

Molecular phylogeny of Trichostomoideae (Pottiaceae, Bryophyta) based on nrITS sequence data

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Bayesian analysis of an alignment of 83 nrITS sequences belonging to 66 taxa of Pottiaceae revealed representatives of subfamily Trichostomoideae in a well supported monophyletic lineage. Two robust clades within Trichostomoideae include species of *Tortella* and *Weissia*, respectively. *Eucladium verticillatum* is part of Trichostomoideae, and *Anoetangium*, *Gymnostomum*, *Hymenostylium*, and *Tuerckheimia* form a robust basal clade. Affinities of *Hyophila* and *Leptobarbula* to Trichostomoideae are unclear. Within Trichostomoideae, *Pleurochaete squarrosa* is resolved in *Tortella* while *Trichostomum tenuirostris*, *Pseudosymblepharis*, and *Chionoloma* are placed as a separate genus or part of a broadly defined *Tortella*. The type species of *Trichostomum*, *T. brachydonium*, is nested in *Weissia* together with *T. brittonianum*, *T. crispulum*, and *T. jamaicensis*. *Trichostomum unguiculatum* and *W. ayresii* are resolved as closely related and separate from the rest of the *Weissia* clade. The genus *Astomum*, erected for cleistocarpous species of *Weissia*, is also nested within *Weissia*. Genetic divergence between selected accessions of *Weissia controversa* and *Astomum* is sometimes smaller than between accessions of *Weissia controversa*.

KEYWORDS: bryophyta, molecular phylogeny, nrITS sequence data, Pottiaceae, Trichostomoideae.

INTRODUCTION

Pottiaceae are a large and taxonomically difficult family of mosses, whose generic delimitations and relationships are complicated and often controversial. Since the description of the family by Bruch & al. (1836–1851), many generic classifications have been proposed based on morphology. Spagnuolo & al. (1999), in a first molecular-phylogenetic study on Pottiaceae, sequenced the nrITS 1 region in a few species and demonstrated the close relationship between *Pleurochaete* Lindb., *Tortella* (Lindb.) Limpr. and *Weissia* Hedw., the placement of *Weissia* in Trichostomoideae rather than Pottioideae, and the separation of *Syntrichia* Brid. from *Tortula* Hedw. Werner & al. (2002a) studied the relationships of *Tortula* and allied genera using *rps4* sequences and confirmed that *Syntrichia* and *Tortula* are clearly separated as observed by Spagnuolo & al. (1999). In addition, they found: that the rhynchostegiate species of *Pottia* (Ehrh. ex Rchb.) Fűrnr. are clearly separated from the conostegiate ones and closer to *Tortula*, that *Crossidium* Jur. is nested within *Tortula*, and that *Desmatodon* Brid., *Pterygoneurum* Jur., *Stegonia* Venturi, and some species of *Phascum* Hedw., are closely related. Using the same marker, Werner & al. (2004a) demonstrated that *Hypodontium* Müll. Hal. and *Timmia* (De Not.) Limpr. should be excluded from Pottiaceae while *Cinclidotus* P.

Beauv., *Dialytrichia* (Schimp.) Limpr., *Ephemerum* Hampe, *Kingiobryum* H. Rob., and *Splachnobryum* Müll. Hal., which are usually treated separately, are members of this family. Later, Werner & al. (2004b) included *Kingiobryum* in the genus *Didymodon* Hedw. based on nrITS1 and nrITS2 sequence data.

At subfamily level, a subdivision into two to seven different subfamilies has been proposed. One of these, subfam. Trichostomoideae, has been variously defined morphologically (e.g., Hilpert, 1933; Chen, 1941; Saito, 1975; Zander, 1993) based on (1) stem sclerodermis poorly differentiated from the central cylinder, (2) hyalodermis commonly present, (3) leaves mostly narrow lanceolate with unistratose, plane or incurved margins, (4) leaf base, when differentiated, separated obliquely from the lamina, (5) upper laminal papillae crowded, (6) upper lamina KOH reaction yellow (seldom yellowish orange or reddish brown), (7) costa with two bands of stereids but lacking a differentiated dorsal epidermis, (8) clavate propagula absent, (9) 5-layered amphitecial derivatives of the capsule and stomata present, and (10) peristome split into two segments from the base, without or with a very short basal membrane.

