

Systematics of the family Pottiaceae (Bryophyta) with special reference to the familial and subfamilial circumscriptions

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The family Pottiaceae is the most generic and species rich family in Bryophyta. The classification of this family is very difficult and controversial due to parallel or convergent evolution and sterility. In this paper, we briefly review the current state of knowledge on phylogeny and classification of the family. Molecular phylogenetic inference based on a new approach using a codon substitution model is also undertaken and confirm four clades within the Pottiaceae, corresponding to Trichostomoideae, Pottioideae, Merceoideae, and a newly proposed subfamily Streblotrichoideae.

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Introduction

The family Pottiaceae Hampe is the most generic and species rich family of Bryophyta Schimp., with around 1,400 species in 83 genera, comprising more than 10 % of the extant moss species (Frey & Stech 2009). Widely distributed in the world, its species have adapted to a wide range of habitat types including xeric, mesic and hydric, growing on various substrata including saxicolous, tericolous and corticolous, and a possessing a variety of life strategies including perennial, annual and ephemeral. The family exhibits a great variety of apparent morphological, physiological and genecological adaptations to their particular environments (Zander 1993). Recent geometric morphometric analyses with evolutionary hypothesis testing revealed that the Pottiaceae was one of the lineages in which multiple evolutionary changes of sporangium shape associated with the types of habitat have occurred (Rose *et al.* 2016).

Among the various types of habitat where they are found, most species exhibit a great tolerance of hot and dry environments, and show numer-

ous adaptations to such harsh environments. The shoots of *Syntrichia caninervis* Mitt. remained viable after exposure to 120°C for 30 min., which is a new upper thermo tolerance record for adult eukaryotic organisms for a minimum 30 min. exposure time (Stark *et al.* 2009), and dried herbarium specimen of *S. ruralis* (Hedw.) F.Weber & D.Mohr retained their viability for 20 years and 3 months, which is the longest record for a moss withstanding continuous desiccation (Stark *et al.* 2016). The Pottiaceae includes all three life strategies which dominate in hot desert bryofloras: the perennial stayer (most of the family), the annual shuttle (*Pottia* Ehrh. ex Fűrnr. and *Phascum* Hedw.) and the perennial shuttle [*Tortula pagorum* (Milde) De Not. and *T. papillosa* Wilson ex Spruce] (Longton 1988).

Adaptation to such harsh selective pressures often leads to the presence of parallel or convergent characters which develop in response to the same environmental stimuli, complicating phyletic assessment. Chen (1941) suggested that the lamellae of *Pterygoneurum* Jur. represented features of convergent evolution and phylogenetically have nothing in common with filaments of

Table 1. History of classification of Pottiaceae and its related families modified from the tables after Saito (1975) and Werner *et al* (2004a).

Aloina Kindb. and *Crossidium* Jur. On the other hand, Delgadillo (1975) argued for the close relationship between *Pterygoneurum* and *Crossidium* since the abnormal filaments of *Crossidium* spp. resemble the lamellae of *Pterygoneurum* spp. Magill (1981) also demonstrated that specialized chlorophyllose marginal cells occur in *Tortula porphyreoneura* (Müll.Hal.) C.C.Towns. and *Barbula arcuata* Griff. growing in arid grasslands in southern Africa. He also suggested that the modification of marginal or costal cells into differentiated photosynthetic tissues is an adaptation to harsh environments, expressed through convergent evolution by several genera in the Pottiaceae: *Acaulon* Müll.Hal., *Aloina*, *Barbula* Hedw., *Crossidium*, *Pterygoneurum* and *Tortula* Hedw.

Mature sporophytes provide taxonomically

important characters in mosses, but many species from xeric habitats produce no, or very few sporophytes, presumably because of the difficulty in effecting fertilization or allowing for the maturation of sporophytes under such xeric conditions. Stark (2002) and Stark *et al.* (2007) showed that in a desert climate, the massive sporophyte abortions seen in *Tortula inermis* (Brid.) Mont. are correlated with unusually heavy summer precipitation events followed by rapid drying, and that the sporophytes are more sensitive to rapid drying than are maternal gametophytes.

These environmental features have made the classification of Pottiaceae very difficult and controversial: the species concepts are often not well understood, and the family has been variously classified without an understanding of its phylogenetic relationships, leaving many ambiguous or

poorly understood taxa unresolved (Satio 1975). A source of independent taxonomic evidence is therefore needed for the revision of this family (Spagnuolo *et al.* 1996, 1997), and during the past 20 years a number of molecular phylogenetic analyses have been conducted in an attempt to resolve relationships within the family (e.g. Spagnuolo *et al.* 1996, 1999; Werner *et al.* 2004a, 2005; Cano *et al.* 2010; Kučera *et al.* 2013; Alonso *et al.* 2016). From the early 19th century, various attempts at a classification of the Pottiaceae and its related families have been proposed by many researchers as summarized in Table 1. The history of classification of the family based on morphological criteria has been overviewed by several authors (e.g. Saito 1975; Zander 1993; Werner *et al.* 2004a), with a limited number of reviews of recent progress in understanding the systematic relationships based on integrated morphological and molecular data (Stech *et al.* 2012).

In the present paper, we review the current state of knowledge on phylogenetic relationships and classification at familial and subfamilial levels within the Pottiaceae. Previous studies are compared with a novel phylogenetic inference based on concatenated sequences of ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) and chloroplast ribosomal protein S4 (*rps4*) genes with codon substitution model. The codon substitution model is a statistically higher precision model than nucleotide and amino acid substitution models for the evolutionary analysis of protein-coding sequences (Seo & Kishino 2008, 2009; Miyazawa 2011).

Materials and methods

Phylogenetic markers and taxon sampling

20 *rbcL* and 23 *rps4* gene sequences were newly obtained. The supposed ingroup species represent all the subfamilies of Pottiaceae recognized by Werner *et al.* (2004a): Trichostomoideae Broth., Pottioidae Broth., Merceyoideae Broth., as well as *Streblotrichum convolutum* (Hedw.) P.Beauv., the type species of the genus *Streblotrichum* P.Beauv., whose phylogenetic position has remained ambiguous (Köckinger & Kučera 2011; Kučera *et al.* 2013). Outgroup species [*Ditrichum heteromallum* (Hedw.) E.Britton and *Pseudophermerum nitidum* (Hedw.) Loeske] were selected

based on the results of Inoue and Tsubota (2014), and Fedosov *et al.* (2016). List of investigated species was shown in Appendix.

