

Molecular phylogeny of Pottiaceae (Musci) based on chloroplast *rps4* sequence data

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Abstract. Comparative sequencing of the chloroplast *rps4* gene was used to reconstruct the phylogenetic relationships within the family Pottiaceae (Musci). The results confirm that *Ephemerum spinulosum*, *Splachnobryum obtusum*, *Goniomitrium acuminatum* and *Cinclidotus fontinaloides* are clearly positioned within the Pottiaceae and that *Hypodontium dregei* is not a member. At subfamily level, the data support the subfamily Pottioideae as being a monophyletic clade. The Trichostomoideae are probably paraphyletic. Neither the subfamily Chionolomideae, represented in this study by *Pseudosymblypharis schimperiana*, nor the subfamily Erythrophylllosoideae, represented by both known species, *Erythrophyllastrum andinum* and *Erythrophyllopsis fuscula*, are supported by the sequence data. The Timmielloideae should be excluded from the Pottiaceae. The Merceyoideae, represented in this study by *Scopelophila cataractae*, might form a sister clade to all other Pottiaceae, but their position is not fully resolved. At the genus level, *Barbula* is clearly polyphyletic since *Barbula bolleana* and *Barbula indica* appear in a clade clearly separated from *Barbula unguiculata*. *Pottiopsis caespitosa* and *Leptobarbula berica* are placed within the Trichostomoideae. Likewise, the genera *Gymnostomum* and *Anoetangium* are excluded from the Pottioideae and placed within the Trichostomoideae. *Leptopascum leptophyllum* is closely related with *Syntrichia*; *Aloina* is not closely

related to *Tortula* or *Crossidium*. Evidence of a clade within the Pottioideae, formed of *Leptodontium* and *Triquetrella*, is provided.

Key words: Musci, Pottiaceae, *rps4*, molecular phylogeny.

The Pottiaceae form the most numerous moss family known, containing nearly 1 500 (Zander 1993) or more than 10% of the 10 000 to 15 000 moss species known (Buck and Goffinet 2000). Many of them are especially adapted to dry climates and they are often the dominant mosses in arid regions of the world. However, taxonomic treatment of the family has been notoriously difficult due to problems of polymorphy, the unclear significance of several anatomical characters, the reduced size of many species, obscure areolation and the sterility of many specimens (Zander 1993). In many cases there is no general agreement on the relative importance of the characters.

Modern treatment of this family started with the work of Chen (1941), who recognised six subfamilies (Eucladioideae, Trichostomoideae, Barbuloideae, Pottioideae, Leptodontioideae and Cinclidotoideae) (Table 1). These were distinguished by characters such as capsule

Table 1. Modern treatments of the family Pottiaceae

Chen (1941)	Saito (1975)	Corley et al. (1981)	Zander (1993)	Werner et al.
Pottiaceae	Pottiaceae	Pottiaceae	Pottiaceae	Pottiaceae
Eucladioideae	Trichostomoideae	Trichostomoideae	Timmielloideae	Merceoideae
Pleuroweisieae	Pottioideae	Pottioideae	Erythrophyllopsoideae	Trichostomoideae
Eucladiaceae	Pleuroweisieae	Pottiae	Gertrudielloideae	Pottioideae
Trichostomoideae	Leptodontiaceae	Merceyeae	Chionolomoideae	Gertrudielloideae?
Tortelleae	Barbuleae	Barbuleae	Trichostomoideae	
Trichostomeae	Pottiae	Pleuroweisieae	Merceoideae	
Barbuloideae		Cinclidotioideae	Tetracoscinodontiaceae	
Hyophileae			Bryoerythrophyllaeae	
Barbuleae			Leptodontiaceae	
Pottioideae			Barbuleae	
Merceyeae			Pottioideae	
Pottiae			Hyophileae	
Leptodontioideae			Pottiae	
Cinclidotioideae				
	Cinclidotaceae		Cinclidotaceae	

and costa anatomy, leaf shape and margin curvature, length of the operculum relative to that of the theca, differentiation of the leaf base and morphology of the laminal papillae.

Saito (1975) provided an extensive discussion on the generic and suprageneric classification of the Pottiaceae. He considered the curvature of the leaf margins, the shape of the area of differentiated basal cells, the occurrence of gemmae, axillary hairs, and sporophyte characters to be the most conservative characters. He observed a reduction series in some genera, leading to simpler sporophytes, and a complication series of certain sporophyte characters (e.g. variations in the peristome) in other genera. He accepted only two subfamilies, Trichostomoideae and Pottioideae, and excluded Cinclidotaceae from the Pottiaceae (Table 1). Trichostomoideae were distinguished from Pottioideae by the presence of the 5-layered amphitecial derivatives of the capsule, a characteristic associated with an incurved upper leaf margin and differentiation of a V-shaped area of hyaline basal cells in the leaf. The taxa in Trichostomoideae are quite uniform as regards most gametophytic characters, and the subfamily is considered to be derived from the ancestral stock of the Pottiaceae because of the increase of the number of amphitecial derivatives in the capsule. In contrast, the Pottioideae differ from the Trichostomoideae in their 4-layered amphitecial derivatives in the capsule, the recurved basal leaf-margin and the differentiation of reverse a V-shaped area of hyaline basal leaf cells.

Corley et al. (1981), in their checklist of the mosses of Europe and the Azores, made a similar classification to that of Saito (1975). They recognised the subfamilies Trichostomoideae and Pottioideae but considered the subfamily Cinclidotioideae instead of segregating the genus *Cinclidotus* P. Beauv. into an independent family (Table 1).

Finally, Zander (1993) proposed a major revision of the family. He identified seven subfamilies (Timmielloideae, Erythrophyllopsoideae, Gertrudielloideae, Chionolomoideae,

Trichostomoideae, Merceyoideae, and Pottioideae) (Table 1). For the separation of the subfamilies this author mainly considered gametophyte characters, the most important of which were whether the sclerodermis and hyalodermis are differentiated in the stem, the leaf shape, margin, apex and base, whether the upper lamina is bistratose or unistratose, the strength of the KOH color reaction in the upper lamina, the differing structure of the leaf costa in cross section, whether the upper laminal cells are ventrally bulging or vertically aligned, and the presence and shape of the propagules. The genera *Splachnobryum* Müll. Hal. and *Cinclidotus* were excluded from the Pottiaceae.

However, the treatment of Zander (1993) is not universally accepted and many questions concerning all taxonomical levels remain to be satisfactorily resolved. For this reason, a molecular approach with an independent set of data was explored to shed some additional light on the phylogenetic relationships within this family. An earlier publication of Spagnuolo et al. (1999) based on ITS1 sequences with a reduced number of taxa of the Trichostomoideae and some Pottioideae pointed to the usefulness of DNA sequences in attempting to clarify the phylogenetic relationships within this family. Other authors were not able to align ITS sequences in Pottiaceae (Colacino and Mishler 1996). On the contrary, chloroplast *rps4* sequences, alone or in combination with others, have been used successfully to resolve the phylogenetic relationships at the family or order level within mosses (i.e. Buck et al. 2000, Hedderson et al. 1999, Pedersen and Hedenäs 2002, La Farge et al. 2002). Based on chloroplast *rps4* sequences, evidence was found supporting the idea that *Syntrichia* Brid. and *Tortula* Hedw. are indeed independent genera within the Pottioideae (Werner et al. 2002) and the taxonomic position of *Tortula inermis* (Brid.) Mont. and *Tortula bolanderi* (Lesq. & James) M. Howe could be clarified (Werner et al. 2003). La Farge et al. (2002) showed that *Kingiobryum paramicola* H. Rob. occupies a position very near to the two

Pottiaceae included in their study and that it probably belongs to this family. We therefore used chloroplast *rps4* sequences to study the phylogenetic relationships within the Pottiaceae and the monophyly of the taxa included traditionally in this family.