Werner & al. (2004a), using *rps4* sequences, accepted three subfamilies in Pottiaceae: Merceyoideae, Trichostomoideae, and Pottioideae. Trichostomoideae were delimited in a very broad sense and included many taxa

not previously assigned to this subfamily on morphological grounds, e.g., *Ephemerum* (previously Ephemeraceae), *Anoetangium* Schwägr., *Aschisma* Lindb., *Gymnostomiella* M. Fleisch., *Gymnostomum* Nees & Hornsch., *Hymenostylium* Brid., *Hyophila* Brid., *Leptobarbula* Schimp., *Pottiopsis* Blockeel & A. J. E. Smith, *Pseudosymblepharis* Broth., *Splachnobryum*, *Weissia* Hedw. and some species of *Barbula* Hedw. (other sequenced species of *Barbula* belonged to Pottioidae). However, Trichostomoideae were not resolved with strong support and the new classification, therefore, remained a provisional one.

In the present paper the delimitation of Trichostomoideae and the relationships among selected genera of this subfamily are explored using new evidence from nrITS sequence data.

MATERIALS AND METHODS

Plant material. — Selected species were sampled from all major lineages of Trichostomoideae. Eighty-three specimens were sequenced, representing 66 taxa. In many cases, more than one sample was sequenced to demonstrate possible sequence variation at species or variety level. The following species of Pottioidae sensu Werner & al. (2004a) were included: *Barbula unguiculata* Hedw., *Bryoerythrophyllum recurvirostrum* (Hedw.) P. C. Chen, *Chenia leptophylla* (Müll. Hal.) R. H. Zander, *Didymodon rigidulus* Hedw., *D. sinuosus* (Mitt.) Delogne, *Pseudocrossidium hornschiianum* (Schultz) R. H. Zander, *Tortula inermis* (Brid.) Mont., *T. muralis* Hedw., *Triquetrella arapilensis* Luisier, and *Tr. tristicha* (Müll. Hal.) Müll. Hal. *Bryoerythrophyllum recurvirostrum* was assigned as outgroup species in the molecular analysis and used to root the tree. Voucher data and GenBank accession numbers are given in the Appendix. Nomenclature follows Zander (1993) except for *Chionoloma bombayensis* (Müll. Hal.) P. Sollman (Sollman, 2001) and *Trichostomum sweetii* (E. B. Bartram) L. R. Stark (Stark, 1996).

DNA extraction. — Total DNA was extracted using the NaOH method of Werner & al. (2002b), in which 5 µl of a crude NaOH extract was diluted by the addition of 45 µl of 100 mM Tris - 1 mM EDTA (pH 8.3), stored frozen at -18°C and used as template for subsequent PCR analysis.

DNA sequencing. — PCR reactions were performed in an Eppendorf Mastercycler using 4 µl of the DNA solution in a 50 µl final volume. The reaction mix contained the primers 18S (5'-GGAGAAGTCGTAA-CAAGGTTTCCG-3'), designed by Spagnuolo & al. (1999) and ITS4 (5'-TCCTCCGCTTATGATATGC-3') by White & al. (1990), at a final concentration of 400

µM, in the presence of 200 µM of each dNTP, 2 mM MgCl₂, 2 units Taq polymerase (Oncor Appligene), 1µl BLOTTO (10% skimmed milk powder and 0.2% NaN₃ in water) and the buffer provided by the supplier of the enzyme. BLOTTO attenuates PCR inhibition caused by plant compounds (De Boer & al., 1995, unpubl.). Amplification started with 3 min denaturation at 94°C, followed by 35 cycles of 15 s at 94°C, 30 s at 50°C, and 1 min at 72°C. Concluding with a final extension step of 7 min at 72°C., 5 µl of amplification products were visualized on a 6% polyacrylamide gel. Successful amplifications were cleaned with the QIAquick purification kit (Qiagen). The amplification primers were used in the sequencing reactions with the Big Dye sequencing kit and separated on an ABI-Prism 3700 sequencing machine using standard protocols.

Sequences were aligned using CLUSTALX (Thompson & al., 1997) with the gap open penalty set to 10 and the gap extension penalty set to 1. BioEdit (Hall, 1999) was used for minor manual adjustments of the alignment. Sequences with high frequency of indels difficult to align were excluded from the analysis [e.g., *Barbula indica* (Hook.) Spreng., *Ephemerum sessile* (Bruch) Müll. Hal., *Splachnobryum obtusum* (Brid.) Müll. Hal.]. The aligned matrix is available on request from the first author. Gaps outside hypervariable regions were coded as present (1) or absent (0). Pairwise distances between sequences were calculated by MEGA 2 (Kumar & al., 2001).

