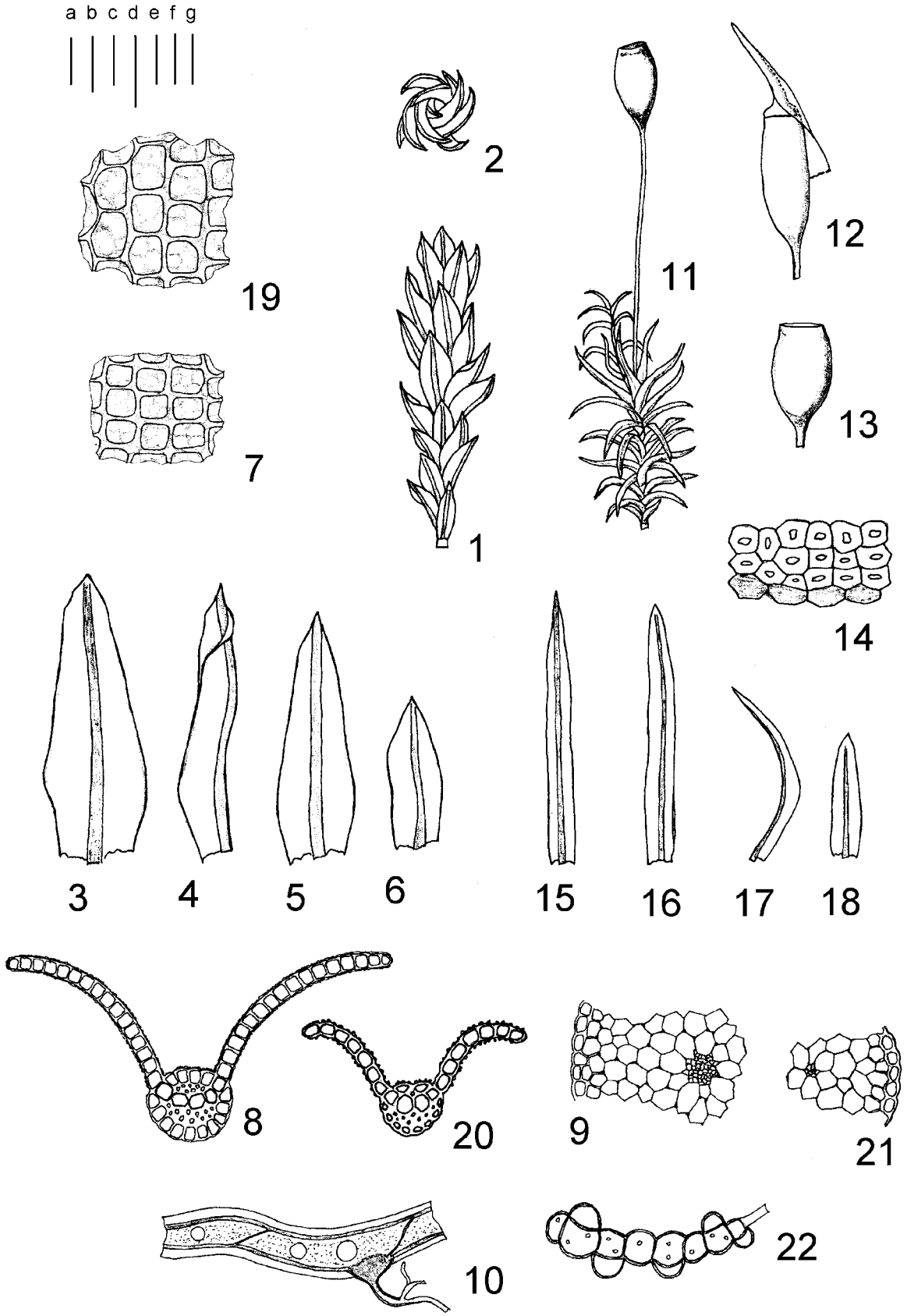

Figure 3 1–10. ***Hymenostylium xerophilum***. 1. Shoot, side view, moist. 2. Shoot apex, top view, dry. 3–6. Leaves. 7. Lamina cells in mid-leaf. 8. Transverse section in mid-leaf. 9. Stem transverse section, portion. 10. Subterranean secondary protonema filament. **11–22.** ***H. gracillimum*.** 11. Shoot with sporophyte, side view, moist. 12. Capsule (with operculum and calyptra). 13. Urn. 14. Annulus. 15–18. Leaves. 19. Lamina cells in mid-leaf. 20. Transverse section in mid-leaf. 21. Stem transverse section, portion. 22. Rhizoidal tuber. (1–10 from holotype, E; 11, 13, 16, 17, 19–21 from *Köckinger 14310*, E; 12, 14, 15 from *Köckinger 14309*, E; 18 from *Köckinger 14319*, E; 22 from *Köckinger 14268*, KL). Scale bars: a: 0.5 mm (1, 2, 11); b: 0.2 mm (3–6, 15–18); c: 0.1 mm (7, 19); d: 50 μm (8, 10, 20); e: 25 μm (9, 21); f: 0.3 mm (12, 13); g: 20 μm (14, 22).Drawings by H. Köckinger.

by means of thicker rhizoids, which *de facto* represent a subterranean secondary protonema, covered by a thick protective layer and filled with oil-droplets and leucoplasts (Köckinger, in prep.).

Distribution. At present, *H. xerophilum* is only known from calcareous areas of the eastern Alps, where it proved to be widespread (although nowhere frequent) and usually appearing in low quantities. The 35 discovered localities are the result of intense and partly also focused fieldwork. Its altitudinal amplitude ranges from the low montane to the alpine zone (*ca* 400–2500 m) with a preference for the upper montane zone. Except for a single German gathering (Meinunger & Schröder, 2007: 167, sub '*Gymnostomum* sp.'), all collections are from Austria. Most of the known localities are situated in the well-explored Austrian provinces of Styria and Carinthia. We are convinced that the real distribution area is much wider, although a search for old herbarium material in Austrian herbaria was not successful.

Selected collections. AUSTRIA: Carinthia: Pöllatal, *HK 14875*, KL; Krastal N of Villach, *HK 14243*, E, KL, CBFS; Weissensee, *HK 12302*, KL, CBFS; Gr. Dürrenbachtal S of Maria Elend, *HK 14876*, KL; SE of Griffen, *HK 12236*, KL, CBFS. Lower Austria: Rax, E of Klobentörl, *HK 14884*, KL. Salzburg: Tweng, *HK 14878*, E, GZU, CBFS; Mosermandl, *HK 14871*, GZU; Zaluβenalm W of Muhr, *HK 14872*, GZU. Styria: Rothenfels SE of Oberwölz, HK 94–216, GZU; Weiβenbachgraben NE of Radmer, *HK 14873*, GZU; Wildfeld, HK 98–931, GZU, Klammkogel SE of Vordernberg, *HK 12291*, E, GZU, CBFS; Hochschwab, Hundswand N of Bodenbauer, *HK 14879*, E, GZU, CBFS; Höllwand N of Mürzsteg, HK 96–1483, GZU, CBFS; Gamskogel W of Kleinstübing, *HK 14874*, E, GZU, CBFS. Upper Austria: Traunstein, *Schlüsslmayr, s.n.* (Schlüsslmayr, 2005, sub '*Gymnostomum* sp.'). Tyrol: Prosseggklamm N of Matrei in Osttirol, *HK 14877*, KL. Vorarlberg: Montafon, Lorüns, *HK 14870*, GZU. GERMANY: Bavaria: Breitenstein W of Ettenhausen, *Meinunger, s.n.* (Meinunger & Schröder, 2007, sub '*Gymnostomum* sp.').

Hymenostylium gracillimum* (Nees & Hornsch.) Köckinger & J.Kučera, *comb. nov. (Figure 3.11–3.22)

Basionym: *Gymnostomum gracillimum* Nees & Hornsch., Bryologia Germanica I: 149. pl. X f. 13. 1823.

Lectotype (designated here): 'Pongau, Alpib. Salisburg. (Hornsch.), Arnott-collection, herb. E, No. 00165014'; isotype: 'Alpib. salisb., Arn., herb. E, No. 00165012'.

Synonyms: *Gymnostomum calcareum* var. *gracillimum* (Nees & Hornsch.) Bruch & Schimp., Bryol. Eur. 1: 78. 33d (fasc. 33–36 Mon. 6. 4d). 1846, *Gymnostomum calcareum* var. *gracile* Breidl. ex G.Roth, Eur. Laubm. 1: 166. 1903, *Gymnostomum boreale* Nyholm & Hedenäs, Lindbergia 12: 41. 1986.

Description. *Plants* in dense, often velvety turfs to flat cushions, living part usually dark green to brownish, not or only moderately glossy, old parts bleached, light brownish. *Stems* slender, up to 25 mm high (but mostly <10 mm), freely branched, smooth, brown, with short-rectangular superficial cells; transverse section circular, with a weak (2–5 cells) to absent central strand, cylinder cells thin-walled, sclerodermis and hyalodermis absent, outermost cell row brown and thicker-walled; a weak greyish to light brownish tomentum rarely present; subterranean rhizoids non-coloured to light yellowish-brown when older, rarely purple when exposed; thicker rhizoids covered with a thick non-coloured integument; brown, irregular rhizoidal tubers on long rhizoids very rare, about 100 μm long, primarily uniseriate with turgid, moderately thick-walled cells and 1-celled protuberances. Axillary hairs of about eight elongated cells, non-coloured, the basal two brownish. *Leaves* in dry condition incurved when narrow and rather long, nearly straight and weakly imbricate when very small, in turgid condition erecto-patent to recurved; weakly to strongly keeled, linear-ligulate or linear-lanceolate, more rarely short-lanceolate, with a sharp, mostly rather narrowly triangular apex, 0.4–1.4 mm long, 0.08–0.18 mm wide, leaf length/width ratio about (3–)4–12 : 1, base non-sheathing and weakly differentiated, not decurrent along stem. *Margin* mostly not recurved, sometimes reflexed in basal part, usually entire, rarely indistinctly toothed near apex, unistratose throughout. *Costa* mostly subpercurrent (more rarely per- to excurrent as a mucro), 20–40 μm wide near base; adaxial superficial cells distally rectangular and papillose, proximally elongate and nearly smooth, abaxial superficial cells mostly narrowly elongate throughout, weakly papillose or smooth; transverse section distally almost circular, at base semi-circular, abaxially with a weak stereid band of 1–2 rows, the adaxial usually absent, usually only two guide cells. *Lamina* in dorsal (abaxial) view concave to plane in mid-leaf, in upper part plane, unistratose, areolation usually well discernible; basal cells short-rectangular, rather thick-walled, hyaline and non-papillose, distal cells not bulging, quadrate or somewhat elongated rectangular or irregular (sometimes throughout), thin- to thick-walled, often with distinct (partly nodular) corner-thickenings, arranged in rows or not, *ca* (8–)10–16 μm wide, larger and often elongate along costa, in mid-leaf about 4–8(–10) cell rows between costa and margin; papillosity moderately fine (rarely absent at irrigated sites), papillae clear, densely set, rounded in outline (rarely irregular to weakly

furcate), about 3–6 per cell but hardly correlated with areolation.

Sexual condition dioicous; perichaetia terminal, perichaetial leaves longer than cauline leaves (up to 1.7 mm long), basal half strongly inflated and hyaline, upper half nearly subulate, gradually narrowing to sharp apex; perigonia terminal, gemmate, perigonial leaves broad-ovate, hyaline throughout. *Seta* yellowish, when old with a tinge of red, 3–4 mm long, twisted clockwise. *Capsules* gymnostomous, pale, rarely chestnut brown, short- to narrowly ovoid, short-necked, rostrate. *Urn* 0.5–0.8 mm long, 1.5–2.5 times longer than wide, widest in the middle, narrowed to reddish-brown mouth, exothecial cells variably thin- to thick-walled, irregularly rectangular, 12–20 × 40–55 μm, few stomata in one row at urn base; annulus of 2–4 cell rows, cells (rounded) quadrate or wider than long, about 12–20 μm wide, moderately vesiculose, lumina small, uppermost row sometimes disintegrating into single cells. *Operculum* not attached to columella, nearly as long as urn, with long, straight or oblique rostrum. *Calyptra* cucullate, pale yellowish, *ca* 1.2 mm long. *Spores ca* 10–16 μm, smooth to finely papillose.

Nomenclatural history. Nees von Esenbeck *et al.* (1823) provided quite an accurate and detailed description of *Gymnostomum gracillimum*, based on a single population from a slate rock-wall near Hüttau in Pongau, Salzburg, Austria. They were, however, not able to give a single character which would allow secure differentiation from similar taxa. Hence, the species was soon reduced to the rank of variety within *G. calcareum* Nees & Hornsch. by Bruch *et al.* (1846), who treated it as an intermediate form between typical plants of the species and var. *viridulum* (Brid.) Bruch & Schimp. (=*G. viridulum* Brid.). The main part of Hornschuch's herbarium was destroyed in Berlin during World War II. Although Zander *et al.* (2007) mentioned an isotype from B, we were informed by the curator that no material of *G. gracillimum* exists in B; no duplicates were found in either STR (herb. Nees), GFW, GZU, or M, where at least small parts of Hornschuch's herbarium are housed. Finally, we acquired two isotypes from E (collection Arnott), also mentioned in Zander *et al.* (2007). The lectotype contains several well-preserved sporophytes. Laminae are characteristically concave in abaxial view, and some leaves with basally distinctly reflexed margins were observed as well. Moreover, the characteristic non-coloured to pale brownish rhizoids with the thick protective layer were found. In general, the material fully corresponds with the protologue and the illustrations in Nees von Esenbeck *et al.* (1823). The exact type locality is not given, although 'Pongau, Alpib. Salisburg. (Hornsch.)' certainly refers to this site. Thus, there can be no doubt of the identity of the lectotypified material. R. Zander revised both specimens as *G. aeruginosum*, having probably concentrated on the exothecium in comparison with *G. calcareum*. Whitehouse & Crundwell (1991) studied another isotype from GL (also from the Arnott collection, not seen by us) and identified it as *G. calcareum*.

In 1885, J Breidler collected a sterile, up to 25 mm high plant at the northern side of the Radstädter Tauern pass (only about 25 km SE of Hüttau) and named it *G. calcareum* var. *gracile* (*in sched.*). It was first mentioned in Limpricht (1890) but as he obviously doubted its taxonomic value, the first valid publication can be regarded as that of Roth (1903). Type material in GJO corresponds well with the recent collection from Obermauern (both from walls, very small-leaved plants with rather large cells). The latter proved to be molecularly identical to other Austrian plants.

More recently, Nyholm & Hedenäs (1986) described *G. boreale* Nyholm & Hedenäs from Northern Europe, which is reduced to synonymy here. The only known locality for the taxon is the Kulmakkapuro ravine (Tuomikoski, 1939; 'Kulmakkapus' in Nyholm & Hedenäs, 1986 is erroneous) in northern Karelia, close to the Oulanka National Park, now in Russian territory but earlier belonging to Finland. However, the authors of *G. boreale* selected another specimen as the holotype, as it was the only one with sporophytes. This specimen, unfortunately, contains no data on its origin. We only know that it came to S in 1883 as a gift (ded.=dedit) from A. Grönvall, determined as *Mollia calcarea* (Nees & Hornsch.) Lindb. (=*G. calcareum*) by an unknown person. The type material fully corresponds with the type of *G. gracillimum*; hypothetically, *G. boreale* may, under the most intriguing scenario, even be homotypic, with its holotype being a putative duplicate of the Hornschuch collection. Nyholm & Hedenäs (1986) described the rhizoids of *G. boreale* as orange to pale orange; we, however, observed only non-coloured to light yellowish-brown (rarely purple) rhizoids in the holotype and the paratypes. This is at least the impression under transmitted light, whereas under incident light, and also depending on the light source, the yellowish-brown rhizoids may, indeed, look more or less orange.

Ecology. *Hymenostylium gracillimum* is an early pioneer of moist and shady rock, preferring easily disintegrating slate, phyllite or schist with a subneutral reaction, where it can develop large and pure, dark green to brownish, velvety colonies on vertical rock faces and in crevices. Only on these rock types was it found with sporophytes (in three of the four sites). Twice it was collected from shady, N-facing dolomite rock crevices. Dolomite is also the rock type given for the Karelian locality; Tuomikoski (1939: 33) even provides a habitat

photo of this site. The two gatherings from artificial walls are remarkable, as the more frequent *H. xerophilum* has never been found in non-natural environments. Among the few associates on schist, *G. aeruginosum*, *Conardia compacta*, or *Scapania verrucosa* could be mentioned, on dolomite *G. calcareum*. Mixed stands with *H. xerophilum* are not known but in one of the dolomitic sites they were found not far from each other, although under very different habitat conditions.

Distribution. *Hymenostylium gracillimum* is a rare, inconspicuous moss, known with certainty only from eight localities in the Alps of Austria and a single site in Russian Karelia. In the Alps, it was found from the low to the middle montane zone (*ca* 500–1300 m). Securely identified material comes from Salzburg (types of *G. gracillimum* and *G. calcareum* var. *gracile*), Carinthia, Styria, and Eastern Tyrol. Additionally, Matouschek (1902) mentioned *G. calcareum* var. *gracile* from a single locality in Northern Tyrol (not seen). Spruce (1849), probably erroneously, reported the species (sub *G. calcareum* var. *gracillimum*) from Rontignon near Pau in southern France (not seen).

Later reports of *G. boreale* (Pilous, 1993 from Slovakia, Boudier, 2003 from Canadian Quebec, Fedosov, 2007 from Northern Siberia, and Hallingbäck *et al.*, 2008 from northern Norway) were all based on misidentifications. The revision of available specimens (*Pilous* — Krakova hol'a, July 1954, S, PR; *Pilous* — Černý kameň, July 1952, PR; *Pilous* — Rozsutec, August 1950, PR, *Boudier* 1491, S; *Hedenäs*, 17 July 2003, S; *Fedosov 05-678*, MW) revealed misidentifications mostly for *G. aeruginosum*, in one case for *Didymodon subandreaeoides* (Kindb.) R.H. Zander (*Pilous*, Nový, July 1952, PR).

Known localities. AUSTRIA: Carinthia: Maltatal, W of Dornbach, *HK 14268*, KL. Trefflinggraben NE of Treffling near Millstättersee, *HK 11386*, KL. Tiboldgraben N of Stockenboi, *HK 14264*, *14308–14322*, E, KL, CBFS; Weissensee, SE of Dolomitenblick, *HK 14650*, KL. Salzburg: 'am Wege vor Hüttau in Pongau', 7 February 1816, *D. H. Hoppe & F. Hornschuch*, in Nees von Esenbeck *et al.* (1823) (type of *G. gracillimum*); 'Straβenmauern an der Nordseite des Radstädter Tauern', *J. Breidler*, 17 August 1885, GJO (type of *G. calcareum* var. *gracile*). Styria: Gamskogel W of Kleinstübing, *HK 14880*, GZU. Tyrol: Virgental, Obermauern, *HK 14267*, KL. RUSSIA: Karelia: Kulmakkapuro, 29 August 1933, *M. J. Kotilainen*, S; 31 July 1937, *M. J. Kotilainen*, S; 6 July 1938, *A. Hülphers*, S.

Differentiation

Hymenostylium xerophilum shares the following main diagnostic characters with *H. gracillimum*: (1) rhizoids non-coloured to light yellowish-brown (rarely purple when exposed); (2) stem with central strand and without sclerodermis; (3) leaves keeled; (4) leaves green to dark-brown and often moderately glossy; (5) lamina concave (to plane) in dorsal view; and (6) lamina cells well discernible and more or less quadrate with several small and low papillae.

Hymenostylium gracillimum differs from *H. xerophilum* in the characters presented in the Key (see below).

Hymenostylium recurvirostrum [excl. var. *cylindricum* (E.B.Bartram) R.H.Zander] differs from both in its red-brown rhizoids, the absence of a stem central strand and presence of a distinct sclerodermis, the often recurved leaf margin, costa usually without adaxial epidermis, often irregular and elongated, porose lamina cells and scattered, high papillae. From *H. gracillimum* it differs in its much coarser sporophytes, with chestnut urns which are widest near the mouth and normally larger spores.

Hymenostylium xanthocarpum (Hook.) Brid. and *H. aurantiacum* Mitt., two problematic Asian taxa, differ mainly in a collenchymatous areolation and scattered, mostly coarse papillae, usually 1–2 per cell (Chen, 1941; Saito, 1975).

Gymnostomum hymenostylioides (Broth. & Dixon) R.H.Zander differs from both in red-brown rhizoids and the obscure areolation with rather small, thin-walled, densely papillose cells; from *H. gracillimum* also in the habit, leaf shape, and costa similar to *H. xerophilum.*

Gymnostomum aeruginosum differs from both in red-brown rhizoids, leaves incurved to curled when dry, lamina convex to plane in dorsal view and margin often bistratose. It differs from *H. xerophilum* in leaves having a usually higher length/width ratio with the apex not broadly triangular, from *H. gracillimum* in normally distinctly coarser leaves, stronger costa and usually larger sporophytes with weakly defined, wider-than-long annulus cells. *Hyophila styriaca* Głow., described by Głowacki (1913) from Styria 'am Salzafall im Stein bei Gröbming, August 1908, *leg*. J. Głowacki, No. 10821, W', resembles *H. xerophilum* in description and illustration. The holotype in W, however, proved to be *G. aeruginosum*, as earlier suggested by R. Düll and L. Loeske according to their revision labels (see also Corley *et al*. 1981).

Gymnostomum calcareum and *G. lanceolatum* differ from both in red-brown to light reddish-brown rhizoids, the light green leaf colouration, much smaller cells in the upper lamina, the lamina convex to plane in dorsal view and the margin often bistratose. Moreover, they differ from *H. xerophilum* in much smaller leaves and narrower costa; from *H. gracillimum* in having longer setae and larger

capsules with much wider, bulging exothecium cells, also in their strict absence from non-calcareous rocks.

Gymnostomum viridulum, which also shows non-coloured (or slightly reddish) rhizoids, differs from both in a light green (rarely reddish-brown) colouration, non-keeled leaves, and the presence of axillary gemmae. It differs from *H. xerophilum*, furthermore, in much smaller, soft leaves with a very weak costa, usually ending before the apex; from *H. gracillimum* in a lower leaf length/width ratio and occurrence in warm and rather xeric habitats.

Anoectangium handelii is differentiated by the same set of characters as the similar *G. viridulum*, except for having somewhat more distinctly keeled leaves and gametangia born on short lateral branches.

Gyroweisia tenuis differs in reddish-brown rhizoids, leaves with variable shape but mostly rounded at the apex with the weak costa vanishing distinctly before the apex. Its sporophytes very much resemble those of *H. gracillimum* in habit; the operculum is, however, conic (not rostrate) and the annulus cells are longer than wide and revoluble, whereas those of *H. gracillimum* are (rounded) quadrate or wider than long and at most irregularly disintegrating in the uppermost row. JK was able to study the type material of *Gyroweisia acutifolia* Philib. from AUT, which, however, proved to be hardly different from usual morphs of *G. tenuis*. The lectotype of *G. acutifolia* is here designated: [Suisse] Bex, 4 août [18]81, [*leg.* H. Philibert], herbarium Philibert in AUT, Planche 69 (bottom).

Key to *Hymenostylium* and the genera of the Pleuroweisieae in Europe (excluding *Gyroweisia* and *Leptobarbula*)

1 Gametangia terminal on main shoots 2
1* Gametangia at the ends of short lateral branches 6
2 Leaves hardly keeled, lamina in dorsal view convex to flat, margins never recurved, costa never excurrent, upper lamina cells usually rather thin-walled (collenchymatic only when cells very small), papillae in upper lamina usually low and densely set, rather opaque, more or less obscuring areolation, never vestigial; plants not glossy . . . . . ***Gymnostomum***
2* Leaves distinctly keeled, lamina in dorsal view concave to flat (except for *Hymenostylium recurvirostrum* var. *insigne*), margins often partially recurved, costa sometimes excurrent, upper lamina cells frequently thick-walled (partly also collenchymatic), papillae in upper lamina loosely (then often high and furcate) to densely set (then low and simple), transparent and not obscuring areolation, in wet habitats vestigial; plants often glossy ***Hymenostylium*** 3
3 Rhizoids red- to dark-brown; stem without central strand, sclerodermis distinct and often strong ***H. recurvirostrum*** 4
3* Rhizoids non-coloured to light yellowish-brown; stem usually with central strand (rarely absent when stem very thin), sclerodermis indistinct 5
4 Leaf base hardly expanded and not distinctly sheathing, lamina in dorsal view concave to flat, margin unistratose, lamina papillae when present rather coarse . . . . . ***H. recurvirostrum* var. *recurvirostrum***
4* Leaf base expanded and distinctly sheathing, lamina in dorsal view convex to flat, margin partially bistratose for one row, lamina papillae when present rather fine . . . . . ***H. recurvirostrum* var. *insigne***
5 Leaves 200–300 μm wide, length/width ratio 2–4(5) : 1 (high ratios only in long-leaved plants), contorted to straight when dry, erecto-patent when moist; number of cell rows between costa and margin 10–20 in mid-leaf, costa 40–80 μm wide near leaf base, margins never recurved; plants hardly branched, not velvety in the field, sporophytes unknown, growing in rather xeric habitats . . . . . ***H. xerophilum***
5* Leaves 80–180 μm wide, length/width ratio (3)4–12 : 1 (low ratios only in extremely small-leaved plants), incurved to straight when dry, patent to recurved when moist; number of cell rows between costa and margin 5–8(10) in mid-leaf, costa 25–40 μm wide near leaf base, basal margin sometimes recurved; plants freely branched, soft and velvety in the field, sporophytes known, growing in rather humid habitats . . . . . ***H. gracillimum***
6 Leaves strongly keeled, usually with a sharp, non-papillose apiculus, lamina in dorsal view concave to flat, ventral costal stereid band absent . . . . . ***Anoectangium***
6* Leaves hardly keeled, apex papillose throughout or (when non-papillose) thickened and deciduous, lamina in dorsal view convex to flat, ventral costal stereid band usually present (absent only in very small-leaved plants). . . . . ***Molendoa***

Discussion

The molecular results generally confirm the phylogenetic relations outlined in Werner *et al.* 2004 (*rps*4 data) and 2005 (ITS data), yet bringing more resolution owing to a wider and more focused sampling. Both datasets seem to be somewhat problematic with respect to the reconstruction of phylogeny in the studied group; the *rps*4 data are relatively little variable, with many well-defined but small clades appearing on relatively long branches, which makes the reconstruction sensitive to the selection of the

analysis method and settings. On the other hand, ITS data are extremely challenging with respect to homology assessment in the alignment. The data from both datasets unequivocally recognize the clade that contains closely related taxa, which forms a core of the tribe Pleuroweisieae. This clade comprises the analysed members of the genera *Hymenostylium*, *Reimersia*, *Molendoa*, *Tuerckheimia*, *Anoectangium*, and the *Gymnostomum aeruginosum/hymenostylioides* clade. The inclusion of the *Gymnostomum calcareum* group and the *Gyroweisia/Leptobarbula* clade in the Pleuroweisieae is positively supported only by the ITS data; however, it is not contradicted by the *rps*4 dataset. This delimitation of the tribe is fully congruent with that of Saito (1975) and essentially identical to the subfamily Eucladioideae of Chen (1941), except for the placement of *Eucladium*, which clearly belongs in the Trichostomeae. The basal position of the *Gyroweisia/Leptobarbula* clade within Pleuroweisieae is morphologically supported by the putatively plesiomorphic presence of the peristome (gradually reduced and finally lost in that clade), whereas the crown taxa of the Pleuroweisieae are completely eperistomate, which seems to be a derived character and a shared synapomorphy of the group. To test this hypothesis, we urgently need to analyse *Tuerckheimia guatemalensis* Broth., which is described as peristomate and therefore is probably not closely related to *T. svihlae* and *T. valeriana*.

The strict translation of the revealed relations in the Pleuroweisieae into classification would probably require either the establishment of a new genus for the *H. gracillimum/xerophilum* clade or synonymization of *Hymenostylium*, *Reimersia*, and *Molendoa* and the transfer of *Tuerckheimia valeriana* to *Hymenostylium*. However, in the absence of sequences of two generitypes (*H. xanthocarpum* and *T. guatemalensis*) and a limited species selection of concerned genera, we consider such a revolutionary change of generic concepts premature and prefer assigning the analysed species to existing genera. The monophyly of the genus *Hymenostylium* cannot be convincingly shown using the available molecular data; however, the potential coherence of the genus is not challenged upon accepting the *H. gracillimum/xerophilum* clade in *Hymenostylium*. On the other hand, the inclusion of the *H. gracillimum/xerophilum* clade in *Gymnostomum* has no support from our molecular data, leaving it polyphyletic, and even wider sampling would probably not change this picture. The close relation of *Leptodontium* and *Triquetrella* to *Hymenostylium*, suggested by Zander (1993), can be refuted, despite the fact that the topology of this particular clade is ambiguous between chloroplast and ITS data.

The molecular integrity of *H. xerophilum* and of *H. gracillimum* from the Alps and their mutual differentiation, despite a close relationship, is unproblematic. On the contrary, the integrity of *H. gracillimum*, as understood here to include '*Gymnostomum boreale*', is supported by the identity of morphological and chloroplast *rps*4 data, but is challenged by the ITS data of the Karelian plant. In this case, two substitutions are shared with *H. xerophilum* and only one with *H. gracillimum* from the Alps, which when translated to the tree topology results in the Karelian plant and *H. xerophilum* forming the sister clade to *H. gracillimum s.str*. The incongruence between chloroplast and nuclear data for the Karelian plant might simply be an artefact resulting from incomplete coverage of the existing molecular variability of the two taxa, or even a sequencing error, given the relatively large weight of single point mutations in this particular case. An eventual hybridization event between *H. gracillimum* and an unknown species of *Hymenostylium* seems to be improbable, as we do not know of any other different but closely related taxon in the region. With respect to the microscopical identity of the Karelian plant with *H. gracillimum* from the Alps, from which it differs only slightly in habit (possibly in response to habitat differences), we are convinced that the inclusion in *H. gracillimum* is justified, as supported by chloroplast sequence data. Moreover, this problem only marginally concerns the usage of the names advocated by us, since the type of *G. boreale* is of unknown geographic origin and morphologically extremely close to the type of *G. gracillimum*, while the molecular identity of the holotype with the Karelian paratypes cannot be proven.

In addition to the molecular data, the majority of the morphological and anatomical characters also favour the placement of our two taxa in a broadly defined *Hymenostylium* and not in the alternative genus *Gymnostomum*, in particular: (1) the keeled leaves; (2) concave lamina in abaxial view; (3) sometimes recurved basal margin; (4) sometimes excurrent costa; (5) frequently thick-walled lamina cells; and (6) translucent and often vestigial papillae, not obscuring the areolation (for comparison with *Gymnostomum* see the Key). With respect to habit, the often glossy, dull green to dark-brown (never yellowish green!) turfs or cushions clearly also point to *Hymenostylium*. The only problematic feature is the usual presence of a stem central strand. Zander (1993) uses the central strand as the main character distinguishing *Hymenostylium* and *Gymnostomum*, which should be absent in the former and present in the latter. However, it should be noted that Zander (1977) allows the occasional presence of a central strand even in *H. recurvirostrum* (var. *cylindricum*) in plants from Central America. The presence of the strand is certainly the ancestral, plesiomorphic character state in the Pottiaceae, a family consisting mainly of xerophytes. In taxa of Trichostomoideae

adapted to moister habitats, e.g. *H. recurvirostrum*, the central strand is often lost and partly replaced by a strengthened sclerodermis to retain the stability of the stem. These alterations can be observed in stem cross-sections of several pairs of xero/mesophytic versus meso/hygrophytic species including *H. xerophilum* versus *H. recurvirostrum*, *Tortella alpicola* versus *T. fragilis* or *Oxystegus tenuirostris s.str.* versus *O. hibernicus*. Hence, we can see no good reason for accepting the presence or absence of the stem central strand as the ultimate diagnostic character separating *Hymenostylium* from *Gymnostomum*. Good pointers to the subtle generic differences are found in the species pair *H. xerophilum* and *G. hymenostylioides*. The latter was formerly thought by HK to possibly be the closest relative of the former. Therefore, the holotype ('India, Simla, on walls, 6900 ft, 26 May 1906, *leg*. E. Long, BM') was loaned and studied by HK. Later, a recent collection by D G Long from the same area permitted a molecular analysis which showed a close relationship of this plant to *G. aeruginosum*. *Hymenostylium xerophilum* and *G. hymenostylioides* are very similar in habit and in most leaf characters (see the section on 'Differentiation') and differ only in a rather thick-walled versus a rather thin-walled areolation and in a faint versus a dense and cell-obscuring papillosity. Here we are obviously confronted with generic differences of real taxonomic importance, although these are certainly less practical in comparison with the absence or presence of a central strand. The species pair *H. xerophilum* and *G. hymenostylioides* represents an excellent example of convergent evolution in adaptation to a rather dry habitat.

The closest relatives of *H. xerophilum* and *H. gracillimum*, based on morphological evidence, may be the as yet poorly known Asian taxa *H. xanthocarpum* and *H. aurantiacum* (Dixon, 1927; Chen, 1941; Saito, 1975; Aziz & Vohra, 1988). The former, sharing with *H. xerophilum* a similar leaf shape, a flat margin, and two stereid bands among other characters, represents the generitype of *Hymenostylium*, which, of course, also supports the placement of the two European taxa in this genus.

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Taxonomic Additions and Changes: *Hymenostylium xerophilum* Köckinger & J.Kučera, *sp. nov*.; *Gymnostomum gracillimum* Nees & Hornsch., lectotype designated; *Hymenostylium gracillimum* (Nees & Hornsch.) Köckinger & J.Kučera, *comb. nov*. (*Gymnostomum calcareum* var. *gracile* Breidl. ex G.Roth, *syn. nov*.; *Gymnostomum boreale* Nyholm & Hedenäs, *syn. nov*.); *Gyroweisia acutifolia* Philib., lectotype designated.

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The identity of *Grimmia andreaeoides* Limpr. and *Didymodon subandreaeoides* (Kindb.) R.H.Zander

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SUMMARY

Didymodon subandreaeoides (Kindb.) R.H.Zander, known to-date from north-western North America and the Beringian part of Arctic Russia, is identical to the earlier described European taxon *Didymodon rigidulus* subsp. *andreaeoides* (Limpr.) Wijk & Margad. (*Grimmia andreaeoides* Limpr.). Nomenclatural history of both taxa and an amended description with illustrations are given, and the variability, differentiation, ecology and distribution are discussed. The typification of all known synonyms is provided.

KEYWORDS: *Grimmia andreaeoides* Limpr., moss taxonomy, nomenclature, typification, ecology.

INTRODUCTION

The results of the first author's taxonomic studies of the *Didymodon rigidulus* group in Europe have led to the conclusion that *D. rigidulus* ssp. *andreaeoides* (Limpr.) Wijk & Margad. is specifically distinct from *D. rigidulus* Hedw. The same opinion, based on field experience in the Eastern Alps, is held by HK. The reasons for separating the two taxa include numerous taxonomically important morphological and anatomical details, contrasting ecology, existence of mixed stands, and distinctive electrophoretic isozyme patterns that will be described in another publication.

The search for the correct species name (*Didymodon andreaeoides* has already been used, *D. andreaeoides* Cardot & Broth., based on a different type) necessitated checking the type of *Didymodon subandreaeoides* (Kindb.) R. H. Zander. The types of the two taxa of Kindberg earlier synonymized by Steere (1938) — *Barbula subandreaeoides* and *Barbula andreaeoides* — were found to be fully identical with the type of the European *Grimmia andreaeoides* Limpr. For nomenclatural reasons, discussed in Zander (1978), the correct name of this taxon is *Didymodon subandreaeoides* (Kindb.) R. H. Zander. It has to be noted that the view that these three taxa could be identical had already been expressed by W. Schultze-Motel on his revision labels (apparently unpublished). He, too, studied the types of *Barbula andreaeoides* and *B. subandreaeoides* in S at the time of his studies on the costate *Andreaea* species (as putative synonyms of *Andreaea rothii* F.Weber & D.Mohr).

NOMENCLATURAL HISTORY

The distinctiveness of *Didymodon subandreaeoides*, under its earlier names, has been repeatedly doubted by European authors. The discoveror of the species, Breidler, having sent two specimens to Limpricht, who later based his description of *G. andreaeoides* on them, labeled one of them (selected here as the lectotype) '*Didymodon rigidulus* forma *gemmipara*?'. In contrast, Sebille (1908), who had found the species in the French Alps, confirmed its position within *Grimmia*. The view that the taxon could merely be a form of *Didymodon rigidulus* was again expressed by Culmann in Amann & Meylan (1918). In the same year Culmann made *G. andreaeoides* a subspecies of *Barbula rigidula* (Hedw.) Mitt. It is interesting to note that Culmann's opinions were evidently based on a misinterpretation. JK was able to study the collections of '*Grimmia andreaeoides*' in Z and found that the specimens interpreted as being transitional between *Didymodon rigidulus* and '*Grimmia andreaeoides*' by Culmann were in fact either pure specimens of *Didymodon subandreaeoides* or mixed stands of both species, without any trace of transitions. Such mixed stands are not rare in the Alps. Loeske (1930) later had the same opinion as Culmann. Jones & Warburg (1950) went still further in viewing '*Grimmia*

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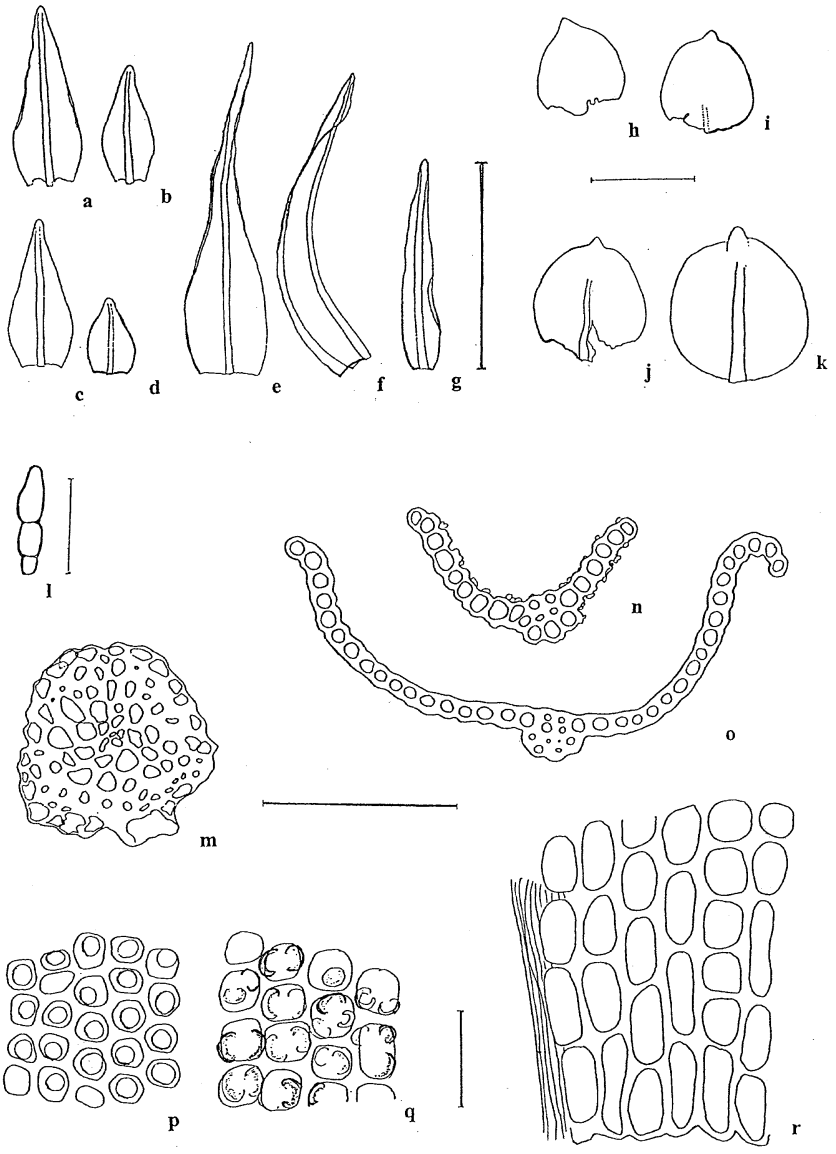

Figure 1. *Didymodon subandreaeoides* (Kindb.) R. H. Zander: a–d, vegetative leaves; e–f, outer perichaetial leaves; g, inner perichaetial leaf; h–k, basal flagellum leaves; l, axillary hair; m, stem cross-section; n, leaf cross-section in the upper part; o, leaf cross-section in the lower part; p–q, upper lamina cells; r, basal lamina cells. [a–b, g: Köckinger 97-626; c–d, r: Köckinger 97-494; e–f, m–o: Kučera E2724; h–l: Kučera E1601; p: Köckinger 97-539; q: Köckinger 97-441]. Scale bars: a–g, 1 mm; h–k, 200 μm; l, 50 μm; m–o, 100 μm; p–r, 20 μm.

andreaeoides' as 'merely an abnormal growth-form of *Barbula rigidula*'. To support their opinion, they noted the presence of gemmae in the leaf axils of plants collected in the Snowdon area (Wales, United Kingdom) originally by D. A. Jones and later by themselves. We also had the opportunity to study the specimen collected on Snowdon by D. A. Jones which is housed at E. This single and unfortunately small specimen indeed combines the characters of both *D. rigidulus* and *D. subandreaeoides* but differs from both taxa in important details. We are convinced that this poor material cannot be unequivocally assigned to either species. Probably the last author who dealt taxonomically with '*Grimmia andreaeoides*' was Pilous (1958) who studied plants from the Belianské Tatry Mts. (Slovakia). He compared them with *Molendoa tenuinervis* Limpr. and came to the conclusion the taxa are closely related and that their morphological characters intergrade to such an extent that '*Grimmia andreaeoides*' should be viewed only as a form of *Molendoa tenuinervis* (the two new combinations he made in his paper were however invalid). It is true that the leaf shape of both taxa is extremely similar, however, the crucial differences between the genera *Molendoa* and *Didymodon*, e.g. the position of gametangia, were unfortunately not taken into account. Since then the existence of '*Grimmia andreaeoides*' has been almost forgotten. British authors (Smith, 1980; Corley *et al.*, 1981) and Maier & Geissler (1995) viewed it as a synonym of *Didymodon rigidulus*. German authors (Düll, 1984; Frahm & Frey 1992; Frey *et al.*, 1995) mostly

neglected the taxon although Düll (1991) later noted that *D. rigidulus* ssp. *andreaeoides* 'is a remarkable taxon'.

Didymodon subandreaeoides had a similar history of neglect and reinstatement in America. In 1905, N. C. Kindberg described, among other taxa from British Columbia, two new members of the genus *Barbula* — *B. andreaeoides* and *B. subandreaeoides*, differing in minor details of leaf shape. They were put into synonymy with *Andreaea rothii* by Steere (1938) and therefore nearly forgotten for the next forty years. Later, however, Steere (1978) changed his mind and pointed out that *Barbula andreaeoides* was a distinct species. Zander (1978) accepted his view and placed the species in the genus *Didymodon*, following the generic concept of Saito (1975). The justification of accepting *Didymodon subandreaeoides* as a distinct species in North America has not been doubted in the last twenty years as far as we know.

AMENDED DESCRIPTION OF *DIDYMODON SUBANDREAEOIDES* (Fig. 1)

Didymodon subandreaeoides (Kindb.) R. H.Zander, Phytologia 41 (1): 23. 1978

Basionym: *Barbula subandreaeoides* Kindb., Rev. Bryol. 32: 36. 1905

Type: Canada, Brit. Columbia, Joho valley, rocks. 6.8.1904 leg. J. Macoun. S, reg. nr. B3378 (lectotype, here designated). Syntype: Canada, Brit. Columbia, Pipestrone Pass, 7000 ft, rocks. 5.7.1904 leg. J. Macoun, three duplicates in S.

Synonyms:

Grimmia andreaeoides Limpr., Die Laubmoose Deutschlands, Oesterreichs und der Schweiz 1: 776–777. 1888, **syn. nov.**

(*Didymodon rigidulus* subsp. *andreaeoides* (Limpr.) Wijk & Margad., Taxon 9: 50. 1960. *Barbula rigidula* subsp. *andreaeoides* (Limpr.) Culm., Revue Bryologique 40: 42. 1912).

Type: [Austria] Tirol: Kitzbühler Horn, Thonschiefer u. Kalk, 1990 m. 13.8.1882 leg. J. Breidler (sub *Didymodon rigidulus* forma *gemmipara*?), BP. (lectotype, here designated).