Material and methods

Plant material. 73 specimens representing 72 taxa were included (20 of them as possible outgroup species), based on the results of Cox and Hedderson (1999), Goffinet and Cox (2000) and La Farge et al. (2002). In the case of six of the included species there is no general agreement on whether or not they belong to the Pottiaceae (*Timmiella crassinervis* (Hampe) L.F. Koch, *Hypodontium dregei* (Hornsch.) Müll. Hal., *Goniomitrium acuminatum* Hook & Wilson, *Ephemerum spinulosum* Schimp., *Splachnobryum obtusum* (Brid.) Müll. Hall., *Cinclidotus fontinaloides* (Hedw.) P. Beauv., *Kingiobryum paramicola*). The supposed ingroup species represent all the subfamilies recognised by Zander (1993) with the exception of the Gertrudielloideae. The only known species of this subfamily is very rare and restricted to the Andes. We tried to borrow some recently collected herbaria specimens of this species from some of the more important U.S.A. herbaria but were unsuccessful. We also tried to represent taxa that are included in different subfamilies by the above mentioned authorities, as is the case with *Weissia* Hedw., *Didymodon* Hedw. and others. 27 sequences were downloaded from Genbank for this study, 15 were presented in a narrower-ranging study treating *Tortula* and allied genera (Werner et al. 2002) and 31 sequences are published here for the first time.

Table 2 lists all the taxa used in this study, the voucher specimen data or reference, the origin of the specimens and GenBank accession numbers. Most of the vouchers are deposited at MUB. The nomenclature follows that of Corley et al. (1981) in most cases.

DNA isolation. Total DNA was extracted from dry material using the DNeasy Plant Mini Kit of Qiagen (Hilden, Germany). The DNA was eluted in 100 µl of 10 mM Tris-buffer (pH 8.5) and stored in the freezer until amplification.

Amplification. The chloroplast *rps4* gene was amplified in 50 µl final volume with the primers *rps5* (Nadot et al. 1994) and *trnas* (Buck et al.

Table 2. Taxa included in the *rps4* sequence analysis. All sequences are submitted to GenBank

Species	Voucher or reference	Origin	GenBank
<i>Acaulon triquetrum</i> (Spruce) Müll. Hal.	MUB 6613	Spain, Almería	AF480969
<i>Aloina brevirostris</i> (Hook. & Grev.) Kindb.	MUB 9705	Spain, Murcia	AF480956
<i>Anoetangium aestivum</i> (Hedw.) Mitt.	MUB 10482	Morocco, Rif	AF480963
<i>Aschisma carniolicum</i> (F. Weber & D. Mohr) Lindb.	MUB 7932	Spain, Huelva	AF480962
<i>Barbula bolleana</i> (Müll. Hal.) Broth.	MUB 11932	Spain, Murcia	AF481033
<i>Barbula indica</i> (Hook.) Spreng	MUB 12234	India, Uttar Pradesh	AF481034
<i>Barbula unguiculata</i> Hedw.	MUB 10325	Germany, Baden-Württemberg	AF480952
<i>Bartramia stricta</i> Brid.	Genbank	–	AF023799
<i>Blindia acuta</i> (Hedw.) Bruch & Schimp.	Hedderson et al. (1999)	–	AF023781
<i>Bryoerythrophyllum recurvirostrum</i> (Hedw.) P.C. Chen	MUB 10529	Morocco, Rif	AF480992
<i>Campylopus atrovirens</i> De Not.	La Farge et al. (2002)	–	AF231270
<i>Ceratodon purpureus</i> (Hedw.) Brid.	La Farge et al. (2002)	–	AF435271
<i>Cinclidotus fontinaloides</i> (Hedw.) P. Beauv.	MUB 6427	Spain, Navarra	AF480975
<i>Crossidium aberrans</i> Holz. & E.B. Bartram	MUB 6089	Spain, Albacete	AF481006
<i>Desmatodon latifolius</i> (Hedw.) Brid.	MUB 11476	Spain, Granada	AF480954
<i>Dialytrichia mucronata</i> (Brid.) Broth.	MUB 11162	Spain, Cádiz	AF480953
<i>Dichodontium pellucidum</i> (Hedw.) Schimp.	La Farge et al. (2002)	–	AF435274
<i>Dicranella heteromalla</i> (Hedw.) Schimp.	La Farge et al. (2002)	–	AF231272
<i>Dicranum scoparium</i> Hedw.	Genbank	–	AF231277
<i>Didymodon luridus</i> Hornsch. ex Spreng.	MUB 8475	Spain, Huesca	AF480951
<i>Distichium capillaceum</i> (Hedw.) Bruch & Schimp.	La Farge et al. (2002)	–	AF435283
<i>Ditrichum flexicaule</i> (Schwägr.) Hampe	La Farge et al. (2002)	–	AF231278
<i>Encalypta rhaptocarpa</i> Schwägr.	Genbank	–	AF023777
<i>Ephemerum spinulosum</i> Schimp.	Goffinet & Cox (2000)	–	AF223055
<i>Erythrophyllastrum andinum</i> (Sull.) R.H. Zander	NY	Bolivia, La Paz	AY121424
<i>Erythrophyllopsis fuscula</i> (Müll. Hal.) Hilp.	NY	Bolivia, Cochabamba	AY121425
<i>Eucladium verticillatum</i> (Brid.) Bruch & Schimp.	MUB 11056	Spain, Guipúzcoa	AF481044
<i>Fissidens subbasilaris</i> Hedw.	Goffinet & Cox (2000)	–	AF233056
<i>Funaria hygrometrica</i> Hedw.	Genbank	–	AF023776
<i>Geheebia gigantea</i> (V.A. Funck) Boulay	MUB 6191	Germany, Bayern	AF480991
<i>Goniomitrium acuminatum</i> Hook & Wilson	Goffinet & Cox (2000)	–	AF223057
<i>Grimmia pulvinata</i> (Hedw.) Sm.	Genbank	–	AF222900
<i>Gymnostomiella monodii</i> P. de la Varde	MUB 11478	Mauritania, Adrar	AF480955
<i>Gymnostomum viridulum</i> Brid.	MUB 10694	Morocco, Rif	AF480968
<i>Hymenostylium recurvirostrum</i> (Hedw.) Dixon	MUB 5394	Spain, Albacete	AF480967
<i>Hyophila involuta</i> (Hook.) A. Jaeger	MUB 12223	India, Rajasthan	AF480948
<i>Hypodontium dregei</i> (Hornsch.) Müll. Hal.	Wheeler et al. unpublished	–	AF226755
<i>Kingiobryum paramicola</i> H. Rob.	La Farge et al. (2002)	Colombia	AF435290

Table 2 (continued)