Bayesian analysis was carried out using MrBayes 3.0 (Huelsenbeck & Ronquist, 2001), with 500,000 generations run, sampling every 100th generation and using the following settings for the sequence data: Nst=6, rates=invgamma (a proportion of the sites are invariable while the rate for the remaining sites corresponds to the general model of DNA substitution with gamma distributed rate variation across sites). Recoded gaps were treated as standard data. Based on empirical evaluation, burn in was set at 100,000 generations. A 50% majority rule tree was constructed using the “sumt” command of MrBayes. The tree was edited using TreeView version 1.6.6 (Page, 1996). Clade credibility values (cv) below 0.75 were treated as “poor” support, between 0.75 and 0.95 as “moderate” and above 0.95 as “good”.

RESULTS

The combined length of the 18S ITS1 - 5.8S rRNA - ITS2 region was 662 [*Trichostomum unguiculatum* (Mitt.) R. H. Zander] to 836 [*Chionoloma bombayensis*] base pairs (bp). The ITS1 region was highly variable with multiple indels and ranged from 233 bp [*Tuerckheimia svihlae* (E. B. Bartram) R. H. Zander] to

360 bp (*C. bombayensis*). Length of the 5.8S rRNA gene was 158 bp in all accessions, that of ITS2 was less variable than ITS1 and measured 264 bp [*T. unguiculatum*] to 337 bp [*Tortella humilis* (Hedw.) Jenn. and *C. bombayensis*].

Bayesian analysis (Fig. 1) resolved members of Trichostomoideae (Werner & al., 2004a) as monophyletic (1.0 cv). Basal positions were occupied by *Hyophila involuta* (Hook.) A. Jaeger and *Leptobarbula berica* (De Not.) Schimp., in a poorly supported clade (0.72 cv), and *Anoetangium aestivum* (Hedw.) Mitt., *Gymnostomum calcareum* Nees & Hornsch., *Hymenostylium hildebrandtii* (Müll. Hal.) R. H. Zander, *Tuerckheimia svihlae*, and *T. valeriana* (E.B. Bartram) R. H. Zander in a well supported clade (1.0 cv).

A core clade including *Eucladium verticillatum* (Hedw.) Bruch & Schimp. and a clade including all species of *Tortella* as well as *Pleurochaete squarrosa* (Brid.) Lindb., *Pseudosymbblepharis schimperiana* (Paris) H. A. Crum, *Chionoloma bombayensis*, *Trichostomum tenuirostris* (Hook. & Taylor) Lindb., *T. caespitosum* (Bruch) Jur., *T. sweetii* and *Weissia triumphans* (De Not.) M. O. Hill, were well supported (1.0 cv). The remaining species of *Weissia* and *Trichostomum* Bruch and *Aschisma carniolicum* (F. Weber & D. Mohr) Lindb. formed the second major clade of Trichostomoideae (1.0 cv). *Trichostomum unguiculatum* and *Weissia ayresii* Schimp. were basal in this clade, separated by at least 25 (*W. ayresii*) and 34 (*T. unguiculatum*) mutational steps from the closest accession (*T. crispulum* Bruch). The next, rather isolated position was occupied by *Aschisma carniolicum* (minimum number of pairwise distances to other species = 31). *Weissia ligulaefolia* (E. B. Bartram) Grout and *W. latuscula* Müll. Hal. were resolved as closely related and isolated from other *Weissia* species. *Trichostomum brachydondium* Bruch, *T. brittonianum* R. H. Zander, *T. crispulum*, and *W. jamaicensis* (Mitt.) Grout were a monophyletic lineage with good support. The majority of the *Weissia*-species together with *Trichostomum austrocrispum* (Beckett) R. H. Zander were very closely related (1.0 cv), with relationships not always being resolved (maximum number of pairwise differences between species in this clade = 23). Cleistocarpous species in this clade, including *W. levieri* (Limpr.) Kindb., *W. longifolia* Mitt., *W. ludoviciana* (Sull.) W. D. Reese & B. A. E. Lemmon, *W. multicapsularis* (Sm.) Mitt., *W. muelhlenbergiana* (Sw.) W. D. Reese & B. A. E. Lemmon, *W. rostellata* (Brid.) Lindb., and *Trichostomum austrocrispum*, did not form a monophyletic group.

DISCUSSION

Sequences of nrITS are widely used to resolve phylogenetic relationships of species at taxonomical levels below the rank of family. Analysis of the ITS region was favored by the development of universal primers (White & al., 1990). In bryophytes, early molecular phylogenetic studies were aimed at resolving relationships at higher taxonomic ranks. In recent years, ITS sequences are increasingly being used to study affinities of closely related species and biogeographical issues. Depending on the taxon examined, ITS sequences may vary considerably and their alignment may be difficult. In *Pyrrhobryum mnioides* (Hook.) Manuel, ITS sequences of New World and Old World populations cannot be reliably aligned (Shaw & al., 2002), but in Amblystegiaceae, Brachytheciaceae and Leskeaceae, sequences can be aligned throughout the family (Vanderpoorten & al., 2001; 2002a, b). In Pottiaceae we have usually been able to successfully align major parts of sequences of species from different subfamilies.