DNA extraction, PCR amplification and DNA sequencing

The protocol for total DNA extraction followed Tsubota *et al.* (1999) and Suzuki *et al.* (2013). Conditions for PCR amplifications for both *rbcL* and *rps4* genes followed Inoue and Tsubota (2014). Direct sequence analyses of the PCR products were performed following Tsubota *et al.* (1999, 2000, 2001) and Inoue *et al.* (2012). Primers used for PCR amplification and DNA sequencing followed Nadot *et al.* (1994), Souza-Chies *et al.* (1997), Tsubota *et al.* (1999, 2001), Masuzaki *et al.* (2010), Inoue *et al.* (2011, 2012), and Inoue and Tsubota (2014). Sequences obtained in the present study have been submitted to DDBJ/EMBL/GenBank International Nucleotide Sequence Database Collaboration (INSDC).

Phylogenetic analyses

Sequences of two genes were aligned separately by using the program MAFFT ver. 7.027 (Kato & Standley 2013) with some manual adjustment on the sequence editor of MEGA ver. 5.2 (Tamura *et al.* 2011). Start and stop codons were removed, and the resulting total length was 2,025 bp.

Phylogenetic analysis using the concatenated sequences of *rbcL* and *rps4* genes was performed based on the maximum likelihood (ML) method (Felsenstein 1981) and the approximate unbiased (AU) test (Shimodaira 2002, 2004) in the final stage of the analysis scheme. Prior to the phylogenetic reconstruction, Kakusan4 (ver. 4.0.2012.12.14; Tanabe 2011) was used to determine the appropriate substitution model and partitioning scheme for our data based on corrected Akaike Information Criterion (AICc; Sugiura 1978). Since the codon substitution model is inappropriate for an heuristic search due to the huge computational burden, phylogenetic trees were constructed using the following three program packages to obtain the candidate topologies: (1) RAxML ver. 8.2.8 (Stamatakis 2014) with ML method using the equal mean rate model among codon positions (GTR + Γ for all codon positions of *rbcL* and *rps4*) with 1,000 heuristic searches;

(2) PAUPRat (Sikes & Lewis 2001) over PAUP* ver. 4.0b10 (Swofford 2002) with the maximum parsimony (MP) method (Fitch 1971) to implement Parsimony Ratchet searches (Nixon 1999) using the Parsimony Ratchet search strategy with random weighting of each character in fifty 200 iteration runs; (3) MrBayes ver. 3.2.5 (Ronquist *et al.* 2012) with Bayesian inference (BI) method using the proportional model among codon positions (GTR + Γ for all codon positions of *rbcL*, HKY85 + Γ for first and second codon positions of *rps4*, GTR + Homogeneous for third codon position of *rps4*) with 10,000,000 generations, sampling trees every 1,000 generations. A 50 % majority-rule consensus tree was calculated after the convergence of the chains and discarding 25 % of the sampled trees as burn-in.

Based on the ML criteria, re-calculation of likelihood values for each tree topology was performed with the codon substitution model which was more or less equivalent to the Goldman-Yang 1994 model implemented in Garli var. 2.01 (Zwickl 2006). The set of candidate topologies was evaluated by the AU test and Bayesian posterior probability (PP) calculated by the BIC approximation (Schwarz 1978; Hasegawa & Kishino 1989) using CONSEL ver. 0.20 (Shimodaira & Hasegawa 2001). A strict condensed tree for the topologies with high ranking log-likelihood values that passed both AU and PP tests was also computed by MEGA. Supporting values more than 50 % obtained by CONSEL were overlaid to assess the robustness of each branch of the highest likelihood and strict condensed topologies: AU test (AU), bootstrap probabilities (NP), and Bayesian posterior probabilities (PP) are shown on or near each branch (AU/NP/PP).

Result

The concatenated data matrix had a total length of 2,025 bp, of which 369 (18.2 %) were variable, and 209 (56.6 % of the variable sites) were parsimony-informative.

A total of 70 topologies were obtained from the three analyses: four ML topologies by RAXML; 65 MP by PAUPRat over PAUP*; and one BI by MrBayes. More detailed topologies were searched through the obtained trees using a log-likelihood measure. The best-supported tree

with the highest likelihood value is shown in Fig. 1. The log-likelihood value for the tree was -7206.803252. One strict condensed tree was also obtained for the six topologies with high-ranking log-likelihood values that passed both AU and PP tests as shown in Fig. 2. Values for the percentage of supported topologies for each branch were superimposed in Figs. 1 and 2.

The best-supported tree with highest likelihood value confirmed the monophyly of the Pottiaceae with four major clades within the family, corresponding to Trichostomoideae (T), Pottioideae (P), Merceyoideae (M), and the newly proposed Streblotrichoideae (S) as shown in Fig. 1. The Merceyoideae was resolved as the most basal clade within the family with high supporting values (100/100/1.00). Pottioideae comprised the sister-group to Trichostomoideae and this clade was sister to Streblotrichoideae. Although the relationships among these three subfamilies were weakly supported (-/50/0.88), the strict condensed tree also supported this branching pattern as shown in Fig. 2.

Discussion

Phylogenetic position and circumscription of Pottiaceae

Earlier phylogenetic studies using molecular markers focused on the Pottiaceae were based on the nuclear internal transcribed spacer (ITS) region (Colacino & Mishler 1996; Spagnuolo *et al.* 1996, 1999). Spagnuolo *et al.* (1999) successfully aligned ITS1 sequences with a reduced number of taxa of Pottiaceae, and showed the usefulness of DNA sequences to clarify the phylogenetic relationships within this family. Their results suggested that the classification of the Pottiaceae based on morphological data did not depict the pattern of descent and therefore the systematics of this group needed to be revised.

In molecular phylogenetic studies focusing on supra-familial relationships within mosses, the Pottiaceae was resolved in the clade of Dicranidae Doweld of the haploleptoid mosses (Cox *et al.* 1999; Goffinet & Cox 2000; Goffinet *et al.* 2001; La Farge *et al.* 2000, 2002; Magombo 2003; Hedderson 2004; Tsubota *et al.* 2004). However the number of genera and species included in these analyses was limited.