<i>Leptobarbula berica</i> (De Not.) Schimp.	MUB 4219	Spain, Alicante	AF480964
<i>Leptodontium flexifolium</i> (Dicks. ex With) Hampe in Lindb.	MUB 2563	Spain, Orense	AF480973
<i>Leptophascum leptophyllum</i> (Müll. Hal.) J. Guerra & M.J. Cano	MUB 10427	Spain, Murcia	AF480960
<i>Mesotus celatus</i> Mitt.	La Farge et al. (2002)	–	AF435270
<i>Oreoweisia erosa</i> (Hampe ex Müll. Hal.) Kindb.	MUB 12219	South Africa, Western Cape Province	AF480985
<i>Oxystegus sinuosus</i> (Mitt.) Hilp.	MUB 5753	Spain, Alicante	AF480966
<i>Phascum curvicolle</i> Hedw.	MUB 8269	Spain, Almería	AF480971
<i>Phascum cuspidatum</i> Hedw.	MUB 11344	Spain, Seville	AF480972
<i>Pleuridium acuminatum</i> Lindb.	La Farge et al. (2002)	–	AF231289
<i>Pleurochaete squarrosa</i> (Brid.) Lindb.	MUB 11686	Spain, Seville	AF480965
<i>Pottia bryoides</i> (Dicks.) Mitt.	MUB 4936	Spain, Alicante	AF480979
<i>Pottia davalliana</i> (Sm.) C.E.O. Jensen	MUB 12537	Spain, Alicante	AF480983
<i>Pottia lanceolata</i> (Hedw.) Müll. Hal.	MUB 10334	Spain, Murcia	AF480988
<i>Pottiopsis caespitosa</i> (Bruch) Blockeel & A.J.E. Smith	MUB 11878	Spain, Murcia	AF480961
<i>Pseudocrossidium hornschurchianum</i> (Schultz) R.H. Zander	MUB 9053	Spain, Almería	AF480979
<i>Pseudocrossidium crinitum</i> (Schultz) R.H. Zander	MUB 12233	South Africa, Western Cape Province	AF480978
<i>Pseudosymblepharis schimperiana</i> (Paris) H.A. Crum	La Farge et al. (2000)	–	AF226756
<i>Pterygoneurum lamellatum</i> (Lindb.) Jur.	MUB 11484	Spain, Murcia	AF480959
<i>Ptychomitrium gardneri</i> Lesq.	Hedderson et al. (1999)	–	AF023831
<i>Scopelophila cataractae</i> (Mitt.) Broth.	MUB 11941	Spain, Seville	AF480974
<i>Splachnobryum obtusum</i> (Brid.) Müll. Hal. 1	Goffinet & Cox (2000)	–	AF223058
<i>Splachnobryum obtusum</i> (Brid.) Müll. Hal. 2	La Farge et al. (2000)	–	AF222901
<i>Stegonia latifolia</i> (Schwägr.) Venturi ex Broth.	La Farge et al. (2000)	–	AF222901
<i>Syntrichia caninervis</i> Mitt.	MUB 10977	Spain, Guadalajara	AF480958
<i>Syntrichia ruralis</i> (Hedw.) Brid.	MUB 10887	Morocco, Rif	AF480980
<i>Tetrapterum tetragonum</i> (Hook.) A.L. Andrews	MUB 12229	South Africa, Western Cape Province	AF480980
<i>Timmiella crassinervis</i> (Hampe) L.F. Koch	La Farge et al. (2000)	–	AF226766
<i>Tortella flavovirens</i> (Bruch) Broth.	MUB 11940	Greece, Pelopónissos	AF481043
<i>Tortula muralis</i> Hedw.	MUB 12540	Spain, Canary Islands, La Palma	AF481016
<i>Trichodontium falcatum</i> (R. Br. bis.) Fife	La Farge et al. (2002)	–	AF435304
<i>Trichostomopsis australasiae</i> (Hook. & Grev.) H. Rob.	MUB 9453	Spain, Almería	AF480950
<i>Trichostomum crispulum</i> Bruch	MUB 9709	Spain, Murcia	AF480977
<i>Triquetrella tristicha</i> (Müll. Hal.) Müll. Hal.	MUB 12218	South Africa, Western Cape Province	AF480949
<i>Wardia hygrometrica</i> Harv. & Hook.	Hedderson et al. (1999)	–	AF023782
<i>Weissia controversa</i> Hedw.	MUB 11704	Spain, Seville	AF480976

2000). 1 µl of stock DNA was added as template. The amplification conditions were as follows: 3 min at 94 °C, 35 cycles of 15 sec at 94 °C, 30 sec at 50 °C and 1 min at 72 °C, and a final 7-min extension step at 72 °C. Amplification products were controlled on 8% PAA gels and successful reactions were cleaned with the help of the High Pure PCR Product Purification Kit of Roche Molecular Biochemicals (Mannheim, Germany).

Sequencing and data analysis. Cycle sequencing was performed with the Big Dyes Sequencing Kit (Perkin Elmer) using a standard protocol and the amplification primers. The annealing temperatures were set to 60 °C in the case of *rps5* and 55 °C in the case of *trnas*. The reaction products were separated on an ABI Prism 3700 automatic sequencer (Perkin Elmer). The sequences were edited using Bioedit 5.0.9 (Hall 1999) and aligned manually. The aligned sequences were analysed using Neighbor-Joining (NJ; Saitou and Nei 1987), Maximum Parsimony (MP; Fitch 1971) and Bayesian inference (Bayes 1763). In the case of NJ, we used PAUP* (Swofford 1998), with distance = hky85, objective = LSFIT, Power = 2. A bootstrap analysis with 1000 replicates was run. The MP analysis, run with PAUP*, used the following settings: RANDOM additions, TBR branch-swapping, MULTREES = yes, steepest descent = no, COLLAPSE = yes. Parsimony analyses were not expected to swap to completion (see Soltis et al. 1998). Maxtrees was set to 100000. All characters were equally weighted and unordered. A bootstrap analysis with 200 replicates was performed with the settings as mentioned above, but with MAXTREES set to 10000. More replicates would improve precision estimates but not accuracy (Hillis and Bull 1993).

The Bayesian approach (Rannala and Yang 1996, Mau and Newton 1997, Mau et al. 1999) is similar to Maximum Likelihood in that the user postulates a model of evolution and the program searches for the best trees that are consistent with both the model and with the data. However it differs from ML in that, while ML seeks the tree that maximises the probability of observing data given that tree, Bayesian analysis seeks the tree that maximises the probability of the tree given the data and the model for evolution. In essence, this rescales likelihoods to true probabilities in that the sum of the probabilities over all trees is 1.0 under the Bayesian approach, which makes it possible to

analyse the data by ordinary probability theory (Hall 2001).

The program MrBayes (Huelsenbeck and Ronquist 2001) was used to estimate the phylogenetic relationships under the Bayesian approach. Three charsets were defined representing the codon position of the coding region. A partition (bycodon) in these three charsets was defined. The following settings were used: lset = nst6, rates = sitespec, site-partition = bycodon. The Markov chain settings were: ngen = 400000, printfreq = 1000, samplefreq = 100, nchains = 4. The first 100000 generations were discarded from the further analysis (burnin = 1000) and contype = halfcompat, which is the equivalent of 50% majority rule in PAUP*. The clade credibility values, as calculated by MrBayes, are given in the cladograms.

Trees were edited using the program Treeview 1.6.6. (Page 1996). This program reads NEXUS tree files such as those produced by PAUP* or MrBayes, and permits easy manipulation of the trees, including rooting with variable outgroups defined by the user. All trees were rooted with *Funaria hygrometrica* Hedw. as outgroup taxon.