The nrITS region is organized in repeated units. Concerted evolution may result in homogenization of the repeats, resulting in the presence of many copies of a single ITS haplotype in the genome (Baldwin, 1992; Baldwin & al., 1995). Nevertheless, multiple ITS haplotypes may sometimes occur in single individuals resulting in sequence chromatograms that are unreadable. In our studies on Pottiaceae, alignment problems due to multiple ITS haplotypes have not arisen; where difficulties occurred, these were due to assumed length variations of ITS sequences. Some genera treated in Trichostomoideae by Werner & al. (2004a) were excluded from the present analysis due to the high frequency of indels, which did not allow reliable alignment of major parts of the ITS sequences. Additional analysis using other markers is needed to assess the taxonomic status of these taxa.

Using *rps4*, Werner & al. (2004a) tentatively included *Anoetangium*, *Gymnostomiella*, *Gymnostomum*, *Hymenostylium*, *Hyophila*, and *Leptobarbula* in Trichostomoideae. The results of the present study do not contradict this taxonomy. In the ITS tree these genera occupy a basal position in Trichostomoideae together with *Tuerckheimia* Broth. (and with exclusion of *Gymnostomiella*, which is omitted because the ITS region in this genus could not be sequenced). The topology indicates that these genera may be included in Trichostomoideae or may be considered to belong to a separate subfamily. A deeper molecular study is necessary to clarify their phylogenetic position. The ITS tree also suggests that *Tuerckheimia svihlae*, *T. valeriana*, and *Hymenostylium hildebrandtii* are closely related. Although the morphological affinities of these two genera have often been