Syntype: Salzburg: Keeskar im Obersulzbachthal im Pinzgau, 26–2700 m. 14.8.1879 leg J. Breidler, BP. isosyntype JE.

Barbula andreaeoides Kindb., Rev. Bryol. 32: 36. 1905.

TYPE: Canada, Brit. Columbia, McArthurs Pass, 7500 ft, rocks. 10.8.1904 leg. J. Macoun, herb. S, reg. Nr. B3375. (Lectotype, here designated; 2 isolectotypes in S!)

Plants in dense low cushions or tufts, dull reddish brown, ferrugineous or dark brown, young or shaded parts dull green. *Stems* ± erect, irregularly branching, with numerous flagelliform innovations, about 5 to 10 mm long including the dead parts of the stems. Cross-section ± rounded pentagonal, only to 0.15 mm in diameter, central strand absent or vestigial, inner cortex formed by thick-walled cells, their walls brownish, sclerodermis absent, weak hyalodermis of enlarged, less thickened cells present. *Axillary hairs* 40–80 μm long, 3–4 celled, basal cell short, brownish. *Leaves* appressed, sometimes slightly spiralled around stem when dry, erect-spreading when moist, (0.3-) 0.5–0.9 (-1.1) mm long and 0.25–0.35 mm wide, 1.5–2.5 times longer than wide, larger leaves from ovate-elongate base obtusely lanceolate, shorter leaves essentially ovate, obtusely keeled to U-shaped in cross-section, apex usually obtuse. Leaves of deciduous flagelliform innovations markedly concave, cochleariform, suborbicular, often wider than long, obtusely apiculate, frequently grading to the normal shape upwards, if the flagellum does not drop off. *Costa* weak, 20–45 μm wide near base, slightly widening towards the upper part of leaf, typically ending 1–4 cells below apex but sometimes percurrent or obtusely excurrent up to 20% of the leaf length, especially in the perichaetial leaves. Costa of the basal flagellum leaves greatly reduced to entirely absent. Costal superficial cells continuous from lamina both ventrally and dorsally, isodiametric in about the upper two-thirds of the leaf length, shortly rectangular in the basal third. Cross section elliptical, in basal part flat on the ventral side, showing ventrally and dorsally developed epidermis, inner cells in usually one row, essentially undifferentiated, of the substereid type or as guide cells, in larger leaves sometimes several dorsal stereids present. *Margins* entire or papillosely or mammillosely crenulate, recurved from about $\frac{1}{4}$ to $\frac{2}{3}$ of the leaf length but often plane (about $\frac{1}{3}$ of the studied leaves), always plane on the flagellum leaves, unistratose or rarely bistratose near apex. Upper lamina *cells* isodiametric, ± rounded, slightly to heavily thick-walled, (6-) 8–12 (-17) μm wide (the walls constituting (10–35% of the cells width), more or less papillose with conical or C-shaped papillae, especially in the apical region, rarely smooth. Cells of the basal flagellum leaves smooth. Basal paracostal cells shortly rectangular, (5-) 8–12 (-19) μm wide and (8-) 9–25 (-35) μm long (the walls constituting 15–40% of the cell width), (0.7-) 1.1–2.0 (-4.0) : 1, mixed with angular cells especially on the transition towards the upper cells, brownish, with thick walls, smooth. Towards margins cells shorter, sometimes wider than long. *Vegetative propagation* by means of deciduous flagella in leaf axils. Apparently dioicous. *Archegonia* terminal, up to *ca* 550 μm long, surrounded by usually conspicuously larger perichaetial leaves (to 1.5 mm) with more excurrent costa. *Antheridia* and *sporophytes* unknown.

VARIABILITY

The plant is usually described as not being variable but it is in fact highly polymorphic in some characters. These include in particular the form and length of the leaves, the

degree of excurrence of the costa, the papillosity of the leaf cells and the width of the cell walls and cell size (see description).

The most constant characters are the cross-section of stem and leaf costa, brownish colour, and the presence of flagelliform innovations in the leaf axils with suborbicular and cochleariform proximal leaves. In the course of our studies, two highly deviant forms were found, which cannot at present be assigned to either *D. subandreaeoides* or another species of the genus with security, due to the scarcity of the available material and the absence of transitive forms. One of them, known to-date from two sites in the Austrian Alps approaches *Didymodon asperifolius* (Mitt.) H. A.Crum, Steere & L. E.Anderson in its habit and dimensions, the other from Clogwyn du'r Arddu (Wales, U.K.) mentioned above is in habit and anatomical and morphological details identical to *D. subandreaeoides* but its numerous axillary gemmae of *Didymodon rigidulus*-type do not fit its known variability.

Differentiation

D. subandreaeoides is most likely to be confused with the most closely related *Didymodon* species — *D. rigidulus* and *D. asperifolius*, with which it also often occurs. It differs from the first mainly in (1) the constant presence of axillary flagella with reduced ovate to suborbicular concave leaves with plane and unistratose margins, (2) the absence of axillary gemmae (provided that the above mentioned specimen from Wales does not belong to *D. subandreaeoides*), (3) the shape of basal cells, which are usually hyaline (including the cell walls) and less thickened in *D. rigidulus*, and (4) the stem cross section, which in *D. rigidulus* shows a distinct central strand, no hyalodermis and ± thin walled cells of the inner cortex. Also the papillosity of the upper cells, costa cross-section, and usually unistratose margins of *D. subandreaeoides* differ from *D. rigidulus* but the evaluation of these characters may require some experience with the variability of both taxa.

Distinction from *D. asperifolius* may prove much more difficult in individual cases (particularly if the deviant plants from the Austrian Alps belong to *D. subandreaeoides*), although this is usually a much coarser plant. In *D. asperifolius*, specialized vegetative propagation is unknown, the leaf bases are more constricted, and the basal paracostal cells are typically much longer. Also, the costa in *D. asperifolius* is somewhat stronger, never excurrent, with well differentiated guide cells, both ventral and dorsal stereids, and the apex is always acute (it may however become eroded in both species). The stem cross-section does not show any trace of hyalodermis. The ferrugineous coloration is essentially identical in both species. Confusion is further possible with other less related or unrelated taxa — *Molendoa tenuinervis* (whether this is a distinct taxon or only a modification of *M. hornschuchiana* (Hook.) Lindb. ex Limpr.), and diverse *Schistidium* and *Grimmia* species. From *Molendoa tenuinervis*, which may have precisely the same leaf shape, it differs mainly in the position of gametangia (which are on short lateral branches in *Molendoa*), in the basal cells, hyaline and less thickened in *Molendoa*, in the upper cells, more heavily papillose and ± thin walled in *Molendoa*, in the specialized vegetative propagation, unknown in *Molendoa tenuinervis*, and the colour, which is usually dull or bluish green in *Molendoa tenuinervis*. From the superficially similar *Schistidium* and *Grimmia* species with muticous leaves, which might occur in similar habitats, it differs among other characters in the costa cross-section (homogeneous in *Schistidium* or with a hydroid strand in *Grimmia*, not biconvex in any of the species with muticous leaves).

Ecology

Didymodon subandreaeoides is an alpine species in Central Europe (the localities usually lie at between 900 and 3000 m, but a single locality at only 550 m is known in Slovakia), in Northern America also growing at low altitudes in tundra. It generally grows on rocks; in the Alps and Carpathians mainly on calcareous schist, marble, limestone, dolomite, greenstone and similar types of base-rich bedrock. It avoids poor siliceous rocks like gneiss or granite. From our experience in Europe, it seems to avoid carbonate rocks without a distinct content of silicates at lower altitudes, whereas in the upper alpine zone its ecological amplitude is much wider, tolerating pure limestone and dolomite. The species prefers dry and sunny, S.-, S.W.- and S.-E.-facing rock walls and ledges. In the upper alpine zone it also occurs on N.-facing slopes, colonizing vertical and inclined rock faces. At somewhat protected sites, particularly below the tree-line, it is able to grow directly on rough surfaces, otherwise it usually becomes established in small fissures. Frequently, especially in exposed alpine habitats, the flagella regenerate within or among the cushions of basiphilous *Grimmia* (*G. tergestina* Tomm. ex Bruch, Schimper & W.Gümbel, *G. poecilostoma* Cardot & Sebille, *G. anodon* Bruch & Schimp.) or *Schistidium* spp. (*S. robustum* (Nees & Hornsch.) H. H.Blom, *S. atrofuscum* (Schimp.) Limpr., *S. brunnescens* Limpr. subsp. *brunnescens* and others). *D. subandreaeoides* is generally restricted to habitats which are not or only temporarily covered by snow in winter. Therefore it avoids boulders unless these are sufficiently large and exposed to the wind. As far as we know, *D. subandreaeoides* has never been found in man-made habitats.

Although *D. subandreaeoides* is a rather delicate plant, it expands its cushions due to the massive development of branches from the leaf axils at the expenses of the 'host' species, which is not infrequently displaced completely. The species is normally not overgrown by larger or competitively stronger mosses but sometimes it might be overgrown by lichens. As the rhizoidal development is limited, the larger and ± isolated cushions become unstable and

drop off. *D. subandreaeoides* is never the dominant species, even in its optimum habitats.

Infrageneric mixed stands occur rather frequently, particularly with *D. rigidulus*, *D. icmadophilus* (Schimp. ex Müll. Hal.) K. Saito and the ecologically close but rare *D. johansenii* (R. S.Williams) H. A.Crum, in the upper alpine zone also with *D. asperifolius*. Other commonly associated species are the above mentioned *Grimmia* and *Schistidium* species, *Tortella tortuosa* (Hedw.) Limpr., *T. bambergeri* (Schimp.) Broth., *Ditrichum flexicaule* (Schwägr.) Hampe, *Orthotrichum cupulatum* Hoffm. ex Brid., *Pseudoleskeella catenulata* (Brid. ex Schrad.) Kindb. or *Hypnum vaucheri* Lesq. On N.-facing slopes in the upper alpine zone of the limestone mountains of the Eastern Alps it is frequently and typically associated with *Schistidium grande* Poelt. On periodically irrigated, sloping rock surfaces it sometimes grows within extensive stands of *Schistidium brunnescens* subsp. *brunnescens*. The companions on subneutral rocks may be moderately acidophilous mosses like *Grimmia unicolor* Hook. or *G. funalis* (Schwägr.) Bruch & Schimp.

Distribution

The distribution area of *D. subandreaeoides* includes the Beringian part of Arctic Russia (Ignatov & Afonina, 1992), north-western North America (from Alaska along the Cordillera mountain range south to Colorado: Zander, 1998), the French, Swiss, German, and Austrian Alps (no specimens yet seen from the Italian territory), and the Carpathians (Belianské Tatry and Malá Fatra Mts. in Slovakia, Făgăraş Mts. in Romania, it can be expected also in the Ukrainian Carpathians). The distribution pattern implies that the taxon is rather old (as it is absent from areas glaciated during the Pleistocene like Scandinavia and Siberia) and seems to prefer areas with continental climatic conditions.

Selected Specimens Studied

CANADA: **British Columbia:** Joho valley, rocks. 6.8.1904 leg. J. Macoun (S); Pipestone Pass, 7000 ft, rocks. 5.7.1904 leg. J. Macoun (S); McArthurs Pass, 7500 ft, rocks. 10.8.1904 leg. J. Macoun (S). **Yukon:** Bonnet Plume Range, Pinguicula Lake: 64°42′N, 133°26′W, 2800–3200 ft elev. On NE facing slope of mtn, at NW end of lake, in calcareous, alpine tundra with mesic limestone outcrops, 21.7.1976 leg. D. H. Vitt (S).

AUSTRIA: **Carinthia:** Hohe Tauern: Franz-Josefs-Höhe, 1904 leg. Nicholson & Dixon (FI) — Winkl, path Gößnitzfall-Bruchetalm, 1400–1450 m, Kučera E1591, E1601 (PR); Gurktaler Alpen: Rinsennock, SE-Seite des Gipfels, 2320–2330 m, Köckinger (GZU). **Upper Austria:** Warscheneck, ca. 2350 m, Köckinger 98-523 (GZU). **Salzburg:** Hohe Tauern: Keeskar im Obersulzbachthal im Pinzgau, 26–2700 m, 1879 leg. J. Breidler (BP, JE); Radstädter Tauern, W Weißeck, Südhang unterh. der Riedlingscharte, ca. 2180 m, Köckinger 97-539 (GZU) — E Zalußenalm, S-Hang der Plankowitzspitze, ca. 1800 m, Köckinger 97-626 (GZU). **Styria:** Schladminger Tauern: Schiedeck, 2300–2330 m, Köckinger 88-112.2 (GZU) — Steinkarhöhe, N der Unt. Klafferscharte, ca. 2250 m, Köckinger 97-135.3 (GZU); Dachstein–Massiv: Eselstein, ca. 2350–2500 m, Köckinger 93-811, 93-817 (GZU); Rottenmanner Tauern: Kl. Geierkogel E. Hochschwung, ca. 1800 m, Köckinger 97-441, 97-494 (GZU); Hochschwab-Gruppe: Polster, SE side, ca. 1650 m, Köckinger 98-495 (GZU); Eisenerzer Alpen: S slopes of Mt. Wildfeld, 1690 m, Kučera E2767, E2774, E2776 (PR), Köckinger 98-929 (GZU); Wölzer Tauern: Gaistrumer Ofen bei Oberwölz, ca. 1000 m, Köckinger 96-302 (GZU). **Tyrol:** Kitzbühler Horn, 1990 m, 1882 leg. J. Breidler (BP); Allgäuer Alpen: Schochenalptal, 1520 m, 1996 leg. A. Schäfer-Verwimp (herb. Schäfer-Verwimp 19633); Hohe Tauern: Granatspitzgruppe: 1 km W Sudetendeutsche Hütte, ca. 2550 m, Köckinger 96-952 (GZU); Venedigergruppe: zwischen Zunagl und Muswand W Hinterbichl, ca. 2350 m, Köckinger 97-1180 (GZU); Glocknergruppe: Rocks NE Lucknerhaus, 2100 m, Kučera E2724 (PR).

FRANCE: **Savoie:** Dans le forêt de Zertan près Pralognan, 1907 leg. Sebelle (Z, PC); Peisey, 1930 leg. Abbé Guillaumet (PC).

GERMANY: **Bavaria:** Gipfel der Hochplatte, 1550 m, 1910 leg. H. Paul (M); Estergebirge, Krottenkopf, 1961 leg. J. Poelt (GZU); Zugspitze, 2900 m, 1997 leg. M. Preußing); Berchtesgaden, bei Funtensee, ca. 1630 m, 1920 leg. Th. Herzog (BP, JE); Kreis Garmisch-Partenkirchen: Osterfelder-Sattel zum Längenfelder, ca. 1860 m, 1989 leg. R. Lotto (herb. Meinunger); Kreis Füssen: Branderschrofen E Hohenschwangau, ca. 1750 m, 1996 leg. L. Meinunger (herb. Meinunger); Kreis Miesbach: Trainsjoch S Bayrischzell, ca. 1700 m, 1995 leg. L. Meinunger (herb. Meinunger).

ROMANIA: Făgăraş Mts., mons Királykő prope Zărneşti, ca. 1500 m, 1962 leg. L. Vajda (BP).

SLOVAKIA: Beseňová, travertines, ca. 550 m, 1958 leg. Z. Pilous (herb. Pilous); Belianské Tatry Mts.: sub monte Muráň, ca. 1680 m, 1946 leg. Z. Pilous (Z, PR); Mons Javorinka prope Podspády, 1500 m, 1962 leg. Á. Boros (BP); Hohe Tatra, Tokarnyn Wrch (= Tokáreň ca. 1200 m), 1906 leg. Györffy (JE). Malá Fatra Mts.: Chleb — skály na vrcholu, vápenec, 1951 leg. Z. Pilous (BRNM).

SWITZERLAND: **Bern:** Am Fuß des Eiger — Rotstock, ca. 2350 m, 1920 leg. Th. Herzog (JE); Kl. Scheidegg, ca. 2100 m, 1920 leg. Th. Herzog (JE); Gipfel des Männlichen, 2340 m, 1909 Culmann (Z); Unterhalb des Lauchenhors am Faulhornweg, 2050 m, 1912 Culmann (Z); Klus bei Kandersteg, 1360 m, 1909 Culmann (Z). **Obwalden:** Schiessplang, 2120 m, 1931 P. Fintan Greter (Z). **Valais:** Chaurion, 2400 m, 1902 leg. Amann (Z); Pont de Nant, 10.6.1894, unsigned (LAU).

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TAXONOMIC ADDITIONS AND CHANGES: *Didymodon subandreaeoides* (Kindb.) R.H.Zander (syn. *Grimmia andreaeoides* Limpr.).

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Appendix 2: Bryoflora of the Czech Republic

Paper 5: Kučera J., Váňa J. & Hradílek Z. 2012. Bryophyte flora of the Czech Republic: updated checklist and Red List and a brief analysis. – Preslia 84: 813–850.

Bryophyte flora of the Czech Republic: updated checklist and Red List and a brief analysis

Bryoflóra České republiky: aktualizace seznamu a červeného seznamu a stručná analýza

Dedicated to the centenary of the Czech Botanical Society (1912–2012)

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Kučera J., Váňa J. & Hradílek Z. (2012): Bryophyte flora of the Czech Republic: updated checklist and Red List and a brief analysis. – Preslia 84: 813–850.

The bryoflora of the Czech Republic is analysed using an updated version of the checklist that includes recent taxonomic and nomenclatural changes. In addition, the baseline data was completely revised using the IUCN 3.1 criteria. The main list includes 863 species of bryophytes (4 hornworts, 207 liverworts and 652 mosses) with 5 additional subspecies and 23 generally recognized varieties; 9 additional species are listed as of doubtful taxonomic status and 17 other species are evaluated as of uncertain occurrence. Of the 892 taxa evaluated, 46% qualified for inclusion in Red List categories (40 taxa in category RE, 70 in CR, 88 in EN, 93 in VU, 66 in LR-nt, 24 in DD-va and 30 in DD), while 54% are considered Least Concern (LC). We discuss the taxonomic problems that influenced our decisions when compiling both the check- and Red Lists, try to identify the alien, invasive and spreading species of bryophytes, and touch upon several phytogeographic aspects, including the questions of relictness and bryophyte endemics in the Czech bryoflora.

Keywords: central Europe, checklist, Czech Republic, endemics, relic, hornworts, liverworts, mosses, phytogeography, Red List

Introduction

The intensity with which the bryophyte flora of the Czech Republic has been studied since the end of 18th century has varied. Fortunately, the last 20 years is one of the periods of greatest bryological activity in the whole history of the states that existed at the territory of the current Czech Republic, which allows us to present a relatively complete checklist of species and assess their frequency of occurrence and list the threatened species categorized in terms of the potential threat to their survival.

Two major versions of the bryophyte checklist for the territory of the Czech Republic have been published over the last 15 years – the first by Váňa (1997, 1998) and a second by Kučera & Váňa (2003), which was followed by an updated version in Czech (Kučera & Váňa 2005). The last two checklists include Red Lists of Czech bryophytes, evaluated using the IUCN criteria in the latest version 3.1 (IUCN 2001). We continue our practice of simultaneously publishing check- and Red Lists, as this is the only way of evaluating all currently known taxa against the Red List criteria and statistically determining the current level of threat to this country's bryoflora.

Methods

For this compilation of an updated checklist, previous versions (Kučera & Váňa 2003, 2005), which were based on extensive revisions of herbarium material of critical taxa, were used as a basis. With respect to nomenclature and taxonomic considerations, such as generic and specific concepts, we attempted to update our previous concepts in accordance with recent published results except for those cases for which the last published treatments still await a broader consensus (notably the moss order *Hypnales* and moss genera *Bryum*, *Pohlia*, *Grimmia* and *Racomitrium*). In the case of mosses, our treatment mostly follows the European checklist of Hill et al. (2006), with the eventual differences listed in the synonymy or to improve the understanding explicitly commented on. The differences from the last published complete European checklists of liverworts and hornworts (Grolle & Long 2000, Söderström et al. 2002) were more numerous as a consequence of recent major systematic rearrangements based on the latest molecular studies.

The name changes of hepatics particularly affected the earlier wide delimitation of the genera *Anastrophyllum*, *Jamesoniella*, *Jungermannia* and *Lophozia* (Yatsentyuk et al. 2004, de Roo et al. 2007, Konstantinova & Vilnet 2009, Feldberg et al. 2010a, 2010b, Vilnet et al. 2011, while the genus *Apometzgeria* is no longer recognized as different from *Metzgeria* (Fuselier et al. 2011), and some of the species of the earlier defined *Marsupella* were transferred to *Gymnomitrion* following the treatment by Váňa et al. (2010). In mosses, the changes in comparison with Hill et al. (2006) applied particularly to the generic delimitations of *Amblystegiaceae*, *Calliergonaceae* (Hedenäs & Rosborg 2009, Vanderpoorten & Hedenäs 2009, Hedenäs 2011) and *Neckeraceae* (Olsson et al. 2011) and in addition different generic concepts applied to *Lescuraea*, *Hygrohypnum* and *Campylophyllum,* following Ignatov et al. (2007), *Polytrichastrum* and *Polytrichum* (Bell & Hyvönen 2010), *Dicranoweisia* (Ochyra et al. 2003), *Barbula* (Köckinger & Kučera 2011) and *Tortula*, which we understand to include *Phascum* and *Protobryum*. Other minor changes are commented on under individual taxa. For the ease of orientation, we have included cross-references (following the ⇒ sign) to generic names that differ from those used in the previous version and to the checklist of European mosses.

Author citations are mostly those used in previous versions of our checklists, over which much effort was spent tracing the correct spelling in cases when the commonly used authoritative sources (Index Muscorum, Index Hepaticarum, Grolle & Long 2000, Ochyra et al. 2003) differed. We have newly adopted the convention of Hill et al. 2006 of not citing the pre-Hedwigian names validated by Hedwig (1801). One new combination is proposed below.

The process by which we evaluated our taxa against the IUCN 3.1 criteria is described by Kučera & Váňa (2003). We continue to recognize the "Vanished" subcategory within Data Deficient taxa (DD-va), i.e. taxa not recorded for a long period of time (more than ≈30 years) but with a realistic chance of being refound, rather than distributing them into other categories, and the 'attention list' as a subcategory of Least Concern taxa (LC-att), which we use for less well known taxa for which there is limited information on their current distribution and the potential threat to them. Such taxa need to be closely monitored in the future as they might either qualify for inclusion in the Red List in future versions of the checklist or might prove not to be threatened.

Results

Composition of the moss flora

The bryoflora of the Czech Republic, based on present taxonomic concepts and current state of knowledge, contains 4 species of hornworts, 207 species of liverworts with two additional subspecific taxa and one additional variety, and 652 species of mosses with 3 additional subspecific taxa and 23 additional varieties. The hornworts are attributed to 3 genera, liverworts to 76 genera and mosses to 194 genera. Nine additional species are listed among the taxonomically problematic taxa, which occur or have been reported from the Czech Republic and 17 species and two additional infraspecific taxa that are reported but the records could not be verified based on the herbarium specimens. We were also able to exclude two additional historically reported species, in addition to 42 species excluded in previous versions of the checklist.

Red List

Of the 892 evaluated taxa, 411 (46%) qualified for Red Listing and included regionally extinct (RE), data deficient (DD) and lower risk (LR) taxa, while 480 taxa (54%) were evaluated as Least Concern and 120 of these are placed on the 'attention list'. Forty taxa are now thought to be extinct and 24 others are regarded Data Deficient-Vanished (DD-va). Thirty taxa are categorized as Data-Deficient in the strict sense (DD), i.e. those with existing recent records and 66 taxa are listed as Lower Risk-Near Threatened (LR-nt). 251 taxa (28%) are regarded as threatened, of which 70 are in the highest, Critically Endangered (CR) category, 88 in the Endangered (EN) category and 93 are regarded as Vulnerable (VU).

List of bryophyte taxa of the Czech Republic as of 2012[1]

(a) Accepted native and naturalized taxa

Hornworts

Anthoceros agrestis Paton **LC**
Anthoceros neesii Prosk. **EN** [C1]
Notothylas orbicularis (Schwein.) A. Gray **CR** [C2a(i)]
Phaeoceros carolinianus (Michx.) Prosk. **LC**

Liverworts

Anastrepta orcadensis (Hook.) Schiffn. **LC-att**
⇒ *Anastrophyllum* p. pte. – see under *Crossocalyx* and *Sphenolobus*
Anastrophyllum michauxii (F. Weber) H. Buch **EN** [B2ab(iii, iv, v); C2a(i)]
Aneura maxima (Schiffn.) Steph. **LR-nt** [D1] (annot. **1**)
Aneura pinguis (L.) Dumort. **LC**
Anthelia julacea (L.) Dumort. **VU** [D2]
Anthelia juratzkana (Limpr.) Trevis. **CR** [B1ab(iii, v)+2ab(iii, v), C2a(i, ii), D]
⇒ *Apometzgeria* – see under *Metzgeria*
⇒ *Asterella* p. pte. – see under *Mannia*
Asterella saccata (Wahlenb.) A. Evans **EN** [B2ab(iii, iv, v); C2a(i, ii); D1]
Barbilophozia barbata (Schmidel ex Schreb.) Loeske (*Lophozia barbata* (Schmidel ex Schreb.) Dumort.) **LC**
Barbilophozia hatcheri (A. Evans) Loeske (*Lophozia hatcheri* (A. Evans) Steph.) **LC**

Barbilophozia lycopodioides (Wallr.) Loeske (*Lophozia lycopodioides* (Wallr.) Cogn.) **LC**
Bazzania flaccida (Dumort.) Grolle **VU** [C1; D1]
Bazzania tricrenata (Wahlenb.) Lindb. **LR-nt** [C1]
Bazzania trilobata (L.) Gray (incl. var. *depauperata* (Müll. Frib.) Grolle) **LC**
Biantheridion undulifolium (Nees) Konst. et Vilnet (*Jamesoniella undulifolia* (Nees) Müll. Frib.) **RE**
Blasia pusilla L. **LC**
Blepharostoma trichophyllum (L.) Dumort. **LC –** only var. *trichophyllum*
Calypogeia azurea Stotler et Crotz **LC**
Calypogeia fissa (L.) Raddi **LR-nt** [D1]
Calypogeia integristipula Steph. **LC**
Calypogeia muelleriana (Schiffn.) Müll. Frib. **LC**
Calypogeia neesiana (C. Massal. et Carestia) Müll. Frib. **LC**
Calypogeia sphagnicola (Arnell et J. Perss.) Warnst. et Loeske **LR-nt** [B2ab(iii, iv, v); D1] (annot. **2**)
Calypogeia suecica (Arnell et J. Perss.) Müll. Frib. **LR-nt** [C1]
Cephalozia bicuspidata (L.) Dumort. **LC**
Cephalozia catenulata (Huebener) Lindb. **LR-nt** [B2ab(iii, iv, v); C1]
Cephalozia connivens (Dicks.) Lindb. **LC**
Cephalozia lacinulata J. B. Jack ex Spruce **RE**
Cephalozia leucantha Spruce **LR-nt** [B2ab(iii, iv, v); C1]
Cephalozia loitlesbergeri Schiffn. **VU** [D1]
Cephalozia lunulifolia (Dumort.) Dumort. **LC**
Cephalozia macrostachya Kaal. **VU** [D1]
Cephalozia pleniceps (Austin) Lindb. **VU** [B2ab(iii, iv, v); D1]
Cephaloziella divaricata (Sm.) Schiffn. **LC**
Cephaloziella elachista (J. B. Jack ex Gottsche et Rabenh.) Schiffn. **EN** [B1+2ab(iii, v); D1]
Cephaloziella elegans (Heeg) Schiffn. **CR** [D1]
Cephaloziella grimsulana (J. B. Jack ex Gottsche et Rabenh.) Lacout. **EN** [D1]
Cephaloziella hampeana (Nees) Schiffn. **LC-att**
Cephaloziella rubella (Nees) Warnst. **LC**
Cephaloziella spinigera (Lindb.) Warnst. **VU** [D1]
Cephaloziella stellulifera (Taylor ex Spruce) Schiffn. **CR** [D1]
Chiloscyphus coadunatus (Sw.) J. J. Engel et R. M. Schust. (*Lophocolea coadunata* (Sw.) Mont., *Chiloscyphus latifolius* (Nees) J. J. Engel et R. M. Schust.) **LC** (annot. **3**)

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[1] For the convenience of the readers, we briefly explain the abbreviations of the IUCN criteria used (IUCN 2001):

Criterion A (only A2a used) – reduction in population size based on (subcriterion A2) an observed, estimated, inferred or suspected population size reduction of ≈30% (category VU) over the last 10 years or 3 generations, whichever is the longer, where the reduction or its causes may not have ceased or may not be understood or may not be reversible, based on (A2a) direct observation.

Criterion B – geographic range in the form of either B1 (extent of occurrence) or B2 (area of occupancy) or both and estimates indicating at least two of the following: (B1/B2a) Severely fragmented or known to exist at only 1 (CR), <5 (EN), <10 (VU) locations; (B1/B2b) Continuing decline, observed, inferred or projected, in any of the following: (i) extent of occurrence, (ii) area of occupancy, (iii) area, extent and/or quality of habitat, (iv) number of locations or subpopulations, (v) number of mature individuals; (B1/B2c) Extreme fluctuations in any of the following: (i) extent of occurrence, (ii) area of occupancy, (iii) number of locations or subpopulations, (iv) number of mature individuals. The limits for qualifying to CR, EN and VU categories are < 100 km^2, < 5000 km^2 and < 20, 000 km^2 in B1 and <10 km^2, < 500 km^2 and < 2000 km^2 in B2, respectively.

Criterion C – population size estimated to be a number fewer than the limiting number of individuals and either (C1) An estimated continuing decline or (C2) A continuing decline, observed, projected, or inferred, in numbers of mature individuals and at least one of the following: (C2a) Population structure in the form of (C2a(i)) no subpopulation estimated to contain more than the limiting number of individuals or (C2a(ii) at least the limiting number of mature individuals in one subpopulation; (C2b) Extreme fluctuations in number of mature individuals. The limits for qualifying for individual categories are set to the following: for CR, C1 = the decline of at least 25% within 3 years or one generation, whichever is longer, C2a(i) < 50 mature individuals, C2a(ii) at least 90% of mature individuals in one subpopulation. For EN, C1 = at least 20% within 5 years or 2 generations, C2a(i) < 250 mature individuals, C2a(ii) at least 95% of mature individuals in one subpopulation. For VU, C1 = at least 10% within 10 years or 3 generations, C2a(i) < 100 mature individuals, C2a(ii) all mature individuals in one subpopulation.

Chiloscyphus cuspidatus (Nees) J. J. Engel et R. M. Schust. (*Lophocolea bidentata* (L.) Dumort., *Lophocolea cuspidata* (Nees) Limpr.) **LC-att** (annot. **3**)
Chiloscyphus minor (Nees) J. J. Engel et R. M. Schust. (*Lophocolea minor* Nees) **LC**
Chiloscyphus pallescens (Ehrh. ex Hoffm.) Dumort. (*Chiloscyphus polyanthos* var. *pallescens* (Ehrh. ex Hoffm.) C. Hartm.) **LC-att** (annot. **3**)
Chiloscyphus polyanthos (L.) Corda **LC**
Chiloscyphus profundus (Nees) J. J. Engel et R. M. Schust. (*Lophocolea heterophylla* (Schrad.) Dumort.) **LC**
Cladopodiella fluitans (Nees) H. Buch **EN** [B2ab(iii, iv, v); C2a(i); D1]
Cladopodiella francisci (Hook.) H. Buch ex Jörg. **CR** [B2ab(v); C2a(i); D1]
Cololejeunea calcarea (Lib.) Schiffn. **VU** [D1]
Cololejeunea rossettiana (C. Massal.) Schiffn. **VU** [D1]
Conocephalum conicum (L.) Dumort. **LC**
Conocephalum salebrosum Szweyk., Buczkowska et Odrzykoski **LC** (annot. **4**)
Crossocalyx hellerianus (Nees ex Lindenb.) Meyl. (*Anastrophyllum hellerianum* (Nees ex Lindenb.) R. M. Schust.) **EN** [B2ab(iv); C2a(i, ii); D1]
Diplophyllum albicans (L.) Dumort. **LC**
Diplophyllum obtusifolium (Hook.) Dumort. **LC**
Diplophyllum taxifolium (Wahlenb.) Dumort. **LC**
Endogemma caespiticia (Lindenb.) Konstant., Vilnet et A.V. Troitsky (*Jungermannia caespiticia* Lindenb.) **LC-att**
Fossombronia angulosa (Dicks.) Raddi **RE**
Fossombronia foveolata Lindb. **EN** [B2ab(iii, iv, v)]
Fossombronia pusilla (L.) Nees **DD-va**
Fossombronia wondraczekii (Corda) Lindb. **LC**
Frullania dilatata (L.) Dumort. **LC**
Frullania fragilifolia (Taylor) Gottsche, Lindenb. et Nees **CR** [C2a(i); D1]
Frullania inflata Gottsche **EN** [B1+2ab(iii, v); C2a(i); D]
Frullania tamarisci (L.) Dumort. **LR-nt** [C1]
Geocalyx graveolens (Schrad.) Nees **VU** [D1]
Gymnocolea inflata (Huds.) Dumort. **LC**
Gymnomitrion adustum Nees (*Marsupella adusta* (Nees) Spruce) **RE**
Gymnomitrion alpinum (Gottsche ex Husn.) Schiffn. (*Marsupella alpina* (Gottsche ex Husn.) Bernet) **EN** [D1]
Gymnomitrion brevissimum (Schleich. ex Dumort.) Warnst. (*Marsupella brevissima* (Dumort.) Grolle) **RE**
Gymnomitrion concinnatum (Lightf.) Corda **LC-att**
Gymnomitrion corallioides Nees **CR** [B2ab(iii, iv, v); C1+C2a(i); D1]
Gymnomitrion obtusum Lindb. **RE**
Haplomitrium hookeri (Sm.) Nees **CR** [B1+2ab(iv, v); C2a(i)]
Harpanthus flotovianus (Nees) Nees **VU** [C2a(i)]
Harpanthus scutatus (F. Weber et D. Mohr) Spruce **EN** [C2a(i)]
Heterogemma capitata (Hook.) Konst. et Vilnet (*Lophozia capitata* (Hook.) Macoun) **VU** [D1+D2]
Hygrobiella laxifolia (Hook.) Spruce **VU** [D2]
Isopaches bicrenatus (Schmidel ex Hoffm.) H. Buch (*Lophozia bicrenata* (Schmidel ex Hoffm.) Dumort.) **LR-nt** [D1]
⇒ *Jamesoniella* – see under *Biantheridion* and *Syzygiella*
⇒ *Jungermannia* p. pte. – see under *Endogemma*, *Liochlaena* and *Solenostoma*
Jungermannia atrovirens Dumort. **VU** [D1+D2]
Jungermannia pumila With. **LR-nt** [D1]
Kurzia pauciflora (Dicks.) Grolle **VU** [D1]
Kurzia sylvatica (A. Evans) Grolle **LC-att**
Kurzia trichoclados (Müll. Frib.) Grolle **EN** [C2a(i, ii); D1]
Leiocolea badensis (Gottsche) Jörg. (*Lophozia badensis* (Gottsche) Schiffn.) **VU** [D2]
Leiocolea bantriensis (Hook.) Jörg. (*Lophozia bantriensis* (Hook.) Steph.) **LC**
Leiocolea heterocolpos (Thed. ex Hartm.) H. Buch (*Lophozia heterocolpos* (Thed. ex Hartm.) M. Howe) **CR** [B2ab(iii, iv, v); C2a(i)]
⇒ *Lejeunea* p. pte. – see under *Microlejeunea*
Lejeunea cavifolia (Ehrh.) Lindb. **LC**
Lepidozia reptans (L.) Dumort. **LC**
Liochlaena lanceolata Nees (*Jungermannia leiantha* Grolle) **LR-nt** [D1]
Liochlaena subulata (A. Evans) Schljakov (*Jungermannia subulata* A. Evans) **CR** [B2ab(v); C2a(i); D1]

⇒ *Lophozia* p. pte. – see under *Barbilophozia*, *Heterogemma*, *Isopaches*, *Leiocolea*, *Lophoziopsis*, *Obtusifolium*, *Orthocaulis*, *Pseudolophozia*, *Schistochilopsis*, *Schljakovia* and *Schljakovianthus*
⇒ *Lophocolea* – see under *Chiloscyphus*
Lophozia ascendens (Warnst.) R. M. Schust. **EN** [C2a(i); D1]
Lophozia guttulata (Lindb.) A. Evans (*Lophozia longiflora* auct.) **LC** (annot. **5**)
Lophozia ventricosa (Dicks.) Dumort.
var. *ventricosa* **LC**
var. *silvicola* (H. Buch) E. W. Jones **LC-att**
Lophozia wenzelii (Nees) Steph. **CR** [B2ab(iii, iv, v)]
Lophoziopsis excisa (Dicks.) Konst. et Vilnet (*Lophozia excisa* (Dicks.) Dumort.) **LC-att**
Lophoziopsis longidens (Lindb.) Konst. et Vilnet (*Lophozia longidens* (Lindb.) Macoun) **LR-nt** [D1]
Lunularia cruciata (L.) Dumort. **LC**
Mannia fragrans (Balbis) Frye et L. Clark **LR-nt** [B2ab(iii, iv, v); C1]
Mannia gracilis (F. Weber) Schill et D. G. Long (*Asterella gracilis* (F. Weber) Underw.) **EN** [B2ab(iii, iv, v); C2a(i)]
Mannia triandra (Scop.) Grolle **CR** [B1ab(iii, v)+2ab(iii, v), C2a(i, ii), D]
Marchantia polymorpha L.
subsp. *polymorpha* **LC**
subsp. *montivagans* Bischl. et Boisselier **LC-att**
subsp. *ruderalis* Bischl. et Boisselier **LC**
⇒ *Marsupella* p. pte. – see under *Gymnomitrion*
Marsupella aquatica (Lindenb.) Schiffn. (*Marsupella emarginata* var. *aquatica* (Lindenb). Dumort.) **LC**
Marsupella emarginata (Ehrh.) Dumort. **LC**
Marsupella funckii (F. Weber et D. Mohr) Dumort. **LR-nt** [D1]
Marsupella sparsifolia (Lindb.) Dumort. **CR** [B2ab(iii, v); C2a(i)]
Marsupella sphacelata (Gieseke ex Lindenb.) Dumort. **LC**
Marsupella sprucei (Limpr.) Bernet **EN** [B2ab(iii, v); C2a(i)]
Metzgeria conjugata Lindb. **LC**
Metzgeria furcata (L.) Dumort. **LC**
Metzgeria pubescens (Schrank) Raddi (*Apometzgeria pubescens* (Schrank) Kuwah.) **LC-att**
Metzgeria violacea (Ach.) Dumort. **VU** [D1]
Microlejeunea ulicina (Taylor) A. Evans (*Lejeunea ulicina* (Taylor) Gottsche, Lindenb. et Nees) **CR** [D1] (annot. **6**)
Moerckia blyttii (Moerch) Brockm. **EN** [C2a(i)]
Moerckia flotoviana (Nees) Schiffn. **CR** [C2a(i); D1] (annot. **7**)
Mylia anomala (Hook.) Gray (*Leiomylia anomala* (Hook.) J. J. Engel et Braggins) **LC**
Mylia taylorii (Hook.) Gray **LC**
Nardia compressa (Hook.) Gray **VU** [D2]
Nardia geoscyphus (De Not.) Lindb. **LC**
Nardia insecta Lindb. **DD-va**
Nardia scalaris Gray **LC**
Nowellia curvifolia (Dicks.) Mitt. **LC-att**
Obtusifolium obtusum (Lindb.) S. W. Arnell (*Lophozia obtusa* (Lindb.) A. Evans) **EN** [C2a(i)]
Odontoschisma denudatum (Mart.) Dumort. **LC-att**
Odontoschisma sphagni (Dicks.) Dumort. **EN** [B2ab(iii, iv, v); C2a(i)]
Orthocaulis atlanticus (Kaal.) H. Buch (*Lophozia atlantica* (Kaal.) Müll. Frib., *Barbilophozia atlantica* (Kaal.) Müll. Frib.) **RE**
Orthocaulis attenuatus (Mart.) A. Evans (*Lophozia attenuata* (Mart.) Dumort., *Neoorthocaulis attenuatus* (Mart.) L. Söderstr., Roo et Hedd., *Barbilophozia attenuata* (Mart.) Loeske) **LC**
Orthocaulis floerkei (F. Weber et D. Mohr) H. Buch (*Lophozia floerkei* (F. Weber et D. Mohr) Schiffn., *Neoorthocaulis floerkei* (F. Weber et D. Mohr) H. Buch, *Barbilophozia floerkei* (F. Weber et D. Mohr) Loeske) **LC**
Oxymitra incrassata (Brot.) Sérgio et Sim-Sim **EN** [B2ab(iii, iv, v)]
Pallavicinia lyellii (Hook.) Carruth. **RE**
Pedinophyllum interruptum (Nees) Kaal. **LC-att**
Pellia endiviifolia (Dicks.) Dumort. **LC**
Pellia epiphylla (L.) Corda **LC**
Pellia neesiana (Gottsche) Limpr. **LC**
Plagiochila asplenioides (L.) Dumort. **LC**

Plagiochila porelloides (Torr. ex Nees) Lindenb. **LC**
Porella arboris-vitae (With.) Grolle **LR-nt** [A2(a); C1+C2a(i); D1]
Porella cordaeana (Huebener) Moore **LR-nt** [C1+C2a(i); D1]
Porella platyphylla (L.) Pfeiff. **LC**
Preissia quadrata (Scop.) Nees **LC**
Pseudolophozia sudetica (Nees ex Huebener) Konst. et Vilnet (*Lophozia sudetica* (Nees ex Huebener) Grolle, *Barbilophozia sudetica* (Nees ex Huebener) L. Söderstr., Roo et Hedd.) **LC**
Ptilidium ciliare (L.) Hampe **LC**
Ptilidium pulcherrimum (G. Weber) Vainio **LC**
Radula complanata (L.) Dumort. **LC**
Radula lindenbergiana Gottsche ex C. Hartm. **VU** [D2]
Reboulia hemisphaerica (L.) Raddi **LR-nt** [C1; D1]
Riccardia chamedryfolia (With.) Grolle **VU** [B2ab(iii, v); D1]
Riccardia incurvata Lindb. **VU** [B2ab(iii, v); D1]
Riccardia latifrons (Lindb.) Lindb. **LC-att**
Riccardia multifida (L.) Gray **LC-att**
Riccardia palmata (Hedw.) Carruth. **LC-att**
Riccia bifurca Hoffm. **LC-att**
Riccia canaliculata Hoffm. **DD-va**
Riccia cavernosa Hoffm. **LR-nt** [B2ab(iii, iv, v)c(iii, iv); C2b]
Riccia ciliata Hoffm. (*R. crinita* Taylor, *R. canescens* Steph., *R. trichocarpa* M. Howe) **LR-nt** [C2a(i)] (annot. **8**)
Riccia ciliifera Link ex Lindenb. **LR-nt** [B2ab(iii, iv, v); C2a(i)]
Riccia fluitans L. **LC**
Riccia glauca L. **LC**
Riccia huebeneriana Lindenb. **EN** [B2ab(iii, iv, v)c(iii, iv); C2a(i)]
Riccia papillosa Moris **CR** [B1+2ab(iii, iv, v)]
Riccia rhenana Lorb. ex Müll. Frib. **LR-nt** [C2a(i)]
Riccia sorocarpa Bisch. **LC**
Riccia warnstorfii Limpr. ex Warnst. **VU** [C2a(i)]
Ricciocarpos natans (L.) Corda **LR-nt** [B2ab(iii, iv, v)c(iii, iv); C2b]
Scapania aequiloba (Schwägr.) Dumort. **LR-nt** [B2ab(iv, v)]
Scapania apiculata Spruce **CR** [B1+2ab(iii, iv, v)] (annot. **9**)
Scapania aspera Bernet et M. Bernet **VU** [B2ab(iv, v); D1]
Scapania calcicola (Arnell et J. Perss.) Ingham **EN** [B2ab(iv, v)]
Scapania carinthiaca J.B. Jack ex Lindb. (only in var. *massalongoi* Müll. Frib.) **RE**
Scapania compacta (A. Roth) Dumort. **DD-va**
Scapania curta (Mart.) Dumort. **LC**
Scapania cuspiduligera (Nees) Müll. Frib. **VU** [B2ab(iii); C2a(i); D1]
Scapania gymnostomophila Kaal. **EN** [C2a(i); D1]
Scapania helvetica Gottsche **CR** [C2a(i)]
Scapania irrigua (Nees) Nees **LC**
Scapania lingulata H. Buch **EN** [D1]
Scapania mucronata H. Buch **DD**
Scapania nemorea (L.) Grolle **LC**
Scapania paludicola Loeske et Müll. Frib. **VU** [B2ab(iii, iv, v); D1]
Scapania paludosa (Müll. Frib.) Müll. Frib. **VU** [D1]
Scapania parvifolia Warnst. **CR** [B1+2ab(iii, v); C2a(i, ii); D1]
Scapania praetervisa Meyl. **VU** [B2ab(iii); D1]
Scapania scandica (Arnell et H. Buch) Macvicar **DD**
Scapania subalpina (Nees ex Lindenb.) Dumort. **LR-nt** [D1]
Scapania uliginosa (Sw. ex Lindenb.) Dumort. **LC**
Scapania umbrosa (Schrad.) Dumort. **LC**
Scapania undulata (L.) Dumort. **LC**
Schistochilopsis grandiretis (Lindb. ex Kaal.) Konst. (*Lophozia grandiretis* (Lindb. ex Kaal.) Schiffn.) **VU** [B2ab(v)]
Schistochilopsis incisa (Schrad.) Konst. (*Lophozia incisa* (Schrad.) Dumort.) **LC**
Schistochilopsis opacifolia (Culm. ex Meyl.) Konst. (*Lophozia opacifolia* Culm. ex Meyl.) **DD-va**