Hillis and Bull (1993) and Suzuki et al. (2002) showed that bootstrap analysis are generally conservative, while Suzuki et al. (2002) found that the posterior probabilities in Bayesian analysis can be excessively liberal. For the purpose of our discussion, robust bootstrap support is presented by values $\geq 70\%$, moderate support is $< 70\%$ and $\geq 50\%$ and poor support is below 50%. In the case of Bayesian clade credibility values, good support was estimated as $\geq 90\%$ clade credibility value and poor support below 70% clade credibility value.

Results

***rps4* sequence characteristics.** The *rps4* sequences of 72 taxa were available for this study. After the exclusion of primers and spacers, the aligned sequences had a length of 588 bp corresponding to the coding region. No indels were observed in the coding region, with the exception of *Funaria hygrometrica* that lacks a single codon triplet. 308 characters of the alignment were constant, 113 were variable but parsimony-uninformative and 167 were parsimony-informative.

Phylogenetic analysis (Figs. 1, 2 and 3). All the analyses (MP, NJ and Bayesian inference) coincided in that *Hypodontium dregei* does not belong to the Pottiaceae. All three types of analysis place it near *Fissidens* Hedw. (54% support with MP, 72% with NJ and 100% clade credibility using MrBayes).

Timmiella crassinervis, too, is placed outside the Pottiaceae by all three analysis methods, although in this case, its position cannot be clearly deduced from the analysis since the various methods applied led to different conclusions. La Farge et al. (2000) also present various trees, where the position of *Timmiella* (De Not.) Limpr. depends on the data set used for the phylogenetic analysis. In part, this problem may be due to long branch attraction (Hendy and Penny 1989).

The position of *Scopelophila cataractae* (Mitt.) Broth. is not clearly resolved. It is placed outside the Pottiaceae by the NJ method, although poorly supported by the bootstrap analysis, while the Bayesian analysis (100% clade credibility), and MP (67% bootstrap support) favour a position as sister clade to all other Pottiaceae, although such support is poor in the case of MP. The topology of all other supposed ingroup taxa did not depend on the choice of the outgroup taxon used for rooting.

The remaining species of the Pottiaceae form a clade with moderate to high support in all three analyses (67% MP, 70% NJ, 100% MrBayes). The Trichostomoideae as defined by Corley et al. (1981) and Saito (1975) are possibly a paraphyletic clade, but since the relationships within this group are not resolved, we think that the classifications proposed by these authors should generally be maintained (with some minor exceptions) until additional data are available (Figs. 1, 2 and 3).

Within the Trichostomoideae in our sense, a clade supported by all analysis types used comprises *Hyophila involuta* (Hook.) A. Jaeger, *Gymnostomiella monodii* P. de la Varde and *Splachnobryum obtusum* (bootstrap values: MP 56%, NJ 58% and clade credibility 100%

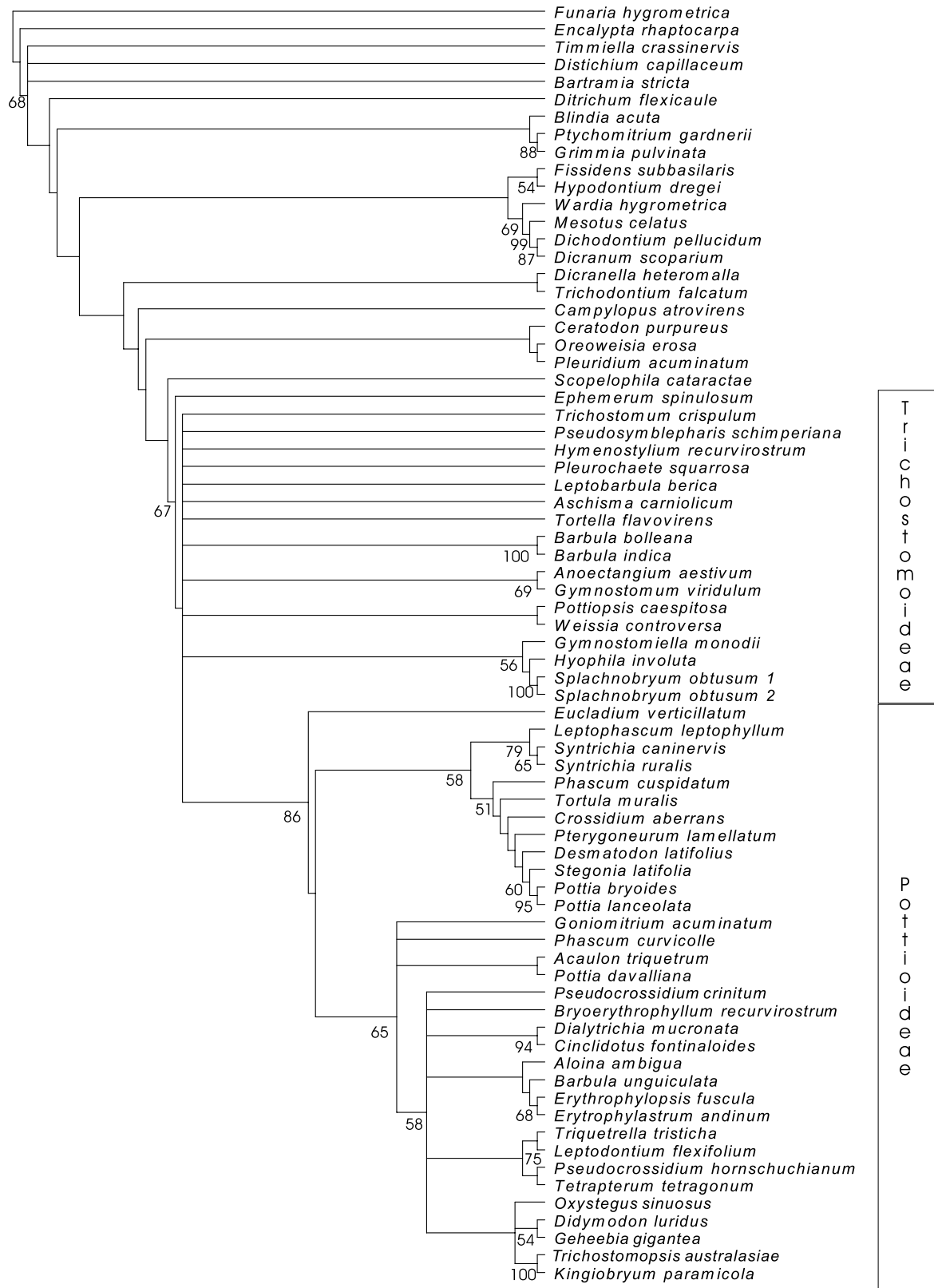
MrBayes). Other subclades with moderate to high support are formed of *Anoectangium aestivum* (Hedw.) Mitt. and *Gymnostomum viridulum* Brid. on the one hand (bootstrap values: MP 69%, NJ 75% and clade credibility 100% MrBayes), and *Barbula bolleana* (Müll. Hal.) Broth. and *B. indica* (Hook.) Spreng. on the other (bootstrap values: MP 100%, NJ 99% and clade credibility 100% MrBayes). The genus *Eucladium* Bruch & Schimp. is in an intermediate position between the Trichostomoideae and the Pottioideae. The position of *Hymenostylium recurvirostrum* (Hedw.) Dixon and *Leptobarbula berica* (De Not.) Schimp. are not resolved but a situation within the Pottioideae, as proposed by Corley et al. (1981) and Zander (1993) is not supported by the *rps4* sequence data. *Aschisma carniolicum* (F. Weber & D. Mohr) Lindb., *Pottiopsis caespitosa* (Brid.) Blockeel & A.J.E. Smith, *Pseudosymblypharis schimperiana* (Paris) H. A. Crum and *Weissia controversa* Hedw. are consistently placed within the Trichostomoideae in our sense.