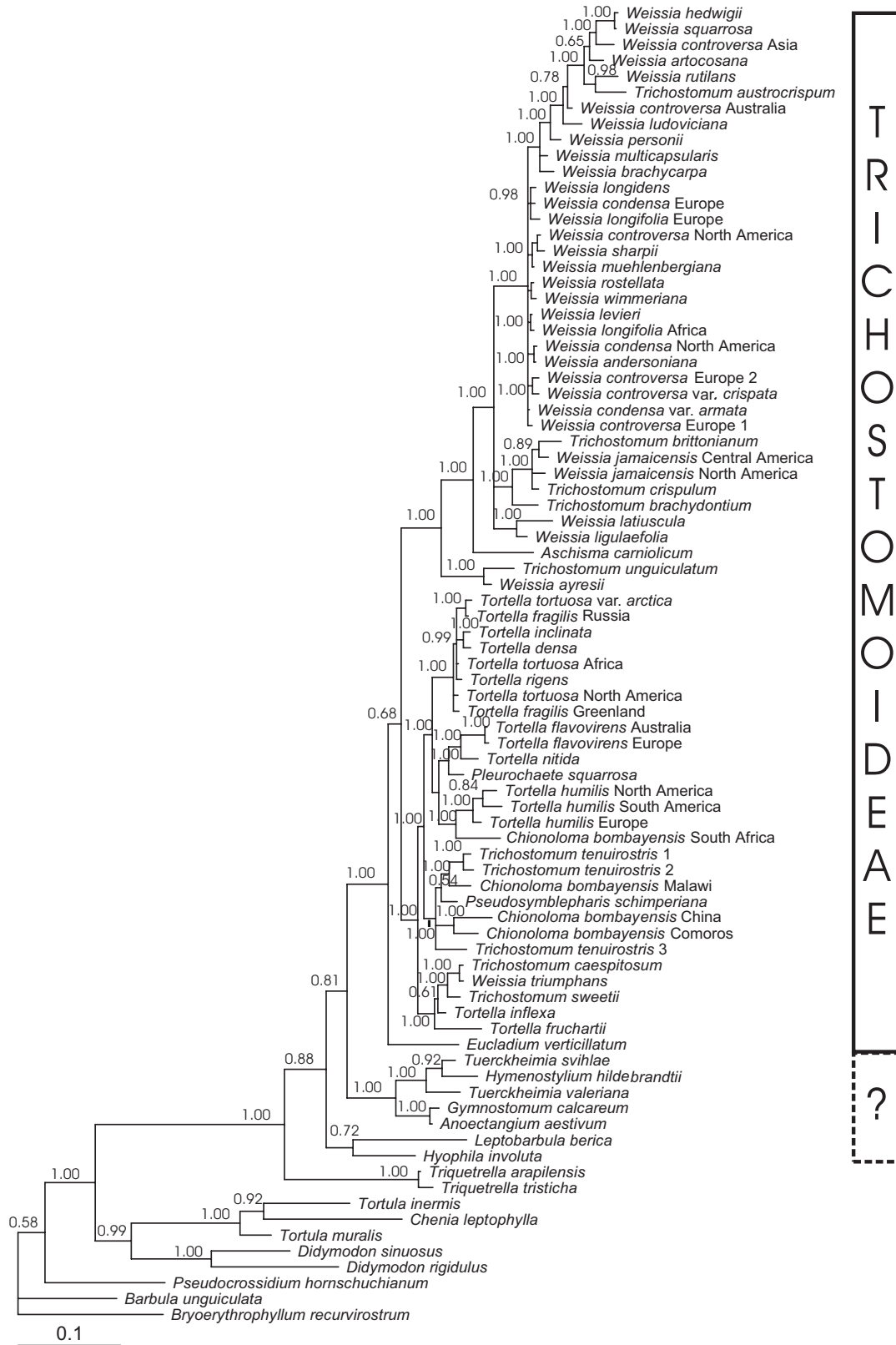


Fig. 1. Phylogram based on Bayesian analysis of nrITS sequences. Numbers indicate the clade credibility values of the nodes. Values below 0.50 are not shown. The tree is rooted with *Bryoerythrophyllum recurvirostrum*.

noted (Zander, 1993), more data are obviously needed to resolve their relationships.

According to the ITS sequences, the core of the subfamily Trichostomoideae is formed by the genera *Eucladium* Bruch & Schimp., *Tortella*, *Pleurochaete*, *Pseudosymblepharis*, *Chionoloma* Dixon, *Trichostomum*, *Weissia*, and *Aschisma*.

Eucladium. — The inclusion of *Eucladium verticillatum* within Pottiaceae is controversial. In the present analysis *Eucladium* is resolved in Trichostomoideae in a basal position, near *Tuerckheimia*. This position agrees with the morphological similarities shared by the two genera (Zander, 1993), including the narrowly lanceolate leaf shape, broad costa, and large laminal cells ornamented by rather large, simple papillae.

Tortella and related taxa. — Most of the *Tortella* species of this study form a monophyletic clade when *Pleurochaete squarrosa* is included. This topology may be due to the segregation of *Pleurochaete* from *Tortella* by the differentiated thin-walled marginal cells in the former, extending from the insertion sometimes to above midleaf but not forming a coherent basal V extending medially to the costa (Zander, 1993).

A clade with solid support (1.0 cv) is formed by *Trichostomum caespitosum*, *T. sweetii*, and *Weissia triumphans*. The three species stand out morphologically by the monoicous condition (autoicous or paroicous), sheathed perichaetial leaves, and a well developed annulus formed by 2–3 rows of inflated cells, characters that could be assumed as synapomorphies. The taxa are clearly separated from other species of *Trichostomum* and *Weissia* and may represent a separate genus. Blockeel & Smith (1998) placed *T. caespitosum* in the new genus *Pottiopsis* based mainly on the sheathed perichaetial leaves. Our study supports this taxonomy and indicates that *T. sweetii* and *Weissia triumphans* should also be referred to *Pottiopsis*. *Tortella fruchartii* (Müll. Hal.) R. H. Zander and *T. inflexa* (Bruch) Broth. are most closely related to the *Pottiopsis* clade and are morphologically distant to most of the other species of *Tortella*. Their phylogenetic relationships need further study, however.

The accessions of *Chionoloma bombayensis* (with one exception, see below), *Pseudosymblepharis schimperiana*, and *Trichostomum tenuirostris*, based on seven specimens from various parts of the world, form a clade with solid support (1.0 cv). Segregation of the latter species in the genus *Oxystegus* (Limpr.) Hilp., usually considered to be controversial, is supported by the molecular data. The close relationship of *T. tenuirostris*, *Pseudosymblepharis*, and *Chionoloma* is confirmed by morphology (Sollman 1996, 2000, 2001) and suggests the need of uniting *Oxystegus*, *Pseudosymblepharis*, and *Chionoloma* into one single genus for which the name *Chionoloma* should be adopted, being the oldest in

generic rank. One accession of *Chionoloma bombayensis* originating from South Africa is not a member of this clade but is resolved near *Tortella humilis*. The identity of this specimen needs further study. Synapomorphies of *Chionoloma* s.l. include the caducous leaf apices, papillae extending over the entire lumen except at the sheathing leaf bases, and the ventral stereid band being larger than the dorsal (Zander, 1993; Li & al., 2001).