Schljakovia kunzeana (Huebener) Konst. et Vilnet (*Lophozia kunzeana* (Huebener) A. Evans, *Barbilophozia kunzeana* (Huebener) Müll. Frib., *Orthocaulis kunzeanus* (Huebener) H. Buch) **EN** [B2ab(iii, iv, c; C2a(i); D1]
Schljakovianthus quadrilobus (Lindb.) Konst. et Vilnet (*Lophozia quadriloba* (Lindb.) A. Evans, *Barbilophozia quadriloba* (Lindb.) Loeske) **EN** [B2ab(iii)]
Solenostoma confertissimum (Nees) Schljakov (*Jungermannia confertissima* Nees) **VU** [D1+D2]
Solenostoma gracillimum (Mitt.) R. M. Schust. (*Jungermannia gracillima* Sm.) **LC**
Solenostoma hyalinum (Lyell) Mitt. (*Jungermannia hyalina* Lyell) **LR-nt** [D1]
Solenostoma obovatum (Nees) C. Massal. (*Jungermannia obovata* Nees) **LC**
Solenostoma sphaerocarpum (Hook.) Steph. (*Jungermannia sphaerocarpa* Hook.) **LC**
Solenostoma subellipticum (Lindb. ex Kaal.) R. M. Schust. (*Jungermannia subelliptica* (Lindb. ex Kaal.) Levier) **VU** [D1]
Sphenolobus minutus (Schreb.) Berggr. (*Anastrophyllum minutum* (Schreb.) R. M. Schust.) **LC** – only in var. *weberi* (Mart.) Schiffn.
Sphenolobus saxicola (Schrad.) Steph. (*Anastrophyllum saxicola* (Schrad.) R. M. Schust.) **VU** [D2]
Syzygiella autumnalis (DC.) Feldberg, Váňa, Hentschel et Heinrichs (*Jamesoniella autumnalis* (DC.) Steph.) **VU** [B2ab(iii, iv, v); C2a(i)]
Targionia hypophylla L. **CR** [B1+2ab(iii, v); C2a(i); D1]
Tetralophozia setiformis (Ehrh.) Schljakov **VU** [D2]
Trichocolea tomentella (Ehrh.) Dumort. **LC-att**
Tritomaria exsecta (Schmidel) Schiffn. ex Loeske **LC**
Tritomaria exsectiformis (Breidl.) Schiffn. ex Loeske **LC-att**
Tritomaria quinquedentata (Huds.) H. Buch **LC**

Mosses

Abietinella abietina (Hedw.) M. Fleisch. (*Thuidium abietinum* (Hedw.) Schimp.)
var. *abietina* **LC**
var. *hystricosa* (Mitt.) Sakurai (*Thuidium abietinum* var. *hystricosum* (Mitt.) Loeske et Lande) **DD**
Acaulon muticum (Hedw.) Müll. Hal. **LC-att**
Acaulon triquetrum (Spruce) Müll. Hal. **VU** [B2ab(iii); C2a(i)]
Alleniella besseri (Lobarzewski) S. Olsson, Enroth et D. Quandt (*Neckera besseri* (Lobarzewski) Jur.) **LC**
Alleniella complanata (Hedw.) S. Olsson, Enroth et D. Quandt (*Neckera complanata* (Hedw.) Huebener) **LC**
Aloina aloides (Koch ex Schultz) Kindb.
var. *aloides* **DD-va**
var. *ambigua* (Bruch et Schimp.) E. J. Craig (*Aloina ambigua* (Bruch et Schimp.) Limpr.) **EN** [B2ab(iii, iv, v)]
Aloina brevirostris (Hook. et Grev.) Kindb. **CR** [B2ab(iii, iv, v); C2a(i, ii)+C2b]
Aloina obliquifolia (Müll. Hal.) Broth. **LC**
Aloina rigida (Hedw.) Limpr. **LC**
Amblyodon dealbatus (Hedw.) P. Beauv. **CR** [B1+2ab(v); C2a(i, ii); D1]
⇒ *Amblystegium* p. pte. – see under *Hygroamblystegium*, *Pseudoamblystegium*, *Pseudocampylium* and *Serpoleskea*
Amblystegium serpens (Hedw.) Schimp. **LC**
Amphidium lapponicum (Hedw.) Schimp. **EN** [B1+2ab(iii, iv, v)]
Amphidium mougeotii (Bruch et Schimp.) Schimp. **LC**
Anacamptodon splachnoides (Froel. ex Brid.) Brid. **EN** [C2a(i)]
Andreaea crassinervia Bruch **CR** [B1+2ab(iii, v)]
Andreaea frigida Huebener **CR** [B1+2ab(iii, v); C2a(ii)]
Andreaea rothii F. Weber et D. Mohr
subsp. *rothii* **EN** [B2ab(iv, v); C1+C2a(i)]
subsp. *falcata* (Schimp.) Lindb. **LC-att**
Andreaea rupestris Hedw. **LC** – only in var. *rupestris*.
Anoectangium aestivum (Hedw.) Mitt. **EN** [B1+2ab(v); C2a(ii)]
Anomobryum concinnatum (Spruce) Lindb. (*Anomobryum julaceum* var. *concinnatum* (Spruce) J. E. Zetterst.) **CR** [B1+2ab(iii, v); C2a(ii)]
Anomodon attenuatus (Hedw.) Huebener **LC**
Anomodon longifolius (Schleich. ex Brid.) Hartm. **LC**
Anomodon rostratus (Hedw.) Schimp. **DD-va**
Anomodon rugelii (Müll. Hal.) Keissl. **VU** [B1+2ab(iii)]

Anomodon viticulosus (Hedw.) Hook. et Taylor **LC**
Antitrichia curtipendula (Hedw.) Brid. **LC-att**
Archidium alternifolium (Hedw.) Mitt. **CR** [B2ab(iii, v)]
Arctoa fulvella (Dicks.) Bruch et Schimp. **RE**
Atrichum angustatum (Brid.) Bruch et Schimp. **EN** [B2ab(iv); C2a(i)]
Atrichum flavisetum Mitt. (*Atrichum undulatum* var. *gracilisetum* Besch.) **DD**
Atrichum tenellum (Röhl.) Bruch et Schimp. **LR-nt** [B2ab(iii); C2a(i)]
Atrichum undulatum (Hedw.) P. Beauv. **LC**
Aulacomnium androgynum (Hedw.) Schwägr. **LC**
Aulacomnium palustre (Hedw.) Schwägr. **LC**
⇒ *Barbula* p. pte. – see under *Streblotrichum*
Barbula crocea (Brid.) F. Weber et D. Mohr) **CR** [C2a(i)] (annot. **10**)
Barbula unguiculata Hedw. **LC**
Bartramia halleriana Hedw. **LR-nt** [B2ab(iii, iv, v); C2a(i)]
Bartramia ithyphylla Brid. **LC-att**
Bartramia pomiformis Hedw. **LC**
Blindia acuta (Hedw.) Bruch et Schimp. **LC**
Brachydontium trichodes (F. Weber) Milde **LC-att**
Brachytheciastrum velutinum (Hedw.) Ignatov et Huttunen (*Brachythecium velutinum* (Hedw.) Schimp.) **LC**
⇒ *Brachythecium* p. pte. – see under *Brachytheciastrum* and *Sciuro-hypnum*
Brachythecium albicans (Hedw.) Schimp. **LC**
Brachythecium campestre (Müll. Hal.) Schimp. **LC-att**
Brachythecium capillaceum (F. Weber et D. Mohr) Giacom. **DD-va**
Brachythecium geheebii Milde **EN** [B2ab(iii, iv, v); C2a(i)]
Brachythecium glareosum (Bruch ex Spruce) Schimp. **LC**
Brachythecium laetum (Brid.) Schimp. **EN** [B2ab(iv, v)]
Brachythecium mildeanum (Schimp.) Schimp. **LC-att** – only in var. *mildeanum*
Brachythecium rivulare Schimp. **LC**
Brachythecium rutabulum (Hedw.) Schimp. **LC** – only in var. *rutabulum*
Brachythecium salebrosum (Hoffm. ex F. Weber et D. Mohr) Schimp. **LC**
Brachythecium tommasinii (Sendtn. ex Boulay) Ignatov et Huttunen (*Cirriphyllum tommasinii* (Sendt. ex Boulay) Grout)
var. *tommasinii* **LC**
var. *fagineum* (H. Müll. ex Milde) Jan Kučera, ***comb. nova***. Basionym: *Eurhynchium vaucheri* var. *fagineum* H. Müll. ex Milde, Bryologia Silesiaca 304. 1869. (*Rhynchostegiella tenuicaulis* (Spruce) Kartt., *Eurhynchium germanicum* Grebe) **CR** [B1+2ab(iii, v)] (annot. **11**)
Breidleria pratensis (W. D. J. Koch ex Spruce) Loeske (*Hypnum pratense* W. D. J. Koch ex Spruce) **LC-att**
Bryoerythrophyllum ferruginascens (Stirt.) Giacom. **LC-att**
Bryoerythrophyllum recurvirostrum (Hedw.) P. C. Chen **LC**
Bryum algovicum Sendtn. ex Müll. Hal. **DD-va**
Bryum alpinum Huds. ex With. **LR-nt** [C1]
Bryum archangelicum Bruch et Schimp. (*Bryum imbricatum* (Schwägr.) Bruch et Schimp.) **EN** [B2ab(iv, v)]
Bryum argenteum Hedw. **LC**
Bryum boreale (F. Weber et D. Mohr) Funck (*Bryum pallescens* Schleich. ex Schwägr., *Ptychostomum boreale* (F. Weber et D. Mohr) Ochyra et Bednarek-Ochyra, *Bryum lonchocaulon* Müll. Hal., *Bryum cirrhatum* Hoppe et Hornsch., *hom. illeg.*) **LC** (annot. **12**)
Bryum caespiticium Hedw. **LC**
Bryum capillare Hedw. **LC**
Bryum creberrimum Taylor **EN** [B2ab(iv, v)]
Bryum cyclophyllum (Schwägr.) Bruch et Schimp. **EN** [B2ab(iii, v)c(iii, iv); C2a(i)]
Bryum dichotomum Hedw. (*Bryum bicolor* Dicks.) **LC**
Bryum elegans Nees **LR-nt** [B2ab(iv, v)]
Bryum funkii Schwägr. (‘*funckii*’ auct.) **DD**
Bryum gemmiferum R. Wilczek et Demaret **LC-att** (annot. **13**)
Bryum intermedium (Brid.) Blandow **CR** [B2ab(v)]
Bryum klinggraeffii Schimp. **LC**
Bryum kunzei Hoppe et Hornsch. **DD**

Bryum longisetum Blandow ex Schwägr. **RE**
Bryum mildeanum Jur. **VU** [D1+D2]
Bryum moravicum Podp. (*Bryum laevifilum* Syed) **LC**
Bryum muehlenbeckii Bruch et Schimp. **LR-nt** [D2]
Bryum pallens Sw. ex Anon. **LC**
Bryum pseudotriquetrum (Hedw.) P. Gaertn., B. Mey. et Scherb.
var. *pseudotriquetrum* **LC**
var. *bimum* (Schreb.) Lilj. (*Bryum bimum* (Schreb.) Turner) **LC-att**
var. *neodamense* (Itzigs.) Buse (*Bryum neodamense* Itzigs.) **RE** (annot. **14**)
Bryum radiculosum Brid. **LC-att**
Bryum rubens Mitt. **LC**
Bryum ruderale Crundw. et Nyholm **DD**
Bryum sauteri Bruch et Schimp. **DD**
Bryum schleicheri Schwägr. **CR** [B1+2ab(v); C2a(ii)]
Bryum subapiculatum Hampe **LC**
Bryum tenuisetum Limpr. **DD**
Bryum torquescens Bruch et Schimp. **DD**
Bryum turbinatum (Hedw.) Turner **EN** [B1+2ab(iii, iv, v); C1+C2a(i); D1]
Bryum uliginosum (Brid.) Bruch et Schimp. **EN** [C2a(i)]
Bryum violaceum Crundw. et Nyholm **LC**
Bryum weigelii Spreng. **LC-att**
Buxbaumia aphylla Hedw. **LR-nt** [C1+C2a(i)]
Buxbaumia viridis (Moug. ex Lam. et DC.) Brid. ex Moug. et Nestl. **VU** [C2a(i)]
*Calliclad*ium *haldanianum* (Grev.) H. A. Crum **VU** [B2ab(iii); C2a(i)]
Calliergon cordifolium (Hedw.) Kindb. **LC**
Calliergon giganteum (Schimp.) Kindb. **VU** [B2ab(iii, iv, v); C2a(i)]
Calliergon megalophyllum Mikut. **RE**
Calliergonella cuspidata (Hedw.) Loeske **LC**
Calliergonella lindbergii (Mitt.) Hedenäs **LC**
Campyliadelphus chrysophyllus (Brid.) R. S. Chopra **LC**
Campyliadelphus elodes (Lindb.) Kanda **DD-va**
Campylidium calcareum (Crundw. et Nyholm) Ochyra (*Campylophyllum calcareum* (Crundw. et Nyholm) Hedenäs) **LC-att**
Campylidium sommerfeltii (Myrin) Ochyra (*Campylophyllum sommerfeltii* (Myrin) Hedenäs) **LC-att** (annot. **15**)
Campylium protensum (Brid.) Kindb. **LC-att**
Campylium stellatum (Hedw.) Lange et C. E. O. Jensen **LR-nt** [B2ab(iii, iv, v)]
⇒ *Campylophyllum* p. pte. – see under *Campylidium*
Campylophyllum halleri (Hedw.) M. Fleisch. **EN** [B2ab(iii, v)]
Campylopus flexuosus (Hedw.) Brid. **LC**
Campylopus fragilis (Brid.) Bruch et Schimp. **LC-att**
Campylopus introflexus (Hedw.) Brid. **LC**
Campylopus pyriformis (Schultz) Brid. **LC-att**
Campylopus subulatus Schimp. ex Milde **VU** [D1+D2]
Campylostelium saxicola (F. Weber et D. Mohr) Bruch et Schimp. **LR-nt** [C2a(i)]
Ceratodon purpureus (Hedw.) Brid. **LC** – only on subsp. *purpureus*
Cinclidotus aquaticus (Hedw.) Bruch et Schimp. **RE**
Cinclidotus fontinaloides (Hedw.) P. Beauv. **CR** [B1+2ab(iii, iv, v)]
Cinclidotus riparius (Host ex Brid.) Arn. **VU** [D2]
⇒ *Cirriphyllum* p. pte. – see under *Brachythecium*
Cirriphyllum crassinervium (Taylor) Loeske et M. Fleisch. (*Eurhynchium crassinervium* (Taylor) Schimp.) **LC**
Cirriphyllum piliferum (Hedw.) Grout **LC**
Cleistocarpidium palustre (Bruch et Schimp.) Ochyra et Bednarek-Ochyra **VU** [B2ab(iii, iv); C2a(i)]
Climacium dendroides (Hedw.) F. Weber et D. Mohr **LC**
Conardia compacta (Müll. Hal.) H. Rob. **EN** [B2ab(iii); C2a(i)]
Coscinodon cribrosus (Hedw.) Spruce **LC**
Cratoneuron filicinum (Hedw.) Spruce **LC**
Crossidium squamiferum (Viv.) Jur. (incl. var. *pottioideum* (De Not.) Mönk.) **CR** [B2ab(iii, v)]

Ctenidium molluscum (Hedw.) Mitt. **LC**
Cynodontium bruntonii (Sm.) Bruch et Schimp. **LC-att**
Cynodontium gracilescens (F. Weber et D. Mohr) Schimp. **VU** [D2]
Cynodontium polycarpon (Hedw.) Schimp. **LC**
Cynodontium strumiferum (Hedw.) Lindb. **LC**
Cynodontium tenellum (Schimp.) Limpr. **VU** [B2ab(iv, v); C2a(i)]
Dichelyma falcatum (Hedw.) Myrin **DD-va**
Dichodontium palustre (Dicks.) M. Stech **LC-att**
Dichodontium pellucidum (Hedw.) Schimp. **LC**
Dicranella cerviculata (Hedw.) Schimp. **LC**
Dicranella crispa (Hedw.) Schimp. **DD-va**
Dicranella heteromalla (Hedw.) Schimp. **LC**
Dicranella humilis R. Ruthe **VU** [D1+D2]
Dicranella rufescens (Dicks.) Schimp. **LC**
Dicranella schreberiana (Hedw.) Dixon **LC**
Dicranella staphylina H. Whitehouse **LC**
Dicranella subulata (Hedw.) Schimp. **VU** [C2a(i)]
Dicranella varia (Hedw.) Schimp. **LC**
Dicranodontium asperulum (Mitt.) Broth. **LR-nt** [B2ab(v); C1+C2a(i)]
Dicranodontium denudatum (Brid.) E. Britton **LC**
Dicranodontium uncinatum (Harv.) A. Jaeger **EN** [B2ab(iii, iv, v); C2a(i)]
⇒ *Dicranoweisia* p. pte. – see under *Hymenoloma*
Dicranoweisia cirrata (Hedw.) Lindb. **LC**
Dicranum bonjeanii De Not. **LR-nt** [B2ab(iii, iv, v); C1]
Dicranum elongatum Schleich. ex Schwägr. **EN** [B1+2ab(iii, iv, v); C2a(i)]
Dicranum flagellare Hedw. **LC-att**
Dicranum flexicaule Brid. **LC**
Dicranum fulvum Hook. **LC-att**
Dicranum fuscescens Sm. **LC**
Dicranum majus Sm. **VU** [B1+2ab(iii, iv, v); C1+2a(i)]
Dicranum montanum Hedw. **LC**
Dicranum muehlenbeckii Bruch et Schimp. **CR** [B1+2ab(iii, iv, v); C2a(ii)]
Dicranum polysetum Sw. ex Anon. **LC**
Dicranum scoparium Hedw. **LC**
Dicranum spadiceum J. E. Zetterst. **CR** [B1+2ab(iii, iv, v); C2a(i, ii)]
Dicranum spurium Hedw. **LC-att**
Dicranum tauricum Sapjegin **LC**
Dicranum undulatum Schrad. ex Brid. **LC-att**
Dicranum viride (Sull. et Lesq.) Lindb. **LR-nt** [B2ab(iii, iv, v)]
Didymodon acutus (Brid.) K. Saito **LC-att**
Didymodon cordatus Jur. **VU** [B2ab(iii)]
Didymodon fallax (Hedw.) R. H. Zander **LC**
Didymodon ferrugineus (Schimp. ex Besch.) M. O. Hill **LC**
Didymodon glaucus Ryan **VU** [C1+C2a(i)]
Didymodon insulanus (De Not.) M. O. Hill **LC**
Didymodon luridus Hornsch. **LR-nt** [B2ab(iii, iv, v)]
Didymodon rigidulus Hedw. **LC**
Didymodon sinuosus (Mitt.) Delogne **VU** [C2a(i)]
Didymodon spadiceus (Mitt.) Limpr. **LR-nt** [B2ab(iii, iv, v); C1]
Didymodon tophaceus (Brid.) Lisa **LC-att**
Didymodon umbrosus (Müll. Hal.) R. H. Zander (*Didymodon australasiae* var. *umbrosus* (Müll. Hal.) R. H. Zander) **NE** (alien; annot. **16**)
Didymodon validus Limpr. (*Didymodon rigidulus* var. *validus* (Limpr.) Düll) **EN** [B2ab(iii, iv, v); C2a(i)] (annot. **17**)
Didymodon vinealis (Brid.) R. H. Zander **EN** [B2ab(iii); C2a(i)]
Diphyscium foliosum (Hedw.) D. Mohr **LC-att**
Discelium nudum (Dicks.) Brid. **VU** [B2ab(iii, iv, v)]
Distichium capillaceum (Hedw.) Bruch et Schimp. **LC**

Distichium inclinatum (Hedw.) Bruch et Schimp. **EN** [B2ab(iii, iv, v)]
Ditrichum flexicaule (Schwägr.) Hampe **LC**
Ditrichum gracile (Mitt.) Kuntze **LC-att**
Ditrichum heteromallum (Hedw.) E. Britton **LC**
Ditrichum lineare (Sw.) Lindb. **LC-att**
Ditrichum pallidum (Hedw.) Hampe **VU** [B2ab(iii, iv, v)]
Ditrichum pusillum (Hedw.) Hampe **LC-att**
Ditrichum zonatum (Brid.) Kindb. **EN** [B2ab(iii, iv, v); C2a(i)]
Drepanocladus aduncus (Hedw.) Warnst. (*Drepanocladus polycarpos* (Blandow ex Voit) Warnst.) **LC**
Drepanocladus longifolius (Mitt.) Paris (*Drepanocladus capillifolius* (Warnst.) Warnst.) **DD-va**
Drepanocladus lycopodioides (Brid.) Warnst. (*Pseudocalliergon lycopodioides* (Brid.) Hedenäs) **RE**
Drepanocladus polygamus (Schimp.) Hedenäs **VU** [B2ab(iii, iv, v); C2a(i)]
Drepanocladus sendtneri (Schimp. ex H. Müll.) Warnst. **CR** [B2ab(iii, v)]
Drepanocladus sordidus (Müll. Hal.) Hedenäs **RE**
Drepanocladus trifarius (F. Weber et D. Mohr) Broth. ex Paris (*Pseudocalliergon trifarium* (F. Weber et D. Mohr) Loeske) **CR** [B2ab(iii, v); C2a(ii)]
Encalypta affinis R. Hedw. **RE**
Encalypta ciliata Hedw. **VU** [C2a(i)]
Encalypta rhaptocarpa Schwägr. (annot. **18**)
  var. *rhaptocarpa* **EN**
  var. *leptodon* Lindb. (*Encalypta trachymitria* Ripart) **DD**
  var. *spathulata* (Müll. Hal.) Husn. (*Encalypta spathulata* Müll. Hal.) **DD-va**
Encalypta streptocarpa Hedw. **LC**
Encalypta vulgaris Hedw. **LC**
Entodon concinnus (De Not.) Paris **LC-att**
Entodon schleicheri (Schimp.) Demet. **DD**
Entosthodon fascicularis (Hedw.) Müll. Hal. **VU** [C2a(i)]
Entosthodon muhlenbergii (Turner) Fife (*Funaria muhlenbergii* Turner) **CR** [B1+2ab(iii)c(iv)]
Entosthodon pulchellus (H. Philib.) Brugués (*Funaria pulchella* H. Philib.) **EN** [B1+2ab(iii)c(iii, iv); C2a(i)] (annot. **19**)
Ephemerum cohaerens (Hedw.) Hampe **DD-va**
Ephemerum minutissimum Lindb. **LC**
Ephemerum recurvifolium (Dicks.) Boulay **VU** [B2ab(iii)c(iii); C2a(i)]
Ephemerum serratum (Hedw.) Hampe **DD**
Eucladium verticillatum (With.) Bruch et Schimp. **LC**
Eurhynchiastrum pulchellum (Hedw.) Ignatov et Huttunen (*Eurhynchium pulchellum* (Hedw.) Jenn.) **LC-att**
⇒ *Eurhynchium* p. pte. – see under *Cirriphyllum*, *Eurhynchiastrum*, *Kindbergia*, *Microeurhynchium*, *Oxyrrhynchium*, *Plasteurhynchium*, and *Sciuro-hypnum*
Eurhynchium angustirete (Broth.) T. J. Kop. **LC**
Eurhynchium striatum (Hedw.) Schimp. **LC-att**
Exsertotheca crispa (Hedw.) S. Olsson, Enroth et D. Quandt (*Neckera crispa* Hedw.) **LC**
Fissidens adianthoides Hedw. **LC-att**
Fissidens arnoldii R. Ruthe **EN** [B1+2ab(iii, iv, v)c(iii, iv); C2a(i, ii)]
Fissidens bambergeri Milde **EN** [B2ab(iii, v); C2a(ii)] (annot. **20**)
Fissidens bryoides Hedw. **LC** – only in var. *bryoides*
Fissidens crassipes Wilson ex Bruch et Schimp. **DD-va** – only in subsp. *crassipes*
Fissidens dubius P. Beauv.
  var. *dubius* **LC**
  var. *mucronatus* (Breidl. ex Limpr.) Kartt., Hedenäs et L. Söderstr. **LC**
Fissidens exilis Hedw. **LC**
Fissidens fontanus (Bach. Pyl.) Steud. (*Octodiceras fontanum* (Bach. Pyl.) Lindb.) **LR-nt** [B2ab(iii)]
Fissidens gracilifolius Brugg.-Nann. et Nyholm **LC**
Fissidens gymnandrus Buse **LC**
Fissidens limbatus Sull. **DD** (annot. **21**)
Fissidens osmundoides Hedw. **LC-att**
Fissidens pusillus (Wilson) Milde **LC-att**
Fissidens rufulus Bruch et Schimp. **LR-nt** [B2ab(iii)c(iii, iv)]

Fissidens taxifolius Hedw. **LC** – only in subsp. *taxifolius*
Fissidens viridulus (Sw. ex Anon.) Wahlenb.
var. *viridulus* **LC**
var. *incurvus* (Starke ex Röhl.) Waldh. (*Fissidens incurvus* Starke ex Röhl.) **LC-att**
Fontinalis antipyretica Hedw. **LC** (annot. **22**)
Fontinalis hypnoides Hartm. **EN** [B2ab(iii)] – only in var. *hypnoides*
Fontinalis squamosa Hedw. **LC**
⇒ *Funaria* p. pte. – see under *Entosthodon*
Funaria hygrometrica Hedw. **LC**
Grimmia alpestris (Schleich. ex F. Weber et D. Mohr) Schleich. **VU**
Grimmia anodon Bruch et Schimp. **EN** [B2ab(iii, iv, v); C2a(i)]
Grimmia anomala Hampe ex Schimp. **VU** [D2]
Grimmia atrata Miel. ex Hornsch. **VU** [D2]
Grimmia caespiticia (Brid.) Jur. **DD**
Grimmia crinita Brid. **EN** [B2ab(iii, iv, v)]
Grimmia dissimulata E. Maier **DD** (annot. **23**)
Grimmia donniana Sm. **LC**
Grimmia elatior Bruch ex Bals.-Criv. et De Not. **CR** [B2ab(iii, v); C1+C2a(i, ii); D1]
Grimmia elongata Kaulf. **LR-nt** [D2]
Grimmia funalis (Schwägr.) Bruch et Schimp. **LC-att**
Grimmia hartmanii Schimp. **LC**
Grimmia incurva Schwägr. **LC**
Grimmia laevigata (Brid.) Brid. **LC**
Grimmia longirostris Hook. **LC**
Grimmia montana Bruch et Schimp. **LC-att**
Grimmia muehlenbeckii Schimp. **LC**
Grimmia orbicularis Bruch ex Wilson **LC-att**
Grimmia ovalis (Hedw.) Lindb. **LC**
Grimmia plagiopodia Hedw. **RE**
Grimmia pulvinata (Hedw.) Sm. **LC**
Grimmia ramondii (Lam. et DC.) Margad. **LC-att**
Grimmia sessitana De Not. (*Grimmia reflexidens* Müll. Hal. fide Muñoz (1998)) **VU** [D2]
Grimmia teretinervis Limpr. **CR** [B1+2ab(v); C2a(i, ii); D1]
Grimmia tergestina Tomm. ex Bruch et Schimp. **LC-att**
Grimmia torquata Hook. ex Drumm. **VU** [C2a(i)]
Grimmia trichophylla Grev. **LC-att**
Grimmia unicolor Hook. **RE**
Gymnostomum aeruginosum Sm. **LC** – only in var. *aeruginosum*
Gymnostomum calcareum Nees et Hornsch. **DD**
Gymnostomum viridulum Brid. **VU** [C2a(i)]
Gyroweisia tenuis (Hedw.) Schimp. **VU** [C2a(i)]
Hamatocaulis vernicosus (Mitt.) Hedenäs **VU** [A2(a); B2ab(iii, iv, v)]
Hedwigia ciliata (Hedw.) P. Beauv. (incl. var. *leucophaea* Bruch et Schimp.) **LC**
Hedwigia stellata Hedenäs **DD**
Helodium blandowii (F. Weber et D. Mohr) Warnst. **EN** [B2ab(iii, iv, v)]
Hennediella heimii (Hedw.) R. H. Zander **DD-va** – only in var. *heimii*
Herzogiella seligeri (Brid.) Z. Iwats. **LC**
Herzogiella striatella (Brid.) Z. Iwats. **LR-nt** [D2]
Heterocladium dimorphum (Brid.) Schimp. **LR-nt** [B2ab(iii)]
Heterocladium heteropterum (Brid.) Schimp. **LC**
Hilpertia velenovskyi (Schiffn.) R. H. Zander **CR** [B1+2ab(v); C2a(ii)]
Homalia trichomanoides (Hedw.) Schimp. **LC**
Homalothecium lutescens (Hedw.) H. Rob. (incl. var. *fallax* H. Philib. ex Schimp.) **LC**
Homalothecium philippeanum (Spruce) Schimp. **LC**
Homalothecium sericeum (Hedw.) Schimp. **LC**
Homomallium incurvatum (Schrad. ex Brid.) Loeske **LC**
Hookeria lucens (Hedw.) Sm. **LR-nt** [B2ab(iii)]

Hygroamblystegium fluviatile (Hedw.) Loeske (*Amblystegium fluviatile* (Hedw.) Schimp.) **LC** (annot. **24**)
Hygroamblystegium humile (P. Beauv.) Vanderp., Goffinet et Hedenäs (*Amblystegium humile* (P. Beauv.) Crundw.) **LC-att**
Hygroamblystegium tenax (Hedw.) Jenn. (*Amblystegium tenax* (Hedw.) C. E. O. Jensen) **LC-att**
Hygroamblystegium varium (Hedw.) Mönk. (*Amblystegium varium* (Hedw.) Lindb.) **LC**
Hygrohypnella ochracea (Turner ex Wilson) Ignatov et Ignatova (*Hygrohypnum ochraceum* (Turner ex Wilson) Loeske) **LC**
⇒ *Hygrohypnum* p. pte. – see under *Hygrohypnella* and *Ochyraea*
Hygrohypnum luridum (Hedw.) Jenn. **LC**
Hylocomiastrum pyrenaicum (Spruce) M. Fleisch. (*Hylocomium pyrenaicum* (Spruce) Lindb.) **VU** [B1+2ab(iii)]
Hylocomiastrum umbratum (Hedw.) M. Fleisch. (*Hylocomium umbratum* ([Ehrh. ex] Hedw.) Schimp.) **LC-att**
⇒ *Hylocomium* p. pte. – see under *Hylocomiastrum* and *Loeskeobryum*
Hylocomium splendens (Hedw.) Schimp. **LC**
Hymenoloma crispulum (Hedw.) Ochyra (*Dicranoweisia crispula* (Hedw.) Milde) **LC**
Hymenostylium recurvirostrum (Hedw.) Dixon **VU** [C2a(i)] – only in var. *recurvirostrum*
⇒ *Hypnum* p. pte. – see under *Breidleria*
Hypnum andoi A. J. E. Sm. **LC**
Hypnum callichroum Brid. **EN** [C2a(i)]
Hypnum cupressiforme Hedw. (annot. **25**)
  var. *cupressiforme* **LC**
  var. *filiforme* Brid. **LC**
  var. *heseleri* (Ando et Higuchi) M. O. Hill (*Hypnum heseleri* Ando et Higuchi) **DD** (annot. **26**)
  var. *lacunosum* Brid. **LC**
  var. *subjulaceum* Molendo **LR-nt** [D1]
Hypnum fertile Sendtn. **CR** [B1+2ab(v); C1+C2a(i, ii); D1]
Hypnum imponens Hedw. **CR** [B1+2ab(v); C1+C2a(i, ii); D1]
Hypnum jutlandicum Holmen et E. Warncke **LC**
Hypnum pallescens (Hedw.) P. Beauv. **LC-att**
Hypnum recurvatum (Lindb. et Arnell) Kindb. **CR** [B1+2ab(v); C1+C2a(i, ii); D1]
Hypnum revolutum (Mitt.) Lindb. **RE** – only in var. *dolomiticum* (Milde) Mönk.
Hypnum sauteri Schimp. **CR** [B1+2ab(v); C1+C2a(i, ii); D1]
Hypnum vaucheri Lesq. **LC-att**
Isopterygiopsis muelleriana (Schimp.) Z. Iwats. **CR**
Isopterygiopsis pulchella (Hedw.) Z. Iwats. **CR**
Isothecium alopecuroides (Lam. ex Dubois) Isov. **LC**
Isothecium myosuroides Brid. **LC-att** – only in var. *myosuroides*
Kiaeria blyttii (Bruch et Schimp.) Broth. **LC**
Kiaeria falcata (Hedw.) I. Hagen **EN** [B1+2ab(iii, iv, v); C1+C2a(i)]
Kiaeria glacialis (Berggr.) I. Hagen **RE**
Kiaeria starkei (F. Weber et D. Mohr) I. Hagen **LC**
Kindbergia praelonga (Hedw.) Ochyra (*Eurhynchium praelongum* (Hedw.) Schimp.) **LC**
Leptobryum pyriforme (Hedw.) Wilson **LC**
Leptodictyum riparium (Hedw.) Warnst. **LC**
Lescuraea incurvata (Hedw.) E. Lawton (*Pseudoleskea incurvata* (Hedw.) Loeske) **LC**
Lescuraea mutabilis (Brid.) Lindb. ex I. Hagen **EN** [C2a(i)]
Lescuraea patens Lindb. (*Pseudoleskea patens* (Lindb.) Kindb.) **EN** [C2a(i); D1]
Lescuraea plicata (Schleich. ex F. Weber et D. Mohr) Lindb. (*Ptychodium plicatum* (Schleich. ex F. Weber et D. Mohr) Schimp.) **EN** [B1+2ab(iii, iv, v)]
Lescuraea radicosa (Mitt.) Mönk. (*Pseudoleskea radicosa* (Mitt.) Macoun et Kindb.) **EN** [B1+2ab(iv, v); C2a(i); D1]
Lescuraea saxicola (Schimp.) Molendo **DD-va**
Leskea polycarpa Hedw. **LC**
Leucobryum glaucum (Hedw.) Ångstr. **LC**
Leucobryum juniperoideum (Brid.) Müll. Hal. **LC** (annot. **27**)
Leucodon sciuroides (Hedw.) Schwägr. **LC** – only in var. *sciuroides*
Loeskeobryum brevirostre (Brid.) M. Fleisch. (*Hylocomium brevirostre* (Brid.) Schimp.) **LR-nt** [D2]
Meesia longiseta Hedw. **RE**
Meesia triquetra (L. ex Jolycl.) Ångstr. **CR** [B2ab(iii, iv, v); C1+C2a(i)]

Meesia uliginosa Hedw. **CR** [B1+2ab(v); C1+C2a(i, ii); D1]
⇒ *Metaneckera* – see under *Neckera*
Microbryum curvicollum (Hedw.) R. H. Zander (‘*curvicolle*’ auct.) **LC-att**
Microbryum davallianum (Sm.) R. H. Zander
var. *davallianum* **VU** [B2ab(iii); C2a(i)]
var. *conicum* (Schleich. ex Schwägr.) R. H. Zander **CR** [B2ab(iii)]
Microbryum floerkeanum (F. Weber et D. Mohr) Schimp. **VU** [C1+C2a(i)]
Microbryum starckeanum (Hedw.) R. H. Zander **DD-va**
Microeurhynchium pumilum (Wilson) Ignatov et Vanderp. (*Oxyrrhynchium pumilum* (Wilson) Loeske, *Eurhynchium pumilum* (Wilson) Schimp.) **DD** (annot. **28**)
Mielichhoferia mielichhoferiana (Funck) Loeske **CR** [B1+2ab(iii, v); C2a(i, ii)]
Mnium hornum Hedw. **LC**
Mnium lycopodioides Schwägr. (*Mnium ambiguum* H. Müll.) **LC-att**
Mnium marginatum (Dicks.) P. Beauv. **LC** – only in var. *marginatum*
Mnium spinosum (Voit) Schwägr. **LC**
Mnium spinulosum Bruch et Schimp. **LC**
Mnium stellare Hedw. **LC**
Mnium thomsonii Schimp. **CR** [B1+2ab(iii, v); C2a(i)]
Myurella julacea (Schwägr.) Schimp. **EN** [B1+2ab(iii, v); C2a(i)]
⇒ *Neckera* p. pte. – see under *Alleniella* and *Exsertotheca*
Neckera menziesii Drumm. (*Metaneckera menziesii* (Drumm.) Steere) **CR** [C2a(i)]
Neckera pennata Hedw. **VU** [C2a(i)]
Neckera pumila Hedw. **RE**
Nyholmiella gymnostoma (Bruch ex Brid.) Holmen et E. Warncke (*Orthotrichum gymnostomum* Bruch ex Brid.) **RE** (annot. **29**)
Nyholmiella obtusifolia (Brid.) Holmen et E. Warncke (*Orthotrichum obtusifolium* Brid.) **LC**
Ochyraea duriuscula (De Not.) Ignatov et Ignatova (*Hygrohypnum duriusculum* (De Not.) D. W. Jamieson, *Hygrohypnella duriuscula* (De Not.) Ignatov et Ignatova) **LR-nt** [C2a(i)] (annot. **30**)
Ochyraea mollis (Hedw.) Ignatov (*Hygrohypnum molle* (Hedw.) Loeske) **DD**
Ochyraea smithii (Sw.) Ignatov et Ignatova (*Hygrohypnum smithii* (Sw.) Broth.) **RE**
⇒ *Octodiceras* – see under *Fissidens*
Oligotrichum hercynicum (Hedw.) Lam. et DC. **LC**
Oncophorus wahlenbergii Brid. **RE** – only in var. *wahlenbergii*
Orthodontium lineare Schwägr. **LC**
Orthothecium intricatum (Hartm.) Schimp. **LC**
Orthothecium rufescens (Dicks. ex Brid.) Schimp. **RE**
⇒ *Orthotrichum* p. pte. – see under *Nyholmiella*
Orthotrichum affine Schrad. ex Brid.
var. *affine* **LC**
var. *bohemicum* Plášek et Sawicki **DD** (annot. **31**)
Orthotrichum alpestre Hornsch. ex Bruch et Schimp. **CR** [B2ab(iii, v); C2a(i, ii); D1]
Orthotrichum anomalum Hedw. **LC**
Orthotrichum cupulatum Hoffm. ex Brid.
var. *cupulatum* **LC**
var. *riparium* Huebener **RE**
Orthotrichum diaphanum Schrad. ex Brid. **LC**
Orthotrichum lyellii Hook. et Taylor **LC-att**
Orthotrichum moravicum Plášek et Sawicki **DD** (annot. **32**)
Orthotrichum pallens Bruch ex Brid. **LC**
Orthotrichum patens Bruch ex Brid. **LR-nt** [D1]
Orthotrichum pulchellum Brunt. ex Sm. **LC-att** (annot. **33**)
Orthotrichum pumilum Sw. ex Anon. **LC**
Orthotrichum rogeri Brid. **VU** [D1]
Orthotrichum rupestre Schleich. ex Schwägr. **VU** [B2ab(iv, v); C2a(i)]
Orthotrichum scanicum Grönvall **CR** [B1+2ab(iii, v); C2a(ii)]
Orthotrichum speciosum Nees **LC**
Orthotrichum stellatum Brid. **CR** [C2a(i)]

Orthotrichum stramineum Hornsch. ex Brid. **LC**
Orthotrichum striatum Hedw. **LC-att**
Orthotrichum tenellum Bruch ex Brid. **DD** (annot. **34**)
Orthotrichum urnigerum Myrin **VU** [B2ab(iii); C1]
Oxyrrhynchium hians (Hedw.) Loeske (*Eurhynchium hians* (Hedw.) Sande Lac.) **LC**
Oxyrrhynchium schleicheri (R. Hedw.) Röll (*Eurhynchium schleicheri* (R. Hedw.) Milde) **LC**
Oxyrrhynchium speciosum (Brid.) Warnst. (*Eurhynchium speciosum* (Brid.) Jur.) **LC-att**
Oxystegus tenuirostris (Hook. et Taylor) A. J. E. Sm. (*Trichostomum tenuirostre* (Hook. et Taylor) Lindb.) **LC-att**
Paludella squarrosa (Hedw.) Brid. **EN** [B2ab(iii, iv, v)]
Palustriella commutata (Hedw.) Ochyra **LC**
Palustriella decipiens (De Not.) Ochyra **LC-att**
Palustriella falcata (Brid.) Hedenäs **LC**
Paraleucobryum longifolium (Hedw.) Loeske **LC**
Paraleucobryum sauteri (Bruch et Schimp.) Loeske **RE** (annot. **35**)
⇒ *Phascum* – see under *Tortula*
Philonotis caespitosa Jur. **LC-att**
Philonotis calcarea (Bruch et Schimp.) Schimp. **LC-att**
Philonotis capillaris Lindb. (*Philonotis arnellii* Husn.) **EN** [B2ab(iii, iv, v); C2a(i)]
Philonotis fontana (Hedw.) Brid. **LC**
Philonotis marchica (Hedw.) Brid. **CR** [B1+2ab(iii, iv, v)c(iii)]
Philonotis seriata Mitt. **LC**
Philonotis tomentella Molendo **VU** [C2a(i); D2]
Physcomitrella patens (Hedw.) Bruch et Schimp. **LC-att**
Physcomitrium eurystomum Sendtn. **VU** [B2ab(iii)c(iii, iv)]
Physcomitrium pyriforme (Hedw.) Brid. **LC**
Physcomitrium sphaericum (C. F. Ludw. ex Schkuhr) Fürnr. **VU** [B2ab(iii)c(iii, iv)]
Plagiobryum zieri (Hedw.) Lindb. **EN** [B2ab(iii, iv, v); C2a(i)]
Plagiomnium affine (Blandow ex Funck) T. J. Kop. **LC**
Plagiomnium cuspidatum (Hedw.) T. J. Kop. **LC**
Plagiomnium elatum (Bruch et Schimp.) T. J. Kop. **LC-att**
Plagiomnium ellipticum (Brid.) T. J. Kop. **LC-att**
Plagiomnium medium (Bruch et Schimp.) T. J. Kop. **LR-nt** [C2a(i)]
Plagiomnium rostratum (Schrad.) T. J. Kop. **LC**
Plagiomnium undulatum (Hedw.) T. J. Kop. **LC** – only in var. *undulatum*
Plagiopus oederianus (Sw.) H. A. Crum et L. E. Anderson **VU** [B2ab(iv, v); C2a(i)]
Plagiothecium cavifolium (Brid.) Z. Iwats. **LC**
Plagiothecium curvifolium Schlieph. ex Limpr. **LC**
Plagiothecium denticulatum (Hedw.) Schimp.
var. *denticulatum* **LC**
var. *obtusifolium* (Turner) Moore (*Plagiothecium donnianum* (Sm.) Mitt.) **VU** [B2ab(iii); C1; D]
var. *undulatum* R. Ruthe ex Geh. (*Plagiothecium ruthei* Limpr.) **LC-att**
Plagiothecium laetum Schimp. **LC**
Plagiothecium latebricola Schimp. **VU** [B2ab(iv, v); D1]
Plagiothecium neckeroideum Schimp. **EN** [B2ab(iii)]
Plagiothecium nemorale (Mitt.) A. Jaeger **LC**
Plagiothecium platyphyllum Mönk. **LC-att**
Plagiothecium succulentum (Wilson) Lindb. **LC**
Plagiothecium undulatum (Hedw.) Schimp. **LC**
Plasteurhynchium striatulum (Spruce) M. Fleisch. (*Eurhynchium striatulum* (Spruce) Schimp.) **LC-att**
Platydictya jungermannioides (Brid.) H. A. Crum **CR** [B1+2ab(iii, v); C2a(i, ii)]
Platygyrium repens (Brid.) Schimp. **LC**
⇒ *Platyhypnidium* – see under *Rhynchostegium*
Pleuridium acuminatum Lindb. **LC-att**
Pleuridium subulatum (Hedw.) Rabenh. **LC**
⇒ *Pleurochaete* – see under *Tortella*
Pleurozium schreberi (Willd. ex Brid.) Mitt. **LC**
Pogonatum aloides (Hedw.) P. Beauv. **LC**