The Pottioideae form a monophyletic group with moderate to good support. The clade receives a moderate bootstrap support of 68% in the NJ analysis (excluding *Eucladium*) and 86% with MP (including *Eucladium*), while a clade credibility value of 84% was assigned by MrBayes (excluding *Eucladium*).

Pottia lanceolata (Hedw.) Müll. Hal. and *Pottia bryoides* (Dicks.) Mitt. are separated from *Pottia davalliana* (Sm.) C.E.O. Jensen. Even more striking is the separation between *Barbula unguiculata* Hedw. and *Barbula bolleana*/*Barbula indica*. The two members of *Phascum* Hedw. are separated.

Aloina seems not to be closely related to *Tortula* or *Crossidium*, but the affinities of this genus are not well resolved.

Another interesting result is the high support for the clade formed by *Dialytrichia mucronata* (Brid.) Broth. and *Cinclidotus fontinaloides*, situated within the Pottioideae and closely related to each other, as shown by the high support of all three analyses (bootstrap



values: MP 94%, NJ 99% and clade credibility 100% MrBayes).

Erythrophyllastrum andinum (Sull.) R. H. Zander and *Erythrophyllopsis fuscula* (Müll. Hal.) Hilp. form a clade with 68% bootstrap support by MP, 82% by NJ and 98% by MrBayes and are placed within the Pottioideae near *Barbula unguiculata*, although the latter poorly supported.

All analyses recognise a clade formed by the genus *Tortula* and related genera in the sense of Zander (1993), including also *Leptophascum* (Müll. Hal.) J. Guerra & M. J. Cano, *Syntrichia*, *Crossidium*, *Pterygoneurum* Jur. and *Stegonia* Venturi. Within this clade, a subclade consisting of *Leptophascum* and *Syntrichia* is differentiated with bootstrap supports of 93% in NJ and 79% with MP, while MrBayes gives a clade credibility value of 99%.

Discussion

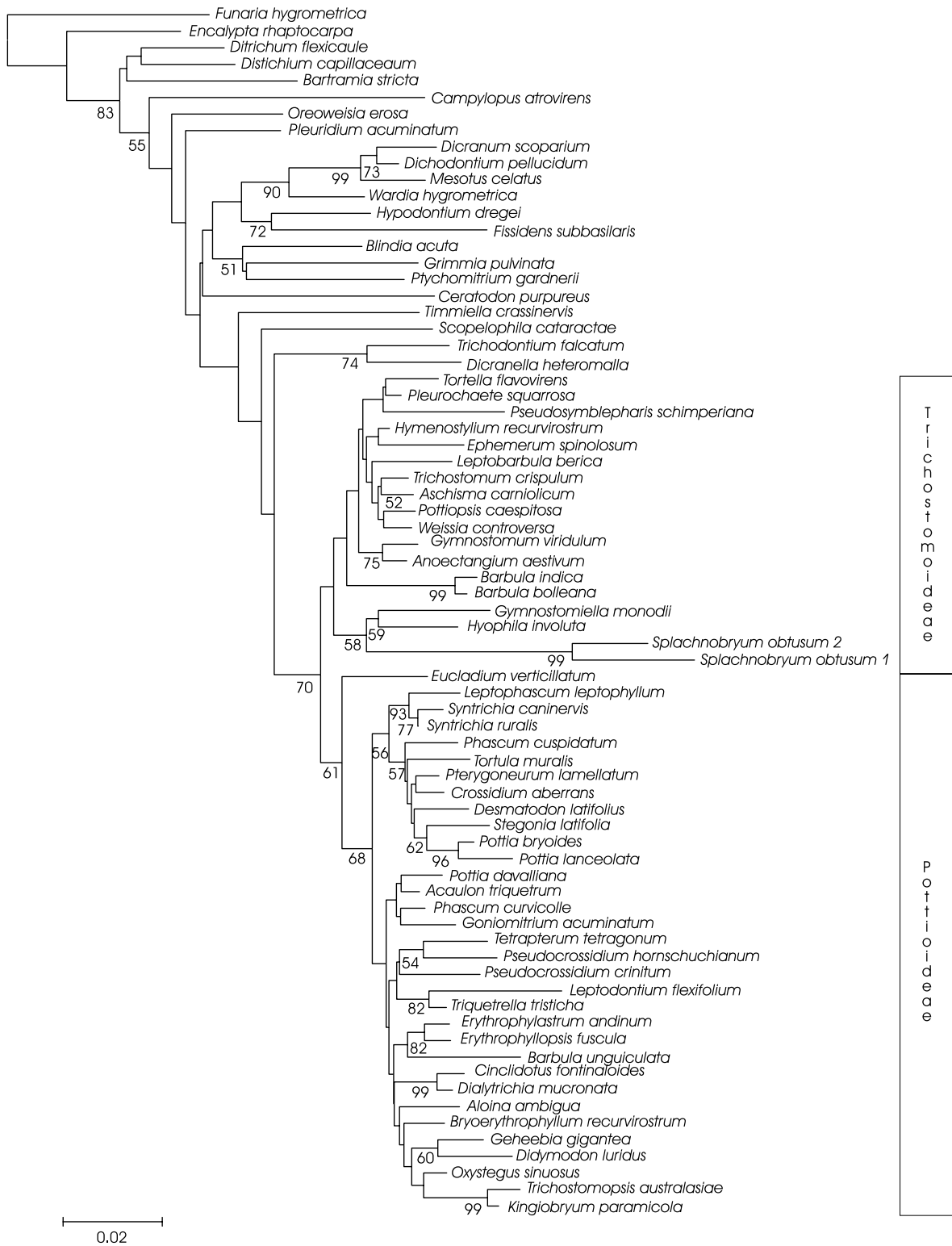
Hypodontium. The monophyly of the Pottiaceae with the exception of *Hypodontium dregei* and very probably *Timmiella* is favoured by our data. The genus *Hypodontium* Müll. Hal. was formerly considered a member of the Calymperaceae (Edwards 1980), but Zander (1993) included it in the subfamily Pottioideae of the Pottiaceae. Unusual in Pottiaceae are the inner perichaetial leaves sheathing below but narrowly subulate or awned apically (Zander 1993). Zander admits that if there were some distinctive autapomorphy or compelling combination of unusual characters, *Hypodontium* might be placed in a family of its own. As mentioned above, our data seem to indicate that the taxonomic position of *Hypodontium* might be near *Fissidens*.