The inclusion of the species of the former clade in the genus *Tortella* should not be ruled out, based on the similarity previously observed by many authors, i.e., the presence of a V-shaped basal area; in *Tortella* it is usually more clearly distinct, however (Mönkemeyer, 1927; Hilpert, 1933; Weber, 1973; Zander, 1993; Gradstein & al., 2001). In this case synapomorphies of *Tortella* could be: plane leaf margins, often inflated basal cells that usually extend up the margins in a more or less distinct V, and a V-shaped transverse section of the dorsal stereid band.

Tortella is resolved as polyphyletic in our analysis. The generitype *T. caespitosa* (Schwägr.) Limpr. (= *T. humilis*) is member of a clade formed by the majority of *Tortella* species and *Pleurochaete squarrosa*, which must be transferred to *Tortella* (as *T. squarrosa* (Brid.) Limpr.). Although the name *Pleurochaete* is older than *Tortella*, the latter name has been conserved and is therefore to be retained (ICBN, appendix IIIA).

Weissia and Trichostomum. — The African species *Weissia ayresii* and *Trichostomum unguiculatum* are resolved in a clade with good support (1.0 cv). They are identical gametophytically and differ in the exerted, non-macrostomous capsule of *W. ayresii* (immersed and macrostomous in *T. unguiculatum*). This clade may deserve recognition at generic level and might possibly be referred to *Phasconica* (the generitype *P. lorentzii* Müll Hal. has not yet been studied, however).

Aschisma carniolicum is situated in a clade with good support (1.0 cv) together with most of the species of *Weissia* and *Trichostomum*. Inclusion of *Aschisma* in Trichostomoideae is confirmed by *rps4* sequences (Werner & al., 2004a). Within this subfamily the genus is morphologically somewhat isolated, although it shares small plant size, differentiated basal leaf parts extending along the margins in a weak V, densely papillose lamina cells, and cleistocarpous condition with some species of *Weissia*. Another clade with good support (1.0 cv) is formed by *W. latiuscula* and *W. ligulaefolia*. The geographical range of the two species differs considerably, with *W. latiuscula* being known from Africa and *W. ligulaefolia* from North America. Nevertheless, the two taxa are morphologically related by the relative short, more or less ligulate or oblong leaves with strongly involute upper margins, and by the lack of a peristome. Further studies into the phylogenetic relationships of these two

species are necessary.

The rest of this clade is exclusively made up of species of *Weissia* and *Trichostomum*, as defined by Zander (1993). These two genera are not resolved as separate genera in our analysis. Accessions of *W. controversa* Hedw., generitype of the genus *Weissia*, are dispersed throughout the clade, and the generitype of *Trichostomum*, *T. brachydontium*, is resolved nested in *Weissia*, in a subclade together with *T. brittonianum*, *T. crispulum*, and *W. jamaicensis*. The only morphological feature characterizing this subclade is the dioicous sexual condition, found in all four species. However, the sexual condition in *Weissia* varies considerably and several species are heteroicous [e.g., *W. ligulaefolia*, *W. brachycarpa* (Nees & Hornsch.) Jur.; see Stoneburner, 1985] or apparently dioicous (*W. ludoviciana*, *W. muehlenbergiana*; see Crum & Anderson, 1981). Thus, our topology clearly supports a broad circumscription of the genus *Weissia*, with inclusion of *Trichostomum*, as previously proposed by Dixon (1913), Andrews (1945), and others. Relevance of cleistocarpy as an important taxonomic character in Pottiaceae is also not revealed by our molecular studies (Werner & al., 2002a, 2004a, this paper). Cleistocarpous species are distributed throughout the *Weissia* clade and recognition of the cleistocarpous genus *Astomum* Hampe is not supported.

Genetic distances within the *Weissia* clade are quite low, suggesting rapid diversification and radiation in this clade. Accessions of single species often do not form monophyletic groups, most notably so in *W. controversa* (see above). This clade is morphologically highly complex and many species are difficult to separate because of the occurrence of intermediate forms. Whether these “species” are merely extreme forms of variation or represent cryptic species is unclear and needs further work.

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Appendix. Collection identification and GenBank accession numbers for the taxa included in the molecular analysis.

Species, Geographic origin, Voucher specimen, GenBank Accession No.