Pogonatum nanum (Hedw.) P. Beauv. **VU** [C2a(i)]
Pogonatum urnigerum (Hedw.) P. Beauv. **LC**
Pohlia andalusica (Höhn.) Broth. **LC-att**
Pohlia annotina (Hedw.) Lindb. **LC**
Pohlia bulbifera (Warnst.) Warnst. **LC**
Pohlia camptotrachela (Renauld et Card.) Broth. **LC-att**
Pohlia cruda (Hedw.) Lindb. **LC**
Pohlia drummondii (Müll. Hal.) A. L. Andrews **LC**
Pohlia elongata Hedw. **LC-att** – only in var. *elongata*
Pohlia filum (Schimp.) Mårtensson **VU** [D2]
Pohlia lescuriana (Sull.) Ochi **LC-att**
Pohlia longicolla (Hedw.) Lindb. (‘*longicollis*’ auct.) **EN** [B2ab(iii, iv, v); C2a(i); D1]
Pohlia ludwigii (Spreng. ex Schwägr.) Broth. **VU** [B1+2ab(iii, iv, v); D2]
Pohlia lutescens (Limpr.) H. Lindb. **DD**
Pohlia melanodon (Brid.) A. J. Shaw **VU** [B2ab(iii, iv, v); C2a(i)]
Pohlia nutans (Hedw.) Lindb.
subsp. *nutans* **LC**
subsp. *schimperi* (Müll. Hal.) Nyholm **LR-nt** [D2]
Pohlia obtusifolia (Vill. ex Brid.) L. F. Koch **RE**
Pohlia proligera (Kindb.) Lindb. ex Broth. **LC**
Pohlia tundrae A. J. Shaw **CR** [B1+2ab(iii, v); C2a(i, ii)] (annot. **36**)
Pohlia wahlenbergii (F. Weber et D. Mohr) A. L. Andrews **LC** (annot. **37**)
⇒ *Polytrichastrum* p. pte. – see under *Polytrichum* (annot. **38**)
Polytrichastrum alpinum (Hedw.) G. L. Sm. **LC**
Polytrichastrum sexangulare (Flörke ex Brid.) G. L. Sm. **RE**
Polytrichum commune Hedw. **LC**
Polytrichum formosum Hedw. (*Polytrichastrum formosum* (Hedw.) G. L. Sm.) **LC**
Polytrichum juniperinum Hedw. **LC**
Polytrichum longisetum Sw. ex Brid. (*Polytrichastrum longisetum* (Sw. ex Brid.) G. L. Sm.) **LC**
Polytrichum pallidisetum Funck (*Polytrichastrum pallidisetum* (Funck) G. L. Sm.) **LC-att**
Polytrichum perigoniale Michx. **LC**
Polytrichum piliferum Hedw. **LC**
Polytrichum strictum Menzies ex Brid. **LC**
Polytrichum uliginosum (Wallr.) Schriebl **LC-att** (annot. **39**)
Pottiopsis caespitosa (Bruch ex Brid.) Blockeel et A. J. E. Sm. (*Trichostomum caespitosum* (Bruch ex Brid.) Jur., *Trichostomum pallidisetum* H. Müll., *Trichostomum triumphans* De Not.) **CR** [B1+2ab(iii, v); C2a(ii)] (annot. **40**)
⇒ *Protobryum* – see under *Tortula*
Pseudephemerum nitidum (Hedw.) Loeske **LC**
Pseudoamblystegium subtile (Hedw.) Vanderp. et Hedenäs (*Serpoleskea subtilis* (Hedw.) Loeske, *Amblystegium subtile* (Hedw.) Schimp.) **LC-att** (annot. **41**)
Pseudobryum cinclidioides (Huebener) T. J. Kop. **EN** [B2ab(iii, iv, v); C2a(i)]
⇒ *Pseudocalliergon* – see under *Drepanocladus*
Pseudocampylium radicale (P. Beauv.) Vanderp. et Hedenäs (*Amblystegium radicale* (P. Beauv.) Schimp.) **LC-att** (annot. **42**)
Pseudocrossidium hornschuchianum (Schultz) R. H. Zander **LC**
Pseudocrossidium revolutum (Brid.) R. H. Zander **EN** [B2ab(iii); C2a(i)]
⇒ *Pseudoleskea* – see under *Lescuraea*
Pseudoleskeella catenulata (Brid. ex Schrad.) Kindb. **LC**
Pseudoleskeella nervosa (Brid.) Nyholm **LC**
Pseudoleskeella rupestris (Berggr.) Hedenäs et L. Söderström **VU** [D2]
Pseudoleskeella tectorum (Funck ex Brid.) Kindb. ex Broth. **CR** [C2a(i)]
Pseudoscleropodium purum (Hedw.) M. Fleisch. (*Scleropodium purum* (Hedw.) Limpr.) **LC**
Pseudotaxiphyllum elegans (Brid.) Z. Iwats. **LC**
Pterigynandrum filiforme Hedw. **LC**
Pterygoneurum lamellatum (Lindb.) Jur. **EN** [B1+2ab(iii)]
Pterygoneurum ovatum (Hedw.) Dixon **LC**

Pterygoneurum subsessile (Brid.) Jur. **VU** [B1+2ab(iii)]
Ptilium crista-castrensis (Hedw.) De Not. **LC-att**
⇒ *Ptychodium* – see under *Lescuraea*
Ptychomitrium polyphyllum (Sw.) Bruch et Schimp. **RE**
Pylaisia polyantha (Hedw.) Schimp. **LC**
Pyramidula tetragona (Brid.) Brid. **CR** [B1+2ab(iii)c(iii, iv)]
Racomitrium aciculare (Hedw.) Brid. **LC**
Racomitrium affine (Schleich. ex F. Weber et D. Mohr) Lindb. **LC-att**
Racomitrium aquaticum (Brid. ex Schrad.) Brid. **LC**
Racomitrium canescens (Hedw.) Brid. **LC** – only in subsp. *canescens*
Racomitrium elongatum Ehrh. ex Frisvoll **LC**
Racomitrium fasciculare (Hedw.) Brid. **LC**
Racomitrium heterostichum (Hedw.) Brid. **LC**
Racomitrium lanuginosum (Hedw.) Brid. **LC**
Racomitrium macounii Kindb.
subsp. *macounii* **EN** [B1+2ab(iii), C2a(i)]
subsp. *alpinum* (E. Lawton) Frisvoll **LC**
Racomitrium microcarpon (Hedw.) Brid. **LC**
Racomitrium sudeticum (Funck) Bruch et Schimp. **LC**
Rhabdoweisia crenulata (Mitt.) H. Jameson **EN** [B1+2ab(v)]
Rhabdoweisia crispata (Dicks.) Lindb. **LR-nt** [C2a(i)]
Rhabdoweisia fugax (Hedw.) Bruch et Schimp. **LC**
Rhizomnium magnifolium (Horik.) T. J. Kop. **LC-att**
Rhizomnium pseudopunctatum (Bruch et Schimp.) T. J. Kop. **EN** [B2ab(iii); C2a(i)]
Rhizomnium punctatum (Hedw.) T. J. Kop. **LC**
Rhodobryum ontariense (Kindb.) Kindb. **LC-att**
Rhodobryum roseum (Hedw.) Limpr. **LC**
⇒ *Rhynchostegiella* p. pte. – see under *Brachythecium* (annot. **11**)
Rhynchostegiella tenella (Dicks.) Limpr. **LR-nt** [C2a(i)] – only in var. *tenella*
Rhynchostegiella teneriffae (Mont.) Dirkse et Bouman **EN** [B2ab(iii, v)]
Rhynchostegium confertum (Dicks.) Schimp. **LC-att**
Rhynchostegium megapolitanum (Blandow ex F. Weber et D. Mohr) Schimp. **VU** [C2a(i)] (annot. **42**)
Rhynchostegium murale (Hedw.) Schimp. **LC**
Rhynchostegium riparioides (Hedw.) Cardot (*Platyhypnidium riparioides* (Hedw.) Dixon) **LC**
Rhynchostegium rotundifolium (Scop. ex Brid.) Schimp. **EN** [B2ab(iii, v); C2a(i)]
Rhytidiadelphus loreus (Hedw.) Warnst. (*Rhytidiastrum loreum* (Hedw.) Ignatov et Ignatova) **LC** (annot. **43**)
Rhytidiadelphus squarrosus (Hedw.) Warnst. (*Rhytidiastrum squarrosum* (Hedw.) Ignatov et Ignatova) **LC**
Rhytidiadelphus subpinnatus (Lindb.) T. J. Kop. (*Rhytidiastrum subpinnatum* (Lindb.) Ignatov et Ignatova) **LC-att**
Rhytidiadelphus triquetrus (Hedw.) Warnst. **LC**
Rhytidium rugosum (Hedw.) Kindb. **LC**
Saelania glaucescens (Hedw.) Broth. **EN** [B2ab(iii, v); C2a(i)]
Sanionia uncinata (Hedw.) Loeske **LC**
Sarmentypnum exannulatum (Schimp.) Hedenäs (*Warnstorfia exannulata* (Schimp.) Loeske) **LC**
Sarmentypnum sarmentosum (Wahlenb.) Tuom. et T. J. Kop. (*Warnstorfia sarmentosa* (Wahlenb.) Hedenäs) **LR-nt** [D2]
Schistidium apocarpum (Hedw.) Bruch et Schimp. **LC**
Schistidium brunnescens Limpr. **LC** – only in subsp. *brunnescens*
Schistidium confertum (Funck) Bruch et Schimp. **VU** [D2]
Schistidium confusum H. H. Blom **LC-att**
Schistidium crassipilum H. H. Blom **LC**
Schistidium dupretii (Thér.) W. A. Weber **LC**
Schistidium elegantulum H. H. Blom **LC-att** – only in subsp. *elegantulum*
Schistidium flaccidum (De Not.) Ochyra **EN** [B2ab(iii, v)]
Schistidium helveticum (Schkuhr) Deguchi (*Schistidium singarense* (Schiffn.) Laz.) **LC-att**
Schistidium lancifolium (Kindb.) H. H. Blom **LC-att**
Schistidium papillosum Culm. **LC**
Schistidium pruinosum (Wilson ex Schimp.) G. Roth **LC-att**

Schistidium rivulare (Brid.) Podp. **LC-att**
Schistidium robustum (Nees et Hornsch.) H. H. Blom **LC**
Schistidium trichodon (Brid.) Poelt
var. *trichodon* **LC-att**
var. *nutans* H. H. Blom **LC-att**
Schistostega pennata (Hedw.) F. Weber et D. Mohr **LC**
Sciuro-hypnum curtum (Lindb.) Ignatov (*Brachythecium curtum* (Lindb.) Limpr.) **LC** (annot. **44**)
Sciuro-hypnum flotowianum (Sendtn.) Ignatov et Huttunen (*Eurhynchium flotowianum* (Sendtn.) Kartt.) **DD**
Sciuro-hypnum plumosum (Hedw.) Ignatov et Huttunen (*Brachythecium plumosum* (Hedw.) Schimp.) **LC**
Sciuro-hypnum populeum (Hedw.) Ignatov et Huttunen (*Brachythecium populeum* (Hedw.) Schimp.) **LC**
Sciuro-hypnum reflexum (Starke) Ignatov et Huttunen (*Brachythecium reflexum* (Starke) Schimp.) **LC**
Sciuro-hypnum starkii (Brid.) Ignatov et Huttunen (*Brachythecium starkii* (Brid.) Schimp.) ('*starkei*' auct.) **LC**
⇒ *Scleropodium* p. pte. – see under *Pseudoscleropodium*
Scorpidium cossonii (Schimp.) Hedenäs **LR-nt** [C2a(i)]
Scorpidium revolvens (Sw. ex Anon.) Hedenäs **EN** [B2ab(iii, v)]
Scorpidium scorpioides (Hedw.) Limpr. **EN** [B2ab(iii, iv); C2a(i)]
Seligeria acutifolia Lindb. **VU** [C2a(i); D2]
Seligeria calcarea (Hedw.) Bruch et Schimp. **EN** [B2ab(iii, iv)]
Seligeria campylopoda Kindb. **VU** [B2ab(iii, iv, v); C2a(i)]
Seligeria donniana (Sm.) Müll. Hal. **LC**
Seligeria patula (Lindb.) I. Hagen **DD-va**
Seligeria pusilla (Hedw.) Bruch et Schimp. **VU** [C2a(i)]
Seligeria recurvata (Hedw.) Bruch et Schimp. **LC**
⇒ *Serpoleskea* p. pte. – see under *Pseudoamblystegium*
Serpoleskea confervoides (Brid.) Loeske (*Amblystegium confervoides* (Brid.) Schimp.) **LC-att**
Sphagnum affine Renauld et Cardot **VU** [B2ab(iii, iv, v)]
Sphagnum angustifolium (C. E. O. Jensen ex Russow) C. E. O. Jensen **LC-att**
Sphagnum auriculatum Schimp. (*Sphagnum denticulatum* Brid.) **LC**
Sphagnum austinii Sull. ex Austin **RE**
Sphagnum balticum (Russow) Russow ex C. E. O. Jensen **LC-att**
Sphagnum capillifolium (Ehrh.) Hedw. **LC**
Sphagnum centrale C. E. O. Jensen **LC-att**
Sphagnum compactum Lam. et DC. **LC**
Sphagnum contortum Schultz **LR-nt** [B2ab(iii); C1]
Sphagnum cuspidatum Ehrh. ex Hoffm. **LC**
Sphagnum fallax (H. Klinggr.) H. Klinggr. (incl. *Sphagnum brevifolium* (Lindb. ex Braithw.) Röll) **LC**
Sphagnum fimbriatum Wilson **LC** – only in subsp. *fimbriatum*
Sphagnum flexuosum Dozy et Molk. **LC**
Sphagnum fuscum (Schimp.) H. Klinggr. **LR-nt** [A2(a); B2ab(iv)]
Sphagnum girgensohnii Russow **LC**
Sphagnum inundatum Russow **DD** (annot. **45**)
Sphagnum lindbergii Schimp. **LC**
Sphagnum magellanicum Brid. **LC**
Sphagnum majus (Russow) C. E. O. Jensen **LC** – only in subsp. *majus*
Sphagnum molle Sull. **RE**
Sphagnum obtusum Warnst. **LR-nt** [B2ab(iii); C1]
Sphagnum palustre L. **LC**
Sphagnum papillosum Lindb. **LC**
Sphagnum platyphyllum (Lindb. ex Braithw.) Sull. ex Warnst. **CR** [B1+2ab(iii, v); C2a(i, ii); D1]
Sphagnum quinquefarium (Lindb. ex Braithw.) Warnst. **LC**
Sphagnum riparium Ångstr. **LC**
Sphagnum rubellum Wilson **LC**
Sphagnum russowii Warnst. **LC**
Sphagnum squarrosum Crome **LC**
Sphagnum subnitens Russow et Warnst. **LC-att** – only in subsp. *subnitens*
Sphagnum subsecundum Nees **LC**
Sphagnum tenellum (Brid.) Pers. ex Brid. **LC**

Sphagnum teres (Schimp.) Ångstr. **LC**
Sphagnum warnstorfii Russow **LC-att**
Splachnum ampullaceum Hedw. **LR-nt** [C2a(i)]
Splachnum sphaericum Hedw. **LR-nt** [C2a(i)]
Stegonia latifolia (Schwägr.) Venturi ex Broth. **RE**
Straminergon stramineum (Dicks. ex Brid.) Hedenäs **LC**
Streblotrichum commutatum (Jur.) Hilp. (*Barbula commutata* Jur., *Barbula convoluta* var. *sardoa* Schimp.) **DD** (annot. **9**, **46**)
Streblotrichum convolutum (Hedw.) P. Beauv. (*Barbula convoluta* Hedw.) **LC**
Streblotrichum enderesii (Garov.) Loeske (*Barbula enderesii* Garov.) **RE**
Syntrichia calcicola J. J. Amann **LC**
Syntrichia caninervis Mitt. **DD-va** – only in var. *gypsophila* (J. J. Amann ex G. Roth) Ochyra (*Syntrichia caninervis* var. *spuria* (J. J. Amann) R. H. Zander)
Syntrichia fragilis (Taylor) Ochyra **CR** [B1+2ab(iii)] (annot. **47**)
Syntrichia laevipila Brid. **DD-va**
Syntrichia latifolia (Bruch ex Hartm.) Huebener **LR-nt** [B2ab(iii)]
Syntrichia montana Nees (*Syntrichia intermedia* Brid.) **LC**
Syntrichia norvegica F. Weber **CR** [C2a(i)]
Syntrichia papillosa (Wilson) Jur. **LC**
Syntrichia ruralis (Hedw.) F. Weber et D. Mohr (*Syntrichia densa* (Velen.) J.-P. Frahm)
var. *ruralis* **LC**
var. *ruraliformis* (Besch.) Delogne (*Syntrichia ruraliformis* (Besch.) Cardot) **LC-att**
Syntrichia virescens (De Not.) Ochyra **LC**
Taxiphyllum wissgrillii (Garov.) Wijk et Margad. **LC**
Tayloria serrata (Hedw.) Bruch et Schimp. **EN** [B2ab(iii, iv, v)]
Tayloria splachnoides (Schleich. ex Schwägr.) Hook. **RE**
Tayloria tenuis (Dicks.) Schimp. **EN** [B2ab(iii, iv, v)]
Tetraphis pellucida Hedw. **LC**
Tetraplodon angustatus (Hedw.) Bruch et Schimp. **VU** [C2a(i); D2]
Tetraplodon mnioides (Hedw.) Bruch et Schimp. **VU** [C2a(i); D2]
Tetrodontium brownianum (Dicks.) Schwägr. **LR-nt** [C2a(i); D2]
Tetrodontium ovatum (Funck) Schwägr. **DD**
Tetrodontium repandum (Funck) Schwägr. **LR-nt** [C2a(i)]
Thamnobryum alopecurum (Hedw.) Gangulee **LC**
Thamnobryum neckeroides (Hook.) E. Lawton **EN** [B1+2ab(iii, v)]
⇒ *Thuidium* p. pte. – see under *Abietinella*
Thuidium assimile (Mitt.) A. Jaeger (*Thuidium philibertii* Limpr.) **LC**
Thuidium delicatulum (Hedw.) Schimp. **LC-att**
Thuidium recognitum (Hedw.) Lindb. **LC**
Thuidium tamariscinum (Hedw.) Schimp. **LC**
Timmia austriaca Hedw. **RE**
Timmia bavarica Hessl. **EN** [B2ab(iii, iv, v); C2a(i)]
Tomentypnum nitens (Hedw.) Loeske **LR-nt** [C1+C2a(i)]
Tortella bambergeri (Schimp.) Broth. **LC**
Tortella inclinata (R. Hedw.) Limpr. **LC** (annot. **48**)
Tortella squarrosa (Brid.) Limpr. (*Pleurochaete squarrosa* (Brid.) Lindb.) **LR-nt** [C1] (annot. **49**)
Tortella tortuosa (Hedw.) Limpr. (incl. var. *fragilifolia* (Jur.) Limpr.) **LC**
Tortula acaulon (With.) R. H. Zander (*Phascum cuspidatum* Hedw.)
var. *acaulon* **LC**
var. *pilifera* (Hedw.) R. H. Zander (*Phascum cuspidatum* var. *piliferum* (Hedw.) Hook. et Taylor) **LC**
Tortula atrovirens (Sm.) Lindb. **CR** [B1+2ab(v)]
Tortula caucasica Lindb. ex Broth. (*Tortula modica* R. H. Zander, *Pottia intermedia* (Turner) Fürnr.) **LC**
Tortula cernua (Huebener) Lindb. (*Desmatodon cernuus* (Huebener) Bruch et Schimp.) **RE**
Tortula hoppeana (Schultz) Ochyra (*Tortula euryphylla* R. H. Zander, *Desmatodon latifolius* (Hedw.) Brid.) **EN** [B2ab(iii, iv, v); C2a(i)] (annot. **50**)
Tortula inermis (Brid.) Mont. **CR** [B1+2ab(iii, v); C2a(i)]
Tortula lindbergii Kindb. ex Broth. (*Tortula lanceola* R. H. Zander, *Pottia lanceolata* (Hedw.) Müll. Hal.) **LC**

Tortula lingulata Lindb. **CR** [B1+2ab(iii, v); C2a(i, ii)] (annot. **51**)
Tortula mucronifolia Schwägr. **CR** [B1+2ab(iii, v); C2a(i, ii)]
Tortula muralis Hedw.
subsp. *muralis* var. *muralis* **LC**
subsp. *muralis* var. *aestiva* Hedw. **LC**
Tortula protobryoides R. H. Zander (*Protobryum bryoides* (Dicks.) J. Guerra et M. J. Cano, *Pottia bryoides* (Dicks.) Mitt.) **LC-att**
Tortula schimperi M. J. Cano, O. Werner et J. Guerra (*Tortula subulata* var. *angustata* (Schimp.) Limpr.) **DD** (annot. **52**)
Tortula subulata Hedw. **LC**
Tortula truncata (Hedw.) Mitt. (*Pottia truncata* (Hedw.) Bruch et Schimp.) **LC**
Trematodon ambiguus (Hedw.) Hornsch. **CR** [B1+2ab(iii, v)]
Trichodon cylindricus (Hedw.) Schimp. **LC**
⇒ *Trichostomum* p. pte. – see under *Oxystegus* and *Pottiopsis*
Trichostomum brachydontium Bruch **DD-va**
Trichostomum crispulum Bruch
var. *crispulum* **LC-att**
var. *angustifolium* Bruch et Schimp. (*Trichostomum viridulum* Bruch) **LC-att** (annot. **53**)
Ulota bruchii Hornsch. ex Brid. **LC**
Ulota coarctata (P. Beauv.) Hammar **CR** [B2ab(iv, v); C2a(i)]
Ulota crispa (Hedw.) Brid. **LC**
Ulota drummondii (Hook. et Grev.) Brid. **RE**
Ulota hutchinsiae (Sm.) Hammar **EN** [B2ab(iii); C2a(i); D1]
⇒ *Warnstorfia* p. pte. – see under *Sarmentypnum*
Warnstorfia fluitans (Hedw.) Loeske **LC**
Warnstorfia pseudostraminea (Müll. Hal.) Tuom. et T. J. Kop. **EN** [C2a(i)]
Weissia brachycarpa (Nees et Hornsch.) Jur. **LC**
Weissia condensa (Voit) Lindb. **LC** – only in var. *condensa*
Weissia controversa Hedw. (incl. var. *densifolia* (Bruch et Schimp.) Wilson) **LC**
Weissia fallax Sehlm. (*Weissia controversa* var. *crispata* (Nees et Hornsch.) Nyholm) **LC-att**
Weissia longifolia Mitt. **LC**
Weissia rostellata (Brid.) Lindb. **DD-va**
Weissia rutilans (Hedw.) Lindb. **EN** [B2ab(iv, v); C2a(i)]
Weissia squarrosa (Nees et Hornsch.) Müll. Hal. **VU** [B2ab(iv, v)]
Weissia wimmeriana (Sendtn.) Bruch et Schimp. (*Weissia controversa* var. *wimmeriana* (Sendtn.) Blockeel et A. J. E. Sm.) **VU** [D2]
Zygodon dentatus (Limpr.) Kartt. **LR-nt** [D2]
Zygodon rupestris Schimp. ex Lorentz **LR-nt** [D2]
Zygodon viridissimus (Dicks.) Brid. **EN** [B2ab(v); C2a(i)]

(b) Doubtful, uncertain and excluded taxa (not evaluated for the Red List)

(i) Doubtful taxonomic status

In addition to *Andreaea alpestris* (Thed.) Schimp., *Bryum dunense* A. J. E. Sm. et H. Whitehouse, *Bryum stirtonii* Schimp., listed, commented on and placed in this category by Kučera & Váňa (2003) and *Bryum badium* (Bruch ex Brid.) Schimp., which was appended in the Erratum part of the list (Preslia 75: 384), following additional taxa need to be taxonomically studied before they can be accepted for inclusion in the checklist:

Metzgeria simplex Lorb. ex Müll. Frib. – this taxon was defined based on the haploid chromosome number (n = 9) and its slightly smaller thallus cells, as opposed to its diploid counterpart (n = 18) *M. conjugata*. Schumacker & Váňa (2005) regard *M. simplex* as conspecific with the Asian-American *M. lindbergii* Schiffn., which needs to be confirmed, and hesitate to distinguish this taxon from *M. conjugata*. Cytometric screening combined with a morphometric evaluation is necessary to ascertain the value of this taxon.

Porella ×*baueri* (Schiffn.) C. E. O. Jensen – this taxon is now thought to be an allopolyploid hybrid of *P. platyphylla* and *P. cordaeana* (Boisselier-Dubayle et al. 1998, Heinrichs et al. 2011). As the reported morphological differences between *P. platyphylla* and *P.* ×*baueri* do not hold for a considerable proportion of our material, the nothotaxon cannot be safely recognized at present and virtually nothing is known about the extent of hybridization between the parental taxa and the occurrence and morphology of hybridogeneous populations.

Riccia gougetiana Durieu et Mont. – this taxon should differ from *R. ciliifera* in its larger dimensions and other essentially quantitative characteristics (with some overlap) despite the same reported chromosome number (n = 8, rarely n = 16). Nevertheless, we discovered only diploid plants (n ≈ 16) during a limited cytometric screening of southern-Moravian populations of *R. ciliifera* s.l. with an intermediate morphology between *R. ciliifera* and *R. gougetiana*. The extent of polyploidization and the morphometric differences between populations need to be evaluated before applying these names to these populations.

Bryum barnesii J. B. Wood ex Schimp. – recent authors differ in their opinion on the value of this taxonomically doubtful species of the *B. dichotomum* complex; while Vanderpoorten & Zartman (2002) and Müller (2004) accept it, the monographer of the genus (Holyoak 2003) is sceptical about its value. Plants corresponding to the description were twice recently reported from the Czech Republic (Kučera et al. 2005, Kučera 2009a).

Platyhypnidium grolleanum Ochyra et Bednarek-Ochyra – a doubtful aquatic taxon described from one historical specimen, based on its multistratose leaves. As recent searches for this plant at the type locality all proved futile, the taxon can probably best be interpreted as a rare mutation of the common *Rhynchostegium riparioides*, as is the case of *Platyhypnidium mutatum* Ochyra et Vanderp. and other pleurocarpous mosses, which develop pluristratose laminae in rheophytic habitats.

(ii) Doubtful or uncertain occurrence

Fossombronia caespitiformis De Not. ex Rabenh., *Riccia beyrichiana* Hampe ex Lehm., *Bryum arcticum* (R. Br.) Bruch et Schimp., *Bryum knowltonii* Barnes, *Bryum warneum* (Röhl.) Blandow ex Brid., *Ceratodon conicus* (Hampe) Lindb., *Cinclidium stygium* Sw., *Cnestrum schisti* (F. Weber et D. Mohr) I. Hagen, *Cynodontium fallax* Limpr., *Cyrtomnium hymenophylloides* (Huebener) T. J. Kop., *Dichodontium flavescens* (Dicks.) Lindb., *Grimmia decipiens* (Schultz) Lindb., *Hypnum cupressiforme* var. *resupinatum* (Taylor) Schimp., *Mnium blyttii* Bruch et Schimp., *Pelekium minutulum* (Hedw.) Touw (*Cyrto-hypnum minutulum* (Hedw.) W. R. Buck et H. A. Crum), *Pohlia sphagnicola* (Bruch et Schimp.) Broth., *Racomitrium ericoides* (Brid.) Brid. and *Syntrichia sinensis* (Müll. Hal.) Ochyra. were included in previous checklists (Kučera & Váňa 2003, 2005) among the taxa for which the historically reported occurrence is regarded as possible but not supported by a correctly identified herbarium specimen. *Microlejeunea ulicina*, *Scapania apiculata*, *Entosthodon pulchellus*, *Orthotrichum tenellum*, *Paraleucobryum sauteri* and *Rhynchostegium megapolitanum* have since been recorded in the Czech Republic (see above). In addition to the preceding species the following taxa are now regarded to be of uncertain occurrence:

Moerckia hibernica (Hook.) Gottsche – as discussed in annotation, we did not find this species among the specimens labelled with this name, after the understanding of this taxon changed following the study by Crandall-Stotler & Stotler (2007). An historical or even recent occurrence of *M. hibernica* is nevertheless possible.

Tortula muralis subsp. *obtusifolia* (Schwägr.) Culm. – its taxonomic status was clarified by Košnar & Kolář (2009) and Košnar et al. (2012). Although the historical occurrence on base-rich sandstones near Kralupy nad Vltavou, reported by Velenovský (1897), is probable, there are no specimens of this species in Czech herbaria. The specimens from the Český kras karst region belong to *T. muralis* var. *aestiva*.

(iii) Newly excluded taxa

Aschisma carniolicum (F. Weber et D. Mohr) Lindb. – reported from nearby Prague by Opiz (1852). Matouschek (1908) published the results of a revision of Opiz's specimens in PR, however the specimen of *A. carniolicum* was not found. We agree with Matouschek's judgment on the probable misidentification of this species based on the distribution pattern of this Mediterranean species.

Tayloria froelichiana (Hedw.) Mitt. ex Broth. – this species was also reported by Opiz (1852) without a particular locality. Matouschek (1906) did not find the original specimen and probably therefore excluded it as subsequently he did not mention this species again. Based on the distribution pattern of this species its occurrence in the Czech Republic is indeed highly improbable.

For information on 42 earlier excluded taxa see Kučera & Váňa (2003).

Annotations:

1 *Aneura maxima* was recently discovered in southern Bohemia by Kučera (2004) and reported at other localities since.

2 Buczkowska et al. (2012) recently published a paper, in which two genetically distinct taxa are recognized within *C. sphagnicola*. While the type corresponds to the haploid taxon and has a markedly northern distribution pattern in Poland (the specimens from outside Poland have not been studied), the diploid (allopolyploid)

taxon, which is called *C. sphagnicola* f. *paludosa* (Warnst.) R. M. Schust. by these authors, which very probably occurs in the Czech Republic, only doubtfully corresponds to Warnstorf's type, and hence its name is uncertain. There is a similar situation with morphologically cryptic or nearly cryptic taxa *Calypogeia muelleriana* (Buczkowska & Bączkiewicz 2011) and *C. fissa* (Buczkowska 2004).

3 The broad concept of *Chiloscyphus* has been advocated in recent molecular studies (He-Nygrén & Piippo 2003, Hentschel et al. 2006a, b), although the subgenus *Lophocolea* is still one of the resonably supported clades, closely related to subg. *Chiloscyphus*. Molecular data also seem to support the specific status of *C. pallescens* (cf. Hentschel et al. 2006b) and *C. cuspidatus* (cf. Hentschel et al. 2007), although the difference in their sexuality probably cannot serve as the sole differentiating character; this was noted by Damsholt (2010), who reported annual variation in sex expression with antheridia and perianths present at different times of the year, and Vogelpoel (1982), who manipulated the sex expression frequency, abundance and vitality of the gametangia by varying day length and light intensity). The application of names within *C. coadunatus* s.l. follows Váňa & Engel (2012), who found the type of *C. coadunatus* to be probably dioicous (containing only female plants), while the type of *Jungermannia bidentata* L. was found to be monoicous (Vogelpoel 1977), contrary to the treatment of Damsholt (2002).

4 *Conocephalum salebrosum* is a newly distinguished taxon (Szweykowski et al. 2005) that occurs widely in the Czech Republic.

5 The identity of *L. guttulata* and *L. longiflora* was recently doubted or rejected by several authors, including the monographer of the genus, V. Bakalin (Bakalin 2001, 2011), hence our acceptance of *L. guttulata* in place of the plant we earlier named *L. longiflora*. Hygric forms of *L. ventricosa* (and possibly also of other closely related taxa including *L. ventricosa* var. *silvicola* and *L. guttulata*) were identified with the types of *L. ventricosa* var. *uliginosa* Breidl. ex Schiffn. (Damsholt 2002) or of *Jungermannia longiflora* Nees. Bakalin (2011), who lists *L. ventricosa* var. *longiflora* (Nees) Macoun does not mention var. *uliginosa* at all). A wide ranging study using molecular markers is needed to resolve this problem.

6 First proven occurrence of *Microlejeunea ulicina* in the Czech Republic was only recently reported (Kučera & Váňa 2011).

7 The distinctness of *Moerckia flotoviana* from *M. hibernica* is discussed and advocated by Crandall-Stotler & Stotler (2007). The two names were commonly misapplied, which was also the case in the earlier identifications in the Czech Republic. The recent and historical collections of material that have been revised belong to *M. flotoviana*, which seems to be generally much commoner than *M. hibernica,* but not all historical collections have been revised.

8 Hugonnot (2010) recently argued for the synonymy of *R. ciliata*, *R. trichocarpa* M. Howe and *R. canescens* Steph., whereas Jovet-Ast (1986) and Schumacker & Váňa (2005) advocate the distinctness of *R. trichocarpa* (syn. *R. canescens*), the latter treatment even synonymized the latter with an older name *R. crinita* Taylor, based on an Australian type.

9 *Scapania apiculata* was listed as of uncertain occurrence in the previous version. Since then it has been twice recorded in the Moravskoslezské Beskydy Mts.

10 Köckinger & Kučera (2011) showed that two of the *Barbula* sect. *Convolutae* Bruch & Schimp. species (*B. convoluta* and *B. commutata*) are phylogenetically very distant from the generitype of *Barbula*, *B. unguiculata*. Hence, their recognition within an earlier recognized genus, *Streblotrichum* P. Beauv. (generitype *S. convolutum*) is appropriate. *Barbula crocea* was also assigned to *Streblotrichum* by older authors including Pilous & Duda (1960) but it has closer genetic affinities with *Hydrogonium* (Müll. Hal.) A. Jaeger (Kučera et al. in prep.).

11 Phylogenetic affinities of this taxon, which is included in the European checklist (Hill et al. 2006) and our previous checklists as *Rhynchostegiella tenuicaulis*, is controversial. While Ignatov & Huttunen (2002) doubt its inclusion in *Brachytheciaceae*, Nebel & Philippi (2001) provide strong arguments that it is only a habitat form of *Brachythecium tommasinii*. Unfortunately, the recent molecular-phylogenetic studies of *Brachytheciaceae* (Huttunen & Ignatov 2004) and *Rhynchostegiella* (Aigoin et al. 2009) do not include this puzzling taxon. We obtained ITS sequences for one specimen of the typical *Brachythecium tommasinii* (JQ814782) that was growing on shaded limestone rocks in the Czech Republic and for one *Rhynchostegiella tenuicaulis* (JQ814783) growing on the bark of *Fagus* at the only Czech locality for this species. These sequences indeed are nearly identical and allow the evaluation as closely related taxa within one genus. For future reference, we prefer to retain the varietal status of the disputed taxon within *B. tommasinii*. The type of *Eurhynchium vaucheri var. fagineum* was studied by Nebel et al. (l.c.) and these authors confirm Limpricht's earlier opinion that it is identical to the type of *Eurhynchium germanicum* Grebe. With respect to the identity of the types of *Eurhynchium germanicum* and *Hypnum tenuicaule* Spruce from the French Pyrenees we refer to the treatment of Karttunen (1990).

12 Ochyra & Bednarek-Ochyra (2011) recently provided arguments for replacing the name *Bryum pallescens* Schleich. ex Schwägr. with the older name *B. boreale* (F. Weber et D. Mohr) Funck.

13 *Bryum gemmiferum* was first reported from the Czech Republic by Soldán & Kučera (2004) but further records continue to be added.

14 Holyoak & Hedenäs (2006) demonstrate the morphological intergradation between *Bryum pseudotriquetrum* var. *pseudotriquetrum* and var. *neodamense* and non-monophyly of the latter taxon.

15 Based on an unpublished revision of JK's collections by O. M. Afonina, the Czech collections of '*Campylidium sommerfeltii*' probably represent a different taxon, closely related to *Hypnum pallescens*. '*C. sommerfeltii*' has been distinguished in the Czech Republic only in recent decades, previous authors confused or merged this taxon with *C. calcareum* and the North American *C. hispidulum*. A revision of this complex is badly needed.

16 *Didymodon umbrosus* was shown to deserve specific status by Jiménez et al. (2005).

17 *Didymodon validus* was recognized as a variety of *D. rigidulus* in our previous checklist and not at all listed by Hill et al. (2006). Later though, Jiménez (2006) and Ochyra et al. (2011) recognized the taxon at the specific level.

18 The taxonomy of *Encalypta rhaptocarpa* agg. is very unsatisfactory. There is not a good match between the development of peristome and other characters of this species mentioned by Horton (1983), Nyholm (1998) and Mogensen (2001), and our application of the name is thus very tentative.

19 Hradílek (2008) clarified the situation with *Entosthodon pulchellus* that was confused with *E. muhlenbergii* in the Czech Republic. Both historical herbarium records and recent collections were cited.

20 We consider that *Fissidens bambergeri* is a good, although only rarely accepted species that cannot be lumped with *F. viridulus*. The distinctness of two Czech and several other central-European populations has been observed for years, although no molecular methods have yet been applied to resolve the genetic background and of course the application of the pattern to existing types may prove problematic.

21 We have applied the name *Fissidens limbatus* only to plants that narrowly correspond to the original description by Sullivant. In this concept, *F. limbatus* is an extremely rare and endangered species in the Czech Republic and should be evaluated similarly to *F. bambergeri*. An eventual broadening of the concept to include *F. crispus* Mont., as understood by Hill et al. (2006), would create problems in delimiting *F. pusillus*, however without an understanding of the underlying genetic pattern the problem cannot be resolved.

22 We are not convinced of the value of infraspecific taxa within *Fontinalis antipyretica*, distinguished pragmatically by Hill et al. (2006). Shaw & Allen (2000) show that the subsp. *gracilis* (Lindb.) Kindb. is paraphyletic and a similar pattern can be expected for other infraspecific taxa. Nevertheless, both the subsp. *gracilis* and subsp. *kindbergii* (Renauld et Cardot) Cardot are reported in the Czech Republic but the underlying genetic differences have never been studied.

23 *Grimmia dissimulata* was newly reported for this country by Kučera (in Ellis et al. 2010).

24 We agree with the authors of the European checklist that the radical treatment of Vanderpoorten (2004), which merged all European species of *Hygroamblystegium* with *H. varium*, needs to be supported by a more extensive study. In a later study Vanderpoorten & Hedenäs (2009) admit *H. humile* is a variety of *H. varium* but maintain the full synonymy of *H. fluviatile* and *H. tenax* with *H. varium*, particularly with respect to the situation in North America.

25 *Hypnum cupressiforme* var. *julaceum* Brid., listed in our previous checklists, is not recognized by Hill et al. (2006). As we could only doubtfully identify some of our plants as of this variety, we have not included it in this list.

26 *Hypnum cupressiforme* var. *heseleri* was recently detected at one locality in southern Moravia (Košnar & Kučera in prep.).

27 Conflicting evidence was presented by Vanderpoorten et al. (2003) and Frahm (2005) about distiguishing *Leucobryum albidum* (Brid. ex P. Beauv.) Lindb. (which is an older name) from *L. juniperoideum*. Therefore, we pragmatically retain the more narrowly defined concept of both taxa until a more convincing conclusion is reached.

28 *Eurhynchium pumilum* was transferred to a newly established monotypic genus *Microeurhynchium* by Aigoin et al. (2009).

29 Goffinet et al. (2004) and Sawicki et al. (2010) argue for accepting the genus *Nyholmiella* as distinct from *Orthotrichum*. Accepting this probably well-defined lineage however renders the rest of *Orthotrichum* paraphyletic, which will necessitate the recognition of further genera within *Orthotrichum* s.l. in the future.

30 Taxonomy of *Hygrohypnum* s.l. partially settled after Oliván et al. (2007) and Ignatov et al. (2007) reached similar conclusions based on different datasets. The only serious conflict is over *Hygrohypnum duriusculum*, which was resolved within *Hygrohypnella* (sequenced specimen from Caucasus) by Ignatov et al., but within *Ochyraea* (sequenced specimen from Norway) by Oliván et al. Our plants seem to match the concept of Oliván et al., which is supported by the nrITS sequence of one Czech specimen (JQ814784). Nevertheless, we

still have problems differentiating *O. mollis* from the closely related *O. duriuscula*, and therefore cannot at present decide on the possible level of threat to *O. mollis*, although it is probable that the latter is very rare, if it occurs at all, in the Czech Republic.

31 *Orthotrichum affine* var. *bohemicum* was recently described (Plášek et al. 2011) based on material from 3 localities in the Czech Republic.

32 *Orthotrichum moravicum* was described from a single locality in Moravia (Plášek et al. 2009) and no other occurrence has been reported.

33 *Orthotrichum pulchellum* was first reported in the Czech Republic in NW Bohemia by Plášek & Marková (2007) and Plášek & Marková in Blockeel et al. (2008) and seems to be spreading.

34 *Orthotrichum tenellum* was listed among the taxa with unproven occurrence in the Czech Republic in previous versions of the checklist. Recently, this species was found in Northern Bohemia (Plášek & Marková 2011, Plášek & Marková in Ellis et al. 2012).

35 *Paraleucobryum sauteri* was listed previously as another taxon of uncertain occurrence in the Czech Republic. During a revision of selected species from the herbarium of the Museum of Upper Austria (LI), JK found one correctly identified specimen of *P. sauteri*, collected by Cypers in 1877 in the valley of Bílé Labe in Krkonoše Mts.

36 *Pohlia tundrae* was first reported from the Czech Republic by Müller (2004) and is still known only from a single locality.

37 We do not recognize the varieties of *Pohlia wahlenbergii* but should they be distinguished they all occur in the Czech Republic and only var. *glacialis* (Brid.) E. F. Warb. has a limited distribution and could qualify for inclusion on the Red List, probably in category LR-nt (D).

38 Bell & Hyvönen (2010) show that species of *Polytrichastrum* sect. *Aporotheca* (*P. formosum*, *P. longisetum* and *P. pallidisetum*) form with *Polytrichum* s.str a well-supported clade.

39 *Polytrichum uliginosum*, re-established by Schriebl (1991), has only recently been shown to be reproductively isolated from *P. commune* (van der Velde & Bijlsma 2004). Nevertheless, there is little information on its distribution in the Czech Republic and elsewhere.

40 Ros & Werner (2007) re-define the genus *Pottiopsis* based on molecular and morphological data and confirm our earlier suspicion (Kučera & Váňa 2003) that *Trichostomum caespitosum* and *T. pallidisetum* are very closely related, as they regard them as synonymous.

41 Vanderpoorten & Hedenäs (2009) describe new genera, *Pseudoamblystegium* and *Pseudocampylium,* to accommodate the phylogenetically isolated species earlier recognized by us as *Serpoleskea subtilis* and *Amblysteium radicale*, respectively.

42 *Rhynchostegium megapolitanum* was listed among uncertain occurrences in the previous version, but has since been recorded several times (see e.g. Kučera et al. 2006).

43 *Rhytidiadelphus loreus, R. squarrosus* and *R. subpinnatus* are tranferred to a newly established genus *Rhytidiastrum* Ignatov et Ignatova in their treatment of pleurocarpous mosses for the Moss flora of the central part of European Russia (Ignatov & Ignatova 2004). This concept needs to be tested using molecular methods.

44 Ignatov & Milyutina (2007) argued for separating *Sciuro-hypnum oedipodium* from *S. curtum*. European records of *S. oedipodium* refer generally to *S. curtum*. *S. oedipodium* s.str. is primarily a western North American taxon with one known disjuct occurrence in the Caucasus, however our material needs to be completely revised.

45 *Sphagnum inundatum* is a problematical taxon both with respect to its morphological definition and genetic background, which is connected with a complicated polyploid and hybridogenous microspeciation pattern (for a summary, see Shaw et al. 2012). While the North American plants that have '*S. inundatum*-morphology'are considered synonymous with either *S. lescurii* Sull., when haploid or with *S. missouricum* Warnst. & Card., when diploid, this pattern cannot be transferred to the situation in Europe, which has not yet been adequately studied, although the type of *S. inundatum* originates from Europe. The European plants of, *S. inundatum*-morphology'studied are allopolyploids derived from *S. subsecundum* (female parent) and haploid *S. auriculatum* (male parent) (Shaw et al. 2008).