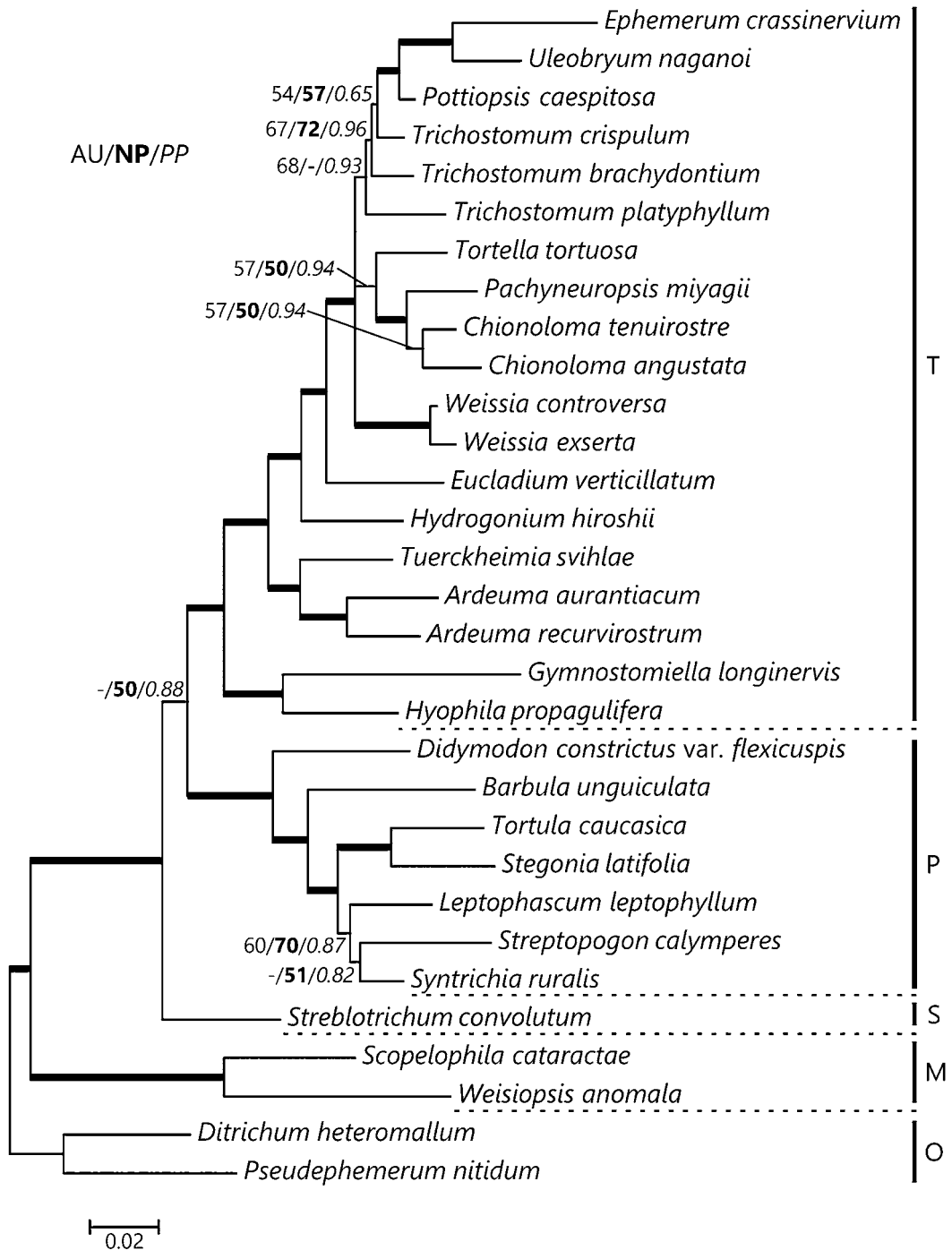


Fig. 1. Phylogenetic tree based on analysis with the concatenated sequences of chloroplast *rbcL* and *rps4* genes, depicted by the best-supported tree with highest likelihood value ($\ln L = -7206.803252$ by Garli). Supporting values more than 50 % obtained by the program CONSEL were overlaid: AU test (AU), bootstrap probabilities (NP), and Bayesian posterior probabilities (PP) are shown on or near each branch (AU/ NP/PP). Thickened branches indicate that all three supporting values are 100 %. The Roman characters correspond to the outgroup species and the subfamilial classification in the present study (T = Trichostomoidae, P = Pottioidae, S = Streblotrichoideae, M = Merceyoideae, O = outgroup).