Anoetangium aestivum (Hedw.) Mitt., Morocco, Rif, MUB 13792, *Cano & Ros s.n.*, AY796280; *Aschisma carniolicum* (F. Weber & D. Mohr) Lindb., Spain, Huelva, MUB 7932, *Cano et al. s.n.*, AY796270; *Barbula unguiculata* Hedw., Germany, Baden-Württemberg, MUB 10325, *Ros & Werner s.n.*, AY437129; *Bryoerythrophyllum recurvirostrum* (Hedw.) P. C. Chen, Italy, Trentino-Alto Adige, MUB 15351, *Ros & Werner s.n.*, AY437130; *Chenia leptophylla* (Müll. Hal.) R. H. Zander, Spain, Murcia, MUB 10427, *Rams s.n.*, AY437134; *Chionoloma bombayensis* (Müll. Hal.) P. Sollman, South Africa, Western Cape Province, MUB 12252, *Arts s.n.*, AY796261; *Chionoloma bombayensis*, China, Guizhou Province, FH, *Tan 91.1096*, AY796278; *Chionoloma bombayensis*, Malawi, BM, *Long 12608*, AY854390; *Chionoloma bombayensis*, Comoros, Mayotte, BM, *Magill & Pocs 13189*, AY854389; *Didymodon rigidulus* Hedw., England, Derbyshire, Herb. Blockeel, *Blockeel s.n.*, AY437106; *Didymodon sinuosus* (Mitt.) Delogne, Italy, Sicily, MUB 13654, *Ros s.n.*, AY437090; *Eucladium verticillatum* (Hedw.) Bruch & Schimp., Germany, Northrhine-Westphalia, BM 000824490, *Schmidt s.n.*, AY854392; *Gymnostomum calcareum* Nees & Hornsch., Spain, Cádiz, MUB 10136, *Guerra et al. s.n.*, AY796279; *Hymenostylium hildebrandtii* (Müll. Hal.) R. H. Zander, Morocco, Anti Atlas, MUB 13690, *Cano et al. s.n.*, AY796282; *Hyophila involuta* (Hook.) A. Jaeger, India, Uttar Pradesh, MUB 12222, *Arts s.n.*, AY796284; *Leptobarbula berica* (De Not.) Schimp., Spain, Balearic Islands, Menorca, MUB 13995, *Cano 1014*, AY796283; *Pleurochaete squarrosa* (Brid.) Lindb., Spain, Sevilla, MUB 11686, *Guerra s.n.*, AY796265; *Pseudocrossidium hornschurchianum* (Schultz) R. H. Zander, Spain, Almería, MUB 9053, *García-Zamora et al. s.n.*, AY437128; *Pseudosymbplepharis schimperiana* (Paris) H. A. Crum, Costa Rica, San José Province, NY, *Holz & Schäfer-Verwimp CR 99-1125*, AY796276; *Tortella densa* (Lorentz & Molendo) Crundw. & Nyholm, Sweden, Gotland, MUB 14121, *Hallingbäck 3780*, AY796267; *Tortella flavovirens* (Bruch) Broth., Australia, South Australia, BM, *Streimann 54900*, AY854413; *Tortella flavovirens*, Greece, Pelopónnisos, MUB 11940, *Cano et al. s.n.*, AY796262; *Tortella fragilis* (Hook. & Wilson) Limpr., Russia, Gorno-Altai, BM, *Ignatov s.n.*, AY854416; *Tortella fragilis*, Greenland, MSUN 0795-869.5, *Daniels s.n.*, AY854417; *Tortella fruchartii* (Mull. Hal.) R. H. Zander, México, Guanajuato, NY 00607643, *Delgadillo s.n.*, AY796269; *Tortella humilis* (Hedw.) Jenn., Spain, Albacete, MUB 17767, *Ros & Werner s.n.*, AY796260; *Tortella humilis*, U.S.A., Arkansas, BM, *Redfearn Jr. s.n.*, AY854418; *Tortella humilis*, Paraguay, Guaira, BM, *Zardini & Aquino 32386*, AY854419; *Tortella inclinata* (R. Hedw.) Limpr., Germany, Northrhine-Westphalia, BM 000824494, *Schmidt s.n.*, AY854420; *Tortella inflexa* (Bruch) Broth., Spain, Alicante, MUB 5892, *Ros & Cano s.n.*, AY796263; *Tortella nitida* (Lindb.) Broth., Spain, Balearic Islands, Mallorca, MUB 14515, *Cano 1061*, AY796264; *Tortella rigens* Alberts., Sweden, Gotland, MUB 14122, *Hallingbäck 38781*, AY796268; *Tortella tortuosa* (Hedw.) Limpr., Morocco, Rif, MUB 10937, *Cano & Ros, s.n.