46 *Streblotrichum commutatum* was earlier not distinguished in the Czech Republic but both historical and recent collections were found during a partial revision of our herbaria and a focused field survey (Kučera unpubl.). Nevertheless, this species seems to be relatively rare and the morphological delimitation from *S. convolutum* is not always straightforward, although the molecular differentiation is considerable (Köckinger & Kučera 2011, Kučera et al. in prep.).

47 *Syntrichia fragilis* was recently first discovered at a single locality in central Bohemia (Müller & Kučera in Blockeel et al. 2006).

48 *Tortella densa* (Lorentz et Molendo) Crundw. & Nyholm, which we accept at the specific level, was listed among the excluded species in the last version of the checklist.

49 The genus *Pleurochaete* Lindb. is nested within *Tortella* (Grundmann et al. 2006).

50 Among the varieties that are traditionaly recognized within *Desmatodon latifolius*, var. *muticus* (Brid.) Brid.) seems to represent a distinct taxon, as mixed stands of clearly separable plants matching both varieties were observed in the Czech Republic (Mt Kotel) and in the Alps. However, we refrain at the moment from combining it within *Tortula hoppeana*, before the problem is addressed using molecular methods.

51 Košnar & Kolář (2009) and Košnar et al. (2012) present arguments for accepting *Tortula lingulata* at the specific level, although this taxon is phylogenetically nested within *T. muralis* s.l. and its acceptance renders *T. muralis* paraphyletic in the strictly cladistic view.

52 *Tortula schimperi* represents a taxon that earlier was mostly recognized as a variety of *T. subulata*. According to Cano et al. (2005), it deserves specific status. There is very little known about its distribution in the Czech Republic, but recently two very small populations were recorded at two localities.

53 While Hill et al. (2006) do not recognize var. *angustifolium* as distinct from *Trichostomum crispulum*, central-European authors (Grims 1999, Müller 2004) usually prefer to distinguish it as a distinct variety or even species. The revision of material in Czech herbaria (Kučera unpublished) at first did not reveal a taxon clearly separable from *T. crispulum* but recently JK realized that there might be a distinguishable taxon matching this variety present in the Czech flora. This problem needs to be addressed in a taxonomic study.

Discussion

Changes in the checklist, and comparison with neighbouring countries and Europe

The total number of accepted and evaluated taxa is 15 more than in the 2003 version. However, of these there are only 12 newly reported taxa for the Czech Republic, while 5 species appeared in the list in the result of a taxonomic reconsideration, and 8 previously listed under uncertain or taxonomically doubtful taxa have since been confirmed as occurring in the Czech Republic. On the other hand, 6 earlier recognized taxa are no longer included, 4 have been moved to the 'taxonomically doubtful' and one to the 'uncertain occurrence' category. From the user's perspective, there has unfortunately been a considerable number of name changes (136 taxa affected, i.e. 15.1%), caused by shifts into different genera (97 taxa), taxonomic rank changes (16 taxa), or changes in (infra)specific epithets for mostly nomenclatural reasons (15 taxa). We corrected the author citation in 31 cases, although mostly only to conform to our 'strategy' of citing pre-Hedwigian moss names to that of Hill et al. (2006). The number of genera increased from the 59 for liverworts and 175 for mosses, recognized in the 2003 version, to 76 and 194, respectively, as a consequence of different generic concepts, mostly based on recent molecular phylogenetic treatments.

The bryoflora of the Czech Republic (78, 867 km^2) comprises roughly half of the European liverworts (423 species listed by Grolle & Long 2000) and mosses (1239 species accepted by Hill et al. 2006). The comparison of numbers with those in neighbouring countries is hampered by the fact that they are either significantly different in area, mostly larger (Germany, Poland), or contain a significantly larger or smaller diversity of ecosystems. For example, the bryoflora of the state of Carinthia in Austria (9536 km^2) exceeds that of the Czech Republic by some 30 species, having 893 species and 48 additional infraspecific taxa (Köckinger et al. 2008), while only 651 species of mosses are listed for the much larger (312,685 km^2) but generally much less diverse Poland (Ochyra et al. 2003). Similarly, the area of Hungary (93,030 km^2) is similar to that of the Czech Republic but the bryophyte flora of Hungary is only three quarters (659 bryophyte species plus 3 subspecies – 2 hornwort, 146 liverwort and 511 moss species according to Papp et al. 2010) of that of the Czech Republic, possibly because the smaller diversity of ecosystems and historically lower intensity of research on bryophytes in the former.

Red List

The relatively great increase in studies on the bryophyte flora over the last ca 20–30 years, together with active monitoring of the bryophytes listed in Annex II of the EC Directive 92/43 and some additional smaller-scale national monitoring projects supported particularly by the Czech Agency for Nature and Landscape Protection (AOPK ČR) enabled us to reconsider the threat status for most of our taxa. This led to changes in the status of 308 bryophyte taxa, i.e. 34.5% of the bryoflora in the 2003 version of the list. As the shifts in the evaluation commonly included both upward and downward reconsiderations of individual taxa, the changes in percentages for individual categories are perhaps less apparent than expected based on the above mentioned rate of change. We particularly addressed the Data Deficient taxa, which resulted in the shift of 102 taxa in total (11.4%) to other categories (including 6 excluded or not evaluated taxa). Of them, 14 were moved from the Vanished to the Regionally Extinct Category but the rest are now either listed among threatened taxa (57) or Lower Risk and Least Concern taxa (25). The high number of recent floristic surveys is best illustrated by the rediscovery of 28 Vanished and even one 'Regionally Extinct' species. New data on earlier non-Data Deficient taxa accounted mostly for a decrease in the threat evaluation, which is the case for 79 taxa (8.8%) in total, but on the other hand, for 41 taxa (4.6%) the threat evaluation was increased.

It is more difficult to compare the percentages in the various threat categories in different countries than to compare checklists. Although the criteria used in different countries to evaluate species for inclusion on the Red List are largely identical (IUCN criteria used in most European countries for which there are Red Lists, although neighbouring Austria and Germany use their own criteria), the baseline data for the different regions vary greatly in both quantity and quality, and even the application of the criteria is very far from being comparable. For example, the authors of the Hungarian Red List (Papp et al. 2010), who use the same criteria as used in the Czech Republic, including the LC-att and DD-va subcategories, use the Data Deficient (21.1%) and Lower Risk (17.3%) categories more frequently, while the authors of the Swiss Red List (Schnyder et al. 2004) treat 259 species (24.4.% of their bryoflora and 62% of their Red-Listed species) as Vulnerable based on criterion D2, i.e. their rarity in terms of very few locations or area of occupancy. Despite the various approaches, the numbers of threatened versus non-threatened species in central-European countries are very similar and substantially higher than in, e.g., the United Kingdom or Sweden (see Table 1).

Analysis of the Czech bryoflora

As reported above, there are 863 accepted species with 5 additional subspecies and 23 varieties, in the modern taxonomic sense, in the Czech bryoflora, i.e. taxa for which there is some genetic background and evolutionary history and which are not just based on phenotypic plasticity. Nine additional species, currently regarded as taxonomically doubtful, might in the future be added to the list if taxonomic studies provide the justification for this and/or the morphological characters that can be used for their identification, and 17 additional taxa if their historical (or eventually recent) occurrence is verified. The composition of the Czech flora can be analysed in several ways as outlined below.

Table 1. – Comparison of the Red Lists of selected countries. Percentages of taxa in particular categories are shown.

Categories	Czech Republic (this study)	Slovakia (Kubinská et al. 2001)	Hungary (Papp et al. 2010)	Switzerland (Schnyder et al. 2004	UK (Hodgetts 2011)	Sweden (www[1])
RE	4.5%	2.9%	0.5%	1.4%	2.4%	1.6%
CR	7.8%	10.5%	3.0%	5.6%	1.5%	0.7%
EN	9.9%	11.4%	13.7%	5.4%	3.8%	3.7%
VU	10.4%	12.3%	9.6%	26.0%	8.2%	5.6%
Sum of Red-Listed extinct and threatened taxa	32.6%	37.1%	26.7%	38.4%	15.9%	11.6%
LR	7.4%	9.4%	17.3%	6.2%	7.4%	6.1%
DD	6.1%	8.1%	21.1%	9.0%	1.8%	4.1%
LC	53.8%	45.4%	34.9%	46.4%	74.9%	78.3%
Sum of evaluated taxa	892	909	659	1083	1056[2]	1072[3]

[1] http://www.artfakta.se/GetSpecies.aspx, accessed on March 20, 2012.
[2] Sum of evaluated taxa inferred from Hill et al. (2008)
[3] Sum of evaluated taxa inferred from Hallingbäck et al. (2006).

Speciation-related problems

Because the structure of bryophytes is simple and some morphological and anatomical characters that can be used to identify them are confined to the ephemeral sporophytic stage, bryologists have always found it difficult to identify species. Only recently, with the advent of molecular techniques, have bryophyte taxonomists realized the extent of two important phenomena, which make it difficult to delimit species. The first is cryptic speciation, which is the molecular divergence and evolution of separate lineages, sometimes showing the characteristics of good biological species, but differing little if at all morphologically. The second is the role of hybridization in the formation of taxa, which is mostly accompanied by polyploidization.

Cryptic speciation is documented, e.g. in the liverwort genera *Pellia*, *Aneura* and *Calypogeia* (Buczkowska 2004, Buczkowska & Bączkiewicz 2011, Wachowiak et al. 2007) and moss genera *Hamatocaulis* and *Rhynchostegium* (Hedenäs & Eldenäs 2007, Hutsemékers et al. 2012), and it is assumed or has been already documented that the formally undescribed sibling species reported in these papers do occur in the Czech Republic, representing moreover probably only the "tip of an iceberg". Morphological characters that can be used for naming the earlier not recognized cryptic lineages have in some cases been successfully identified (e.g., *Conocephalum salebrosum*, see annot. 4 above) and this process is likely to continue in the future.

Moss hybrids have rarely been identified and formally described in the past and have generally been omitted from checklists, including the European list of Hill et al. (2006). The Polish catalogue (Ochyra et al. 2003) represents a rare exception, listing the putatively hybridogeneous taxa *Funaria* ×*hybrida* R. Ruthe ex Limpr. and *Physcomitrella* ×*hampei* Limpr., which are also likely to occur in the Czech Republic. Recent studies have shown that there are allopolyploid hybrids, commonly of polytopic origin, not only in taxa that have traditionally been regarded as difficult (*Porella* ×*baueri*, removed from the main list, see under Not Evaluated – taxonomically doubtful taxa) but also in taxa that have the characteristics of typical species, with clear morphological characters, ecology and pattern of

distribution (*Plagiomnium medium*, *Polytrichum longisetum*, *Sphagnum auriculatum*, *S. majus*, *S. papillosum* and others). The situation needs to be clarified e.g. in the *Metzgeria conjugata/simplex* and *Riccia ciliifera/gougetiana* complexes, which are no longer included in the main list, and also in *Sphagnum inundatum*, in which the equivocal morphological delimitation is obviously related to its complex speciation pattern (Shaw et al. 2008, 2012).

Native status, invasive and spreading species

Of the taxa that are known to occur in the Czech Republic the majority are native, with new records of bryophytes being published nearly every year, depending on the level of bryofloristic activity and application of latest taxonomic treatments, which recognize new taxa. With respect to non-native (exotic) taxa, bryophytes differ from vascular plants mainly in the fact that such species are hardly ever deliberately introduced and the recording of such accidental introductions is poor (Essl & Lambdon 2009). Most of the unintentional introductions are ephemeral escapes of tropical and subtropical species from greenhouses that are usually not included on lists of non-native plants of individual regions and are also not included in this list, which is in accordance with the practice adopted by Pyšek et al. (2002). Bryophytes may not only commonly be cryptogenic in the sense of Carlton (1996), i.e. not clearly native or exotic (alien), because of the way species that enter the Czech Republic from a neighbouring region and spread are evaluated. They are categorized as non-native if they arrived from an area in which they are also non-native but native if they are native in the area from which they spread (Pyšek et al. 2004). However, in bryophytes this differentiation may be less straightforward or even arbitrary, because native status in the area of origin may be disputed (cf. the status of *Didymodon umbrosus* in the British Isles, Smith 2006) and moreover the character of the spontaneous spreading/invasion of individual bryophyte species hardly differs between putatively 'native in the neighbouring/next-to-neighbouring area' and non-native taxa. Hence we have summarized the available information for known cases of non-native and recently spreading species and explain the particular circumstances in each case. For the definition of the terms see Pyšek et al. (2004).

Non-native species

Lunularia cruciata – probably a casual alien, widespread in the Mediterranean area and western Europe, which regularly occurs in botanical gardens and parks and sometimes it is reported for extended periods of time in natural biotopes in the Czech Republic (Prokopské údolí valley in Prague), probably dependent on the repeated adventive supply of diaspores.

Campylopus introflexus – invasive, introduced to the British Isles from Southern Hemisphere, first recorded in the Czech Republic in 1988 (Novotný 1990) and currently spreading rapidly (Mikulášková 2006). *Campylopus introflexus* is probably the only Czech non-native species that depends on human activity for its spread (exploited peatlands or other easily colonizable substrates).

Orthodontium lineare – invasive non-native species, first recorded in the Czech Republic in 1964 (Futschig & Kurková 1977), rapidly spreading throughout the country (Soldán 1996) in natural habitats.

Didymodon umbrosus – probably a naturalized non-invasive species, first recorded in 1997 (Kučera 1999). Not yet reported from any other than its initial locality near Prague, revisited by JK in 1998 and 2000.

Native species that are extending their ranges and cryptogenic species

Campylopus flexuosus – probably a native species, which was regarded as very rare by older authors (e.g. Velenovský 1897), is nowadays widely distributed in sandstone regions and dry pine woods throughout the country and seems to be spreading.

Campylopus pyriformis – was first reported from the Czech Republic in the 2003 checklist, although the revision of herbarium material showed that it was collected earlier (one from 1899 and another from 1968). It is currently widely scattered in south-western and the southern part of the country and is perhaps still spreading.

Bryoerythrophyllum ferruginascens – first reported from this country by Pilous (1993), based on the adventive occurrence in an abandoned limestone pit. Since then, the species seems to be spreading in similar habitats and along the roads and interestingly, the revision of unidentified herbarium material of *Pottiaceae*, revealed earlier collections, among others the probably native occurrence on rocks in the Hrubý Jeseník Mts.

Dicranum tauricum – according to the bryoflora of Czechoslovakia (Pilous & Duda 1960), this species was reported to occur only 'rarely in eastern Slovakia'. First Czech reports started to appear in early 1990s (Anonymous 1993). Franklová (1997) summarized the known distribution, based on an old herbarium record from 1927, two records from 1977–1978 and an increasing number of records since 1989.

Dicranoweisia cirrata – known from the Czech Republic since the time of the early bryological studies but recorded only extremely sporadically between the first record in 1884 and early 1980s (Plášek 2001). Since then, the species has spread widely, particularly as an epiphyte.

Orthotrichum pulchellum – apparently native in western Europe and otherwise occurring only in western North America but now a cryptogenic species spreading in many countries of western to central Europe. Its rapid expansion after apparently completely vanishing in Germany started in early 1990s (Frahm 2002), together with other (sub)oceanic taxa (*Ulota phyllantha*, *Zygodon conoideus*, *Dendrocryphaea lamyana*, *Orthotrichum consimile*, *Metzgeria temperata*), of which the latter three have already been recorded in neighbouring Saxony and Bavaria (Müller 2004, Meinunger & Schröder 2007). The rate of spread of *O. pulchellum* is moderate and no adverse effect on native epiphytes has been observed.

Orthotrichum rogeri – regarded as native, historically known from a single locality in northern Moravia near Šumperk. Spreading at a moderate rate from Saxony since 2008 (Kučera 2009b) in a way comparable to that of *Orthotrichum pulchellum.* The source of recolonization lies obviously outside the Czech Republic.

Uncertain cases

Zygodon dentatus, *Orthotrichum patens*, *Metzgeria violacea*, *Orthotrichum tenellum* and *Microlejeunea ulicina* and many other epiphytes might belong among taxa that have started to spread in this country, although in the case of the latter two species there is only a single recent record, and their eventual spread is only inferred from the situation in neighbouring regions of Germany (Seifert 2009). The spread of epiphytes following the improvement in air quality in recent decades occurred in all central-European countries. It is interesting that the restored habitat is not simply being reclaimed by earlier occurring epiphytes but rather earlier unknown or extremely rarely occurring species emerge, often using migratory routes different from the historical ones (the above mentioned *Orthotrichum rogeri, Zygodon viridissimus*). The cases of recently spreading terrestrial bryophytes are less clearly documented but *Endogemma caespiticia* is an example; whether the terrestrial species of *Bryum* and *Pohlia* with rhizoidal and axillary gemmae are spreading, is not known, as they were recognized only in the last three decades.

Phytogeographic considerations

Phytogeographic aspects of the bryophytes occurring in the Czech Republic have never been studied in a comprehensive way and this task goes far beyond the scope of this article. The main problem is the incomplete knowledge of the world-wide distribution of those bryophytes occurring in Europe, and also the generally broad distribution pattern of most European bryophytes, which is very difficult to simplify and abstract in a way that could be easily used in regional bryophytogeographic analyses. Dierßen (2001) tried to summarize the available phytogeographic information on European bryophytes, based largely on earlier works by Düll, but his evaluation is difficult to apply for the above mentioned reasons and in many cases his evaluation is very different from our experience, hence we have refrained from presenting a general phytogeographic analysis of the Czech bryoflora and a comparison with that of neighbouring countries.

The geographic position of the Czech Republic in central Europe, which is influenced both by oceanic and continental climatic conditions but at the same time is protected from

their more extreme effects, latitudinally belongs to the middle of the temperate zone and altitudinally mostly occupies the lower and middle altitudes, barely touching the lower alpine zone in the highest mountain ranges. The presence of individual species and their distribution has historically been determined by climate changes, particularly numerous and severe during the Pleistocene, although significant climate changes have occurred throughout the Holocene, local geology and geomorphology (influencing the microclimatic condition), human activity and the dispersal and establishment abilities. Logically, the Czech bryoflora contains the majority of the broadly distributed, temperate, or boreo-montane elements.

With respect to the gradients of oceanity and continentality, the Czech bryoflora has several dozens of suboceanic elements, which more or less reach their eastern-European limit of distribution in the Czech Republic or at last markedly decrease in abundance further east – *Anastrepta orcadensis*, *Cephalozia macrostachya*, *Kurzia* spp., *Microlejeunea ulicina*, *Nardia compressa*, *Odontoschisma sphagni* and *Scapania compacta* may be named among the liverworts and *Campylopus* and *Dicranodontium* species, *Kindbergia praelonga*, *Fissidens rufulus*, *Hookeria lucens*, *Hypnum imponens*, *Isothecium myosuroides*, *Mnium hornum*, *Plagiothecium undulatum*, *Rhabdoweisia crenulata*, *Thamnobryum alopecurum* and *Zygodon dentatus* among the mosses, to name just a few examples. The more pronouncedly oceanic species commonly do not occur in the Czech Republic, although recorded in Germany or Austria, sometimes even close to their border with the Czech Republic (e.g. *Metzgeria temperata*, *Solenostoma paroicum*, *Frullania microphylla*, *Leptodontium flexifolium*, *Syntrichia pagorum*, *Racomitrium obtusum*, *Zygodon conoideus*, *Pterogonium gracile*, *Isothecium holtii* and *Hygrohypnum eugyrium*). Interestingly, while there are several suboceanic bryophytes among them, which are now regarded extinct or vanished from Czech Republic (*Gymnomitrion obtusum*, *Pallavicinia lyellii*, *Neckera pumila*, *Ptychomitrium polyphyllum*, *Sphagnum austinii*, *Ulota drummondii*), another group of suboceanic taxa is now spreading eastwards, particularly but not solely, the epiphytes (*Orthotrichum pulchellum*, *Microlejeunea ulicina*, *Campylopus introflexus*, *C. pyriformis*). Subcontinental elements in the Czech flora are much rarer and mostly can be attributed to the Pannonian migration route (*Hilpertia velenovskyi*, *Syntrichia caninervis*) but there are also rare examples of eastern boreal elements (*Callicladium haldanianum* and also the common *Eurhynchium angustirete*, which is increasingly rare west of the Czech border). *Tortula lingulata* is another example of a taxon with a very limited (subendemic) distribution centred in the eastern Baltic region.

The Czech Republic is also the region, where several circumboreal species reach their southern limit of distribution and a few southern taxa are at their northern limit. Well-known examples of circumarctic or circumboreal taxa at their southern limit in the Czech Republic are *Sphagnum lindbergii*, *Discelium nudum* and the vanished *Dichelyma falcatum* and *Sphagnum jensenii* (known from Poland just a few dozen metres from our boundary) can be added to these examples if we do not limit our considerations to political boundaries. Southern species generally do not reach their northern limit in the Czech Republic but mostly extend to the warm, subcontinental regions of Germany via the Pannonian route (*Didymodon acutus*, *D. cordatus*, *Hilpertia velenovskyi*) or have reached the oceanically influenced regions in north-western Europe in the case of the species spreading from the southwest. A rare and remarkable example of a subcontinental south-eastern element is the probably extinct *Syntrichia caninervis,* with one historical locality

in southern Moravia, and the only known example of the extant Illyric-Insubric element is *Frullania inflata*, known from several close by locations in southern Moravia. Two primarily Alpine species that occur in the Czech Republic are *Plagiothecium neckeroideum*, occurring only in the Šumava Mts (Bohemian Forest) and *Streblotrichum enderesii*, known from one historical locality in the Krkonoše (Giant) Mts.

There are very few examples of convincingly stenoendemic bryophyte species in Europe, because the ability of bryophytes to disperse is considerable and the rate of speciation accompanied by observable morphological changes is relatively low. Therefore, despite the fact that many originally believed endemic species are described for Europe, they have later either been synonymized with earlier described, broadly distributed taxa, or have been recorded from other localities in Europe or beyond. Several dozens of broadly distributed species were originally described from the Czech Republic, including e.g. *Racomitrium sudeticum* described from Krkonoše Mts and *Fossombronia wondraczekii* and *Hilpertia velenovskyi*, from localities in what is now Prague. *Bryum moravicum*, described by Podpěra as a southern-Moravian endemic from one locality near Řeznovice, was recently shown to be the oldest name for a widely distributed species, which has been known under several different names (Kučera & Holyoak 2005). Three taxa were described recently from the Czech Republic: *Platyhypnidium grolleanum* Ochyra, which probably represents only a rheophytic modification of *Rhynchostegium riparioides*, and two *Orthotrichum* taxa – *O. moravicum* and *O. affine* var. *bohemicum*. It is likely that further localities of the latter two taxa will be reported from adjacent countries in the near future, as the latter taxon has been recorded in the USA (Plášek in Ellis et al. 2012). An interesting example of a relatively stenoendemic species that was described from the Czech Republic and not so far recorded elsewhere than in central Europe, is *Anthoceros neesii*. Although it occurs in the common, broadly distributed biotope of stubble fields in submontane regions on non-calcareous substrates, it seems to be surprisingly rare and was long regarded as having vanished from our bryoflora, until its rediscovery in 2010 (Koval & Zmrhalová 2010).

With respect to relic taxa, the reasons for their scarcity and the problems with their identification are the same as for the regional endemics. It can be assumed that species of severely fragmented fen biotopes, which are entirely dependent on non-specific vegetative propagation, can be considered to be relics from the Ice Age. These species are generally under strong threat (*Drepanocladus sendtneri*, *D. trifarius*, *Helodium blandowii*, *Meesia triquetra*, *Paludella squarrosa*, *Scorpidium scorpioides*) or have already become extinct (*Bryum longisetum*, *Drepanocladus lycopodioides*, *Meesia longiseta*). Glacial relics can also be identified among the arctic-alpine elements, although these are more often species that sporulate and hence it cannot be excluded that their populations were sometimes boosted by propagules from the Alps or other mountain ranges during the Holocene. Nevertheless, this group of species seems to be currently declining in abundance (*Anthelia juratzkana*, *Gymnomitrion corallioides*, *Lophozia wenzelii*, *Dicranum elongatum*, *Grimmia elatior*, *Kiaeria falcata*) or such species have apparently become extinct in the past few decades (*Gymnomitrion adustum*, *G. brevissimum*, *Arctoa fulvella*, *Grimmia unicolor*, *Ochyraea smithii*, *Pohlia obtusifolia*, *Polytrichastrum sexangulare*), as a consequence of successional changes connected with the warming of the climate in the recent century.

Earlier authors speculated about the possibility of pre-glacial relics. This seems to be particularly tempting in cases of bryophytes occurring in biotopes where the level of competition from vascular plants is very low and which are believed not to have grown by woods during the last climatic optima or were climatically stable with respect to specific geomorphological and geological conditions. Suza (1938) believed that *Oxymitra incrassata*, *Riccia ciliifera* and *R. ciliata*, which occur in the valleys of larger rivers in southern Moravia, might be Tertiary relics, Pospíšil (1962) suggests a similar scenario for the occurrence of *Frullania inflata* near Znojmo and later (Pospíšil 1968) for Pleistocene refugia for *Homalothecium lutescens*, *Entodon concinnus*, *Rhytidium rugosum* and *Abietinella abietina*. Similarly the occurrence of *Targionia hypophylla* at the ventaroles on Boreč hill was regarded as a relict population that goes back to the Tertiary (Pilous 1959). Nevertheless, sound evidence of the length of time these bryophytes have been present at these localities is missing.

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Souhrn

Předkládáme stručnou analýzu bryoflóry České republiky založenou na aktualizované verzi seznamu a červeného seznamu mechorostů České republiky. Do soupisu druhů byly zahrnuty veškeré nové nálezy a revize vztahující se k našemu území a taxonomická pojetí rodů, druhů i poddruhových taxonů byla přizpůsobena nejnovějším taxonomickým a fylogenetickým studiím. Hlavní seznam nyní obsahuje 863 druhy mechorostů (4 hlevíky, 207 játrovek a 652 mechů) s 5 dalšími poddruhy a 23 všeobecně uznávanými varietami; 9 dalších druhů je uvedeno jako taxonomicky problematických a nejistý či neprokázaný výskyt je dokumentován pro 17 dalších druhů. Zároveň jsme znovu kompletně přehodnotili podkladová data pro aplikaci IUCN 3.1 kritérií pro vytvoření revidovaného červeného seznamu mechorostů, který předkládáme zároveň se seznamem. Z 892 hodnocených taxonů bylo 46 % vyhodnoceno jako splňující některé z kritérií pro zařazení do červeného seznamu (40 taxonů v kategorii RE, 70 v CR, 88 v EN, 93 ve VU, 66 v LR-nt, 24 v DD-va a 30 v DD), 54 % bylo hodnocených jako neohrožených, z nich ovšem 120 zůstává v seznamu druhů vyžadujících pozornost (podkategorie LC-att). V analýze bryoflóry diskutujeme taxonomické problémy, které ovlivnily naše rozhodování v hodnocení oprávněnosti rozeznávání druhů i hodnocení kritérií potenciální ohroženosti, pokusili jsme se sestavit seznam nepůvodních, invazních a expanzních mechorostů ČR a rozebíráme specifické problémy mechorostů z hlediska původu a invazivnosti. Dotýkáme se také fytogeografických aspektů reliktnosti, okrajů areálu, endemismu a uvádíme významné elementy z hlediska kontinua kontinentality a oceanity.

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Appendix 3: Biology of rare and threatened species (supervision of students' theses)

Paper 6: Holá E., Košnar J. & Kučera J. 2015. Comparison of Genetic Structure of Epixylic Liverwort *Crossocalyx hellerianus* between Central European and Fennoscandian Populations. – PLoS ONE 10: e0133134.

PLOS ONE

RESEARCH ARTICLE

Comparison of Genetic Structure of Epixylic Liverwort *Crossocalyx hellerianus* between Central European and Fennoscandian Populations

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Abstract

Patterns of genetic variation and spatial genetic structure (SGS) were investigated in *Crossocalyx hellerianus*, a strictly epixylic dioicous liverwort (Scapaniaceae *s.l.*, Marchantiophyta). Studied populations were located in Fennoscandia and Central Europe, with localities differing in availability of substrate and the population connectivity, and their populations consequently different in size, density, and prevailing reproductive mode. A set of nine polymorphic microsatellites was successfully developed and used. Identical individuals were only found within populations. Especially in large populations, the majority of the individuals were genetically unique. Resampled number of genotypes, mean number of observed alleles per locus after rarefaction, and Nei's gene diversity in large populations reached high values and ranged between 4.41–4.97, 3.13–4.45, and 0.94–0.99, respectively. On the contrary, the values in small populations were lower and ranged between 1.00–4.42, 1.00–2.73, and 0.00–0.95, respectively. As expected, large populations were found to be more genetically diverse than small populations but relatively big diversity of genotypes was also found in small populations. This indicated that even small populations are important sources of genetic variation in bryophytes and processes causing loss of genetic variation might be compensated by other sources of variability, of which somatic mutations might play an important role. The presence of SGS was discovered in all populations. Large populations possessed less SGS, with individuals showing a pronounced decrease in kinship over 50 cm of distance. Apparent SGS of small populations even at distances up to 16 meters suggests the aggregation of similar genotypes, caused predominantly by the deposition of asexually formed gemmae. Although no strong kinship was detectable at the distances over 16 meters in both small and large populations, identical genotypes were occasionally detected at longer distances (20–80 m), suggesting effective dispersal of asexual propagules.

Introduction

The structure of genetic diversity on fine scales within populations and on larger scales among populations may bring valuable insights into the reproductive systems of studied organisms including the assessment of reproductive effort, rates of sexual and vegetative reproduction, dispersal capacity of diaspores and levels of gene flow among populations. Bryophytes are generally considered to possess high dispersal capacity of their sexually originating spores [1, 2], which however is often impaired by the relatively low reproductive effort allocated into the production of energetically costly sporophytes. In dioicous bryophytes, which constitute a significant proportion as opposed to the situation in remaining land plants [3, 4], the sexual reproduction is further complicated by the necessity of spatial proximity of male and female gametangia, as the dispersal range for sperm is generally very short [5, 6] On the other hand, most bryophytes also propagate by means of vegetative fragments, and a notable proportion of bryophytes produce specialized vegetative diaspores, such as the gametophytic gemmae, which were proven to possess a dispersal capacity comparable to spores and even effectively contributing to gene flow among populations [7]. Recruitment of progeny is nevertheless not only dependent on the formation and dispersal capacity of diaspores, but also on the diaspore establishment and sustainable growth conditions for mature plants. A significant proportion of bryophytes are known to be strictly specialized in particular substrates or habitats [8], and one such examples of habitat specialization are the epixylic species, i.e. species growing on decomposing wood matter. Decomposing wood supports a rich community of plants, fungi and animals [9]. Decaying logs are a very dynamic substratum with a non-random patchy distribution, restricted duration and time-variable quality [8, 10] where composition of bryophyte communities changes following the decay stage of logs [11]. Moreover, a sufficient amount of decomposing wood is missing from most human-managed forests and is only present in natural and old-growth forests. These unfortunately belong to prime examples of habitats under globally strong anthropogenic pressure [12]. Epixylic species are thus handicapped on two scales. The suitable substrate is not continuously available, as exemplified in a study of the epixylic liverwort *Ptilidium pulcherrimum*, which showed that less than 1% of produced spores were deposited on substrate suitable for establishment [13]. On the landscape scale, extensive forestry has resulted in considerable decrease and fragmentation of forest habitats, in which the specific substrate occurs. To date, no strictly epixylic bryophyte has been studied, although the genetic diversity and structure of epiphytic forest bryophytes has been addressed in several studies [14–16]. Genetic variation in wood living fungi and beetles, to our knowledge the only studied epixylic organisms, showed low gene flow and low genetic variation among isolated and fragmented populations similarly as it was the case in other forest dwelling species [16–18].

Recent studies of population genetic variability and spatial genetic structure using DNA fingerprinting methods have shown a remarkable variability of results, showing the uniqueness of parameters of individual reproduction systems in different taxa. One of the most interesting findings is that the level of genetic differentiation among bryophytes reproducing mostly or exclusively vegetatively was in several cases surprisingly high [19–21]. The genetic variability in mostly non-sexual populations can be maintained by migration from neighboring populations, occasional sporophyte production, or by the accumulation of somatic mutations [19, 20, 22]. Studies of spatial genetic structure (SGS) in bryophyte populations are also relatively rare [14, 23–26]. Only one study [23] focused on small-scale pattern of SGS in the liverwort species *Barbilophozia attenuata* Mart. (Loeske), which is a species closely related to our object of study, possessing a similar reproduction mode.

We have studied *Crossocalyx hellerianus* (Nees ex Lindenb.) Meyl., a minute, circumboreally distributed dioicous epixylic liverwort (Fig 1) of the family Scapaniaceae *s.l.* (Anastrophyllaceae

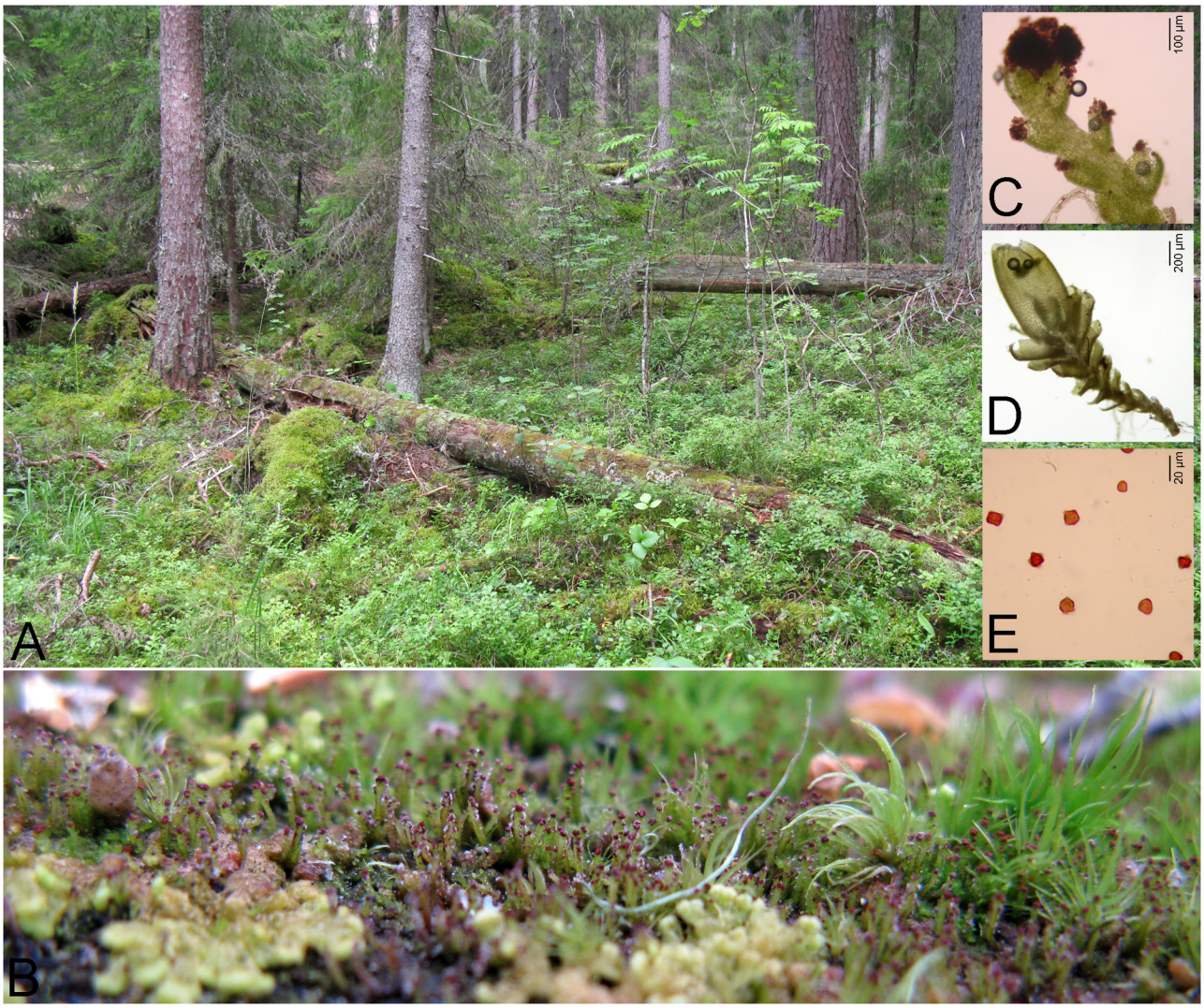

Fig 1. The studied species *Crossocalyx hellerianus*. Pictures from Vesijako Strict Nature Reserve (A) overgrown log of *C. hellerianus*, (B) *C. hellerianus* in detail. Light microscope pictures (C) gemmiparous shoot, (D) perianth, (E) gemmae.

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[27, 28]). Both sexually formed spores and asexual gemmae are produced, with both being approximately 10–12 μm in diameter. Sexual reproduction is described as occasional in Nordic countries (sporophyte formation was observed in 2.5–12% of the colonies [29]), whereas in other parts of European distribution area it might be much rarer, e.g. they were never reported from Ireland and Britain [30, 31]. On the contrary, gemmae are always present and generally abundant. It is considered to be a colonist species with the potential life span of only a few years [32], inhabiting decaying logs (mostly of spruce) of intermediate decay stages [33]. With respect to its habitat preference, it usually occurs in old-growth spruce forests with high amounts of coarse woody debris [33] and therefore it is relatively rare in all parts of its distribution area. In the countries of this study, it has been classified as Near Threatened (NT) in Finland [34], and Endangered (EN) in the Czech Republic [35] according to IUCN criteria. In the latter country, only 8 populations are recently known, with only one population classified as large (see below for definitions).

The study populations, located in Scandinavia and Central Europe, differ in size, density, prevailing reproductive mode, and population connectivity. Thus, these populations represent a suitable study system for investigations on patterns of genetic variation with regard to the above mentioned population characteristics. The studied liverwort moreover produces sexual and vegetative diaspores of potentially very similar dispersal capacities with respect to their size, which facilitates the inference on dispersal efficiency. We hypothesized that the population size or density and prevailing reproductive mode would be mirrored in the population genetic diversity and fine-scale spatial genetic structure. Microsatellite markers, which have been developed for his study, further allowed for the assessment of gene flow levels among populations and rates between sexual and asexual reproduction.

Material and Methods

Study sites and sampling

Sampling was performed in Finland (FI, 4 populations) and in the Czech Republic (CZ, 6 populations; Table 1 and S1 Fig). Mean geographic distances among CZ populations amounted to 55 km, those among FI populations 62 km, and the distances among CZ and FI populations averaged 1500 km (S1 Fig). Studied Finnish populations are located in the boreal zone of southern Finland, representing only a part of regional populations [34]. Czech populations are located in South Bohemia within the temperate zone and represent all known Czech localities as of 2012. The Finnish forests are mainly old virgin forests dominated by spruce with several canopy layers (pines, birches and aspens), characterized by huge amounts of decaying conifer wood, which is reflected in the relatively common occurrence of *Crossocalyx hellerianus*. The Czech forests represent small extant fragments of herb-rich and acidophilous montane mixed old-growth forests with the tree composition and herb vegetation approaching the natural one, dominated mostly by beech with spruce admixtures. The amount of suitable decaying wood is only high in the Boubínský prales National Nature Reserve among the Czech forests. Consequently, *C. hellerianus* is relatively common only in this reserve, while the other Czech localities support only very small populations of the liverwort (Table 1).

In populations, where *C. hellerianus* was abundant (with more than 10 logs supporting the species, further on assigned as 'large' populations, Table 1), 8–9 logs were sampled. In smaller populations ('small', Table 1), all logs supporting the occurrence of *C. hellerianus* were sampled and surroundings of these logs (up to 0.5 km around) were investigated for possible occurrence.

Approximately 0.5×0.5 cm was sampled from every occurrence of *C. hellerianus* at a minimum distance of 20 cm; the maximum distance depended on the patchy distribution of species on each sampled log (Fig 2). For detection of genetic structure at the smallest spatial distances, three shoots were taken from four pairs of neighboring patches (one pair on each log) in large populations and three shoots from two pairs of neighboring patches in small populations. One shoot was taken from each of the other patches. Distances among shoots that originated from the same patch were arbitrary equaled to one centimeter and distances among the sampled patches were measured. The small size of the population Nová Bystřice (10×15 cm) allowed for removal of only five shoots.

All studied populations were searched for the production of sporophytes. As these are ephemeral and we were not able to record them at the time of visit, perianths were considered as the indication of the sexual reproduction. Perianths (Fig 1D) of the leafy liverworts are gametophytic structures of foliar origin around the archegonium which serve the protection of developing capsule. Perianths were searched in all sampled patches, using a stereo-microscope.

Table 1. List of study populations with quantitative data.

Locality abbr.	Population	Coordinates [WGS 84]	Country	Population size	Number of sampled logs	Date of sampling (DD. MM.YY)
Z	Boubínský prales National Nature Reserve	48°58'32"N, 13° 48'54"E	CZ	LARGE	9	17.11.12
G	Kamenná hill	48°49'08"N, 13° 48'50"E	CZ	SMALL	3	22.11.12
M	Medvědí hora Nature Monument	48°37'13"N, 14° 13'40"E	CZ	SMALL	2	16.09.12
Y	Milešický prales Nature Reserve	48°59'06"N, 13° 50'19"E	CZ	SMALL	5	17.11.12
R	Nová Bystřice	49°01'13"N, 15° 01'16"E	CZ	SMALL	1	08.05.12
P	Žofínský prales National Nature Reserve	48°40'10"N, 14° 42'20"E	CZ	SMALL	2	13.10.12
N	Nuuksio National Park	60°18'36"N, 24° 29'57"E	FI	LARGE	8	11.08.12
S	Sudenpesänkangas Nature Reserve	61°12'15"N, 25° 11'49"E	FI	LARGE	8	08.08.12
K	Kotinen Nature Reserve	61°14'28"N, 25° 03'47"E	FI	LARGE	8	08.08.12
V	Vesijako Strict Nature Reserve	61°21'00"N, 25° 06'04"E	FI	LARGE	8	09.08.12

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Ethics statement

All necessary permits were obtained for field studies to collect species material. Metsähallitus issued the permission for entry into Finish localities and Nature Conservation Agency of the Czech Republic issued the entry permission into Czech localities. No special permission is required for sampling of *Crossocalyx hellerianus* in the respective countries, although it is considered is Endangered (EN) species in the Czech Republic according to IUCN criteria [32],

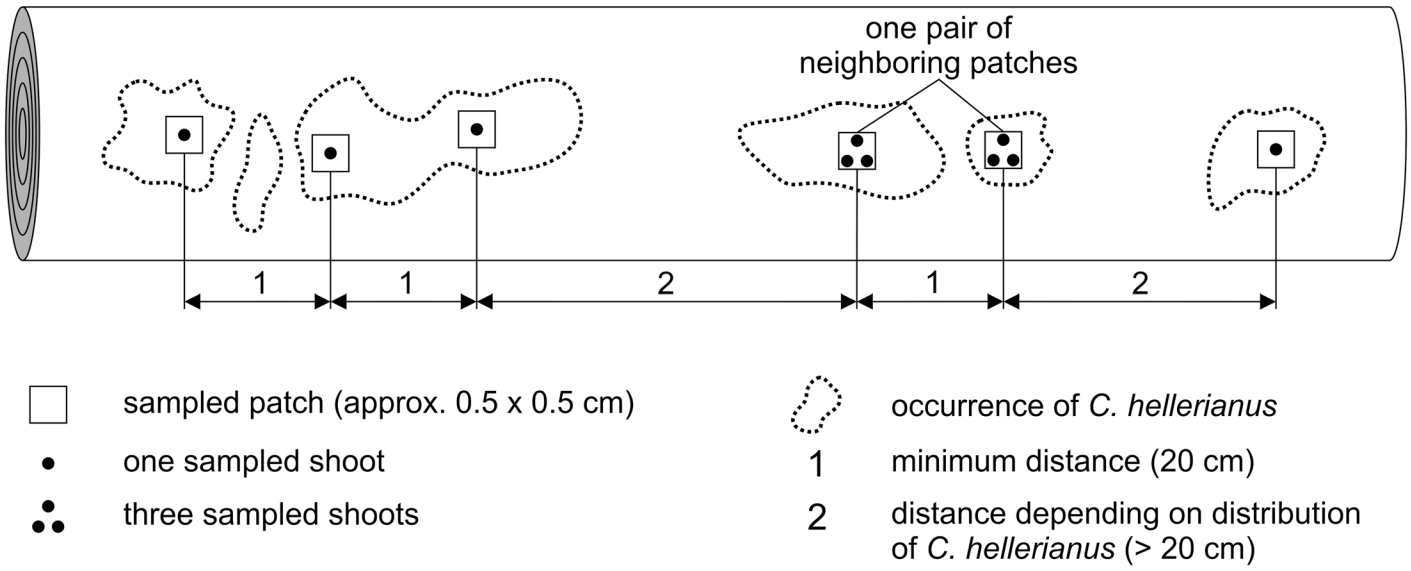

Fig 2. Schematic illustration of *Crossocalyx hellerianus* sampling on logs.