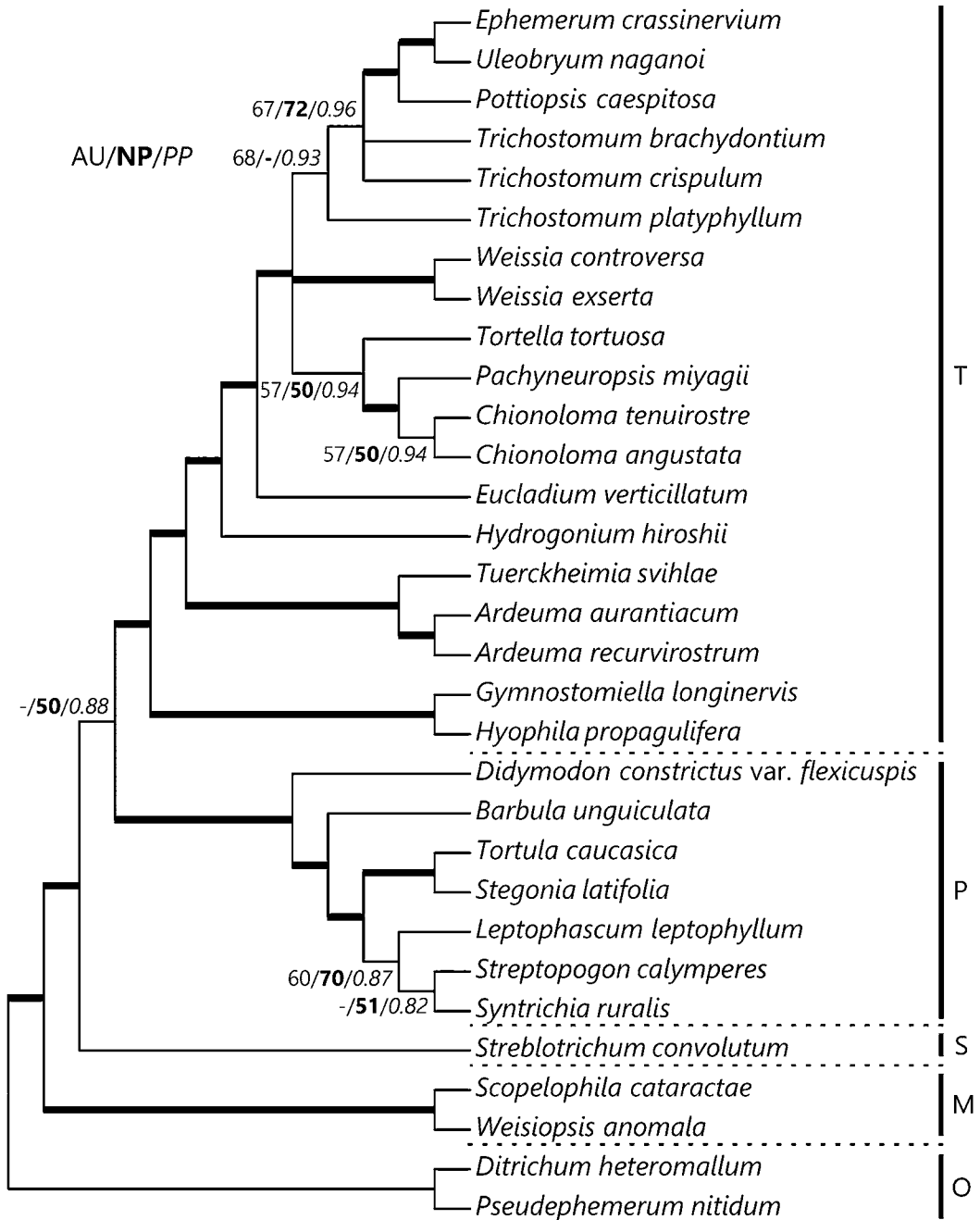


Fig. 2. Phylogenetic tree based on analysis with the concatenated sequences of chloroplast *rbcL* and *rps4* genes, depicted by the strict condensed tree for six topologies passing both AU and PP tests. Supporting values more than 50% obtained by the program CONSEL were overlaid: AU test (AU), bootstrap probabilities (NP), and Bayesian posterior probabilities (PP) are shown on or near each branch (AU/NP/PP). Thickened branches indicate that all three supporting values are 100%. The Roman characters correspond to the out-group species and the subfamilial classification in the present study (T = Trichostomoideae, P = Pottioideae, S = Streblotrichoideae, M = Merceoideae, O = outgroup).

Werner *et al.* (2004a) conducted the first comprehensive molecular phylogenetic analysis of the family using chloroplast *rps4* gene sequences which had been used successfully to resolve the phylogenetic relationships at species or generic level within the family (Werner *et al.* 2002, 2003). The Pottiaceae was almost monophyletic in its traditional circumscription, but with some exceptions as discussed below. Some genera, without general agreement on whether or not they belonged to Pottiaceae, were positioned in Pottiaceae (Werner *et al.* 2004a): *Cinclidotus* P.Beauv. (Cinclidotaceae Schimp.), *Ephemerum* Hampe (Ephemeraceae J.W.Griff. & Henfr.), *Goniomitrium* Hook & Wilson (Funariaceae Schwägr.), *Kingiobryum* H.Rob. (Dicranaceae Schimp.) and *Splachnobryum* Müll.Hal. (Splachnobryaceae A.K.Kop.). The systematic position of *Cinclidotus*, *Ephemerum*, *Kingiobryum*, and *Splachnobryum* within Pottiaceae was also supported by other molecular phylogenetic studies (e.g. Goffinet *et al.* 2001; Sato *et al.* 2004; Werner *et al.* 2004b, 2007; Cox *et al.* 2010; Inoue *et al.* 2011, 2012). On the other hand, Werner *et al.* (2007) concluded that *Goniomitrium* should be excluded from Pottiaceae and placed again in Funariaceae, and they also showed that the name used in the previous studies resulted from misidentification. The inclusion of *Tridontium tasmanicum* Hook.f. (Grimmiaceae Arn./Scouleriaceae S.P.Churchill) within Pottiaceae was also suggested by Cox *et al.* (2010) and Goffinet *et al.* (2011). *Hypodontium* Müll.Hal. and *Timmiella* (De Not.) Limpr. were resolved outside Pottiaceae (Werner *et al.* 2004a). Other molecular data also rejected the hypothesis of the taxonomic position of *Hypodontium* in Pottiaceae (Hedderson *et al.* 2004; Tsubota *et al.* 2004), and the family Hypodontiaceae M.Stech, as a distinct family, was segregated from Pottiaceae to accommodate *Hypodontium* (Stech & Frey 2008). In the most recent analysis (Fedosov *et al.* 2016) the Hypodontiaceae was included in the clade represented by Aongstroemiaceae De Not., Dicranaceae Schimp., Dicranellaceae M. Stech, Fissidentaceae Schimp. and Serpotortellaceae W.D.Reese & R.H.Zander. Although many molecular data rejected the hypothesis on the taxonomic position of *Timmiella* in Pottiaceae and supported its repositioning as an early-diverging clade within the Dicranidae (La Farge

et al. 2000, 2002; Hedderson *et al.* 2004; Tsubota *et al.* 2004; Wahrmund *et al.* 2009, 2010; Cox *et al.* 2010), it was retained as a member of the Pottiaceae because of its morphological affinity to the family, especially the presence of the characteristic twisted peristome. Zander (2006) argued that the complex twisted peristome was scattered among the lineages of the Pottiaceae *sensu stricto* and resulted from the re-activation of a silenced gene cluster involved in major organs that is highly adaptive, and that the same phenotype found in *Timmiella* and Pottiaceae *s. str.* was suggested to be homoiologous. Based on their comprehensive taxon sampling of basal haplolepidous taxa, Inoue and Tsubota (2014) showed the more sound phylogenetic position of *Timmiella* and showed its close relationship with *Luisierella* Thér. & P.de la Varde which had been placed in the Pottiaceae *s. str.* They further argued that the direction of twist of the operculum cells and of the peristome was a significant character that discriminated the genera from Pottiaceae and the other haplolepidous moss families, and proposed a new family Timmiellaceae Y.Inoue & H.Tsubota to accommodate the genera *Timmiella* and *Luisierella*. Their study also supported the monophyletic circumscription of the family Pottiaceae with a close relationship to Ditrichaceae Limpr. *pro parte*. Phylogenetic trees using extensive taxon sampling of Dicranidae have showed that the Pottiaceae was resolved in the clade intermingled with genera of Bruchiaaceae Schimp., Ditrichaceae *p.p.* and Erpodiaceae Broth. (Fedosov *et al.* 2015, 2016).

Subfamilial relationships within Pottiaceae

In his extensive revision of the family Pottiaceae, Zander (1993) recognized seven subfamilies based on phylogenetic analyses using morphological data as shown in Table 1. The molecular phylogenetic analyses by Werner *et al.* (2004a) based on cp *rps4* gene sequences included all the subfamilies recognized by Zander (1993), with the exception of Gertrudielloideae R.H.Zander. Based on the inferred trees they recognized three subfamilies: Trichostomoideae, Pottioideae and Merceyoideae. Their analyses supported the most basal position of Merceyoideae in the Pottiaceae. The remaining species of Pottiaceae formed the clade corresponding to Trichosto-

moideae and Pottioideae. The Trichostomoideae formed a paraphyletic group, and the Pottioideae was monophyletic. The genus *Eucladium* Bruch & Schimp. was placed in an intermediate position between these two subfamilies. The phylogenetic analysis by Werner *et al.* (2005) based on comprehensive taxon sampling of Trichostomoideae and nr ITS sequences supported the monophyly of the subfamily and *Eucladium* was resolved in the Trichostomoideae. Jiménez *et al.* (2012) first obtained DNA data for *Gertrudiella* Broth., and the genus was resolved in the Pottioideae *sensu* Werner *et al.* (2004a). Zander (2006) revised the classification of the family based on molecular and morphologically based phylogenies, and recognized five subfamilies [Timmielloideae R.H.Zander, Trichostomoideae (syn. Chionolomoideae R.H.Zander), Barbuloideae Hilp. (syn. Erythrophyllopsidoideae R.H.Zander, Gertrudielloideae R.H.Zander), Pottioideae and Merceyoideae]. The latest classification of subfamilies in Pottiaceae (Frey & Stech 2009) also adopted the five subfamilies recognized by Zander (2006). The present analyses also supported the most basal position of Merceyoideae within Pottiaceae, the monophyly of Trichostomoideae with inclusion of *Eucladium*, and the monophyly of Pottioideae (Figs. 1 & 2).