Subfamily Timmielloideae. The subfamily Timmielloideae was described by Zander (1993) and consists of the genus *Timmiella*

with 13 species characterised by a stem with a very strong central strand, lamina plane to weakly incurved, denticulate to dentate margins, very wide costa, with multiple hydroid strands, bistratose but not vertically aligned near the costa laminal cells, epapillose, ventrally bulging and dorsally nearly flat, straight peristome or twisted counterclockwise. Morphologically this genus is characterised by a high number of plesiomorphic characters (Zander 1993). Corley et al. (1981) included *Timmiella* in the subfamily Trichostomoideae while Zander (1993) separated the genus in a monogeneric subfamily. The *rps4* sequence data support an isolated position of this genus, very probably outside the Pottiaceae. Only NJ assigns a position as sister group to the Pottiaceae, but with poor bootstrap support. A recent study on the Dicranaceae based on the chloroplast *rps4* and *trnL-trnF* regions also supports the separation of *Timmiella* from the Pottiaceae (La Farge et al. 2002)

Subfamily Merceyoideae. The genus *Scopelophila* (Mitt.) Lindb. is the type of this subfamily. *Scopelophila cataractae* is a copper moss, colonising soils contaminated by heavy metals. It is characterised by its blue-green colour, acute leaves without papillae on the lamina, and a costa with adaxial epidermal cells, among other features (Sotiaux and De Zuttere 1987). It is supposed to have its origin in North America and to have spread recently to Europe, where it has experienced rapid dispersal (Sotiaux and De Zuttere 1987, Shaw 1995). Corley et al. (1981) put this genus in the tribe Merceyae within the subfamily Pottioideae, Saito (1975) included it in the tribe Pottiae, also in the subfamily Pottioideae, while Zander (1993) considers this tribe to be a subfamily of its own, Merceyoideae, characterised by the presence of a stem sclerodermis which is commonly well differentiated from the cells of the

Fig. 1. Strict consensus of 753 most parsimonious trees with tree length of 854 steps (RI = 0.665, CI = 0.434). Bootstrap values above 50% are given below the clades (200 replicates). *Eucladium verticillatum* is tentatively included in the subfamily Pottioideae. The tree is rooted with *Funaria hygrometrica*



central cylinder (which have abruptly larger lumens), leaves usually broadly lanceolate to narrowly elliptical, a costa with usually two stereid bands, a leaf base commonly differentiated in shape and ovate or rectangular, upper laminal cells equally convex on both surfaces and, in some genera, frequently clavate axillary propagula. This taxon includes, in his sense, also genera, such as *Gymnostomum* Nees & Hornsch., *Barbula* Hedw., and *Anoetangium* Schwägr. Our data support the isolated position of *Scopelophila*, probably as sister group to the remaining Pottiaceae. Alternatively, it might be placed in a family of its own. But since NJ even places this species outside the Pottiaceae and MP separates it moderately well from the Pottiaceae, but provides no clear support for its position with respect to the outgroup species, further molecular studies including more taxa may change this posture.

Subfamily Trichostomoideae. For the remaining taxa, at the subfamily level the Trichostomoideae in the sense of Corley et al. (1981) and Saito (1975) might be a paraphyletic clade, since in none of the analysis does this subfamily receive 50% or more support. But until more data will be available to resolve the relationships within this group, we think that a definition of this subfamily as given in Figs. 1, 2 and 3 might serve as a basis.

The tribe Pleuroweisiae sensu Corley et al. (1981) and Saito (1975) was treated as part of the subfamily Pottioideae by these authors but was assigned to the Eucladioideae, according to Chen (1941). However, most of our samples of this tribe (*Gymnostomum viridulum*, *Anoetangium aestivum*, and *Hymenostylium recurvirostrum*) are included within the Trichostomoideae, while the genus *Eucladium* is in an intermediate position between the Trichostomoideae and the Pottioideae. Our analysis does not resolve the relations at the tribe level,

but supports a close relationship between *A. aestivum* and *G. viridulum*.

Hyophila Brid. is one of the most complex genera of the Pottiaceae, as is reflected by its abundant synonymy and combinations. Many species are morphologically similar to *Trichostomum* Bruch. Chen (1941) considered it within the Barbuloideae, which was later corroborated by Norris and Koponen (1989) who felt that this genus is closely related to *Barbula*. Saito (1975), Corley et al. (1981) and Zander (1993) included it in the Pottioideae (tribe Barbuleae according to the two first works and tribe Hyophileae according to Zander). Here, only the generitype *Hyophila involuta* is included, while other species might give different results in future studies due to the uncertainties mentioned above. *Hyophila involuta* is placed with moderate to good support near *Gymnostomiella monodii* and *Splachnobryum obtusum*. The phylogenetic placement of *Gymnostomiella* M. Fleisch. has not been sure up to now. These very delicate plants grow in dense tufts or mats and their stems are only 1.5 to 6 mm long. The main reason for placing it in the Pottiaceae are its papillose, obovate or spatulate leaves. Phylogenetic relationships with *Chenia* R. H. Zander, *Henediella* Paris or *Barbula* were discussed by Zander (1993), while Andrews (1949), Crum (1949) and Koponen (1981) discussed its possible relationship with the Splachnobryaceae. Former investigations have shown that *Splachnobryum* should be placed within the Pottiaceae (Goffinet and Cox 2000). Our data with a broader data base of Pottiaceae species confirm that *Splachnobryum* is indeed a Pottiaceae and that it is closely related to *Gymnostomiella monodii*.

Trichostomum is a large genus generally supposed to be related with *Tortella* (Lindb.) Limpr. Some species are difficult to distinguish

←
Fig. 2. Neighbor-Joining phylogram of the *rps4* sequences. Arabic numbers on the branches indicate percentage support in 1000 bootstrap replications. Bootstrap values below 50% are not shown. *Eucladium verticillatum* is tentatively included in the subfamily Pottioideae. The tree is rooted with *Funaria hygrometrica*