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which however does constitute the basis for legal protection in that country (see S1 File). The species sampled are not listed by CITES (Convention on the International Trade in Endangered Species). All studied localities are on public lands.

Genetic analysis

A SSR-enriched genomic library was constructed using a biotin-streptavidin capture method [36]. Screening of SSR-enriched genomic library was performed using combined approach involving traditional cloning and Sanger sequencing of the library, together with direct 454 pyrosequencing of the library on a GS Junior System (454 Life Sciences, Branford, USA) as described in [37]. Specific primers were designed using Primer3 [38, 39], see Table 2.

Total genomic DNA was extracted from each of the analyzed shoots using the NaOH method [40]. PCRs were performed in a reaction mixture containing 0.5 μL of genomic DNA, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.3 μM primers, 0.25 U *Taq* polymerase (Top-Bio, Prague, Czech Republic) in the manufacturer's reaction buffer, and sterile water to make up a final volume of 5 μL. Amplifications were performed with an initial denaturation of 3 min at 94°C, followed by 45 cycles of 1 min denaturation at 94°C, 30 s at primer-specific annealing temperature (Table 2), 15–30 s extension at 72°C, and a final extension of 10 min at 72°C. PCR products were pooled and analyzed using fragment analysis on an ABI 3730xl DNA Analyser (Applied Biosystems) with GeneScan 600 LIZ (Applied Biosystems, Foster City, USA) as the internal size standard. Microsatellite alleles were scored using GeneMarker v1.80 (SoftGenetics LLC, State College, USA) and were coded as a number of repeats of the SSR motif. Samples in which amplification of more than three loci failed were omitted. Allelic data are available in S2 File.

Data analysis

Nei's gene diversity ($\hat{H}$) was calculated using Arlequin v3.5 [41]. Number of genotypes—N_g and number of recurrent genotypes—N_{rg} were calculated in the GenClone 2.0 program [42]. With respect to different sample size of populations, values of N_g were resampled using GenClone 2.0, and HP-Rare software [43] was used for rarefaction of mean number of observed alleles per locus—N_a. For both N_g and N_a calculations, the sample sizes were adjusted to five (the smallest sample in the comparison). The probability that individuals shared the same multilocus genotypes (MLG) were derived from sexual reproduction involving recombination (P_{sex}) calculated in the GenClone 2.0. Samples with missing data were excluded from all above mentioned computations.

In addition to P_{sex} assessment, linkage disequilibrium analysis was performed to assess whether marker distributions resulted from sexual or asexual reproduction. Multilocus linkage

Table 2. Characterization of the nine microsatellite loci developed for *Crossocalyx hellerianus*.

Repeat motif	Ta [°C]	Forward primer (5'- 3')	Reverse primer (5'- 3')	No. of alleles	Size range [bp]	GenBank accession no.
(TG)13	58	CCACTTTCCATTGTGACCTTT	AGTTTCTTCTCCGCCATCA	7	148–160	KM065844
(AC)10	54	GGACGCACTAACTCGTTTTCTC	GGTCCAGCATGAGGTTGATT	33	246–314	KM065843
(TG)24	54	TTCTGTCATTTTCGGATTTGG	GTGGGCAACTTCTTTGGACT	18	384–426	KM065842
(TC)24	54	TTGGGATGAGAAAAGTGA	CCTCGTATTGATTGTGGGTAT	24	486–536	KM065838
(GT)10	54	CCTTGCAGCTCATATCTTGTT	CCTTTCGTCCACCATAAGTCC	14	205–237	KM065837
(CA)11	54	CCAAGCATGAACTAATCCCATC	GCAAAGGTAACACCAAAGTGAG	5	158–172	KM065839
(CA)21	58	TCAAGAACCTTACATCCAAACC	GCATCACTCACTCCTCACCA	25	307–357	KM065840
(AC)13	54	CGTGGAAAGACTGTTGAGGA	GGATTTGAGGCGAGGGATAG	7	173–185	KM065845
(GT)13	54	CAAGCCAACAAGGAGAGAGATT	AAGCCCAATGTGAAGAAGGA	12	226–260	KM065841

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disequilibrium was tested using the index of association modified to remove the effect of number of loci analyzed (r_d [44]) and calculated for each population using Multilocus v 1.3. Significance was tested by comparing the observed dataset against the null hypothesis of infinite amount of sex and recombination by random shuffling the alleles amongst individuals using 1,000 randomizations.

Hierarchical structure of genetic variation was examined using analysis of molecular variance (AMOVA) in Arlequin v 3.5 [41] with calculations based on the R_{ST}-like method, using the sum of squared size differences. The R_{ST}-like method was preferred because a preliminary allele permutation test performed in SPAGeDi 1.4 software [45] was significant, indicating that an allele size-based statistic was informative for population differentiation and may contain more information than allele identity measures such as F_{ST}, which is likely to provide a biased estimate of gene flow [46]. The following partitioning of genetic variation was tested: between distant geographic regions (Czech Republic and Finland) and among localities within the regions. The analysis based on F_{ST}-like method showed that variation among populations within regions was slightly higher than variation between the two geographic regions (CZ vs. FI). In addition, the pairwise R_{ST} values for all populations were computed. The significance of AMOVA components and of pairwise R_{ST} values was tested using 10,000 permutations.

To reveal the fine-scale spatial genetic structure (SGS), a spatial autocorrelation analysis was conducted in SPAGeDi 1.4 software [45]. Distance classes with upper boundaries of 0.01, 0.5, 1, 2, 4, 8, 16 and 500 m were used (spatial sampling information is available in S3 File). Multilocus pairwise kinship coefficients (F_{ij}) based on Nason's kinship coefficient [47] were calculated. To test the influence of population size on SGS, populations were further assigned into three groups: small CZ populations, large FI populations and the large CZ population (see Table 1). For each group of populations, mean multilocus pairwise kinship coefficient values were plotted against the upper boundaries of geographic distance classes. Significance of the mean F_{ij} per distance class was tested using 1,000 random permutations of individuals.

The spatial extent of clonal dispersal was quantified using distance classes and population assignment defined as above. The percentage of clones within each of the distance classes was calculated using pairwise comparisons which included identical genotypes and they were plotted against the upper boundaries of classes. In addition, the maximum distance among samples of the same genotype was recorded for each population.

Results

Population genetic analyses

Nine polymorphic microsatellite markers from the liverwort *Crossocalyx hellerianus* were developed (Table 2 and S2 Fig). All genotyped material was haploid and the microsatellite loci contained between 5 and 33 alleles (Table 2). The final dataset of 393 successfully genotyped samples contained two samples with missing data for three loci, four samples with missing data for two loci, and 52 samples with missing data for one locus, respectively. 243 MLGs were found among the 335 genotyped individuals (without missing data). Identical genotypes were only rarely detected inside large FI and CZ populations, while in small CZ populations recurrent genotypes occurred at higher rates (Fig 3 and S3 Fig). Identical genotypes were relatively frequently detected only within individual logs (see below). No identical genotype has been found among populations.

Resampled number of genotypes (N_g), mean number of observed alleles per locus (N_a) after rarefaction, and Nei's gene diversity ($\hat{H}$) varied from 1.00 to 4.97, 1.00 to 4.45, and 0.233 to 0.995, respectively (Fig 3). Lower values of N_a, N_g and $\hat{H}$ were detected in small CZ populations; the small CZ population R contained a single MLG.

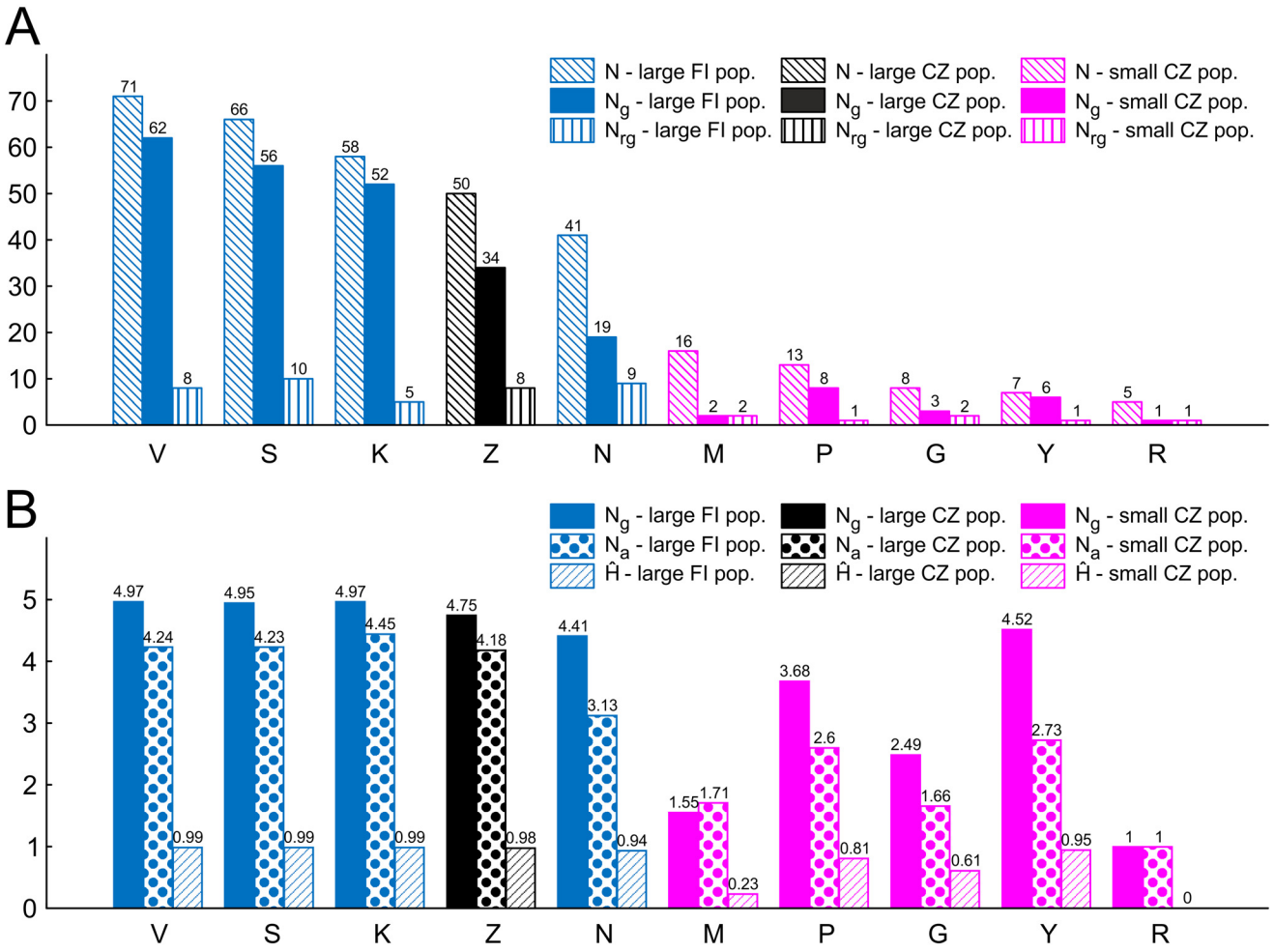

Fig 3. Genetic diversity indices for *Crossocalyx hellerianus* populations. (A) Sample size (N), number of genotypes (N_g) and number of recurrent genotypes (N_{rg}, i.e. those occurring more than once) computed for all samples in each population. (B) Resampled values of number of genotypes (N_g), mean number of observed alleles per locus (N_a) after rarefaction, and Nei's gene diversity ($\hat{H}$). Abbreviations of localities correspond to Table 1.

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The analysis of molecular variance based on R_{ST}-like method (Table 3) showed that the highest proportion of genetic variation occurred within populations (67.7%), followed by the variation between the two geographic regions (CZ vs. FI; 25.3%), and the variation among populations (7.0%). Separate analyses of both regional datasets found higher rate of variation among CZ populations (18.5%) than among FI populations (6.2%).

The highest pairwise R_{ST} values were usually observed between CZ and FI populations (Fig 4 and S1 Table), which is in agreement with geographic distances separating both regions (ca. 1,500 km). Nevertheless, considerable divergence was also found among most of the CZ populations, with pairwise R_{ST} values usually higher than 0.1 (11 out of 15 values). On the contrary, the pairwise R_{ST} values between FI populations except the most remote population N did not exceed the value of 0.1. Even in case of population N, the pairwise comparisons with the remaining FI populations (K, S and V) revealed generally lower R_{ST} values than those observed among CZ populations separated by even shorter geographic distances (the distances between N and other FI populations spanned 106–120 km, whereas 18–92 km separated CZ populations, respectively). The pairwise R_{ST} values among geographically close populations (separated

Table 3. The distribution of genetic variation based on the analysis of molecular variance (AMOVA).

Source of variation	d.f.	Variance component	Variance %	Fixation index
Between CZ and FI groups of populations	1	53.5	25.3	F_{CT} = 0.253**
Among populations within groups	8	14.9	7	F_{SC} = 0.094***
Within populations	382	143.3	67.7	F_{ST} = 0.323***
Total (CZ and FI)	391	211.7		
Among CZ populations	5	21.9	18.5	F_{ST} = 0.185***
Within CZ populations	129	96.8	81.5	
Total (CZ)	134	118.7		
Among FI populations	3	11.8	6.2	F_{ST} = 0.062***
Within FI populations	254	180.0	93.9	
Total (FI)	257	191.8		

Significance of *F* values is marked as *** $P < 0.001$; ** $P < 0.01$.

doi:10.1371/journal.pone.0133134.t003

by distances not exceeding 18 km, i.e. CZ populations G, Z, Y, and FI populations S, K, V, respectively) were higher among CZ populations (see S1 Fig).

Significant and high r_d values indicating linkage disequilibrium were found in all small CZ populations (Table 4). Only the value for the large FI population N was comparable to values

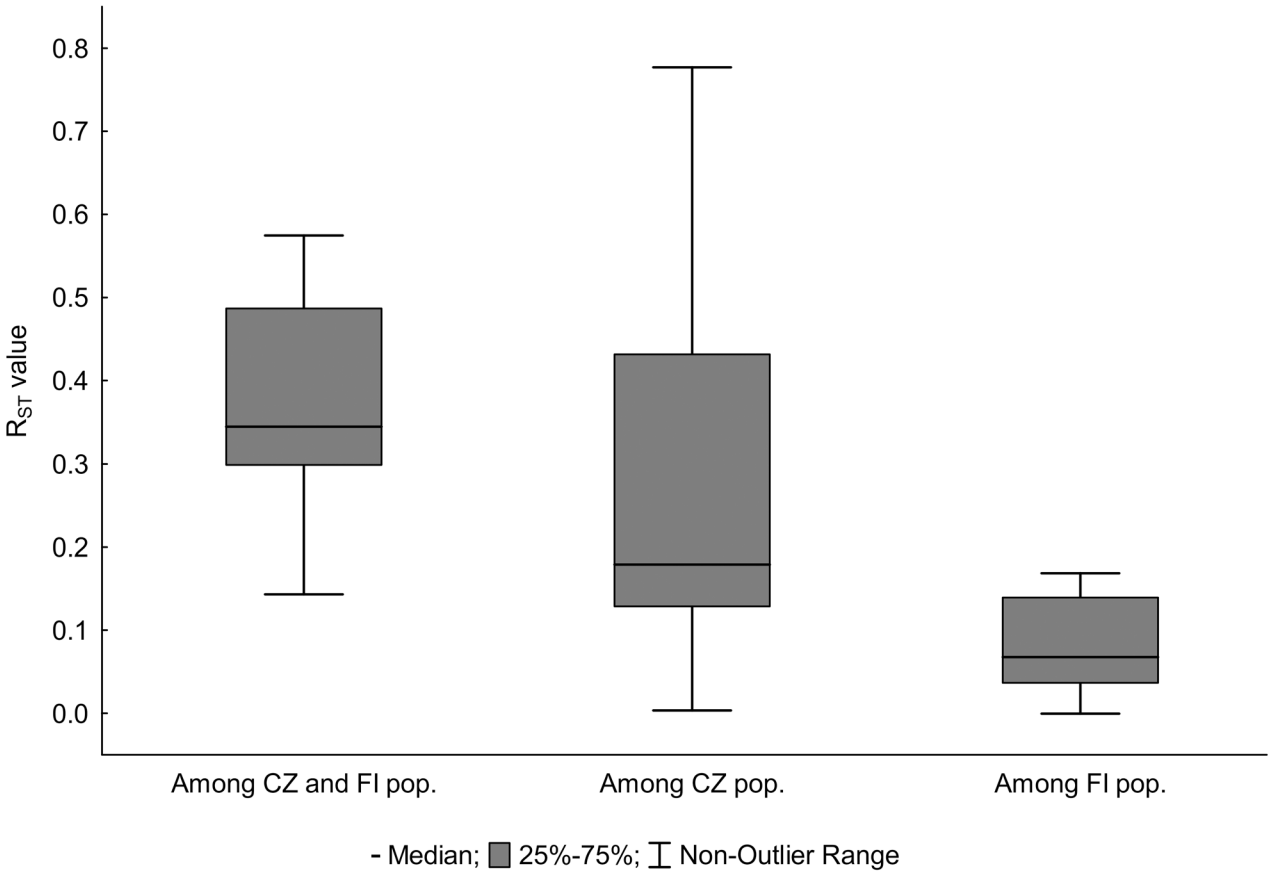

Fig 4. Genetic differentiation. Genetic differentiation among populations between and within the two geographic areas based on pairwise R_{ST} values.

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Table 4. Linkage disequilibrium, maximum distance between the same MLG, % of patches with perianths.

Locality	Linkage disequilibrium (r_d)	Max. distance between samples of the same genotype [m]	% of patches with perianths
Z	0.06***	6.8	7.5
G	0.44***	15	0
M	0.87***	10	8.3
Y	0.20***	0.01	0
R	–	0.01	0
P	0.26***	3.5	0
N	0.22***	50	25.9
S	0.01	20	8.6
K	0.03**	62	24.5
V	0.02	80	25

Linkage disequilibrium (significance of r_d values is marked as *** $P < 0.001$; ** $P < 0.01$) based on data at nine microsatellite loci in *Crossocalyx hellerianus*, maximum distance between samples of the same multilocus genotype, and percentage of patches with perianths. Locality R comprised a single multilocus genotype.

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of small CZ populations. Non-significant or low values of linkage disequilibrium were observed in populations with high N_g, N_a, $\hat{H}$. These populations also contained a high number of observed patches with perianths, indicating the production of sporophytes.

Spatial genetic structure

Kinship coefficient in small CZ populations reached initial values of 0.77 on distances up to 1 cm, and varied from 0.48 to 0.72 on distances between 50 cm and 16 m (Fig 5). On the other hand, kinship coefficients in large populations were considerably lower, reaching the initial values of 0.47 and 0.37 on distances up to 1 cm, respectively, and varied from 0.02 to 0.28 on distances between 50 cm and 16 m. On distances exceeding 16 m, the kinship coefficient decreased and dropped below zero in all population groups.

The spatial extent of clonal dispersal differed between small and large populations (Fig 6 and S4 Fig). In small CZ populations, the percentage of pairwise comparisons with observed identical genotypes sustained high values (31.0–75.9%) for the first six distance classes (1 cm–8 m), and started to decrease at the distances exceeding 16 m. The pattern found in large FI and CZ populations were rather similar to each other. High initial values of clonality were observed only in the first two distance classes (< 1 and 1–50 cm), then suddenly dropped in the third class (50–100 cm), and decreased more or less gradually at longer distances. However, the percentage of clonality was higher in the large CZ population than in all large FI populations. The probability of sexual origin (P_{sex}) was relatively high for some of the putative clones from small CZ populations, but negligible for majority of individuals from large CZ and FI populations (S4 Fig).

The maximum extent of clonal dispersal was found in the population V, with shoots sharing the same genotype separated by 80 m (Table 4). Nevertheless, considerably long distances among shoots of identical genotypes ($\geq$ 20 m) were found in all FI populations sampled (see Table 4). The maximum distance value for small CZ population was 15 m in population G, with clones always confined to one log.

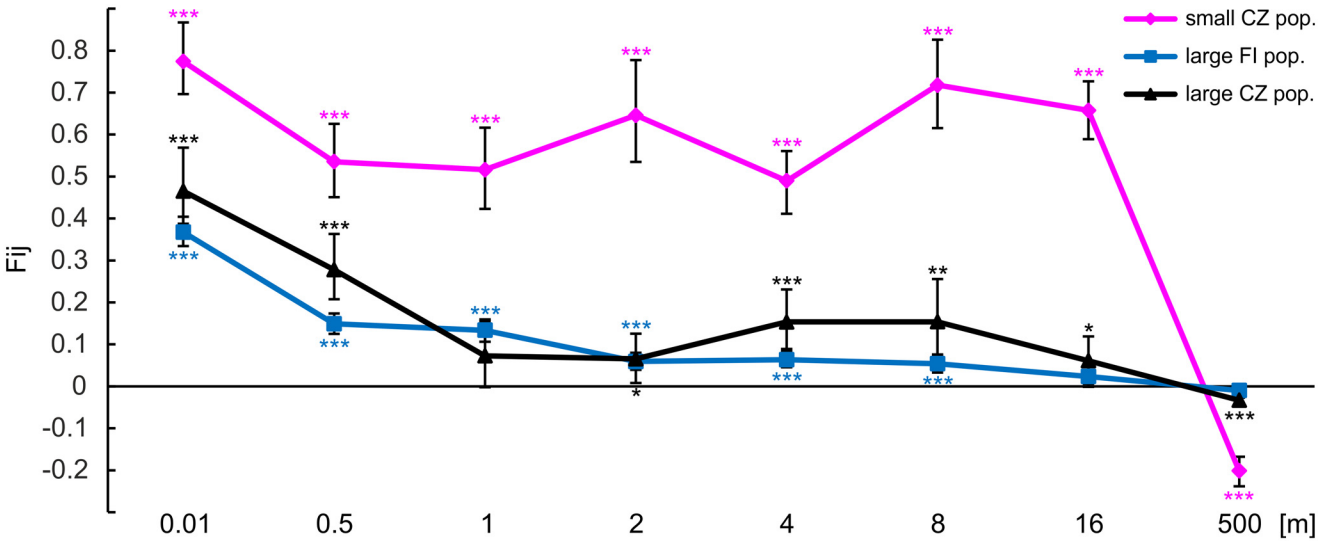

Fig 5. Spatial autocorrelation analysis based on microsatellite data. Populations of *Crossocalyx hellerianus* were divided into three categories (Table 1): small CZ pop., large FI pop., large CZ pop. The Nason's kinship coefficients (F_{ij}) are positioned along the X-axis at the mean pairwise distance within each distance class. Vertical bars show standard errors. Significance of average F values is marked as *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

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Discussion

Genetic variability

The observed pattern of genetic variation in studied populations of *Crossocalyx hellerianus*, as documented by values of N_g, N_a and $\hat{H}$, is congruent with the general assumption that larger populations (here FI populations N, S, K, V and CZ population Z) tend to have bigger pool of genotypes/alleles. In large populations, the majority of the individuals were genetically unique, whereas small populations showed higher ratio between N/N_g. The reduced variation in

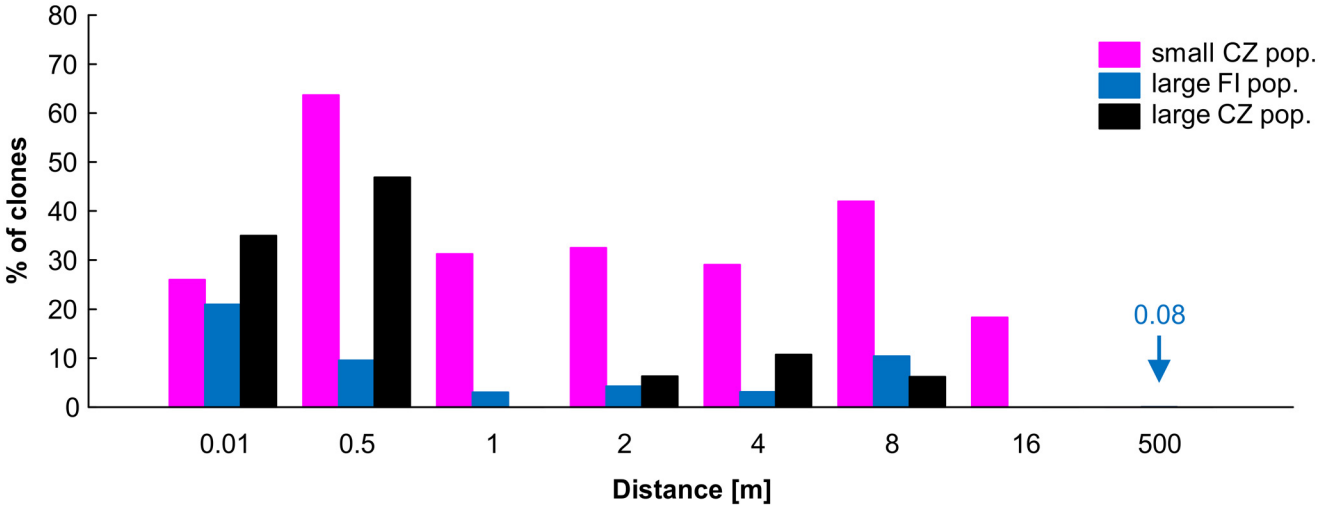

Fig 6. Percentages of clones within distance classes. Number of all pairwise comparisons in each distance: 0.01 m– 58, 96, 24; 0.5 m– 54, 211, 35; 1 m– 43, 166, 30; 2 m– 31, 274, 39; 4 m– 75, 321, 39; 8 m– 36, 266, 48; 16 m– 87, 168, 48; 500 m– 270, 6558, 948 for small CZ pop., large FI pop., large CZ pop., respectively. Long distances among clones (> 16 m) were found only in all FI populations (blue arrow).

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smaller populations may result from processes such as bottleneck, genetic drift or inbreeding [48, 49]. Nevertheless, several bryophyte studies found no relation between population size and genetic variation [15, 19, 50]. Moderate levels of genetic diversity found in small CZ populations of *C. hellerianus* support the earlier views that even small populations are important sources of genetic variation in bryophytes [19, 50] and that such populations may not be drastically threatened by processes causing loss of genetic variation (genetic risk [51]). Interestingly, genetic diversity of the small CZ populations Y and P is somewhat higher than those found in other small CZ populations. Possible explanation could include the history, in course of which these populations experienced significant reduction of population size as a consequence of a severe drop in the availability of substrate. It is known that the tree species composition in the Žofín forest (population P) changed significantly from *Abies alba* dominated forest towards broad-leaved forest dominated by *Fagus sylvatica* with only a minor percentage of spruce (*Picea abies* 15% [52]). The population Y could have benefited from the past or recent gene flow from nearby large population Z, as evidenced by the lowest detected genetic differentiation based on pairwise R_{ST} values (S1 Table).

Fully identical individuals were only found within populations. A large diversity of multilocus genotypes within populations appears to be common in both liverworts [19, 20, 23] and mosses [24, 53], irrespective of the prevailing reproductive mode. The unexpected genetic variation found in taxa with rare sexual reproduction or even in asexually reproducing populations [19–21] implies other sources of genetic diversity than recombination events. The authors mostly suggest neutral somatic mutations, originating in various vegetative parts as the probably most important source. According to Weismann's doctrine [54], only the germ line (i.e. cells giving rise to gametes) has evolutionary significance and somatic variation within individuals is not transmitted to progeny [55]. However, this is not the true for majority of land plants including bryophytes, as the sequestration of somatic cells and germ line is incomplete, and the extent to which cells or tissues become irreversibly excluded from propagation is rather low [55]. Both sexual organs and asexual propagules are formed in later ontogenetic phases from somatic stem cells, leading to transmission of mutations originated in somatic tissues directly to gametes and/or asexual gemmae or vegetative fragments. In other words, the nature and relative contribution to novel alleles is basically indistinguishable for both sexual and asexual propagules. The propagation of somatic mutations is further enhanced by consistently greater mutation rates in somatic tissues than in germ lines [56]. In plants, as well as in other clonal or modular organisms, such as aphids, freshwater snails, bryozoans, or reef corals, the somatic cells in bryophytes undergo high number of cell divisions before gametes and/or asexual propagules are formed, providing relatively high probability of mutation during numerous DNA replications [57]. In liverworts, a single apical cell is responsible for the shoot growth, and each somatic mutation in this cell is propagated to all thallus parts, which originated from mitotic divisions following the mutation event. Similarly, any somatic mutation that occurred in leaf cells that gave rise to the asexual propagules (gemmae) of liverworts, which often are only 1–2 celled, can easily be directly expressed in the progeny.

Other explanations of remarkable genetic diversity in predominantly and/or seemingly asexual bryophytes may involve e.g. population establishment by multiple genotypes, or periodical occurrence of sexual reproduction generating novel recombinant genotypes. Recruitment of new genotypes from neighboring populations seems to be a rather improbable and rare event in the studied system, as no identical MLG were shared among populations, not even between the spatially closest populations Z and Y, distant only 4 km. Occasional and unobserved sexual reproduction, which might be a major source of variation in large populations with stable reproductive system even with only small number of reproducing individuals per generation [58], also probably plays a minor role in generating the genetic diversity of

Crossocalyx hellerianus, as the frequency of these events is massively outweighed by the gemmae production. The study [59] reported only 32% of bisexual colonies, and only 12% of colonies producing sporophytes and even these numbers are much higher than in studied Central European populations (only two out of six populations producing perianths at all and 8% of perianth-forming patches in these populations; Table 4). Moreover, the estimated gemmae output per square centimeter of *C. hellerianus* colony exceeded the spore production nearly five times, while the ability to germinate in both types of propagules was similar [59]. Prevailing asexual reproduction and absence of recombination in small CZ populations of *C. hellerianus* is also indicated by high values of linkage disequilibrium (or P_{sex} values). Significant and rather high linkage disequilibrium was also found in the large FI population N, although the percentage of patches with perianths (25.9%) was comparable with other large FI populations. Nevertheless, the slightly lower genetic variation as inferred from N_g, N_a and $\hat{H}$ values was congruent with linkage disequilibrium. This pattern could be explained by low portion of gametophytes arising from sexually produced spores or as a result of inbreeding. Mating may occur among haploid siblings originating from the same sporophyte as a result of non-existing mechanism to distinguish among differently related gametes [60]. Inbreeding would further reduce the relative contribution of otherwise rare sexual reproduction for genetic variation in *C. hellerianus*. Especially small CZ populations showed high values of linkage disequilibrium, rather low number of genotypes and aggregation of similar genotypes, which is consistent with the assumption of low recombination efficiency. Therefore, genetic variation in small CZ populations has most likely been caused by somatic mutations, past genetic variation prior to population reduction, and/or establishment by multiple genotypes, although we cannot rule out the contribution of sexual reproduction with respect to the facts discussed above.

Estimates of genetic differentiation among populations reflect the amount of gene flow between them [61]. Isolation by distance inferred from pairwise R_{ST} values was found in most of the studied populations. Genetic differentiation was rather low among the FI populations (R_{ST} values usually < 0.1), whereas the values among the CZ populations mostly exceeded 0.1 (Fig 4). This implies greater gene flow among Finnish populations than it is the case in the Czech Republic, which might be explained by the less fragmented landscape of forests with better availability of decaying wood substrate in Finland. Lesser extent of gene flow among CZ populations can be demonstrated in comparison of genetic differentiation between similarly distant FI and CZ populations. The small CZ population G was considerably differentiated from the 18 km distant Y and Z populations, which is in contrast with low R_{ST} values among FI populations V, S and K, respectively, separated by similar spatial distances (7–18 km). We suppose that suitable substrate, enabling step-by-step dispersal [14, 62] supports gene flow among FI populations in contrast to the complete lack of 'substrate bridges' among the CZ populations. Our results are in accordance with other studies of genetic differentiation in wood living fungi and beetles [17, 18]. Generally, habitat loss and fragmentation have negative effect on the genetic structure of populations with respect to the restricted level of gene flow. The combination of reduced gene flow among isolated populations and their reduced size leads to genetic drift and the fixation of different alleles, which brings strong genetic differentiation among populations [48, 49].

Spatial genetic structure

Direct observations of propagule dispersal in *Crossocalyx hellerianum* [7] showed that a proportion of propagules deposit within few meters from source colonies but a considerable proportion may disperse over farther distances. In the absence of any specialized dispersal adaptations, the wind probably serves as the main dispersal vector, and the deposition of

propagules may be further enhanced by water during rainy days. Dispersal by animal vectors such as the ants, hardly has an important role [23]. Anyway, the direct methods have limited use for large spatial scale studies (few hundreds of meters) or for studies on short timescale. In these cases, indirect methods revealing the spatial genetic structure can bring a reasonable assessment of propagule dispersal.

High values of kinship coefficients observed in most of the small populations provided the evidence for aggregation of similar genotypes. This can be caused by the relatively low level of genetic diversity resulting from bottleneck and/or founder effect, prevailing asexual reproduction or breeding of related individuals, as discussed above. Spatial distribution reflects both substrate availability and the mode of reproduction. If suitable habitats are evenly distributed and spore production is frequent, allowing effective dispersal at the middle and long distances, randomness in distribution, reflected in the absence of SGS, can be achieved [33]. This is not the case at localities with small populations of *C. hellerianus*, where the amount of decaying wood is generally low and essentially all available substrate is occupied. Random SGS cannot be achieved in the absence of sexual reproduction, evidenced by high values of linkage disequilibrium and absence of perianths in small CZ populations. Asexual reproduction by gemmae represents here the most important and efficient role in maintaining the populations. This is in agreement with previously postulated conclusions in vascular plant studies [63, 64].

Recent investigations of SGS in seed plants, reviewed in [65] showed that its presence is positively correlated with self-compatibility, low population densities, and poorly dispersed seeds. In *C. hellerianus*, large populations possessed less SGS than small populations, with their individuals showing marked decrease in kinship over 50 cm distances and appearing to be without any obvious kinship on distances exceeding 16 m. This result reflects higher population density and more frequent spore production observed in large populations, both allowing more efficient dispersal of different or novel MLGs on farther distances, which reduces the pattern of SGS. Anyway, even in large populations, the plants continue to produce gemmae massively, contributing to aggregation of genotypes and presence of SGS over short distances. Vegetative reproduction by gemmae obviously contributes to economic balance avoiding the costly production of sporophytes [59].

Comparison of SGS shape between studied populations of *C. hellerianus* and the small-scale pattern of SGS in a closely related liverwort species, *Barbilophozia attenuata* [23] shows similar patterns between large CZ and FI populations and the shape for *B. attenuata*, whereas small CZ populations of *C. hellerianus* differed in noticeably strong SGS. Whereas the kinship coefficients reached zero over 8–10 m in *B. attenuata*, they approached zero not earlier that at distance of 16 m and turned negative at distance of 500 m in *C. hellerianus*, reflecting the aggregation of genotypes over larger distances in the latter species. This might infer that *B. attenuata* produces sporophytes more often or the gemmae of *C. hellerianus* have better dispersal capacity. The latter explanation can be supported by the difference in propagule weight, because the smaller gemmae of *C. hellerianus* have about eight times smaller volume than the gemmae of *B. attenuata*.

In our study, clones, probably arising from gemmae, were detected even at distances of 20, 50, 62, and 80 m. Although some of the identical MLG may have arisen from sexual reproduction, the probability of such events was negligible in large FI and CZ populations (S4 Fig). Higher frequency of clones distributed over long distances in FI populations thus probably reflects the larger spatial extent of these populations. The observation of clones spanning long distances is consistent with the results of an earlier experiment [7], who found considerable potential for long-distance dispersal of gemmae in *C. hellerianus*. We observed most of clones to be dispersed only within logs at short distances in large populations, whereas small CZ populations showed significant portion of clones dispersed at distances up to 10 m (Fig 6). The

apparently more efficient dispersal of clones in small CZ populations might however rather be the consequence of the absent sporophyte production. On the other hand, the clonal pattern of FI populations seemingly involving long distance dispersal might be a consequence of several successive step-by-step dispersal events over much shorter distances, as the continuous availability of epixylic substratum in space and time at Finnish localities increases the probability of successful establishment.

Conclusions

Genetic diversity in populations of the dioicous epixylic liverwort *Crossocalyx hellerianus* was related to population size but even the small populations were found to be important sources of genetic variation. Recombination connected with sexual reproduction only plays a significant role in generating the genetic diversity in large populations of *C. hellerianus*, whereas smaller populations are maintained by vegetative diaspores and their main source of genetic diversity are probably the somatic mutations. We were able to demonstrate notably low levels of gene flow among populations in Central Europe, where habitat fragmentation poses a significant barrier to dispersal of diaspores. Populations from southern Finland show lower levels of inter-population differentiation at the same distances, which can probably be explained by the presence of step-by-step dispersal. The fine scale study of SGS revealed a strong aggregation of genotypes, particularly in smaller populations, and at the same time showed that asexual reproduction is an efficient mean of maintaining the population at not only the short distances, given the spatial extent of clones spanning dozens of meters. On the other hand, strong SGS in large populations seems to be reduced by the relatively efficient dispersal of both spores and gemmae.

Supporting Information

S1 Fig. Sampling sites in the Czech Republic and Finland. Abbreviations of localities correspond to Table 1. Made with Natural Earth. Free vector and raster map data @ naturalearthdata.com.
(TIF)

S2 Fig. Multilocus genotypic resolution of microsatellites in the data set of *Crossocalyx hellerianus*. The plot was generated using 1,000 random samples of 1 to 9 loci. Resampling of loci indicated that our set of nine loci had sufficient haplotypic resolution, as even the use of approximately 7 loci would reveal the majority of MLGs detected in this study.
(TIF)

S3 Fig. Number of distinct multilocus genotypes (MLGs) plotted against the number of individuals (A and B). Plots were generated for each population separately (A) small populations and (B) large populations, using 1,000 random samples of individuals to see if the relationship reached a plateau. Resampling of individuals indicated that increased sampling would yield higher number of MLGs in large populations (B), whereas in small populations the number of MLGs mostly tended to reach a plateau (A). The estimated number of MLGs was substantially lower in small populations (1–8 MLGs) than in large populations (5–15 MLGs, grey part of B) at smaller sampling sizes (N ranging from 5 to 16), corresponding to the maximum sampling size in small populations. Therefore, sampling in small populations was probably rather comprehensive despite lower number of individuals in population, whereas in large populations the clonal diversity estimates could be underestimated.
(TIF)

S4 Fig. The probability of sexual reproductive events. Probability of sexual reproduction (P_{sex}) was plotted against the particular repeated multilocus genotypes (MLG) for populations

(A) small CZ populations, (B) large CZ population and (C) large FI populations. If the probability is below significance threshold ($P_{sex} < 0.05$), the respective individual is not likely to be the result of a distinct event of sexual reproduction. Thus we can conclude that individuals with identical genotypes, which occur more than once in the population and their $P_{sex} < 0.05$, were probably established from asexual propagules (predominantly found in large CZ and FI populations—Z, N, S, K and V).
(TIF)

S1 File. Certification of Ethics statement.
(DOCX)

S2 File. Allelic data for all samples. Abbreviations of localities correspond to Table 1. Samples within each population were numbered, and individuals collected within a single patch were indexed by letters A-F. Missing data were assigned as '-1'.
(XLSX)

S3 File. Distances among sampled individuals within each population. Abbreviations of localities correspond to Table 1. Samples within each population were numbered, and individuals collected within a single patch were indexed by letters A-F.
(XLSX)

S1 Table. The pairwise R_{ST} values calculated between all populations. Significance of *F* values is marked as *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.
(DOCX)

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Author Contributions

Conceived and designed the experiments: EH J. Košnar J. Kučera. Performed the experiments: EH J. Košnar. Analyzed the data: EH J. Košnar. Contributed reagents/materials/analysis tools: EH J. Košnar J. Kučera. Wrote the paper: EH J. Košnar J. Kučera.

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SYSTEMATICS AND PHYLOGENY

A case study of intragenomic ITS variation in bryophytes: Assessment of gene flow and role of polyploidy in the origin of European taxa of the *Tortula muralis* (Musci: Pottiaceae) complex

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Abstract For the first time in bryophyte studies, we performed comprehensive cloning of the ITS region to reveal intraindividual variation of ITS sequences. We assessed relationships among morphologically defined taxa of the polyploid complex of the moss *Tortula muralis*. Our results detected a monophyletic *T. muralis* complex comprising *T. muralis* subsp. *muralis, T. muralis* subsp. *obtusifolia*, *T. lingulata, T. israelis,* and *T. edentula*. The single accession of *T. edentula* was found nested within *T. obtusifolia*, and biphyletic *T. israelis* was found to be nested within *T. muralis*. With the exception of *T. lingulata*, intragenomic ITS sequence variation was high in the *T. muralis* complex. Most intraindividual sequences were nevertheless only weakly divergent, suggesting their origin via mutations exceeding the rates of concerted evolution. Markedly divergent sequences found within a single individual most probably resulted from gene flow among distant lineages of the complex. Such pattern of ITS variation challenges the traditional morphology-based taxonomy. No phylogenetic signal was associated with ploidy-level variation, suggesting a polytopic origin of the diploids. Interestingly, the pattern of ITS variation together with morphological evidence indicate the autopolyploid origin of some lineages, which renders the *T. muralis* complex the first group of mosses in which autopolyploidy is implied by molecular markers.

Keywords bryophytes; gene flow; intragenomic variation; ITS; *Tortula*

Supplementary Material Figures S1–S3 (in the Electronic Supplement) and the alignment are available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

■ INTRODUCTION

The internal transcribed spacer (ITS) of 18S-26S nuclear ribosomal DNA is one of the most widely used sequence markers in bryophyte studies (Stech & Quandt, 2010). As a non-coding part of the 18S-26S operon, the ITS region is a true multi-copy marker with hundreds to thousands of copies arranged in tandem arrays of the operon (Álvarez & Wendel, 2003). Despite its multi-copy nature, the homogeneity of individual ITS copies is driven by concerted evolution (Arnheim, 1983; Elder & Turner, 1995). However, the rate of concerted evolution varies greatly, and intragenomic variation of ITS copies (ITS paralogs sensu Álvarez & Wendel, 2003) is not exceptional (Buckler & al., 1997; Álvarez & Wendel, 2003; Nieto Feliner & Rosselló, 2007).

There are two main possible explanations for the occurrence of intragenomic ITS variation, both assuming incomplete concerted evolution of nrDNA arrays. First, the occurrence of intragenomic ITS variation might result from the hybridization between parents containing different ITS sequences (Baldwin & al., 1995; Sang & al., 1995). Second, divergent intraindividual sequences might arise by molecular processes unrelated to hybridization, such as the accumulation of mutations that exceeds the rate of concerted evolution, nrDNA array multiplication, or pseudogenization (Álvarez & Wendel, 2003; Nieto Feliner & Rosselló, 2007). These molecular mechanisms might result in polymorphisms which together with incomplete lineage sorting processes may obscure phylogenetic analysis, especially when non-orthologous sequences or apparent pseudogenes are not recognized (Buckler & al., 1997). The intragenomic variation of ITS sequences is challenging, because the assumption of orthology is crucial for the correct reconstruction of phylogeny. Numerous studies addressed intragenomic ITS variation in vascular plants (Álvarez & Wendel, 2003). However, little is known about intragenomic ITS variation in bryophytes. To the best of our knowledge, this phenomenon has been detected only in the genus *Plagiomnium* T.J. Kop. (Harris, 2008).