Recent phylogenetic analyses (Köckinger & Kučera 2011; Kučera *et al.* 2013) have indicated the isolated position of some species traditionally assigned to the genus *Streblotrichum* P.Beauv. The phylogenetic position of this group is, however, ambiguous because it is: (1) sister to the clade Pottioideae-Trichostomoideae (cp *rps4*), (2) basal within the Trichostomoideae (cp *rps4* + *trnM-V*), or (3) even polyphyletic (nr ITS) with low support for any of these placements (Kučera *et al.* 2013). The sister position of *Streblotrichum* to the clade Trichostomoideae-Pottioideae was supported by the ML tree inferred from the concatenated *rbcL* and *rps4* gene sequences with codon substitution model, and the strict condensed tree (Figs. 1 & 2). *Streblotrichum* was originally established by Palisot de Beauvois (1804) and it has traditionally been recognized to be included in *Barbula* *sensu lato* at generic, subgeneric or sectional rank (e.g. Limpricht 1888; Saito 1975). According to Kučera *et al.* (2013), the following combination of characters: (1) strongly differen-

tiated convolute perichaetial leaves, (2) yellow seta, (3) revoluble annulus, (4) well-developed twisted peristome and (5) brown, spherical, rhizoidal gemmae, supports the molecular groupings and re-delimits *Streblotrichum*, with the acceptance of three species in the genus: *S. convolutum* (Hedw.) P.Beauv. (type species), *S. commutatum* (Jur.) Hilp. and *S. enderesii* (Garov.) Loeske. We conclude that the unique position of the genus *Streblotrichum* requires the recognition of a new subfamily, based on its morpho-molecular distinction.

As suggested by Stech *et al.* (2012), recent findings of several new species and genera based on morpho-molecular data indicate that the total diversity within the Pottiaceae remains insufficiently known or understood. In the present study, we have succeeded in obtaining a more robust topology based on the codon substitution model. A more complete phylogenetic analysis could provide a better understanding of the diversity and evolutionary history of the family based on comprehensive taxon- and marker- samplings, as well as a proper analysis scheme.

Taxonomic treatment

Subfamily **Streblotrichoideae** Y.Inoue & H.Tsubota, *stat. nov.*

Basionym: *Streblotrichum* P.Beauv., Mag. Encycl. 9 (5): 317. 1804.

- ≡ *Tortula* subg. *Streblotrichum* (P.Beauv.) A.Chev., Fl. Gén. Env. Paris 2: 51. 1827. ≡ *Barbula* sect. *Streblotrichum* (P. Beauv.) Limpr., Laubm. Deutschl. 1: 626. 1888. ≡ *Barbula* subg. *Streblotrichum* (P.Beauv.) K.Saito, J. Hattori Bot. Lab. 39: 499. 1975.
- = *Tortula* sect. *Convolutae* De Not., Mem. Reale Accad. Sci. Torino 40: 287. 1838. ≡ *Barbula* sect. *Convolutae* (De Not.) Bruch & Schimp., Bruch *et al.*, Bryol. Europ. 2: 91 (fasc. 13–15. Mon. 29). 1842.

Type: *Streblotrichum* P.Beauv. Included genus: *Streblotrichum* P.Beauv. [with *S. convolutum* (Hedw.) P.Beauv. as Type species]

Diagnosis: The subfamily Streblotrichoideae can be characterized by the combination of the following traits which discriminates it from the other subfamilies of Pottiaceae: Trichostomoidae, Pottioideae and Merceyoideae: (1) strongly

convolute perichaetial leaves, (2) yellow seta, (3) revoluble annulus, (4) well-developed twisted peristome, and (5) brown, spherical, rhizoidal gemmae.

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Literature cited

- Alonso, M., Jiménez, J. A., Nylinder, S., Hedenäs, L. & Cano, M. J. 2016. Disentangling generic limits in *Chionoloma*, *Oxystegus*, *Pachyneuropsis* and *Pseudosymblypharis* (Bryophyta: Pottiaceae): an inquiry into their phylogenetic relationships. *Taxon* 65: 3–18.
- Brotherus, V. F. 1902. Pottiaceae. In Engler, A. & Prantl, K. (eds.), *Natürlichen Pflanzenfamilien* 1 (3), pp. 380–439. Wilhelm Engelmann, Leipzig.
- . 1924. Pottiaceae. In Engler, A. & Prantl, K. (eds.), *Natürlichen Pflanzenfamilien* ed. 2, 10, pp. 243–302. Wilhelm Engelmann, Leipzig.
- Cano, M. J., Jiménez, J. A., Gallego, M. T. & Jiménez, J. F. 2010. *Guerramontesia microdonta* (Pottiaceae, Bryophyta) a new monotypic genus from South America. *Syst. Bot.* 35: 453–460.
- Chen, P. C. 1941. Studien über die ostasiatischen arten der Pottiaceae I & II. *Hedwigia* 80: 1–76, 141–322.
- Colacino, C. & Mishler, B. D. 1996. Preliminary results from ITS sequence data analysis in the genus *Syntrichia* (Musci: Pottiaceae). *Am. J. Bot.* 83: 8.
- Corley, M. F. V., Crundwell, A. C., Düll, R., Hill, M. O. & Smith, A. J. E. 1981. Mosses of Europe and the Azores; an annotated list of species, with synonyms from the recent literature. *J. Bryol.* 11: 609–689.
- Cox, C. J. & Hedderson, A. J. 1999. Phylogenetic relationships among the ciliate arthrodontous mosses: evidence from chloroplast and nuclear DNA sequences. *Pl. Syst. Evol.* 215: 119–139.
- , Goffinet, B., Wickett, N. J., Boles, S. B. & Shaw, A. J. 2010. Moss diversity: a molecular phylogenetic analysis of genera. *Phytotaxa* 9: 175–195.
- Delgadillo, M. C. 1975. Taxonomic revision of *Aloina*, *Aloinella* and *Crossidium* (Musci). *Bryologist* 78: 245–303.
- Fedosov, V. E., Fedorova, A. V., Ignatova, E. A., Bobrova, V. K. & Troitsky, A. V. 2015. *Rps4* and *NAD5* sequences indicate the polyphyly of Ditrichaceae and parallelisms in the evolution of haplolepideous mosses. *Molecular Biology* 49: 890–894.
- , Fedorova, A. V., Fedosov, A. E. & Ignatov, M. S. 2016. Phylogenetic inference and peristome evolution in haplolepideous mosses, focusing on Pseudoditrichaceae and Ditrichaceae *s.l.* *Bot. J. Linn. Soc.* 181: 139–155.
- Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* 17: 368–376.
- Fitch, W. M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* 20: 406–416.
- Frey, W. & Stech, M. 2009. Marchantiophyta, Bryophyta, Anthocerotophyta. In Frey, W. (ed.) *Syllabus of Plant Families. A. Engler's Syllabus der Pflanzenfamilien*, 13th ed., Part 3 Bryophytes and seedless Vascular Plants., pp. 1–257. Gebr. Borntraeger, Stuttgart.
- Goffinet, B. & Cox, C. J. 2000. Phylogenetic relationships among basal-most arthrodontous mosses with special emphasis on the evolutionary significance of the Funariinae. *Bryologist* 103: 212–223.
- , Budke, J. M. & Newman, L. C. 2011. Micromitriaceae: a new family of highly reduced mosses. *Taxon* 60: 1245–1254.
- , Cox, C. J., Shaw, A. J. & Hedderson, T. A. J. 2001. The Bryophyta (Mosses): systematic and evolutionary inferences from an *rps4* gene (cpDNA) phylogeny. *Ann. Bot.* 87: 191–208.
- Hasegawa, M. & Kishino, H. 1989. Confidence limits on the maximum likelihood estimate of the homi-