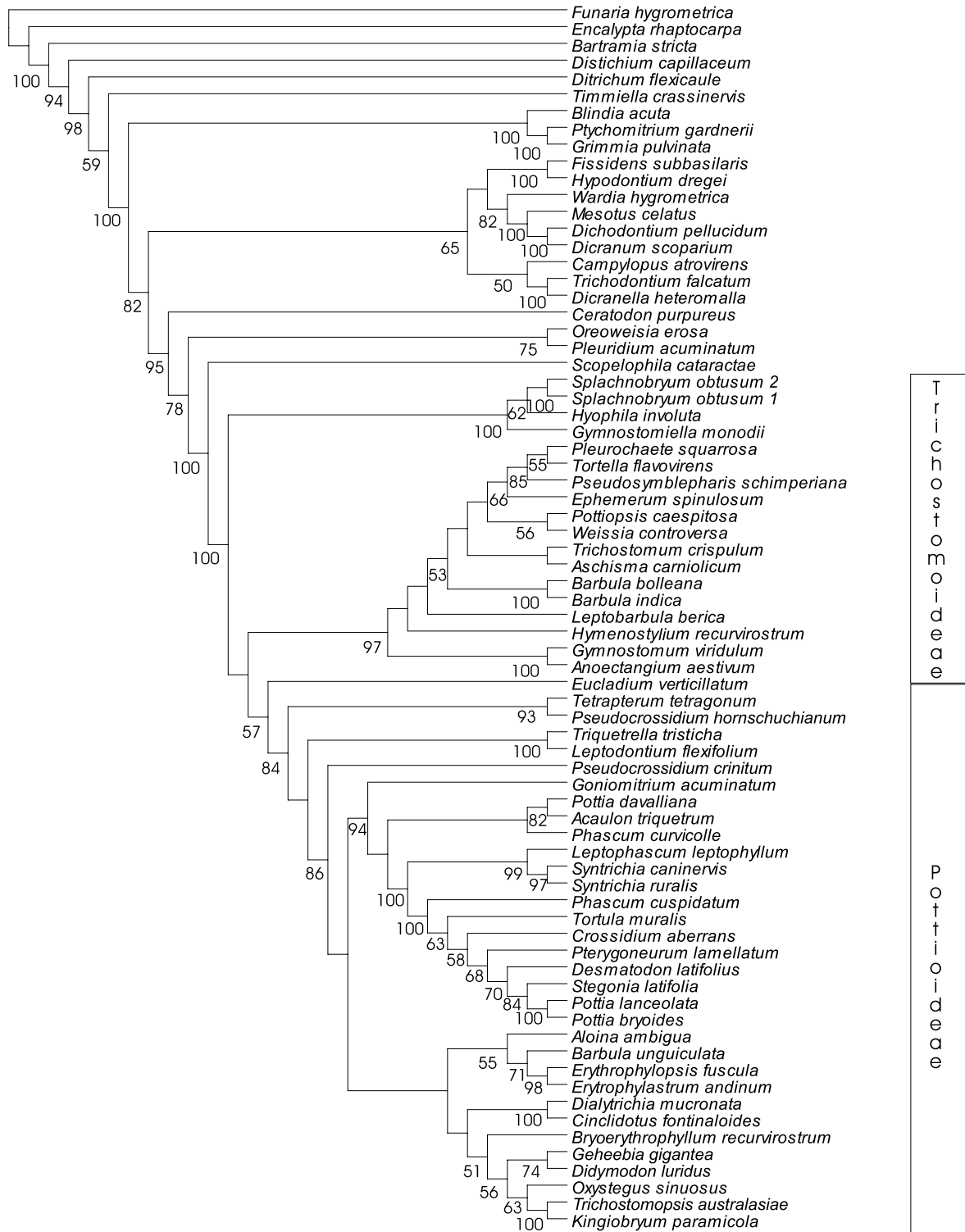


Fig. 3. Cladogram based on the Bayesian approach for 73 Pottiaceae *rps4* sequences. Arabic numbers indicate the clade credibility values of the nodes. Values below 50% are not shown. *Eucladium verticillatum* is tentatively included in the subfamily Pottioidae. The tree is rooted with *Funaria hygrometrica*

from *Trichostomum*, as is the case with *Pseudosymblepharis* Broth., *Oxystegus* (Limpr.) Hilp., and *Weissia* (Saito 1975, Crum and Anderson 1981, Eddy 1990). Nevertheless, Zander (1993) placed *Weissia*, *Trichostomum* and *Pseudosymblepharis* in three different subfamilies. Despite the fact it is sometimes virtually impossible to distinguish sterile specimens of *Weissia* and *Trichostomum*. Our data indicate that the above mentioned genera do not belong to different subfamilies, with the exception of the sequenced species of *Oxystegus sinuosus* (Mitt.) Hilp., which seems to be closely related to *Didymodon*, although the case of the other species included in the genus *Oxystegus* as *Oxystegus tenuirostre* (Hook. & Taylor) Lindb. could be different. The ITS1 sequence data of Spagnuolo et al. (1999) also demonstrate the close relationship of *Weissia* and *Trichostomum*.

Pottiopsis caespitosa is characterised by a reduced peristome, its incrassate laminal cells and distinctly differentiated perichaetial leaves. It differs from *Trichostomum* in its ephemeral habit, its autoicous nature and its production of abundant capsules. This species has suffered various taxonomic changes in the recent past. Smith (1978) and Corley et al. (1981) accepted its inclusion in *Pottia* Ehrh. ex Furnr., while Zander (1993) placed it in *Trichostomum*, and finally Blockeel and Smith (1998) described the genus *Pottiopsis* Blockeel & A.J.E. Smith to accommodate this species. Our data exclude a close relationship with the species of *Pottia* and suggest a closer relationship with *Weissia*, *Trichostomum* and the genus *Aschisma* Lindb.

Aschisma carniolicum is a very small species with a reduced cleistocarpous sporophyte adapted to dry habitats. The plants are similar to *Acaulon* Mull. Hal. or *Phascum*, but the laminal cells are smaller and densely papillose. Zander (1993) placed this genus within the Pottioideae. Our analysis indicates a position near *Trichostomum*, *Weissia*, or *Pottiopsis* in the Trichostomoideae.

Barbula bolleana (= *B. ehrenbergii* (Lorentz) M. Fleisch.) and *B. indica* are two species from moist habitats. *Barbula bolleana* is

the type species of the sect. *Hydrogonium* (Mull. Hal.) K. Saito of the genus *Barbula*. Zander (1993) suggested that *Hydrogonium* (Mull. Hal.) A. Jaeger should be recognised at the generic level, as it has been done by several authors. In this study, *Barbula unguiculata* represents a more typical species of the genus *Barbula*. The sequence data show that *B. bolleana* and *B. indica* on the one hand, and *B. unguiculata* on the other, are not related at the genus level and should most probably be placed in different subfamilies, since *Barbula unguiculata* is clearly a member of the Pottioideae and *B. bolleana* and *B. indica* of the Trichostomoideae. The genus *Barbula* is one of the largest within the Pottiaceae with 205 species recognised by Zander (1993). Consequently the phylogenetic relationships are sometimes difficult to resolve. Ties with the genera *Didymodon* and *Trichostomum* are discussed (Saito 1975, Norris and Koponen 1989, Zander 1993). A broader sequence study including various members of the different sections might provide even give more surprises.

Leptobarbula berica is the only representative of this monotypic genus. This is a small moss with a local occurrence in the Mediterranean region of Europe, the Middle East and North Africa. It was first thought to be related with *Barbula*, but Appleyard et al. (1985) felt that it was "allied to *Gymnostomum* and *Gyroweissia*, differing apparently only in the revolute anulus and well-developed peristome". Our analysis does not resolve clearly the exact systematic position of this moss, but a position within the Pottioideae as proposed by Corley et al. (1981) and Zander (1993) is not supported by the *rps4* sequence data.

Recently, Goffinet and Cox (2000) published sequence data for *Ephemerum spinulosum* that showed that *Ephemerum* Hampe has strong affinities with the Pottiaceae. Their data set only included one Pottiaceae and therefore it was not possible to draw a final conclusion concerning the position of this genus. Our data set consistently places *Ephemerum* within the

Pottiaceae, as suspected by the mentioned authors.

Eucladium. The monotypic genus *Eucladium* is found on calcareous rocks in wet places across Europe, Asia, Africa and Northern America. It has a somewhat isolated position between the Trichostomoideae and Pottioideae and, depending on the type of analysis, it appears to be more related to one or other of the two subfamilies. However our data do not allow any definitive conclusion about which subfamily it belongs to or whether it might be treated as representative of a subfamily of its own. Sequence data of the nuclear ITS1 region (Spagnuolo et al. 1999) favour a position closer to the Trichostomoideae. Chen (1941) proposed an independent subfamily Eucladioideae derived from the Trichostomoideae and considered the tribe Eucladieae for the genera *Eucladium*, *Gyroweisia* Schimp., *Gymnostomum* and *Hymenostylium* Brid. among other genera, which does not reflect our data.