Recently, we have found intragenomic ITS variation in the European taxa of the *Tortula muralis* complex. According to a morphological study by Košnar & Kolář (2009), the complex was defined to include *T. muralis* Hedw. subsp. *muralis* with var. *muralis* and var. *aestiva* Brid. ex Hedw., *T. muralis* subsp. *obtusifolia* (Schwägr.) Culm., and *T. lingulata* Lindb. The detected clinal variation and poor morphological differentiation

among the taxa of the *T. muralis* complex might result from gene flow among taxa, or might reflect cryptic speciation, i.e., the existence of additional, genetically divergent lineages that are poorly or not at all defined morphologically, as has been revealed frequently in all major groups of bryophytes studied using molecular markers (Shaw, 2001). The latter hypothesis was proposed in a study of molecular variation in *Tortula muralis* using *rps4* sequences (Werner & Guerra, 2004), where several morphologically undefined lineages were detected. These lineages were hypothesized to represent putative cryptic species because one of the nested clades included the morphologically well-defined and generally accepted *Tortula vahliana* (Schultz) Mont. Unfortunately, low variability of chloroplast *rps4* sequences poorly reflects patterns of genetic variability in closely related taxa of Pottiaceae (Köckinger & Kučera, 2011). Therefore, such hypothesis needs to be substantiated using more variable molecular markers.

In addition, a distinct pattern of ploidy variation and habitat preferences has been detected among subspecies and varieties of *T. muralis* (Košnar & Kolář, 2009). Plants evaluated as subsp. *obtusifolia* were exclusively haploid, whereas both haploid and diploid cytotypes were found in both varieties of *T. muralis* subsp. *muralis*. The morphological variability in the broader distribution area in Eurasia comprises several other taxa, including *T. israelis* Bizot & F. Bilewski, known from the Mediterranean region and the Near East, and the recently described *T. edentula* Ignatova & Ignatov from the Kuril Islands. Other putatively closely related taxa, including, e.g., *T. vahliana* and *T. brevissima* Schiffn. (Werner & al., 2002a; Werner & Guerra, 2004), were also included for further consideration, as described below.

The objectives of the current study were to: (i) evaluate intragenomic ITS variation in the *T. muralis* complex and related taxa; (ii) determine the phylogeny of the *T. muralis* complex, including putatively related Eurasian species of *Tortula* and related genera; and (iii) determine the relationship between ploidy level and genetic lineages in the *T. muralis* complex, i.e., determine whether diploids arose recurrently from different haploid ancestors.

■ MATERIALS AND METHODS

Plant material. — A total of 159 herbarium specimens were selected for molecular analysis (Appendix). Most specimens were collected in Europe but a few were from Asia. Definition of the taxa in the *T. muralis* complex followed the morphological concept suggested in our previous study (Košnar & Kolář, 2009). In cases when plants from a single collection were markedly heterogeneous morphologically, plants of each analysed morphotype were considered a separate sample. **S**amples of morphologically uniform plants collected at one locality were treated as a population.

To incorporate our data into a broader phylogenetic context, we included samples of other species of *Tortula* sensu Zander (1993), together with selected taxa of *Crossidium* Jur., *Pterygoneurum* Jur. and *Stegonia latifolia* (Schwägr.) Venturi ex Broth. The nomenclature follows Zander (1993) and Cano (2006).

Flow cytometry. — Ploidy levels of plants tentatively assigned to the *T. muralis* complex were determined using flow cytometry (FCM). Usually 1 to 3 moss shoots were chopped together with the internal standard (*Glycine max* (L.) Merr. 'Polanka', 2C = 2.50 pg) in LB01 buffer (Doležel & al., 1989) containing 4,6-diamidino-2-phenylindol (DAPI). Analyses were performed on a Partec PA II flow cytometer (Partec, Münster, Germany), and data were processed using Partec FloMax v.2.4d software. For details on the FCM protocols, see Košnar & Kolář (2009).

Molecular protocols. — Total genomic DNA was extracted from one moss shoot or occasionally from 2 to 10 shoots (see Appendix) using the NaOH method (Werner & al., 2002b) or the Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany). In addition to ITS, 17 samples including all morphologically defined taxa of the *T. muralis* complex were selected for preliminary analysis of the *rps4* chloroplast region. The PCRs for ITS were performed according to the protocol by Köckinger & Kučera (2011), and the protocol by Werner & Guerra (2004) for *rps4*. Direct sequencing was performed as described in Köckinger & Kučera (2011).

When data obtained from direct ITS sequencing indicated a mixed template, and more than two polymorphic positions within one sequence were detected, molecular cloning was performed. For approximately half of the cloned samples, both DNA extraction and PCR reactions were repeated on a different day to ensure reproducibility (see below). Repeated PCR reactions were performed as above, except that only 30 cycles and a 2-minute cycle extension step were used in order to reduce formation of chimeric sequences. PCR products were cloned using the pGEM-T Vector System I (Promega, Madison, Wisconsin, U.S.A.). Clone sampling and sequencing were usually performed until all variation detectable on direct sequences was recovered. No differences were found between sequences and clones obtained from repeated DNA extractions and PCR reactions of the same sample, indicating the absence of artificial ITS variation originating from sample cross-contaminations or other sources.

Data analysis. — Sequences were edited using BioEdit v.7.0.9.0 (Hall, 1999) and preliminarily aligned using Clustal W v.1.4 with default options (Thompson & al., 1994). The raw alignments were trimmed according to the shortest sequence in the dataset. This led to exclusion of the first 9 bp of ITS1 and the last 7 bp of ITS2, which could not be aligned with certainty. The first 22 bp of the *rps4* amplicon were excluded because of the shorter length of some of the sequences. The ITS dataset was subsequently aligned by MAFFT v.6 (Katoh & al., 2002; available online at http://mafft.cbrc.jp/alignment/server/) using the Q-INS-i algorithm with the 200PAM / κ = 2 scoring matrix. The gap opening penalty was set to 1, and the offset value was set to 0.0. For accessions in which up to two polymorphic sites within one direct sequence were detectable in both forward and reverse directions, reconstructed sequences with all possible combinations of polymorphic sites were used. For accessions obtained by cloning, autapomorphic changes unique to a single accession at a non-variable position of the alignment were considered *Taq* errors (Hengen, 1995) and were overwritten

according to the direct sequence. The *rps4* dataset was aligned manually, and sequences were assigned to haplotypes following Werner & Guerra (2004).

Using ITS data, phylogenetic relationships were assessed using maximum parsimony (MP) as implemented in TNT v.1.1 (Goloboff & al., 2008) and Bayesian inference as implemented in MrBayes v.3.1.2. (Huelsenbeck & al., 2001). All characters were given equal weight, and gaps were coded as missing data. The MP analysis was run using the heuristic New Technology search with the following settings: Sectional Search = ON (including active RSS, CSS, and XSS), Ratchet = ON, Drift = ON, Tree Fusing = ON, Maxtrees = 10,000, random additions with 10,000 replicates. A bootstrap analysis (Felsenstein, 1985) was performed with 1000 replicates using the heuristic search strategy as described, except for random addition with 20 replicates. For Bayesian inference, the best-fit model of sequence evolution was selected using the Bayesian information criterion (Schwarz, 1978) calculated in jModelTest v.0.1.1 (Posada, 2008). The general time-reversible model (Rodríguez & al., 1990) with a discrete gamma distribution was selected. Two runs with 10,000,000 generations starting with a random tree and employing 12 simultaneous chains each (one hot, eleven cold) were executed. The temperature of a hot chain was set empirically to 0.01, and every 100th tree was saved. The analysis was considered to be completed when the average standard deviation of split frequencies dropped below 0.01. The first 25,000 trees (25%) were discarded as the burn-in phase, and the remaining 75,000 trees were used for construction of a 50% majority consensus tree. Based on recent phylogenetic studies (Werner & al., 2002a, 2004) and our preliminary analysis of ITS data of related taxa, *Chenia leptophylla* was used as outgroup. To test the phylogenetic signal in intragenomic ITS variation, alternative topological hypotheses were evaluated. For Bayesian inference, monophyly of markedly polyphyletic intraindividual ITS sequences (see Appendix) was tested by calculating the posterior probability (PP) of the set of trees containing such monophyly (Huelsenbeck & Imennov, 2002).

TCS v.1.18 (Clement & al., 2000) was used to produce a parsimony network of *rps4* haplotypes with a 95% confidence limit. Based on results by Werner & Guerra (2004), suggesting that *rps4* sequences of *T. muralis* and *T. vahliana* are closely related, the *rps4* dataset included taxa of the *T. muralis* complex together with *T. vahliana*. Gaps were treated as missing data, but potentially informative indels were scored (present/absent) and the data were added to the matrix.

■ RESULTS

All products of the ITS amplification were full length, spanning the ITS1 region, the 5.8S rDNA gene, and the ITS2 region. The aligned sequences had a length of 1036 bp, of which 382 characters were variable and 300 parsimony-informative. The lowest variation was observed in the 5.8S gene, which had only two variable positions. The strict consensus tree obtained from MP was generally more resolved than the 50% consensus Bayesian tree (Figs. S1–S2 in the Electronic Supplement; and Figs. 1–2, respectively). Both trees showed similar general topologies and differed only in poorly supported internal branches, which were better resolved by MP. For simplicity, only the Bayesian tree is presented here (Figs. 1–2), and only those groups resolved by both methods are discussed.

The aligned *rps4* data matrix contained 655 characters, of which 37 were variable and 17 parsimony-informative.

Occurrence of intragenomic ITS variation. — Intragenomic variation was detected in approximately 46% of the samples belonging to the *T. muralis* complex and in 50% of the samples of the taxa related to the complex. For the *T. muralis* complex, the intraindividual ITS sequences of 22 samples (16%) were markedly polyphyletic and caused eight reticulations among the most distinct lineages (Fig. 2; see below). As evaluated using posterior probability, hypotheses assuming monophyly of such markedly polyphyletic sequences were found to be significantly worse than the topology observed in the 50% consensus Bayesian tree. The highest PP of monophyly of intraindividual ITS sequences was found in sample M37 (PP = 0.026), and in other samples the PP was lower than 0.000 (for list of analysed samples, see Appendix).

Delimitation of the *T. muralis* complex based on ITS data. — Taxa of the *T. muralis* complex together with *T. israelis* and *T. edentula* form a poorly supported (PP = 0.92, BS = 51%) monophyletic group, here called the "*T. muralis* clade" (Figs. 1–2). This clade notably does not include *T. vahliana* and *T. brevissima*, and is sister to a clade comprising the remaining taxa of *Tortula* and related genera (PP = 0.81, BS < 50%) with the exception of *T. marginata*. The genera *Tortula, Crossidium,* and *Pterygoneurum* are apparently polyphyletic. The most distinct lineage in the ITS tree is a long and well-supported "*Pottia* clade" (PP = 1.00, BS = 69%), comprising *Crossidium squamiferum, Stegonia latifolia, Pterygoneurum* taxa, and several terricolous *Tortula* taxa, belonging to section *Pottia* (Rchb.) Kindb., together with *Hilpertia velenovskyi, T. brevissima,* and *T. mucronifolia*. Interestingly, ITS sequences of *T. brevissima* appeared to be polyphyletic. Although three of the four cloned sequences obtained from two Spanish samples of *T. brevissima* cluster together in a well-supported clade, the remaining sequence is sister to a clade consisting of *T. acaulon, T. mucronifolia, Crossidium squamiferum, Stegonia latifolia,* and *Pterygoneurum* taxa.

Relationships within the *T. muralis* complex based on ITS data. — The pattern of relationships based on the analysis of ITS sequences (Fig. 2) does not agree with the previously suggested classification based on a morphometric analysis. An exception to this is *T. lingulata*, which forms a monophyletic clade (PP = 0.98, BS = 69%) consisting of two haplotypes that differ by a single nucleotide substitution. No intragenomic ITS variation was detected in *T. lingulata*.

The most distinct ITS clade, hereafter called the "*obtusifolia* 1 clade", is a well-supported branch (PP = 0.98, BS = 95%) that contains a high frequency of *T. muralis* subsp. *obtusifolia* morphotypes (Fig. 2). Sequences from 70% of the populations identified morphologically as subsp. *obtusifolia* belong here, together with sequences from 23% of populations of morphs intermediate between *T. muralis* subsp. *obtusifolia* and *T. muralis*

subsp. *muralis* var. *aestiva*. Nevertheless, the *obtusifolia* 1 clade also contains sequences from 30% of the populations of *T. muralis* subsp. *muralis* morphs (both varieties and irrespective of ploidy level). The single sequence of *T. edentula*, which morphologically resembles *T. muralis* subsp. *obtusifolia,* is also nested in the *obtusifolia* 1 clade. ITS sequences of *T. muralis* subsp. *muralis* and *T. muralis* subsp. *obtusifolia* commonly were part of markedly polyphyletic assemblages of intragenomic ITS variation from individual amplifications. Thus, 36% of *T. muralis* subsp. *muralis* and one sample of *T. muralis* subsp. *obtusifolia* nested in the *obtusifolia* 1 clade are parts of intraindividual ITS variation appearing on distant branches of the *T. muralis* clade. Those polyphyletic sequences were strongly divergent, sharing a rather low number of identical nucleotides with *obtusifolia* 1 sequences (86.2%–92.2%).

Tortula muralis subsp. *obtusifolia* is clearly polyphyletic because accessions not contained in the *obtusifolia* 1 clade appear in other lineages (Fig. 2). Although most accessions from the "*obtusifolia* 2 clade" contain the sequences from morphs of subsp. *obtusifolia*, the frequency of plants with the clear morphology of subsp. *obtusifolia* in this clade (sequences from 30% of its populations) was lower than in the *obtusifolia* 1 clade (sequences from 70% of its populations; Fig. 2), while the frequency of plants intermediate between subsp. *obtusifolia*

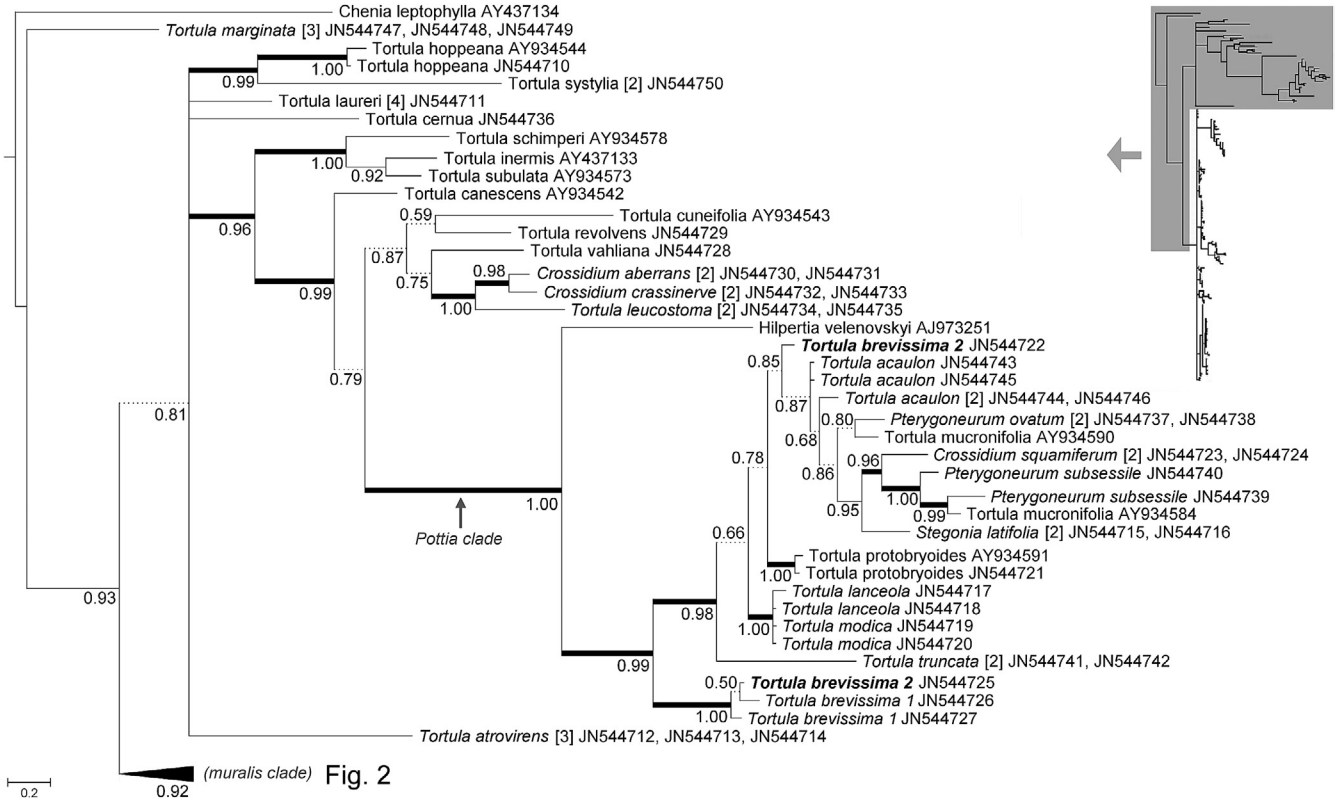

Fig. 1. Phylogenetic tree of the *Tortula muralis* complex and related taxa based on ITS sequence data. The tree was constructed using Bayesian inference and was rooted with *Chenia leptophylla*. Numbers on branches indicate posterior probabilities. Dotted lines indicate branches with posterior probabilities <0.90, and bold lines indicate branches with posterior probabilities >0.95. Sequences obtained by molecular cloning are in italics. Samples containing polyphyletic intragenomic sequences belonging to different major clades are in bold. Monophyletic clades containing sequences that originated from a single specimen with intragenomic ITS variation were compressed and considered a single sequence; numbers in square brackets indicate the number of such monophyletic sequences. Numbers after taxa correspond to GenBank accession numbers. For detailed voucher information, see Appendix.

Fig. 2. Subtree showing the *Tortula muralis* clade of the ITS tree. The tree was constructed using Bayesian inference. Numbers on branches of major lineages indicate posterior probabilities. Dotted lines indicate branches with posterior probabilities <0.90, and bold lines indicate branches with posterior probabilities >0.95. Graphs indicate the percentage of populations of a given morphotype containing the ITS sequence of each particular group (only percentages >10% are shown). Sequences obtained by molecular cloning are in italics. Samples containing polyphyletic intragenomic sequences belonging to different major clades are in bold. Lines in the right part of the figure indicate reticulations among main groups caused by samples containing markedly polyphyletic intragenomic sequences of different clades of the tree (numbers refer to number of such samples). Monophyletic clades containing sequences that originated from a single specimen with intragenomic ITS variation were compressed and considered a single sequence; numbers in square brackets indicate the number of such monophyletic sequences. Known *rps4* haplotypes are underlined and in parentheses. "x" indicates haploid cytotypes, and "2x" diploid cytotypes (for detailed voucher information, see Appendix). ▶

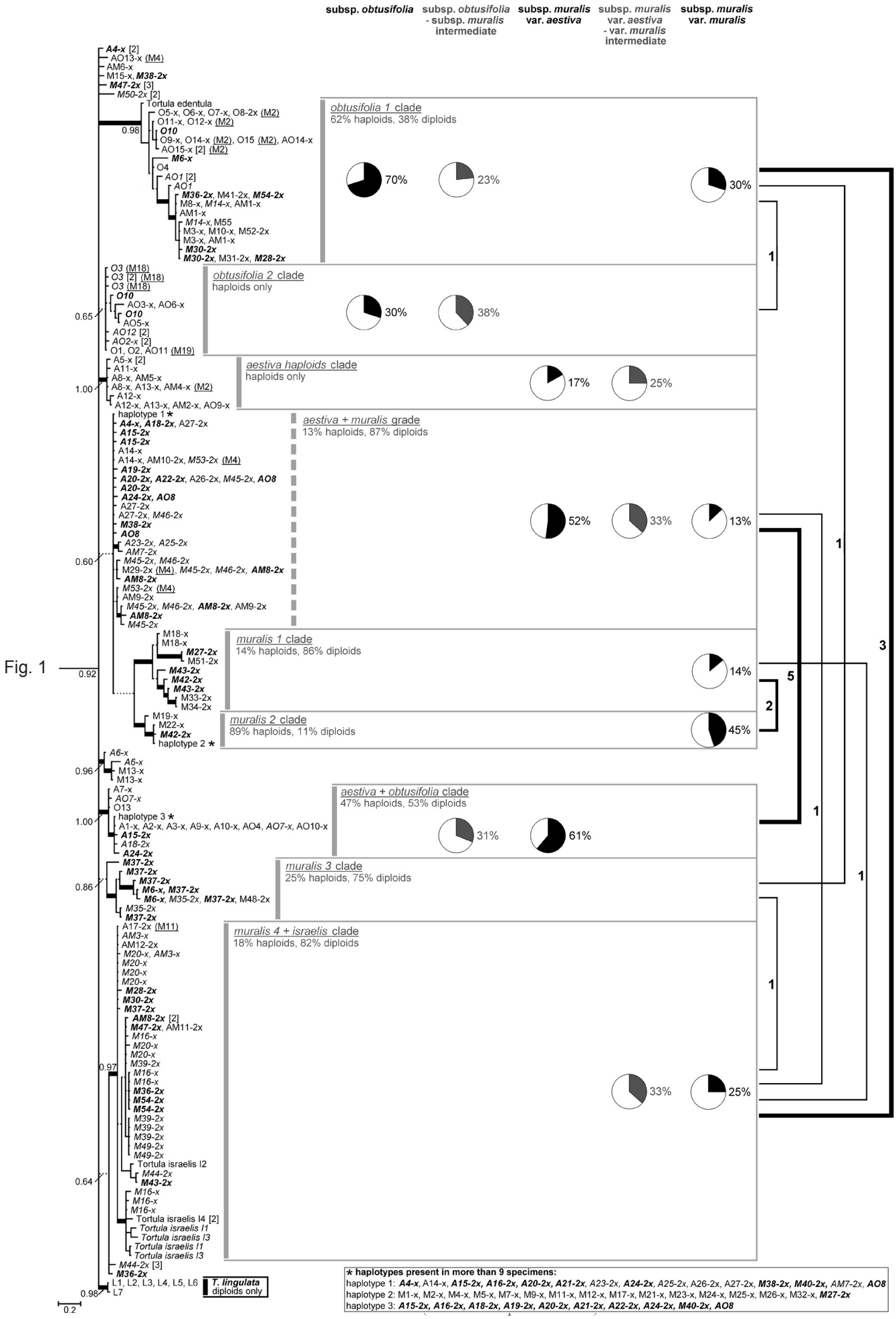
subsp. obtusifolia
subsp. obtusifolia - subsp. muralis intermediate
subsp. muralis var. aestiva
subsp. muralis var. aestiva - var. muralis intermediate
subsp. muralis var. muralis
obtusifolia 1 clade
62% haploids, 38% diploids
70%
23%
30%
obtusifolia 2 clade
haploids only
30%
38%
aestiva haploids clade
haploids only
17%
25%
aestiva + muralis grade
13% haploids, 87% diploids
52%
33%
13%
muralis 1 clade
14% haploids, 86% diploids
14%
muralis 2 clade
89% haploids, 11% diploids
45%
aestiva + obtusifolia clade
47% haploids, 53% diploids
31%
61%
muralis 3 clade
25% haploids, 75% diploids
muralis 4 + israelis clade
18% haploids, 82% diploids
33%
25%
A4-x [2]
AO13-x (M4)
AM6-x
M15-x, M38-2x
M47-2x [3]
M50-2x [2]
Tortula edentula
O5-x, O6-x, O7-x, O8-2x (M2)
O11-x, O12-x (M2)
O10
O9-x, O14-x (M2), O15 (M2), AO14-x
AO15-x [2] (M2)
M6-x
O4
AO1 [2]
AO1
M36-2x, M41-2x, M54-2x
M8-x, M14-x, AM1-x
AM1-x
M14-x, M55
M3-x, M10-x, M52-2x
M3-x, AM1-x
M30-2x
M30-2x, M31-2x, M28-2x
O3 (M18)
O3 [2] (M18)
O3 (M18)
O10
AO3-x, AO6-x
O10
AO5-x
AO12 [2]
AO2-x [2]
O1, O2, AO11 (M19)
A5-x [2]
A11-x
A8-x, AM5-x
A8-x, A13-x, AM4-x (M2)
A12-x
A12-x, A13-x, AM2-x, AO9-x
haplotype 1 *
A4-x, A18-2x, A27-2x
A15-2x
A15-2x
A14-x
A14-x, AM10-2x, M53-2x (M4)
A19-2x
A20-2x, A22-2x, A26-2x, M45-2x, AO8
A20-2x
A24-2x, AO8
A27-2x
A27-2x, M46-2x
M38-2x
AO8
A23-2x, A25-2x
AM7-2x
M45-2x, M46-2x
M29-2x (M4), M45-2x, M46-2x, AM8-2x
AM8-2x
M53-2x (M4)
AM9-2x
M45-2x, M46-2x, AM8-2x, AM9-2x
AM8-2x
M45-2x
M18-x
M18-x
M27-2x
M51-2x
M43-2x
M42-2x
M43-2x
M33-2x
M34-2x
M19-x
M22-x
M42-2x
haplotype 2 *
A6-x
A6-x
M13-x
M13-x
A7-x
AO7-x
O13
haplotype 3 *
A1-x, A2-x, A3-x, A9-x, A10-x, AO4, AO7-x, AO10-x
A15-2x
A18-2x
A24-2x
M37-2x
M37-2x
M37-2x
M6-x, M37-2x
M6-x, M35-2x, M37-2x, M48-2x
M35-2x
M37-2x
A17-2x (M11)
AM3-x
AM12-2x
M20-x, AM3-x
M20-x
M20-x
M20-x
M28-2x
M30-2x
M37-2x
AM8-2x [2]
M47-2x, AM11-2x
M16-x
M20-x
M20-x
M39-2x
M16-x
M16-x
M36-2x
M54-2x
M54-2x
M39-2x
M39-2x
M39-2x
M49-2x
M49-2x
Tortula israelis I2
M44-2x
M43-2x
M16-x
M16-x
M16-x
Tortula israelis I4 [2]
Tortula israelis I1
Tortula israelis I3
Tortula israelis I1
Tortula israelis I3
M44-2x [3]
M36-2x
L1, L2, L3, L4, L5, L6
L7
T. lingulata diploids only
0.92
0.60
0.65
1.00
0.96
1.00
0.86
0.97
0.64
0.98
0.2
1
1
5
2
3
1
1
1
* haplotypes present in more than 9 specimens:
haplotype 1: A4-x, A14-x, A15-2x, A16-2x, A20-2x, A21-2x, A23-2x, A24-2x, A25-2x, A26-2x, A27-2x, M38-2x, M40-2x, AM7-2x, AO8
haplotype 2: M1-x, M2-x, M4-x, M5-x, M7-x, M9-x, M11-x, M12-x, M17-x, M21-x, M23-x, M24-x, M25-x, M26-x, M32-x, M27-2x
haplotype 3: A15-2x, A16-2x, A18-2x, A19-2x, A20-2x, A21-2x, A22-2x, A24-2x, M40-2x, AO8

Fig. 1

and subsp. *muralis* (sequences from approximately 38% of its populations) was somewhat higher than in the *obtusifolia* 1 clade (sequences from 23% of its populations, Fig. 2). In a single collection from France, plants of both the *obtusifolia* 1 and *obtusifolia* 2 clades were detected. This collection was morphologically heterogeneous, containing plants with the morphology of subsp. *obtusifolia* (O4; *obtusifolia* 1 clade) together with plants intermediate between subsp. *obtusifolia* and subsp. *muralis* (AO12; *obtusifolia* 2 clade).

Although some clades contained plants with the morphology of var. *muralis* ("*muralis* 1 clade", "*muralis* 2 clade", "*muralis* 3 clade"), both varieties of *T. muralis* subsp. *muralis* are apparently polyphyletic. Moreover, several ITS sequences were shared by plants which morphologically belonged to one or the other variety.

A biphyletic nature was observed for *T. israelis*, which is nested within one of the moderately supported *T. muralis* subsp. *muralis* clades that contained mostly var. *muralis* morphotypes ("*muralis* 4+*israelis* clade", PP = 0.97, BS = 52%).

Only one major clade (considering those with sequences from more than two samples) was completely free of reticulations caused by intragenomic ITS variation. This clade, here called the "*aestiva* haploids clade" (PP = 1.00, BS = 84%), consists predominantly of var. *aestiva* samples. Interestingly, plants of this clade tend to occur in natural habitats (base-rich rocks).

No geographical pattern was detected in the phylogenetic relationships based on ITS sequences of the *Tortula muralis* complex. The only exception to this was the clade that contained predominantly eastern European samples of *T. lingulata*.

Distribution of ploidy levels on the ITS tree of the *T. muralis* complex. — No phylogenetic pattern was detected in the distribution of haploids and diploids on the phylogenetic tree constructed with ITS data (Fig. 2). Both cytotypes were detected in six of the nine major subclades of the *T. muralis* clade. Moreover, nine haplotypes were shared by haploid and diploid individuals, including four diploid samples without intragenomic variation of ITS.

Intragenomic variation in ITS was more frequent in diploids (71% of the analysed samples) than in haploids (30%). The same was also true for markedly polyphyletic intragenomic ITS sequences (i.e., sequences of the major well-supported lineages).

No intermediate (triploid) ploidy level was detected in the *T. muralis* clade.

Variation in the chloroplast *rps4* region. — Among the 17 samples sequenced, six *rps4* haplotypes were revealed. Interestingly, two of them (M18, M19) were not recorded in the earlier study by Werner & Guerra (2004), while the remaining four had been previously recorded among the 17 haplotypes detected among samples of the world-wide distribution area. The distribution of *rps4* haplotypes is not consistent with the ITS tree (Fig. 2; Fig. S3 in the Electronic Supplement). The most common haplotype M2 was found in 10 samples that included both cytotypes and morphotypes of *T. muralis* subsp. *obtusifolia* and *T. muralis* subsp. *muralis* var. *aestiva*, morphotypes intermediate between *T. muralis* subsp. *obtusifolia* and *T. muralis* subsp. *muralis* var. *aestiva*, morphotypes intermediate between both varieties of *T. muralis* subsp. *muralis*, and *T. lingulata*. Similarly, haplotype M4 (differing by a single mutation from M2) was found in three samples from two independent ITS lineages, including both cytotypes and plants of different morphotypes. Haplotypes M1 and M11 were each found in a single sample.

■ DISCUSSION

Origin of intragenomic ITS variation in *Tortula* and related taxa. — When investigating intragenomic ITS variation, it is necessary to use a single individual for molecular analysis. Even in small bryophytes, one shoot is usually sufficient for DNA extraction. In our study we used a single moss shoot for most DNA extractions, and it is therefore unlikely that variation in sequences was caused by sampling of several individuals with different genotypes. This is especially evident for those samples in which markedly polyphyletic intraindividual ITS sequences were detected; in all these cases, only one shoot was used for DNA extraction (see Appendix for details).

Sampling of pseudogenes is also improbable in our study, because all the obtained sequences have signs of functional nrDNA, including a conserved 5.8S gene (Harpke & Peterson, 2008). In approximately 50% of our samples, the non-identical ITS sequences from a single sample proved to be more or less closely related and often were resolved within a monophyletic clade. This pattern indicates a rather recent differentiation, which resulted from only few mutations within nrDNA arrays. In other cases, however, we observed relatively large differences among intragenomic ITS sequences, which are difficult to explain by stepwise molecular processes or ancestral polymorphism and rather might result from hybridization. According to Nieto Feliner & al. (2004), the existence of concerted evolution affecting multicopy regions reduces the possibility of incomplete lineage sorting of ancestral polymorphisms. The presence of concerted evolution in our case can be inferred from the existence of plants lacking intragenomic ITS variation. The probable existence of gene flow among ITS lineages is in accordance with the usually sexual reproduction within the *T. muralis* complex. In addition, the poorly resolved topologies with low support that were detected in our dataset might also be caused by occasional ITS recombination following hybridization, because recombinant signal in some cases may result in more trees with a larger number of polytomies (Funk, 1985; McDade, 1992).

Remarks on the phylogeny of *Tortula* and related taxa inferred from ITS data. — The phylogeny inferred from the ITS sequences was partly different from that based on *rps4* (Werner & al., 2002a). Both phylogenies contain a well-supported *Pottia* clade, which comprises *Tortula* sect. *Pottia* sensu Zander (1993), i.e., a clade that includes *Protobryum* sensu Guerra & Cano (2000) together with *Stegonia latifolia*. According to the ITS data, this clade moreover contains *Hilpertia, Tortula mucronifolia, Crossidium squamiferum* (type of *Crossidium*), *Pterygoneurum ovatum* (type of *Pterygoneurum*), and *P. subsessile*, which were not analysed by Werner & al. (2000a). However, several taxa had different relationships in

the two phylogenies. Discrepancies between ITS and *rps4* data notably include *Tortula brevissima* and *T. acaulon* (*Phascum cuspidatum* sensu Guerra & Cano, 2000, the type species of *Phascum*), which are nested within *Pottia* according to ITS but appear in a sister clade (*T. acaulon*) or even in different clades of Pottioideae (*T. brevissima*) according to *rps4*.

Evolution of the *T. muralis* complex and taxonomic implications. — ITS data demonstrated that the morphologically defined *T. muralis* complex, as delimited by Košnar & Kolář (2009), is indeed monophyletic. The complex further includes *T. israelis* and *T. edentula* but not *T. vahliana*, as postulated by Werner & Guerra (2004). Taxa of the complex share the usually epilithic growth, small (9–12 µm) and densely papillose leaf cells, markedly revolute leaf margins, isodiametric marginal leaf cells, absence of photosynthetic outgrowths on the ventral side of the costa, and rather small spores (8.5–12.0 µm, but 11–15 µm in *T. lingulata*). These characters allow to distinguish superficially similar but phylogenetically distant taxa, such as *T. brevissima, T. vahliana*, or *T. marginata*. Although the monophyly of the *T. muralis* complex received poor statistical support in the ITS analysis, it is supported by the pattern of intragenomic ITS variation. Even though the intraindividual sequences detected in taxa within the *T. muralis* clade were commonly recorded on distant branches within this clade, they never occurred in other clades of *Tortula*.

As discussed above, phylogenetic analysis of ITS data resulted in a complex pattern suggesting the existence of gene flow among lineages of the *T. muralis* complex, together with some level of ancestral polymorphism. Thus, with the exception of *T. lingulata*, the taxonomic status of the taxa analysed remains critical. The variability of chloroplast *rps4* sequences was too low for reconstructing the species-level phylogeny of the *T. muralis* complex. Our sampling, however, did not include non-European plants (except for *T. edentula*, which was nested within *T. muralis* subsp. *obtusifolia* in the ITS tree). In consequence, we refrain from drawing conclusions about possible cryptic speciation within *T. muralis*, as hypothesized by Werner & Guerra (2004). On the other hand, the virtual absence of reproductive isolation among lineages can be considered important evidence contradicting the cryptic speciation hypothesis in the *T. muralis* complex, at least within the geographical scope of our analysis.

Evolutionary relationships between haploids and diploids in the *T. muralis* complex. — In most cases, both haploids and diploids were found in individual subclades (Fig. 2), which suggests a polytopic and recurrent origin of diploids. Recurrent polyploidization enhances unidirectional inter-ploidy gene flow, which might be followed by homoploid hybridization among the distinct polyploid (in our case gametophytic diploid) lineages, further increasing their variability (Soltis & Soltis, 1999). Such processes might have further obscured the relationships within the *T. muralis* complex.

In some clades, one cytotype prevails. *Tortula lingulata,* as discussed above, seems to be strictly diploid. Interestingly, one German population, previously considered to be probably *T. lingulata* by Meinunger & Schröder (2007), contains both haploids and diploids. These plants were collected far from the distribution centre of *T. lingulata*, which lies in the eastern Baltic region. Their morphology is intermediate between *T. muralis* subsp. *obtusifolia* and *T. lingulata*, but the spores are heterogeneous in size. Spore size was found to be the most important character for distinguishing between the two taxa (Košnar & Kolář, 2009). The spore size of haploid plants was within the range of *T. muralis* subsp. *obtusifolia,* whereas the diploid plants had the larger spores typical of *T. lingulata*. The ITS haplotype of both cytotypes was identical. Therefore, the likely explanation is that the German population consists of haploid plants of *T. muralis* subsp. *obtusifolia* that in situ gave rise to autodiploid progeny. The same explanation might apply to *T. edentula*, which is reported to differ from *T. muralis* subsp. *obtusifolia* by having larger spores (typical for diploids) and by lacking a peristome. Unfortunately, the *T. edentula* material was too old to provide FCM data, but the variation of all important morphological characters, including the absence of a peristome, is identical to that of the above-described German '*T. lingulata*'. An autodiploid origin is thus a plausible hypothesis to explain the larger spores. Moreover, the phylogenetic analysis places *T. edentula* within the *obtusifolia* 1 clade, and we therefore consider *T. edentula* to be identical with *T. muralis* subsp. *obtusifolia* (see Taxonomic Changes below).

The overall frequency of markedly divergent intragenomic ITS sequences was considerably higher in diploids (38% of the samples) than in haploids (3%). Diploids with intragenomic ITS variation are most likely hybrids of different lineages of the ITS tree; although divergent, all are nested within the *T. muralis* clade. On the other hand, approximately 29% of the diploids lacked intragenomic ITS variation, and four of them shared ITS sequences with haploids. This is consistent with the autopolyploid origin of diploids from closely related haploids. Autopolyploidy is clearly evident at least in two cases of mixed populations of both cytotypes sharing the same ITS sequence: the above discussed German population of *T. muralis* subsp. *obtusifolia*, and a Czech population of *T. muralis* var. *muralis,* i.e., samples M9 and M32, respectively. Even when the intragenomic ITS sequences isolated from diploid individuals were not identical, they had not diverged much, which also indicates an autopolyploid origin. Autopolyploidy is further supported by the almost identical morphology of both cytotypes (Košnar & Kolář, 2009) and the frequent existence of populations with mixed ploidy (J. Košnar & al., unpub. data). Based on these facts, we consider the *T. muralis* complex to be the first case of autopolyploidy in mosses that is supported by molecular marker data. The demonstration of autopolyploidy in mosses contrasts with the allopolyploid (i.e., hybrid polyploid) origin proposed for almost all other bryophyte groups that have been studied by molecular markers (Såstad, 2005; Shaw, 2009).

■ TAXONOMIC CHANGES

Tortula muralis subsp. *obtusifolia* (Schwägr.) Culm. in Rev. Bryol. 48: 22. 1921 = *Tortula edentula* Ignatova & Ignatov in Arctoa 18: 135. 2010 ('2009').

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Appendix. List of herbarium specimens used for sequencing and FCM analysis. Samples with ITS paralogs appearing markedly polyphyletic in the ITS phylogeny are in bold. +, more than one moss shoot used for DNA extraction; *, sample tested for monophyly of intraindividual ITS sequences by calculating the posterior probability of monophyly using Bayesian inference; *x*, haploid gametophyte; 2*x*, diploid gametophyte; 3*x*, triploid gametophyte. GenBank accession numbers of ITS are in normal font, *rps4* sequences are in italics, with haplotype designations in brackets; for accession numbers of previously published sequences, see Fig. 1 and Fig. S1. Specimens collected by Košnar and Kučera are deposited in CBFS.

Sample	Ploidy	GenBank accession	Locality	Substrate	Voucher
Tortula edentula					
E1	–	JN544826	Russia: Shikotan Island	Cliffs on sea coast	*Bakalin K-49-2-07* (MHA)
Tortula israelis					
I1	–	JN544880, JN544882	Greece: Athens	Nitrophilous vegetation	*Cano & al. 12104* (MUB)
I2	–	JN544879	Spain: Pontevedra		*Gallego 11866* (MUB)
I3	–	JN544881, JN544883	Spain: Cádiz	Wall	*Cano 1386* (MUB)
I4	–	JN544897	Spain: Murcia		*Rams 10421* (MUB)
Tortula lingulata					
L1+	–	JN544837	Czech Rep.: Peruc	Sandstone boulder	*Košnar 577*
L2+	–	JN544837	Latvia: Krimulda	Sandstone rock	*Košnar 772*
L3+	–	JN544837	Latvia: Sigulda	Sandstone rock	*Košnar 786*
L4+	–	JN544837	Latvia: Ieriķi	Sandstone rock	*Košnar 795*
L5+	2*x*	JN544837	Latvia: Kārļi	Sandstone rock	*Košnar 797*
L6+	–	JN544837	Estonia: Toila	Wall (sandstone)	*Ingerpuu* 24.6.2005 (TU)
L7+	–	JN544838	Russia: Sablino	Sandstone rock	*Abramov & Abramova s.n.* (TAM)
L8+	2*x*	*JN581668 (M2)*	Latvia: Cīrulīši	Sandstone rock	*Košnar 802*
Tortula muralis subsp. *muralis* var. *aestiva*					
A1+	*x*	JN544804, *JN581673 (M2)*	Czech Rep.: Dolní Adršpach	Wall (sandstone)	*Košnar 724*
A2	*x*	JN544804	Czech Rep.: České Žleby	Wall (granite)	*Košnar 1647*
A3	*x*	JN544804	Czech Rep.: Vilémovice	Limestone rock	*Košnar 1713*
A4	*x*	JN544771, JN544789, JN544790, JN544793	Czech Rep.: Trhanov	Bridge (concrete)	*Košnar 1888*
A5	*x*	JN544763	Czech Rep.: Velké Hydčice	Limestone rock	*Košnar 1904*
A6	*x*	JN544773, JN544774	Germany: Neusatz	Wall (granite)	*Košnar 1601*
A7	*x*	JN544808	Hungary: Dömös	Andesite rock	*Košnar 746*
A8	*x*	JN544766, JN544768	Hungary: Hont	Andesite rock	*Košnar 1825*
A9	*x*	JN544804	Hungary: Királyháza	Wall (andesite)	*Košnar 1838*
A10	*x*	JN544804	Latvia: Krimulda	Wall (limestone)	*Košnar 775*
A11	*x*	JN544764	Romania: Băile Olăneşti	Wall	*Košnar 1918*
A12	*x*	JN544765, JN544767	Romania: Cozia	Sandstone rock	*Košnar 1920*
A13	*x*	JN544767, JN544768	Romania: Cozia	Sandstone rock	*Košnar 1921*
A14	*x*	JN544771, JN544781, JN544782	Slovakia: Čabraď	Wall (andesite?)	*Košnar 635*
A15	2*x*	JN544769, JN544770, JN544771, JN544775, JN544776	Czech Rep.: Nebákov	Wall (Sandstone)	*Košnar 560*
A16*	2*x*	JN544771, JN544775	Czech Rep.: Kost	Wall (sandstone)	*Košnar 561*
A17	2*x*	JN544845, *JN581680 (M11)*	Czech Rep.: Kralupy n. Vltavou	Wall (sandstone)	*Košnar 817*
A18	2*x*	JN544775, JN544793, JN544805	Czech Rep.: Bohumilice	Wall (concrete)	*Košnar 1294*
A19	2*x*	JN544775, JN544890	Czech Rep.: Bílek	Wall (mortar)	*Košnar 1508*
A20	2*x*	JN544771, JN544775, JN544785, JN544815	Czech Rep.: Rabštejn n. Střelou	Phyllitic schist rock	*Košnar 1572*
A21	2*x*	JN544771, JN544775	Czech Rep.: Josefov	Wall (mortar)	*Košnar 1723*
A22	2*x*	JN544775, JN544785	Hungary: Mt. Csóványos	Andesite boulder	*Košnar 1842*
A23+	2*x*	JN544771, JN544777	Hungary: Mt. Csóványos	Andesite rock	*Košnar 1847*
A24	2*x*	JN544771, JN544775, JN544786, JN544787	Latvia: Krimulda	Wall (limestone)	*Košnar 778*
A25+	2*x*	JN544771, JN544777, *JN581667 (M2)*	Slovakia: Čabraď	Wall (andesite?)	*Košnar 648*
A26	2*x*	JN544771, JN544785	Slovakia: Kečovo	Wall (concrete)	*Košnar 1007*
A27	2*x*	JN544771, JN544778, JN544793, JN544814	Slovakia: Buková	Wall (limestone)	*Košnar 1017*
Tortula muralis subsp. *muralis* var. *muralis*					
M1	*x*	JN544812	Bosnia and Hercegovina: Vlasenica	Limestone rock	*Košnar 1360*
M2	*x*	JN544813	Bosnia and Hercegovina: Police	Limestone rock	*Košnar 1363*
M3	*x*	JN544829, JN544831	Czech Rep.: Templštejn	Wall (concrete)	*Košnar 418*

Appendix. Continued.