- noid tree from mitochondrial DNA sequences. *Evolution* 43: 672–677.
- Hedderson, T. A., Murray, D. J., Cox, C. J. & Nowell, T. L. 2004. Phylogenetic relationships of haplolepidous mosses (Dicranidae) inferred from *rps4* gene sequences. *Syst. Bot.* 29: 29–41.
- Hilpert, F. 1933. Studien zur Systematik der Trichostomaceen. *Beih. Bot. Centralbl.* 50: 585–706.
- Inoue, Y., Tsubota, H., Kubo, H., Uchida, S., Mukai, S., Shimamura, M. & Deguchi, H. 2011. A note on *Pottia intermedia* (Turner) Fűrnr. (Pottiaceae, Bryopsida) with special reference to its phylogeny and new localities in SW Japan. *Hikobia* 16: 67–78.
- , ———, Sato, H. & Yamaguchi, T. 2012. Phylogenetic note on *Pachyneuroopsis miyagii* T.Yamag. (Pottiaceae, Bryophyta). *Hikobia* 16: 221–228.
- , ———. 2014. On the systematic position of the genus *Timmiella* (Dicranidae, Bryopsida) and its allied genera, with the description of a new family Timmiellaceae. *Phytotaxa* 181: 151–162.
- Jiménez, J. A., Cano, M. J. & Jiménez, J. F. 2012. Taxonomy and phylogeny of *Andina* (Pottiaceae, Bryophyta): a new moss genus from the tropical Andes. *Syst. Bot.* 37: 293–306.
- Katoh, K. & Standley, D. M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30: 772–780.
- Köckinger, H. & Kučera, J. 2011. *Hymenostylium xerophilum*, spec. nov., and *H. gracillimum*, comb. nov., two neglected European mosses and their molecular affinities. *J. Bryol.* 33: 195–209.
- Kučera, J., Košnar, J. & Werner, O. 2013. Partial generic revision of *Barbula* (Musci: Pottiaceae): re-establishment of *Hydrogonium* and *Streblotrichum*, and the new genus *Gymnobarbula*. *Taxon* 62: 21–39.
- La Farge, C., Mishler, B. D., Wheeler, J. A., Wall, D. P., Johannes, K., Schaffer, S. & Shaw, A. J. 2000. Phylogenetic relationships within the haplolepidous mosses. *Bryologist* 103: 257–276.
- , Shaw, A. J. & Vitt, D. H. 2002. The circumscription of the Dicranaceae (Bryopsida) based on the chloroplast regions *trnL-trnF* and *rps4*. *Syst. Bot.* 27: 435–452.
- Limpricht, K. G. 1885–1889. Die Laubmoose Deutschlands, Österreichs und der Schweiz 1. 836 pp. Eduard Kummer, Leipzig.
- Longton, R. E. 1988. Life-history strategies among bryophytes of arid regions. *J. Hattori Bot. Lab.* 64: 15–28.
- Magombo, Z. L. K. 2003. The phylogeny of basal peristomate mosses: evidence from cpDNA, and implications for peristome evolution. *Syst. Bot.* 28: 24–38.
- Magill, R. E. 1981. Flora of Southern Africa, Bryophyta I, Mosses, Fascicle I, Sphagnaceae–Grimmiaceae. xv + 291 pp. Botanical Research Institute, Department of Agriculture and Fisheries, Pretoria.
- Masuzaki, H., Shimamura, M., Furuki, T., Tsubota, H., Yamaguchi, T., Haji Mohamed, A. M. & Deguchi, H. 2010. Systematic position of the enigmatic liverwort *Mizutania* (Mizutaniaceae, Marchantiophyta) inferred from molecular phylogenetic analyses. *Taxon* 59: 448–458.
- Miyazawa, S. 2011. Selective constraints on amino acids estimated by a mechanistic codon substitution model with multiple nucleotide changes. *PLoS One* 10: 1371.
- Nadot, S., Bajon, R. & Lejeune, B. 1994. The chloroplast gene *rps4* as a tool for the study of Poaceae phylogeny. *Pl. Syst. Evol.* 191: 27–38.
- Nixon, K. C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414.
- Palisot de Beauvois, A. M. F. J. 1804. Prodrome de l'aéthéogramie ou d'un traité sur les familles de plantes dont la fructification est extraordinaire. *Mag. Encycl.* 9 (5): 289–330.
- Podpěra, J. 1954. *Conspectus Muscorum Europaeorum*. 697 pp. Československé akademie věd, Praha.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61: 539–542.
- Rose, J. P., Kriebel, R. & Sytsma, K. J. 2016. Shape analysis of moss (Bryophyta) sporophytes: insights into land plant evolution. *Am. J. Bot.* 103: 652–662.
- Saito, K. 1975. A monograph of Japanese Pottiaceae (Musci). *J. Hattori Bot. Lab.* 39: 373–537.
- Sato, H., Tsubota, H., Yamaguchi, T. & Deguchi, H. 2004. Phylogenetic and morphological notes on *Uleobryum naganoi* Kiguchi et al. (Pottiaceae, Musci). *Hikobia* 14: 143–147.
- Schimper, W. P. 1856. *Corollarium Bryologiae Europaeae*. 140 pp. E. Schweizerbart, Stuttgart.
- Schwarz, G. 1978. Estimating the dimension of a model. *Ann. Stat.* 6: 461–464.