Subfamily Pottioideae. Our data coincide almost fully with the position of Corley et al. (1981) and Saito (1975) concerning the circumscription of the subfamily Pottioideae. The exceptions discussed in the preceding paragraph are the tribe Pleuroweisiae and *Leptobarbula berica*. Another important exception are the genera *Dialytrichia* (Schimp.) Limpr. and *Cinclidotus*. Corley et al. (1981) unite these two genera in one (a position that is well supported on the basis of our data) and put it in a subfamily of its own, Cinclidotoideae. Zander (1993) includes *Dialytrichia* in the subfamily Merceyoideae together with *Didymodon*, *Barbula*, *Hymenostylium* and others, but excludes *Cinclidotus* from the Pottiaceae, a point of view clearly at odds with our data but shared by Saito (1975). Chen (1941) accepted both *Cinclidotus* and *Dialytrichia* genera as members of the subfamily Cinclidotoideae although he did not study them. The peristome and the laminal areolation of *Dialytrichia* are clearly of the type found within the Pottiaceae. The relationship of *Dialytrichia*/*Cinclidotus* with other members of the Pottioideae remains ambiguous.

Tetrapterum Hampe ex A. Jaeger is a genus with unclear affinities and there are contradictory opinions as to which species should be included. But there seems to be a general agreement that the sequenced species, *T. tetragonum* (Hook.) A.L. Andrews, belongs to this genus (Andrews 1945, Zander 1993). Andrews felt that this genus was ultimately related to *Trichostomum*, but on the basis of our data, we agree with Zander (1993) that it should be placed within the Pottioideae.

Leptodontium (Müll. Hal.) Hampe ex Lindb. and *Triquetrella* Müll. Hal. are two genera that are similar in their 16 smooth or nearly smooth peristome teeth, strongly differentiated perichaetial leaves, a stem usually without a central strand, squarrose strongly reflexed cauline leaves and the absence of a differentiated epidermal layer on the ventral surface of the costa. Chen (1941) placed these two genera in the subfamily Leptodontioideae, while Zander (1993) recognised them within the Merceyoideae. Our data support their close relationship but would place them within the Pottioideae, as suggested by Corley et al. (1981) and Saito (1975).

The position of *Aloina* Kindb. within the Pottioideae cannot be clarified. Placing this genus very near *Tortula* or *Crossidium* Jur., as suggested by Chen (1941), Corley et al. (1981) and Zander (1993) on the basis of a few morphological data (e.g. photosynthetic filaments on the leaf surface), seems inappropriate since the development of these filaments seems to be an example of convergent evolution, probably under dry habitat conditions.

The genus *Pseudocrossidium* R. S. Williams was placed by Zander (1993) in the subfamily Merceyoideae in clear contradiction with our data, which support a position within the Pottioideae, as Corley et al. (1981) thought.

The genus *Didymodon* is often considered as “a synthetic genus composed of discordant elements originating in several genera of the Pottiaceae” (Steere 1947). Consequently, some taxa are sometimes included or excluded from this genus, examples being *Oxystegus sinuosus*, *Geheebia gigantea* (Funck) Boulay and *Tricho-*

stomopsis australasiae (Hook. & Grev.) H. Rob. Nevertheless, the NJ analysis seems to support the monophyly of the taxa included in this study. But the clade confidence value of MrBayes is only 56% and the bootstrap support using NJ and MP below 50%. A more intense sampling of this large genus (more than 100 species) is needed before this question can be answered. Interestingly, *Kingiobryum paramicola* is placed with very good support near *Trichostomopsis australasiae*. This species was originally included in the Seligeriaceae by Robinson (1967), but recent evidence (La Farge et al. 2002) suggests that it belongs to the Pottiaceae.

Goniomitrium acuminatum was traditionally included in the Funariaceae (Fife 1985) but recent molecular evidence strongly suggested its inclusion in the Pottiaceae (Goffinet and Cox 2000). Our data confirm this point of view and place it within the Pottioideae.

Zander (1993) did not recognise the genera *Phascum* and *Pottia* but, instead, included the species of *Pottia* with rostrate lid (named by Warnstorf (1916) as *Rhynchostegiae* and represented in this work by *Pottia lanceolata*), together with *Pottia bryoides*, in *Tortula*, while those with a conic lid (named by Warnstorf (1916) as *Conostegiae*, represented in this work by *Pottia davalliana*) were placed in *Microbryum*. As for the genus *Phascum*, Zander (1993) included the species of the subgenus *Microbryum* (Schimp.) G. Roth (*P. curvicolle* Hedw. was the representative taken for this work) in the genus of the same name, *Microbryum*, and the species of the subgenus *Euphascum* Limpr. (represented in this work by *Phascum cuspidatum* Hedw.) in the genus *Tortula*. The sequence data support a close relationship of the *Rhynchostegiae* species of *Pottia* with *Tortula*, although a final conclusion of the best taxonomic treatment cannot be given at this point. The inclusion of the *Conostegiae* species of *Pottia* and those of the subgenus *Microbryum* of *Phascum* (excluding *Acaulon*) in the genus *Microbryum* seems to somewhat problematic, since *Acaulon* appears as closest neighbour of *Pottia davalliana*. There is strong

evidence to separate *P. davalliana* from *Pottia bryoides* and *Pottia lanceolata*. *Phascum cuspidatum* belongs to the *Tortula* clade and *Phascum curvicolle* is more related to *Pottia davalliana* and *Acaulon triquetrum* (Spruce) Müll. Hal., although with low bootstrap support.

Leptophascum leptophyllum (Müll. Hal) J. Guerra & M.J. Cano has undergone various taxonomic treatments in the past and has been classified within *Tortula* (Corley et al. 1981), *Phascum* (Arts and Sollmann 1991), *Chenia* (Zander 1989) and *Leptophascum* (Guerra and Cano 2000). Its small size and the general absence of sporophytes make its taxonomic treatment difficult. The phylogenetic analysis of Zander (1993) based on morphological data indicated a close relationship with *Syntrichia*, which is clearly supported by our data.

Syntrichia and *Tortula* have long been placed in one genus (*Tortula*). Zander (1989) and Ochya (1992) supported the concept of *Syntrichia* as “a natural group that deserves recognition as a genus of its own”. The *rps4* sequence data point in the same direction, since *T. muralis* Hedw. seems to be more closely related to species of *Crossidium*, *Stegonia*, *Desmatodon* Brid., *Pterygoneurum* and some *Pottia* than to *Syntrichia*.

A study concentrating on the phylogenetic relationships of “*Syntrichia-Tortula*”-clade, based on *rps4* sequence data and including more related taxa, has been published recently (Werner et al. 2002).

Erythrophyllastrum andinum and *Erythrophyllopsis fuscula* are included by Zander (1993) in the subfamily Erythrophylllopoideae, characterised by having lanceolate leaves, plane to weakly incurved margins, an acute apex, base sheathing, an upper lamina that turn red when treated with 2% (w/v) KOH, a costa with two stereid bands, 4–6 guide cells and 10–16 rows of cells across the ventral surface of the costa. It is represented by only two rare species from the Andes in South America. By our analysis they are placed within the Pottioideae near *Barbula unguiculata*, although the latter poorly

supported. We therefore conclude that these species should be included within the Pottiaceae.

We are grateful to the late Theo Arts for the gift of some Pottiaceae specimens from South Africa and India, and to the curator of the NY Herbarium for the loan of some specimens of *Erythrophyllastrum andinum* and *Erythrophyllopsis fuscula*. This work was carried out with financial support from the Spanish Government (Ministerio de Ciencia y Tecnología, project BOS 2000-0296-C03-01 and BOS 2001-0276).

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