Sample	Ploidy	GenBank accession	Locality	Substrate	Voucher
M4+	*x*	JN544813	Czech Rep.: Žďárky	Concrete	*Košnar 741*
M5	*x*	JN544813	Czech Rep.: Zlatý kůň	Limestone rock	*Košnar 1263*
M6*	*x*	JN544791, JN544792, JN544828	Czech Rep.: Srbsko	Limestone rock	*Košnar 1280*
M7	*x*	JN544813	Czech Rep.: Sudslavice	Limestone rock	*Košnar 1301*
M8	*x*	JN544830	Czech Rep.: České Žleby	Wall (granite)	*Košnar 1648*
M9	*x*	JN544813	Czech Rep.: Bechyně	Granite rock	*Košnar 1897*
M10	*x*	JN544829	Czech Rep.: Nerestce	Limestone rock	*Košnar 1899*
M11	*x*	JN544813	Czech Rep.: Nerestce	Limestone rock	*Košnar 1900*
M12	*x*	JN544812	Switzerland: Meiringen	Bridge (concrete)	*Košnar 990*
M13	*x*	JN544772	Germany: Neusatz	Wall (granite)	*Košnar 1599*
M14	*x*	JN544827, JN544830	Hungary: Drégelyvár	Wall (andesite)	*Košnar 1831*
M15	*x*	JN544817	Italy: Anguillara Sabazia		*Košnar 1907*
M16	*x*	JN544847, JN544848, JN544854, JN544855, JN544856, JN544857	Montenegro: Mratinje	Wall (concrete)	*Košnar 1365*
M17	*x*	JN544812	Montenegro: Plav	Wall (concrete)	*Košnar 1392*
M18	*x*	JN544839	Montenegro: Djurkovići	Wall (mortar)	*Košnar 1405*
M19	*x*	JN544816	Montenegro: Žabljak	Wall (concrete)	*Košnar 1409*
M20	*x*	JN544862, JN544870, JN544871, JN544872, JN544873, JN544874	Norway: Runde	Concrete	*Košnar 1906*
M21	*x*	JN544813	Romania: Măcin	Granite rock	*Košnar 1188*
M22	*x*	JN544811	Romania: Răstoliţa	Bridge (concrete)	*Košnar 1348*
M23	*x*	JN544813	Slovakia: Čenkov	Wall (concrete)	*Košnar 993*
M24	*x*	JN544813	Slovakia: Turňa n. Bodvou	Wall (limestone)	*Košnar 1010*
M25	*x*	JN544813, *JN581666 (M1)*	Slovakia: Buková	Limestone rock	*Košnar 1016*
M26	*x*	JN544813	Switzerland: Luzern	Wall (mortar)	*Košnar 991*
M27	2*x*	JN544813, JN544843	Armenia: Tatev	Wall	*Košnar 1646*
M28	2*x*	JN544836, JN544846	Czech Rep.: Senorady	Wall (concrete)	*Košnar 416*
M29	2*x*	JN544795, *JN581679 (M4)*	Czech Rep.: Tachov	Wall (concrete)	*Košnar 771*
M30*	2*x*	JN544834, JN544835, JN544836	Czech Rep.: Peruc	Sandstone rock	*Košnar 874*
M31	2*x*	JN544836	Czech Rep.: Český Krumlov	Wall (mortar)	*Košnar 885*
M32	2*x*	JN544812	Czech Rep.: Bechyně	Granite rock	*Košnar 1898*
M33	2*x*	JN544842	Czech Rep.: Nerestce	Limestone rock	*Košnar 1901*
M34	2*x*	JN544841	Czech Rep.: Nerestce	Limestone rock	*Košnar 1902*
M35	2*x*	JN544792, JN544889	France: Montpellier	Wall	*Košnar 1033*
M36	2*x*	JN544833, JN544875, JN544876	Hungary: Drégelyvár	Wall (andesite)	*Košnar 1832*
M37*	2*x*	JN544791, JN544792, JN544892, JN544893, JN544894, JN544895, JN544896	Hungary: Poroszló		*Košnar 1912*
M38	2*x*	JN544771, JN544794, JN544817	Italy: Monte Chianti		*Košnar 1908*
M39	2*x*	JN544865, JN544866, JN544867, JN544868	Italy: Sicily, Police		*Košnar 1909*
M40	2*x*	JN544771, JN544775	Latvia: Krimulda	Wall (limestone)	*Košnar 777*
M41	2*x*	JN544833	Montenegro: Mratinje	Wall (concrete)	*Košnar 1367*
M42*	2*x*	JN544810, JN544840	Montenegro: Djurkovići	Wall (limestone)	*Košnar 1404*
M43*	2*x*	JN544878, JN544884, JN544885	Montenegro: Žabljak	Wall (concrete)	*Košnar 1408*
M44	2*x*	JN544858, JN544859, JN544860, JN544861	Montenegro: Riječani	Wall (concrete)	*Košnar 1417*
M45	2*x*	JN544779, JN544780, JN544785, JN544795, JN544891	Poland: Wisełka	Concrete	*Košnar 1905*
M46	2*x*	JN544778, JN544779, JN544780, JN544795	Romania: Răstoliţa	Bridge (concrete)	*Košnar 1347*
M47	2*x*	JN544869, JN544886, JN544887, JN544888	Romania: Capaţini Mts., Stogsoara	Limestone rock	*Košnar 1916*
M48	2*x*	JN544792	Spain: Madrid	Wall (concrete)	*Košnar 1255*
M49	2*x*	JN544863, JN544864	Spain: Bullas, Río Mula	Concrete	*Kučera 13671*
M50	2*x*	JN544761, JN544762	Slovakia: Čenkov	Brick	*Košnar 992*
M51+	2*x*	JN544844	Slovakia: Turňa n. Bodvou	Wall (limestone)	*Košnar 1009*

Appendix. Continued.

Sample	Ploidy	GenBank accession	Locality	Substrate	Voucher
M52	2*x*	JN544829	Slovakia: Stakčín	Wall (concrete)	*Košnar 1018*
M53	2*x*	JN544782, JN544783, *JN581678 (M4)*	Slovakia: Belina	Wall (concrete)	*Košnar 1021*
M54	2*x*	JN544833, JN544852, JN544853	Slovakia: Hajnáčka	Basalt rock	*Košnar 1023*
M55	–	JN544827	Slovakia: Devín	Limestone boulder	*Košnar 1042*
Tortula muralis subsp. *muralis*—plants intermediate between var. *aestiva* and var. *muralis*					
AM1	*x*	JN544830, JN544831, JN544832	Czech Rep.: Luže	Wall (brick)	*Košnar 466*
AM2	*x*	JN544767	Czech Rep.: Karlštejn	Limestone rock	*Košnar 1287*
AM3	*x*	JN544862, JN544877	Germany: Neusatz	Wall (granite)	*Košnar 1600*
AM4	*x*	JN544768, *JN581674 (M2)*	Hungary: Dömös	Andesite rock	*Košnar 747*
AM5	*x*	JN544766	Romania: Capaţini, Stogsoara	Limestone rock	*Košnar 1917*
AM6	*x*	JN544798	Slovakia: Stožok	Andesite rock	*Košnar 630*
AM7	2*x*	JN544771, JN544788	Czech Rep.: Hrubá Vrbka	Concrete	*Košnar 710*
AM8*	2*x*	JN544780, JN544795, JN544796, JN544797, JN544850, JN544851	Czech Rep.: Kralupy n. Vltavou	Sandstone rock	*Košnar 832*
AM9	2*x*	JN544780, JN544799	Germany: Ruhestein	Wall (sandstone)	*Košnar 1598*
AM10	2*x*	JN544782	Hungary: Dobogókö	Andesite rock	*Košnar 744*
AM11	2*x*	JN544869	Romania: Băile Olăneşti	Wall	*Košnar 1919*
AM12	2*x*	JN544849	Romania: Oradea	Wall (concrete)	*Košnar 1353*
Tortula muralis subsp. *obtusifolia*					
O1	*x*	JN544751	Austria: Zalußenalm	Base-rich schist rock	*Košnar 926*
O2	–	JN544751	France: Mt. Cenis		*De Zuttere 22169* (priv. herb.)
O3	–	JN544800, JN544801, JN544802, JN544803, *JN581681 (M18)*	France: Mt. Cenis		*Skrzypczak 03424* (priv. herb.)
O4	–	JN544821	France: Mt. Cenis, Grotte percée		*Skrzypczak 98395* (priv. herb.)
O5	*x*	JN544825	Germany: Schwarzwald	Sandstone rock	*Košnar 1586*
O6	*x*	JN544825	Germany: Schwarzwald	Sandstone rock	*Košnar 1588*
O7	*x*	JN544825	Germany: Schwarzwald	Sandstone rock	*Košnar 1589*
O8+	2*x*	JN544825, *JN581676 (M2)*	Germany: Schwarzwald	Sandstone rock	*Košnar 1587*
O9+	*x*	JN544824	Hungary: Mt. Csóványos	Andesite rock	*Košnar 1845*
O10*	–	JN544758, JN544759, JN544760	Iceland: Rangárvallasýsla	Rock	*Johansson s.n.* (S)
O11	*x*	JN544822	Romania: Călimani Mts.	Andesite rock	*Košnar 1324*
O12+	*x*	JN544822, *JN581675 (M2)*	Romania: Călimani Mts.	Andesite rock	*Košnar 1330*
O13	–	JN544807	Romania: Răstoliţa	Andesite rock	*Košnar 1349*
O14	*x*	JN544824, *JN581671 (M2)*	Slovakia: Stožok	Andesite rock	*Košnar 631*
O15	–	JN544824, *JN581669 (M2)*	Slovakia: Čabraď	Andesite rock	*Košnar 639*
Plants intermediate between *Tortula muralis* subsp. *muralis* var. *aestiva* and *Tortula muralis* subsp. *obtusifolia*					
AO1+	–	JN544818, JN544819, JN544820	Armenia: Garni		*Vašák s.n.* (B)
AO2	*x*	JN544752, JN544753	Armenia: Tatev	Wall	*Košnar 1646*
AO3	*x*	JN544757	Austria: Mt. Leiterkopf	Base-rich schist rock	*Košnar 1543*
AO4	*x*	JN544804	Austria: Leiterbach	Base-rich schist rock	*Košnar 1551*
AO5	*x*	JN544756	Austria: Kleinfleißbach	Base-rich schist rock	*Košnar 1556*
AO6+	*x*	JN544757	Austria: Kleinfleißbach	Base-rich schist rock	*Košnar 1565*
AO7+	*x*	JN544804, JN544806, *JN581670 (M2)*	Czech Rep.: Lažánky	Limestone rock	*Košnar 601*
AO8	–	JN544771, JN544775, JN544784, JN544785, JN544785, JN544786	Czech Rep.: Kralupy	Sandstone rock	*Košnar 824*
AO9	*x*	JN544767	Czech Rep.: Holštejn	Limestone rock	*Košnar 1533*
AO10	*x*	JN544804	Czech Rep.: Příběnice	Erlan rock	*Košnar 1903*
AO11	–	JN544751, *JN581682 (M19)*	France: Mt. Cenis	Rock	*Skrzypczak 03455* (priv. herb.)
AO12	–	JN544754, JN544755	France: Mt. Cenis, Grotte percée		*Skrzypczak 98395* (priv. herb.)
AO13	*x*	JN544809, *JN581677 (M4)*	Hungary: Dömös	Andesite rock	*Košnar 749*
AO14	*x*	JN544824	Hungary: Dömös	Andesite rock	*Košnar 750*
AO15	*x*	JN544823, *JN581672 (M2)*	Hungary: Visegrád	Andesite rock	*Košnar 756*

Appendix. Continued.

Sample	Ploidy	GenBank accession	Locality	Substrate	Voucher
Crossidium aberrans	–	JN544730, JN544731	Spain: Sierra de Cazorla	Rock	*Kučera 5747*
C. crassinerve	–	JN544732, JN544733	Spain: Las Torres de Cotillas	Calcareous soil	*Kučera 13662*
C. squamiferum	–	JN544723, JN544724	Montenegro: Virpazar	Limestone rock	*Košnar 1414*
Pterygoneurum ovatum	–	JN544737, JN544738	Czech Rep.: Němčičky	Loess	*Košnar 319*
P. subsessile	–	JN544739, JN544740	Czech Rep.: Čejkovice	Loess	*Košnar 1913*
Stegonia latifolia	–	JN544715, JN544716	Austria: Mt. Hohe Dock	Bare soil	*Košnar 1448*
Tortula acaulon	–	JN544743, JN544744, JN544745, JN544746	Czech Rep.: Horní Bojanovice	Bare soil	*Košnar 317*
T. atrovirens	–	JN544712, JN544713, JN544714	Spain: Cabo de Gata		*Kučera 5338*
T. brevissima 1	3*x*	JN544726, JN544727	Spain: Las Torres de Cotillas	Calcareous soil	*Kučera 13662*
***T. brevissima* 2**+	–	JN544722, JN544725	Spain: Cabo de Gata	Soil	*Kučera 5332*
T. cernua	–	JN544736	Norway: Svalbard, Petuniabukta	Soil	*Košnar 1914*
T. hoppeana	–	JN544710	Austria: Mt. Waldhorn	Gneiss rock	*Kučera 12892*
T. lanceola	–	JN544717, JN544718	Czech Rep.: Nové Dobrkovice	Soil	*Košnar 245*
T. laureri	–	JN544711	Austria: Mt. Scharnock	Soil	*Kučera 9218*
T. leucostoma	–	JN544734, JN544735	Norway: Svalbard, Petuniabukta	Soil	*Košnar 1915*
T. marginata	–	JN544747, JN544748, JN544749	Italy: Sicily, Scopello	Wall	*Košnar 1910*
T. modica	–	JN544719, JN544720	Czech Rep.: Nové Dobrkovice	Soil	*Košnar 250*
T. protobryoides	–	JN544721	Czech Rep.: Horní Němčí	Soil	*Košnar 1245*
T. revolvens	–	JN544729	Spain: Rambla de Tabernas		*Kučera 5386*
T. systylia	–	JN544750	Italy: Mt. Col del Cuc	Soil	*Kučera 7278*
T. truncata	–	JN544741, JN544742	Germany: Hub	Soil	*Košnar 1605*
T. vahliana	–	JN544728, *JN581683 (V2)*	Netherlands		*Vanderpoorten 4835* (priv. herb.)

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ORIGINAL PAPER

Factors affecting population size and vitality of *Hamatocaulis vernicosus* (Mitt.) Hedenäs (Calliergonaceae, Musci)

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Abstract *Hamatocaulis vernicosus*, a rare moss species, was monitored in 33 fens in the Czech Republic for five to six years. Population size, vitality and trends of population development were recorded. Water chemistry, water level fluctuation, vegetation type and cover, as well as mowing regime were assessed and the effect of these potential predictors on the species populations was examined. Populations of *H. vernicosus* were affected mainly by the density of vascular plants, the species thrived best in habitats with sparse herb and abundant "brown moss" cover. Other important factors included water table fluctuation and water concentration of iron. Populations were more vital and prospered better in sites with a stable water table and more iron-rich conditions. Dependence of population parameters on other measured characteristics of water chemistry was not detected.

Keywords Bryophytes · Fens · Management · pH · Water chemistry

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Introduction

Bryophytes are important components of mire ecosystems. Due to the degradation of peatlands during the last decades, many moss species have been rapidly decreasing and their existence has been threatened (Kooijman 1992; Mälson and Rydin 2007; Štechová and Štech 2007). Species of rich fens have been among the most severely threatened due to general rarity as well as the specific dynamics of this habitat (Hájek et al. 2006; Vitt and Wieder 2008). Additionally, from the Central European perspective, they have been at risk due to their occurrence in densely populated and heavily exploited landscapes.

Hamatocaulis vernicosus (Calliergonaceae) is a typical representative of threatened rich fen mosses. Due to its general rarity in Europe, it has been recommended for special attention within the European Union, being listed in Appendix II of the Bern Convention (Council Directive 92/43/EEC 1992). The coherence of *H. vernicosus* from a phylogenetic species perspective was challenged recently after molecular markers pointed toward the existence of two separate lineages (Hedenäs and Eldenäs 2007). Both of these occurred in Central Europe (as of this writing confirmed in Switzerland and Austria); it was suggested that they be interpreted as cryptic species.

Hamatocaulis vernicosus occurs in Central Europe predominantly in rich and moderately rich fens of the alliance *Sphagno warnstorfiani-Tomenthypnion* (Štechová et al. 2008). Less often it is able to grow in extremely rich fens of the alliance *Caricion*

davallianae and very rarely in the poorer communities of *Caricion fuscae* (Hájek et al. 2006). Site conditions of *H. vernicosus* were studied earlier by Hedenäs and Kooijman (1996), who reported mean values for the concentration of chemical components at localities in Sweden. Interestingly, the representatives of the two phylogenetic lineages were not shown to have significantly different ecological requirements (Hedenäs and Eldenäs 2007). It may be inferred that the earlier reported narrow niche of *H. vernicosus* can thus largely be applied to both cryptic taxa.

Information on the sites of *H. vernicosus* in the Czech Republic has been revised since 2001 within the framework of the NATURA 2000 project; their most recent occurrences have been monitored since 2003. In our previous study (Štechová and Kučera 2007), we investigated several key aspects of *H. vernicosus* ecology—the vegetation composition at its localities, detailed chemistry of water samples in a limited selection of seven localities and the effect of management represented by experimental mowing at three localities. Our attempt at testing the influence of water chemistry on the dynamics of populations in the Czech Republic did not reveal any dependence.

Building on the above information, we designed an experiment with broader-scaled monitoring that spanned 33 sites, representing about 65 % of the recently identified locations of *H. vernicosus* in the Czech Republic. Monitoring included detailed water chemistry and underground water table recording at fixed plots. In addition, we noted the vegetation composition, the dynamics of *H. vernicosus* populations and the type of management applied by the owners or conservation authorities over the course of five to six years.

We asked two questions:

(1) What is the influence of water chemistry and water level on population size, vitality and dynamics?
(2) How do habitat type, density of vegetation cover and intensity of management affect species populations?

Materials and methods

Field sampling and laboratory analyses

Data were sampled at 33 localities of *H. vernicosus* in the Czech Republic in the years 2005–2010 (at three localities, data were sampled only in the years 2006–2010—see Table 1). The sampled localities were all identified as of autumn 2006, when the water samples for analyses were collected (with the exception of three sites that were not visited due to their difficult accessibility). The studied sites were situated across most of the areas with natural fen occurrence in the Czech Republic at the altitudes between 250 and 960 m. The average annual temperature at these sites was reported to be in the range 5–8 °C and annual precipitation in the range 550–1,100 mm (Tolasz 2007).

One permanent plot (4 × 4 m) was fixed at each site. It was located to include the largest part of the population of *H. vernicosus* at each locality. The sites were visited twice a year, though in a few cases only once. The timing of visits was planned to ensure an approximately identical degree of vegetation development in the course of the whole vegetation season. This meant May and June for spring visits, autumn visits in September and October, depending on the altitude. Only the first visits in the first year, during which we recorded the vegetation samples, occurred in early summer (June and July) to ensure the recording of most of the vascular plant species. The vegetation samples enabled the analysis of the bryophyte, herb and shrub cover.

During most of the visits, we measured pH and conductivity; however, some readings were missing, particularly the conductivity readings in 2007 and 2008, caused by device failures. Both chemical characteristics were measured in situ at three points in each plot using a portable device (Vario pH, WTW, Germany; Snail Instruments, Czech Republic). Underground water was sampled for detailed analyses of water chemistry (NH_4^+, NO_3^-, PO_4^{3-}, Ca^{2+}, Fe); these were collected directly in the *H. vernicosus* patches in 2006 in late summer to early autumn (September/October), when the chemical gradients are more stable (Tahvanainen et al. 2003).

The samples were filtered over a glass filter and frozen within 24 h for later analysis. NH_4^+, NO_3^- and PO_4^{3-} were determined colorimetrically by flow injection analysis (FIA Lachat QC8500, Lachat Instruments, USA), total N (LiquiTOC), Ca^{2+} and Fe concentrations were analyzed spectrophotometrically (SpectrAA 640, Australia). The water table (minimum and maximum) was measured mostly over the course of the whole vegetation season using the

Table 1 Description of studied sites

	Population size	Vitality	Trend of population development	Observation period (years)	Coordinates (WGS 84)	pH	Conductivity (uS cm^{-1})	Fe (mg/l)	Ca (mg/l)	NH_4 (ug/l)
Bažiny	2	2.4	2.3	6	N50°17′47″ E016°17′59″	6.4	152	0.26	22.1	145
Červený rybník	5	3	2	6	N50°44′07″ E014°33′10″	6.8	193	2.07	13.6	15.7
Dolejší rybník	3	1.7	1.5	6	N49°25′57″ E013°49′17″	5.7	508	3.35	37.8	78.3
Hrádecká bahna	3	2.1	1.7	6	N49°42′47″ E013°39′32″	7.0	185	0.82	19.6	9.98
Hůrky	3	2.2	1.7	6	N49°53′45″ E013°11′01″	6.1	67	1.13	6.28	16.9
Chvojnov	2	3	3	6	N49°24′26″ E15°25′09″	6.3	102	0.95	14.2	19.4
Jezdovické rašeliniště	1	1.4	1.2	6	N49°19′25″ E015°27′42″	6.3	265	0.43	22.6	33.1
Kaliště	1	1.1	1.4	6	N49°19′25″ E015°27′42″	6.5	123	1.94	11	53.5
Klátov	3	2.9	2.8	6	N49°08′15″ E015°27′08″	7.0	301	0.1	31	245
Křemelná 2	5	3	3	5	N49°10′13″ E013°19′58″	6.6	45	0.42	4.2	161
Louky u Č. lesa	3	2.7	3	6	N49°35′09″ E015°56′34″	6.2	148	0.91	18.4	11.2
Louky v Jeníkově	2	3	3	5	N49°44′18″ E015°57′52″	6.3	209	1.27	9.45	61
Matenský rybník	2	1.6	1.2	6	N49°09′04″ E014°55′51″	6.2	192	5.82	9.0	257
Na Oklice	4	2.6	1.7	6	N49°24′15″ E015°23′40″	6.5	77	54.1	16.7	147
Nad Svitákem	1	1.2	1.3	6	N49°23′48″ E015°24′17″	6.7	177	0.55	16.6	4.79
Novozámecký rybník	2	3	2	6	N50°36′45″ E014°35′07″	7.2	230	1.17	32.7	1
Nový rybník u Rohozné	4	1.9	1.3	6	N49°48′13″ E015°49′11″	6.3	153	3.61	12.2	391
Odměny u rybníka Svět	2	1.8	2	6	N48°59′31″ E014°43′33″	5.9	51	1.17	4.46	19.2
Podtrosecká údolí	5	2.5	2.3	6	N50°31′28″ E015°13′02″	7.0	241	1.97	41.3	7.3
Prameny Klíčavy	3	2.7	3	6	N50°08′45″ E013°49′43″	6.7	227	0.4	25	4.17
Rašeliniště u Suchdola	3	2	2.3	6	N49°07′55″ E015°14′18″	6.1	151	1.07	47.9	204
Ratajské rybníky	3	1.7	1.5	6	N49°46′09″ E015°56′02″	6.8	219	0.08	14.9	39.3
Ruda jih	3	3	2.8	6	N49°08′43″ E014°41′26″	6.6	166	2.99	14.6	917
Ruda sever	2	1.3	1.8	6	N49°09′05″ E014°41′21″	6.3	173	1.14	14.7	300
Řeka	5	3	3	6	N49°39′59″ E015°51′10″	7.1	247	0.52	46.4	165
Řežabinec	3	1.8	2	6	N49°15′04″ E014°04′58″	6.3	129	1.90	9.5	42.1
Staré jezero	4	2.3	2.3	6	N48°58′45″ E014°53′51″	5.3	60	13.4	12.4	349
Strádovka	2	1.9	2.3	6	N49°48′33″ E015°48′12″	6.5	98	2.39	15.1	26.1
Šimanovské rašeliniště	2	1.5	1.5	6	N49°27′01″ E015°26′48″	6.4	108	1.33	17.9	15
V Lisovech	4	2.3	1.6	6	N49°14′49″ E015°16′44″	5.7	104	0.33	11.9	29.4
V rájích	2	3	2	5	N48°59′11″ E014°42′31″	6.5	205	0.06	39.3	44.7

Table 1 continued

	Population size	Vitality	Trend of population development	Observation period (years)	Coordinates (WGS 84)	pH	Conductivity (uS cm^{-1})	Fe (mg/l)	Ca (mg/l)	NH_4 (ug/l)
Velká Kuš	2	1.8	2	5	N49°23′39″ E013°47′41″	6.3	208	6.53	13.1	351
Zlámanec	2	2.7	2.8	6	N49°42′19″ E015°55′56″	6.0	147	3.07	16.2	143

	NO_3 (ug/l)	PO_4 (mg/l)	Altitude (m asl)	Average water table (cm)	Water fluctuation (cm)	Management intensity	Moss cover (%)	Herb cover (%)	Shrub cover (%)	*Sphagnum* cover (%)	Habitat type
Bažiny	66.4	12.5	620	−3	6	2	60	70	0	20	Spring
Červený rybník	33.4	21	300	−10	7	0	90	50	5	25	Fishpond margin
Dolejší rybník	43.4	7.59	450	−11	30	1	60	60	0	2	Fishpond margin
Hrádecká bahna	74.2	11.5	400	−8	10	2	90	90	0	50	Fen meadow
Hůrky	79.4	7.71	550	−5	4	0	60	60	2	50	Spring
Chvojnov	53.2	13.8	605	−6	4	2	60	50	0	25	Fen
Jezdovické rašeliniště	52.6	9.97	575	−2	5	2	40	75	0	20	Spring
Kaliště	49.1	9.08	655	−7	2	2	80	80	0	65	Fen meadow
Klátov	105	16.6	485	−6	5	2	80	60	0	0	Spring
Křemelná 2	143	18.3	960	−2	1	0	90	40	0	60	Spring
Louky u Č. lesa	30.7	11.6	570	−5	3	2	60	60	0	30	Fen
Louky v Jeníkově	44	22	630	−8	3	2	60	80	0	25	Fen meadow
Matenský rybník	37.7	66.6	525	−10	15	2	60	80	0	5	Fen meadow
Na Oklice	51.2	11.7	660	−9	5	1	80	70	0	20	Spring
Nad Svitákem	69.8	12.2	630	−2	2	0	10	90	5	10	Fishpond margin
Novozámecký rybník	33	8.53	255	−2	2	2	70	60	0	0	Fen meadow
Nový rybník u Rohozné	45.4	12.8	560	−8	9	1	40	60	3	2	Fishpond margin
Odměny u rybníka Svět	84.3	10.8	435	−2	20	0	80	60	5	80	Fen
Podtrosecká údolí	56.7	9.48	280	−3	10	2	50	60	0	0	Fen
Prameny Klíčavy	72.1	16	430	−5	2	2	70	60	0	40	Spring
Rašeliniště u Suchdola	68	7.55	625	−5	3	2	90	70	0	40	Spring
Ratajské rybníky	45	29.2	590	−11	19	1	60	50	0	1	Fishpond margin
Ruda jih	108	18.1	415	−7	4	0	50	50	0	35	Fen
Ruda sever	75.1	10.6	415	−6	7	0	40	45	0	20	Fen
Řeka	48.8	11.3	555	−6	8	2	80	50	0	0	Spring
Řežabinec	29.1	19.1	370	−4	5	2	80	60	0	60	Fen meadow

Table 1 continued

	NO_3 (ug/l)	PO_4 (mg/l)	Altitude (m asl)	Average water table (cm)	Water fluctuation (cm)	Management intensity	Moss cover (%)	Herb cover (%)	Shrub cover (%)	*Sphagnum* cover (%)	Habitat type
Staré jezero	152	13.7	440	−6	6	0	60	60	1	40	Fen
Strádovka	44.7	9.36	580	−10	21	0	35	60	0	10	Fishpond margin
Šimanovské rašeliniště	43.8	9.52	605	−4	4	2	70	50	0	30	Spring
V Lisovech	61.1	14.2	650	−6	6	1	70	60	1	40	Fen meadow
V rájích	390	31.6	445	−7	5	2	70	50	0	15	Spring
Velká Kuš	62	36	482	−17	5	1	80	80	0	0	Fen meadow
Zlámanec	41.8	46.5	620	−6	3	2	60	70	0	35	Fen meadow

PVC discoloration method (Belyea 1999; Navrátilová and Hájek 2005). In each permanent plot, a vegetation relevé was recorded in June or July with respect to the altitude of the site by making a visual estimate of the cover of all species.

Management intensity applied by the owners or conservation authorities at the localities was estimated on a three-grade scale: 0, no management; 1, sporadic mowing once every two or three years; 2, regular yearly mowing. No management was recorded at nine sites (fens, fishpond margins and springs), sporadic management at six sites (fen meadows, fishpond margins and springs) and regular management at 18 sites (fens, fen meadows and springs)—see Table 1. Fen meadows without management, fens with sporadic management and fishpond margins with regular yearly mowing where *H. vernicosus* occurs were unknown in the Czech Republic. Mowing was mostly done using brush cutters. All sites which were recently regularly mown shared a similar management history from the mid-20th century. They were gradually abandoned until late 1990s or early 2000s. Then management was resumed, albeit no longer for agricultural but rather for conservation purposes, with brush-cutters replacing mowers.

The habitats were classified into four groups. These were: fishpond margins (fens developed along the banks of fishponds, which were affected by pond water), springs (habitats with flowing spring water), fen meadows (drier habitats with peat thickness <1 m) and fens (wetter habitats with peat thickness >1 m).

Three characteristics of *H. vernicosus* populations were evaluated at each locality: population size, vitality and trend of population development.

Population size was estimated on a five degree scale. 1, <100 stems; 2, no more than 0.25 m^2; 3, 0.25–1 m^2; 4, 1–5 m^2 and 5, more than 5 m^2.

Vitality was recorded on each visit during four- to six-year observations. It was assessed on a three degree scale: 1, the majority of stems faded, partially rotten or very thin and lean; 2, most stems with normal vitality, green, faded and rotten stems rare; 3, all stems vital and sturdy. The vitality of the moss was a quite variable characteristic, which often fluctuated widely between visits, probably depending on the actual water table depth and herb shading. As no notable increase or decrease in vitality was evident at any site, we assessed the vitality of the population as the mean value of all observations.

The trend of population development was recorded every year and was evaluated on the basis of changes

Table 2 Median and quartile range of all analyzed site characteristics. Predictors selected on the basis of AIC statistics in one of the considered models are shown in bold. Shrub cover had nonzero value only at seven localities (ranging from 1 to 5 % cover), its average value is 0.67 %

	Median	Lower quartile	Upper quartile
pH	6.3	6.2	6.8
Conductivity (μS/cm)	139	87	180
Fe (mg/l)	1.17	0.52	2.39
Ca^{2+} (mg/l)	15.10	12.20	22.61
NH_4^+ (μg/l)	44.7	16.9	165
NO_3^- (μg/l)	53.2	43.8	74.2
PO_4^{3-} (μg/l)	12.5	10.0	18.3
Altitude (asl)	555	435	620
Average water table (cm under stem apex)	−6	−7	−4
Water fluctuation (cm)	5	3	8
Bryophyte cover (%)	60	60	80
Herb cover (%)	60	50	70
Shrub cover (%)	0	0	0
Sphagnum cover (%)	25	5	40

Table 3 Summary of the linear models fitted for the three parameters of *H. vernicosus* populations. Full model selected based on AIC value is shown, the presented F statistic values and type I error estimates (p) only supplement the parsimony-based results. The order of selected predictors represents the order of their selection into model, based on the AIC statistics

Population size			
Predictor	Regression coefficient	$F_{1,27}$	p
Herb cover	−0.0376	7.19	0.012
Fe content	+0.5394	3.82	0.061
Bryophyte cover	+0.0249	2.66	0.115
Sphagnum cover	−0.0165	2.64	0.116
Shrub cover	+0.1590	1.99	0.170
Population vitality			
Predictor	Regression coefficient	$F_{1,28}$	p
Herb cover	−0.0179	7.05	0.013
Water table fluctuation	−0.0348	5.87	0.022
Bryophyte cover	+0.0126	3.05	0.092
Sphagnum cover	−0.0086	3.55	0.070
Trend of population development			
Predictor	Regression coefficient	$F_{1,29}$	p
Herb cover	−0.0166	4.59	0.041
Water table fluctuation	−0.0295	4.70	0.038
Shrub cover	−0.0917	2.13	0.156

of population size between the visits. This characteristic was estimated by using sketch micromaps of populations or their parts (according to population sizes). We used a three degree scale: 1, the population size decreasing; 2, the population size not changing (change within ca 10 %); 3, the population size increasing. The definitive value of this characteristic was the mean value of all observations.

Data analysis

Variables representing the concentration of ions and conductivity values were log-transformed.

The effects of considered factors, listed in Table 2, on the three recorded population characteristics were modelled by a general linear model. Final models were selected based on their parsimony, measured by the AIC statistic (Akaike, 1974). This approach usually provided a more liberal selection of predictors (Chambers and Hastie 1992) and was also difficult to compare with studies, where parametric approaches to model selection were adopted. We have therefore re-evaluated the individual term of the models selected with AIC using parametric analysis of variance and F statistic. Statistical analyses were performed using the R program, version 2.8 (R Development Core Team, 2008).

Results

According to the fitted models, the size of *H. vernicosus* populations is negatively affected by herb and *Sphagnum* cover, yet positively affected by

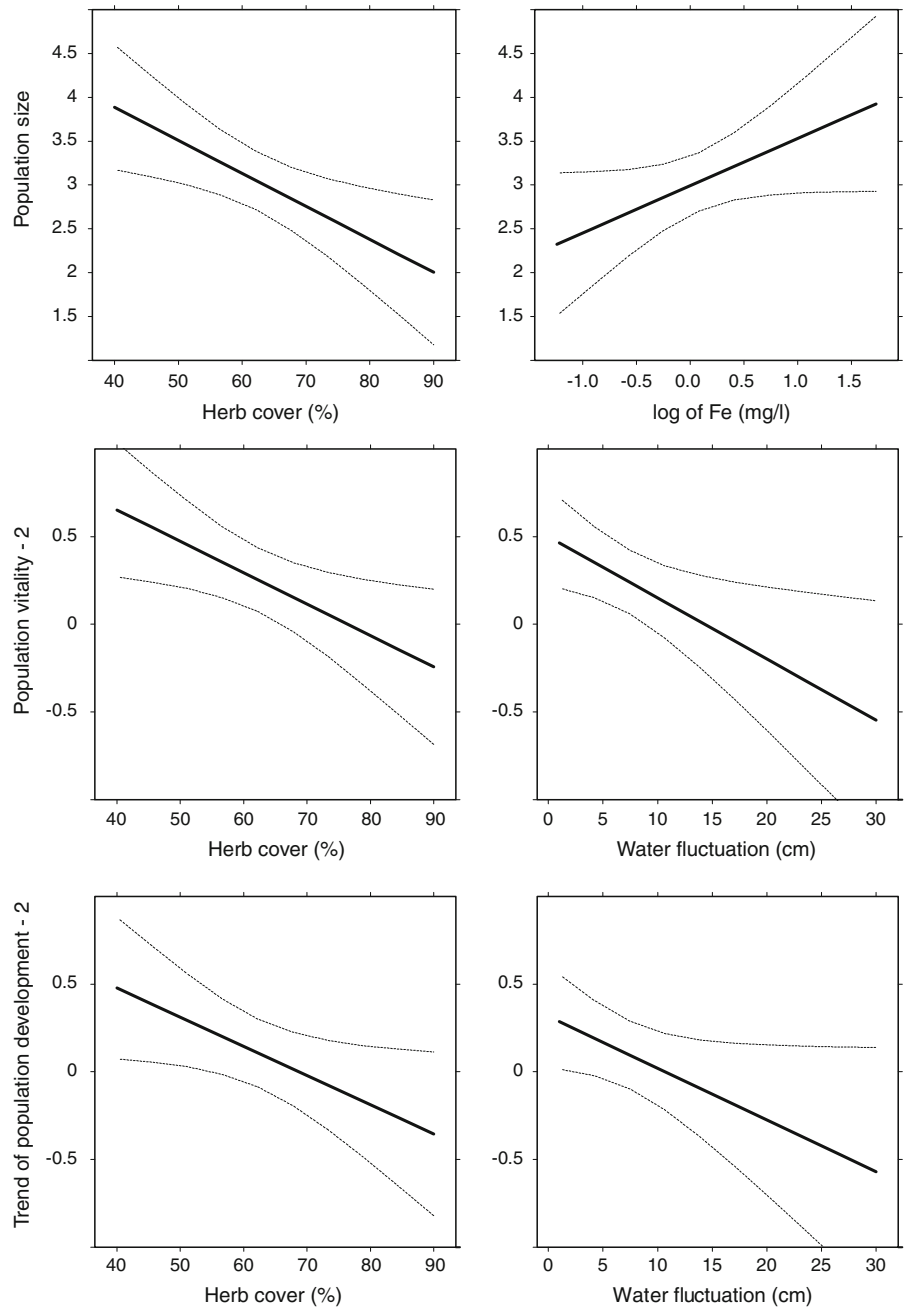

Fig. 1 Visual presentation of the partial effects of significant predictors from the three regression models summarized in Table 3. *Dashed lines* delineate 95 % confidence regions (or intervals, in the case of categorical predictor). In the *first row*, the effects on population size are presented, and the effects on the average population vitality and on the population size increase are shown, respectively, in the *second* and *third row*

increasing iron concentration and density of bryophyte and shrub cover (Table 3, Fig. 1). However, the effects of bryophyte, *Sphagnum* and shrub cover are not significant in the parametric test.

The vitality of populations is positively correlated with the density of moss layer, whereas it is negatively correlated with the herb and *Sphagnum* cover and the degree of water level fluctuation (Table 3, Fig. 1). The

trend of population development seems to be negatively correlated with the density of herb cover, water table fluctuation and density of shrub cover, which is positively correlated with population size (Table 3, Fig. 1). The effect of shrub cover was, however, not significant in the parametric tests.

Except for the iron content, which positively affected population size, no significant influence of chemical composition of water on *H. vernicosus* populations was shown.

Discussion

According to our results, herb cover is the most important factor negatively affecting all three measured characteristics, which included the size of *H. vernicosus* populations, their vitality and population development trend. Generally, the moss layer is held down by a high cover of vascular plants (Hájková et al. 2009), but each species reacts differently to this factor. Herb cover sensitively responds to the intensity of management at *H. vernicosus* localities. Hence regular mowing is necessary at localities where the water table does not keep the cover of vascular plants low (Štechová and Kučera 2007). No correlation between the management intensity and vascular plant cover in this study could be found given the existence of few localities where the water table is high and herb cover sparse despite the absence of management. Moreover, the mowing is often not sufficient to keep aboveground vascular plant biomass low in the case of increased eutrophication and decreased water level (Bergamini et al. 2009).

No correlation was detected between the phosphate content and herb layer cover, contrary to the positive correlation in most other studies (e.g. Venterink et al. 2009, Gerdol et al. 2010). The interactions between phosphate content and other factors enhancing or suppressing the herb layer (management intensity, water table fluctuation) might be too complicated to render the positive effect of phosphate on herb layer. Also, the single phosphate determination might not have been sufficient for revealing the existing trends due to the possible variance in the readings. Moreover, the phosphate content available to the plants might have differed considerably from the total content measured, as argued by Kooijman and Hedenäs (2009).

A probably misleading effect was the significant positive correlation between population size of *H. vernicosus* and the cover of shrubs. The species preferred open-canopy microsites (Bauer et al. 2007), which was also confirmed in our other studies. The trend of *H. vernicosus* population development was negatively affected by the cover of higher shrubs (Table 3). The shrub cover was recorded at only 7 of 33 localities (Table 1). A significant positive effect from shrubs on the population size was likely caused by the unusual situation at the "Červený rybník" site, where *H. vernicosus* grew in very favourable conditions (permanently wet, sparse herb cover); its population was large, and it had not yet responded to the recent gradual expansion of *Salix cinerea* shrubs. The positive effect of shrubs was not significant in the parametric test.

A cover of *Sphagna*, which were the most serious bryophyte competitors for other fen mosses (Paulissen et al. 2004), had a negative effect on population size and vitality. Habitats dominated by *Sphagna* were generally less suitable for *H. vernicosus* (and other brown mosses) due to adverse chemical conditions (Hájek et al. 2006), as well as the direct competition for space, light, and possibly nutrients. Plants of *Sphagnum* benefitted from their more robust constitution and higher growth rate, as evidenced already by Kooijman (1993). *H. vernicosus* stems growing among *Sphagna* were thin and lean probably due to lack of light.

Another advantage for *Sphagnum* species was their ability to acidify habitats, handicapping the more calcicole species. During natural succession with associated acidification, rich fen bryophytes were replaced by calcium-tolerant *Sphagna*, and then by *Sphagna* with an optimal occurrence in mineral-poor fens or bogs (Glime et al. 1982, Bragazza and Gerdol 1999). In our study, pH and mineral richness were not selected as significant factors affecting *H. vernicosus* populations. However, higher *Sphagnum* cover and lower pH were significantly correlated.

Water table fluctuation also had a significant impact on the vitality of the target species and the trend of its development in our study. The best vitality was recorded in fen and spring habitats, where the water table was high and relatively stable. The moss was found to be less vital at fishpond margins, where water conditions were very unstable (cf. Navrátilová and Navrátil 2005; Štechová and Štech 2009), as the water

table fluctuated according to the water table of the managed fishpond. The worst vitality was generally recorded in populations from majority of fen meadows, where the moss often suffered from the lack of water, caused by frequent artificial drainage.

The reduction of *H. vernicosus* populations was recorded at majority of sites with high water table fluctuation. That was expected at localities where the water level repeatedly decreases deep below the surface this situation often has led to the extinction of many rich fen bryophytes (Mälson et al. 2008). However, population reduction was observed also at several localities where the water table was often a few centimetres above the surface during the vegetation season and mosses were inundated. This negative effect confirmed that the studied moss does not tolerate long-term inundation, as noted already by Janssens (1983).

The positive correlation of the iron content with population size was very interesting for us, as it contradicted the results from our previous studies (Štechová and Kučera 2007; Štechová et al. 2010). On the other hand, this result was supported by earlier data from Hedenäs and Kooijman (1996), who stated that *H. vernicosus* preferred iron-rich habitats. Different iron contents at the same localities can be caused by different sampling times, because it was known that iron concentrations fluctuate widely in time (Hájek and Hekera 2004). This could have influenced our previous study, for which most samples were taken in spring, when the chemical gradients were believed to be quite variable and weakly definable (Tahvanainen et al. 2003). The positive iron effect on *H. vernicosus* population size may be explained by the lower uptake of Ca ions in conditions of higher iron content (cf. Zohlen and Tyler 2000), disadvantaging the competing calcicolous moss species (e.g. *Scorpidium cossonii*, *Campylium stellatum*, *Palustriella commutata*).

We found no statistically significant effect from nutrient concentrations on *H. vernicosus* populations. This was a surprising result because an increase in nutrients together with a decrease in the water table has been considered one of the main factors causing the retreat of many fen bryophytes (e.g. Kooijman and Bakker 1995; Paulissen et al. 2004, 2005, Bergamini et al. 2009). We must consider the possibility that the nutrient content in the water did not always correspond to the amount of nutrients available to the mosses. Especially in the more calcareous fens, net N and P mineralization was found to be relatively low by Kooijman and Hedenäs (2009). Therefore the simple measuring of nutrient content in the surrounding water might have been insufficient for true detection of a nutrient influence on the studied species.

The nutrient contents in our study were quite variable within the monitored plots. The average NH_4^+ content from 33 localities in this study was roughly half of that measured in our previous study (about 220 μg/l in samples from seven localities, Štechová and Kučera 2007), although all seven sites from the last study were included in this study, too. This discrepancy can be explained by a high seasonal variation in water chemistry (Tahvanainen et al. 2003), because the sampling term was different in this and the last study. The average value reported by Hedenäs and Kooijman (1996) from the Swedish sites was even higher (350 μg/l). Likewise the average NO_3^- content about 70 μg/l was almost half of that of the previous study, whereas the Swedish average was 90 μg/l. Concentrations of PO_4^{3-} were not analysed in our previous study, but according to the results of this study the average content was about 20 μg/l, which was the same value as that reported by the Swedish sites.

We must of course acknowledge that a part of the unexplained variability might relate to the possible occurrence of two recently discovered phylogenetic lineages (Hedenäs and Eldenäs 2007) with non-identical realized niches in the investigated area. However, that study proved that no difference exists in habitat preferences between the two cryptic species as represented by the basic factors of water chemistry (pH and conductivity). This seems to indicate that our results might be applied irrespective of the precise genetic identity of the studied populations, at present only identifiable by genetic barcoding.

We conclude that the population size and the vitality of *H. vernicosus* are affected mainly by the density of vascular plant cover. A higher cover of *Sphagna* also has a negative effect on population performance. Water conditions are very important for the vitality of the moss and the development of its populations; the species thrives best in habitats with a relatively stable water table. Another important factor affecting *H. vernicosus* populations is the content of Fe ions, as the populations prosper better in iron-rich conditions. Based on this research, population characteristics do not appear to be dependent upon nutrient contents. Future studies should probably consider the

identity of the studied populations within the recognized cryptic species.

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