- Seo, T.-K. & Kishino, H. 2008. Synonymous substitutions substantially improve evolutionary inference from highly diverged proteins. *Syst. Biol.* 57: 367–377.
- & ———. 2009. Statistical comparison of nucleotide, amino acid, and codon substitution models for evolutionary analysis of protein-coding sequences. *Syst. Biol.* 58: 199–210.
- Shimodaira, H. & Hasegawa, M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17: 1246–1247.
- . 2002. An approximately unbiased test of phylogenetic tree selection. *Syst. Biol.* 51: 492–508.
- . 2004. Approximately unbiased tests of regions using multistep-multiscale bootstrap resampling. *Ann. Stat.* 32: 2616–2641.
- Sikes, D. S. & Lewis, P. O. 2001. Software manual for PAUPRat: a tool to implement Parsimony Ratchet searches using PAUP*. 12 pp. University of Connecticut, Storrs.
- Souza-Chies, T. T., Bittar, G., Nadot, S., Carter, L., Besin, E. & Lejeune, B. 1997. Phylogenetic analysis of Iridaceae with parsimony and distance methods using the plastid gene *rps4*. *Pl. Syst. Evol.* 204: 109–123.
- Spagnuolo, V., Caputo, P., Cozzolino, S., Castaldo, R. & De Luca, P. 1996. ITS1 sequence in some Pottiaceae (Pottiales, Musci). *Giorn. Bot. Ital.* 130: 373.
- , ———, ———, ——— & ———. 1997. Length polymorphism in the intragenic spacer 1 of the nuclear ribosomal DNA of some Pottiaceae (Pottiales, Musci). *Cryptog. Bryol. Lichenol.* 18: 55–61.
- , ———, ———, ——— & ———. 1999. Patterns of relationships in Trichostomoideae (Pottiaceae, Musci). *Pl. Syst. Evol.* 216: 69–79.
- Stamatakis, A. 2014. RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Stark, L. R. 2002. Skipped reproductive cycles and extensive sporophyte abortion in the desert moss *Tortula inermis* correspond to unusual rainfall patterns. *Can. J. Bot.* 80: 533–542.
- , Oliver, M. J., Mishler, B. D. & Mclethie, D. N. 2007. Generational differences in response to desiccation stress in the desert moss *Tortula inermis*. *Am. J. Bot.* 99: 53–60.
- , Nicholas M. D. & Roberts, S. P. 2009. Gender differences and a new adult eukaryotic record for upper thermal tolerance in the desert moss *Syntrichia caninervis*. *J. Therm. Biol.* 34: 131–137.
- , Greenwood, J. L. & Brinda, J. C. 2016. Desiccated *Syntrichia ruralis* shoots regenerate after 20 years in the herbarium. *J. Bryol.*, Doi: 10.1080/03736687.2016.1176307, 30 June 2016.
- Stech, M. & Frey, W. 2008. A morpho-molecular classification of the mosses (Bryophyta). *Nova Hedwigia* 86: 1–21.
- , McDaniel, S. F., Hernández-Maqueda, R., Ros, R. M., Werner, O., Muñoz, J. & Quandt, D. 2012. Phylogeny of haplolepidous mosses—challenges and perspectives. *J. Bryol.* 34: 173–186.
- Sugiura, N. 1978. Further analysis of the data by Akaike's information criterion and the finite corrections. *Commun. Stat. Theory Methods* 7: 13–26.
- Suzuki, T., Inoue, Y., Tsubota, H. & Iwatsuki, Z. 2013. Notes on *Aptychella* (Sematophyllaceae, Bryopsida): *Yakushimabryum longissimum*, syn. nov. *Hattoria* 4: 107–118.
- Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0 beta version. 142 + ii pp. Sinauer Associates, Massachusetts.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28: 2731–2739.
- Tanabe, A. S. 2011. Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Mol. Ecol. Resour.* 11: 914–921.
- Tsubota, H., Nakao, N., Arikawa, T., Yamaguchi, T., Higuchi, M., Deguchi, H. & Seki, T. 1999. A preliminary phylogeny of Hypnales (Musci) as inferred from chloroplast *rbcL* sequence data. *Hikobia* 7: 233–248.
- , ———, Yamaguchi, T., Seki, T. & Deguchi, H. 2000. Preliminary phylogenetic relationships of the genus *Brotherella* and its allied genera (Hypnales, Musci) based on chloroplast *rbcL* sequence data. *J. Hattori Bot. Lab.* 88: 77–99.
- , Akiyama, H., Yamaguchi, T. & Deguchi, H. 2001. Molecular phylogeny of the genus *Trismegistia* and related genera (Sematophyllaceae, Musci) based on chloroplast *rbcL* sequences. *Hikobia* 13: 529–549.
- , De Luna, E., González, D., Ignatov, M. S. & Deguchi, H. 2004. Molecular phylogenetics and

- ordinal relationships based on analyses of a large-scale data set of 600 *rbcL* sequences of mosses. *Hikobia* 14: 149–169.
- Wahrmund, U., Rein, T., Müller, K. F., Groth-Maloney, M. & Knoop, V. 2009. Fifty mosses on five trees: comparing phylogenetic information in three types of non-coding mitochondrial DNA and two chloroplast loci. *Pl. Syst. Evol.* 282: 241–255.
- , Quandt, D. & Knoop, V. 2010. The phylogeny of mosses—addressing open issues with a new mitochondrial locus: group 1 intron *cob1420*. *Mol. Phylogenet. Evol.* 54: 417–426.
- Walther, K. 1983. Brophytina. Laubmoose. In Gerloff, J. & Poelt, A. (eds.), *A. Engler's Syllabus der Pflanzenfamilien : mit besonderer Berücksichtigung der Nutzpflanzen nebst einer Übersicht über die Florenreiche und Florengebiete der Erde, Kapitel V, 2*, pp. 1–108. Gebrüder Borntraeger, Berlin.
- Werner, O., Ros, R. M., Cano, M. J. & Guerra, J. 2002. *Tortula* and some related genera (Pottiaceae, Musci): phylogenetic relationships based on chloroplast *rps4* sequences. *Pl. Syst. Evol.* 235: 197–207.
- , ———, ——— & ———. 2003. On the systematic position of *Tortula inermis* and *Tortula bolanderi* (Pottiaceae, Musci) based on chloroplast *rps4* sequences. *Nova Hedwigia* 76: 137–145.
- , Ros, R. M., Cano, M. J. & Guerra, J. 2004a. Molecular phylogeny of Pottiaceae (Musci) based on chloroplast *rps4* sequence data. *Pl. Syst. Evol.* 243: 147–164.
- , Jiménez, J. A. & Ros, R. M. 2004b. The systematic position of the moss *Kingiobryum paramicola* (Pottiaceae) based on molecular and morphological data. *Bryologist* 107: 215–221.
- , Ros, R. M., & Grundmann, M. 2005. Molecular phylogeny of Trichostomoideae (Pottiaceae, Bryophyta) based on nrITS sequence data. *Taxon* 54: 361–368.
- , Ros, R. M. & Goffinet, B. 2007. A reconsideration of the systematic position of *Goniomitrium* (Funariaceae) based on chloroplast sequence markers. *Bryologist* 110: 108–114.
- Zander, R. H. 1993. Genera of the Pottiaceae: mosses of harsh environments. *Bull. Buffalo Soc. Nat. Sci.* 32: 1–378.
- . 2006. The Pottiaceae *s. str.* as an evolutionary Lazarus taxon. *J. Hattori Bot. Lab.* 100: 581–602.
- Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. x + 115 pp. Ph.D. dissertation, The University of Texas at Austin, Austin.

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Appendix

List of species investigated for *rbcL* and *rps4* gene sequences with the voucher information and the accession number. Bold accession numbers indicate newly obtained sequences for the present study.

Ingroup species: *Ardeuma aurantiacum* (Mitt.) R.H.Zander & Hedd., **LC176249/LC176270**, Japan, Yamaguchi Pref., *Y. Inoue 4007* (HIRO); *Ardeuma recurvirostrum* (Hedw.) R.H.Zander & Hedd., **LC176251/LC176272**, Japan, Nagano Pref., *Y. Inoue 1323* (HIRO); *Barbula unguiculata* Hedw., AB670696/**LC176265**, Japan, Hiroshima Pref., *Y. Inoue 113* (HIRO); *Chionoloma angustata* (Mitt.) M.Menzel, **LC176254/LC176276**, Japan, Miyazaki Pref., *Y. Inoue 3238* (HIRO); *Chionoloma tenuirostre* (Hook. & Taylor) M.Alonso, M.J.Cano & J.A.Jiménez, **LC176252/LC176274**, Japan, Hiroshima Pref., *Y. Inoue 3218* (HIRO); *Didymodon constrictus* (Mitt.) K.Saito var. *flexicuspis* (P.C.Chen) K.Saito, **LC176245/LC176266**, Japan, Nagano Pref., *Y. Inoue 4040* (HIRO); *Ephemerum crassinervium* (Schwägr.) Hampe, **LC176246/LC176267**, Japan, Tochigi Pref., *T. Kamiyama 8980* (HIRO); *Eucladium verticillatum* (With.) Bruch & Schimp., **LC176247/LC176268**, Japan, Kanagawa Pref., *Y. Inoue 1803* (HIRO); *Gymnostomiella longinervis* Broth., **LC176248/LC176269**, Japan, Okinawa Pref., *Y. Inoue 3902* (HIRO); *Hydrogonium hirosii* (K.Saito) Jan Kučera, **LC176250/LC176271**, Japan, Shizuoka Pref., *T. Suzuki 61397* (HIRO); *Hyophila propagulifera* Broth., AB853074/AB853084, Japan, Hiroshima Pref., *Y. Inoue 1745* (HIRO); *Leptophascum leptophyllum* (Müll.Hal.) J.Guerra & M.J.Cano, AB670695/**LC176273**, Japan, Ehime Pref., *Y. Inoue 57* (HIRO); *Pachyneuropsis miyagii* T.Yamag., AB853078/AB759969, Japan, Okinawa Pref., *T. Yamaguchi 34243* (HIRO); *Pottiopsis caespitosa* (Bruch ex Brid.) Blockeel & A.J.E.Sm., **LC176253/LC176275**, Czech Republic, S Moravia, CBFS 14602 (duplicate in HIRO); *Scopelophila cataractae* (Mitt.) Broth., AB853075/AB853087, Japan, Kochi Pref., *Y. Inoue 318* (HIRO); *Stegonia latifolia* (Schwägr.) Venturi ex Broth., AF231314/AF222901, Canada, Alberta, *La Farge s.n.* (ALTA); *Streblotrichum convolutum* (Hedw.) P.Beauv., **LC176255/LC176277**, Japan, Hiroshima Pref., *H. Tsubota 7997* (HIRO); *Streptopogon calymperes* Müll.Hal., AF478231/

- AF478285, Bolivia, La Paz, Z. L. K. Magombo 5695 (MO); *Syntrichia ruralis* (Hedw.) F.Weber & D.Mohr, FJ546412/FJ546412, Canada, Alberta (CAVA); *Tortella tortuosa* (Schrad. ex Hedw.) Limpr., AB853080/AB853089, Japan, Nagano Pref., Y. Inoue 1297 (HIRO); *Tortula caucasica* Lindb., AB670694/AB759970, Japan, Ehime Pref., Y. Inoue 56 (HIRO); *Trichostomum brachydontium* Bruch, **LC176256/LC176278**, Spain, Murcia, CBFS13652 (duplicate in HIRO); *Trichostomum crispulum* Bruch, **LC176257/LC176279**, Spain, Asturias, MUB 45068 (duplicate in HIRO); *Trichostomum platyphyllum* (Broth. ex Iisiba) P.C.Chen, **LC176258/LC176280**, Japan, Okinawa Pref., Y. Inoue 3869 (HIRO); *Tuerckheimia svihlae* (E.B.Bartram) R.H.Zander, **LC176259/LC176281**, Japan, Fukuoka Pref., T. Suzuki 61444 (HIRO); *Uleobryum naganoi* Kiguchi, I.G.Stone & Z.Iwats., AB194717/**LC176282**, Japan, Kagawa Pref., H. Sato 377 (HIRO); *Weisiopsis anomala* (Broth. & Paris) Broth. & Paris, **LC176260/LC176283**, Japan, Tokyo Pref., Y. Inoue 2812 (HIRO); *Weissia controversa* Hedw., **LC176261/LC176284**, Japan, Hiroshima Pref., Y. Inoue 2568 (HIRO); *Weissia exserta* (Broth.) P.C.Chen, **LC176262/LC176285**, Japan, Hiroshima Pref., Y. Inoue 794 (HIRO). Outgroup species: *Ditrichum heteromallum* (Hedw.) E.Britton, **LC176243/LC176263**, Japan, Niigata Pref., H. Sato 284 (HIRO); *Pseudephemerum nitidum* (Hedw.) Loeske, **LC176244/LC176264**, Japan, Hiroshima Pref., H. Sato 820 (HIRO).