*, AY796266; *Tortella tortuosa*, Canada, Newfoundland, BM, *Hedderston 5548*, AY854422; *Tortella tortuosa* var. *arctica* (Arnold) Broth., Greenland, MSUN 0795-867.1, *Daniels s.n.*, AY854411; *Tortula inermis* (Brid.) Mont., Greece, Steréa Ellas, MUB 14049, *Cano et al. s.n.*, AY437133; *Tortula muralis* Hedw., Yugoslavia, Serbia, MUB 13827, *Sabovljevic s.n.*, AY437132; *Trichostomum austrocrispum* (Beckett) R. H. Zander, New Zealand, South Island, Canterbury, NY, *MacMillan 82/35 & Fife s.n.*, AY796246; *Trichostomum brachydontium* Bruch, Spain, Cádiz, MUB 12650, *Guerra & Ros s.n.*, AY796252; *Trichostomum brittonianum* R. H. Zander, The Bahamas, San Salvador, NY 00607637, *Nash 297*, AY796251; *Trichostomum caespitosum* (Bruch) Jur., Spain, Zaragoza, MUB 15128, *Cano 1138*, AY796271; *Trichostomum crispulum* Bruch, Spain, Murcia, MUB 14239, *Ros s.n.*, AY796249; *Trichostomum sweetii* (E. B. Bartram) L. R. Stark, U.S.A., Nevada, UNLV, *Stark NV-143B*, AY796273; *Trichostomum tenuirostris* (Hook. & Taylor) Lindb., Germany, Northrhine-Westphalia, BM 000824488, *Schmidt s.n.*, AY854429; *Trichostomum tenuirostris* 2, Germany, Northrhine-Westphalia, BM 000824487, *Schmidt s.n.*, AY854428; *Trichostomum tenuirostris* 3, U.K., Peeblesshire, BBSUK C.2001.019.4175, *Long s.n.*, AY796277; *Trichostomum unguiculatum* (Mitt.) R. H. Zander, South Africa, Western Cape Province, MUB 12254, *Arts s.n.*, AY437127; *Triquetrella arapilensis* Luisier, Spain, Ciudad Real, MUB 6465, *Fuertes s.n.*, AY437127; *Triquetrella tristicha* (Müll. Hal.) Müll. Hal., South Africa, Western Cape province, MUB 12218, *Arts s.n.*, AY437125; *Tuerckheimia svihlae* (E. B. Bartram) R. H. Zander, U.S.A., Tennessee, DUKE, *Anderson 24791*, AY796281; *Tuerckheimia valeriana* (E. B. Bartram) R. H. Zander, Costa Rica, San José Province, GOET, *Holz & Schaefer s.n.*, AY854431; *Weissia andersoniana* R. H. Zander, U.S.A., Arizona, DUKE, *Anderson 24574 & Shaw*, AY796230; *Weissia artocosa* R. H. Zander, Yemen, Socotra Island, Herb. Kürschner, *Kilian YP 2557*, AY796240; *Weissia ayresii* Schimp., Mauritius, NY, *Guého 94*, AY796275; *Weissia brachycarpa* (Nees & Hornsch.) Jur., Spain, Ávila, MUB 15609, *Ros & Werner s.n.*, AY796243; *Weissia condensata* (Voit.) Lindb. Europe, Spain, Murcia, MUB 14112, *Ros & Werner s.n.*, AY796226; *Weissia condensata*, U.S.A., Arizona, NY 00364695, *Talbot T0001-4*, AY803774; *Weissia condensata* var. *armata* (Thér. & Trab.) M. J. Cano et al., Spain, Granada, MUB 12922, *Cano 791*, AY796241; *Weissia controversa* Hedw., China, Guizhou Province, NY, *Tan 91-659*, AY796242; *Weissia controversa*, Australia, New South Wales, NY, *Streimann 52993*, AY796257; *Weissia controversa*, [Europe 1], Spain, Albacete, MUB 17754, *Werner s.n.*, AY796222; *Weissia controversa*, [Europe 2], Spain, Albacete, MUB 17753, *Werner s.n.*, AY796223; *Weissia controversa*, U.S.A., Virginia, NY 00615040, *Majestyk 3321*, AY796227; *Weissia controversa* var. *crispata* (Nees & Hornsch.) Nyholm, Spain, Murcia, MUB 13805, *Cano et al. s.n.*, AY796225; *Weissia hedwigii* H. A. Crum, British Columbia, DUKE 115564, *Schofield & Belland 88813*, AY796245; *Weissia jamaicensis* (Mitt.) Grout, Central America, Dominican Republic, Pedernales Province, NY 00607642, *Thompson 9180 et al.*, AY796254; *Weissia jamaicensis*, U.S.A., Missouri, DUKE 26024, *Anderson 26024*, AY796253; *Weissia ligulaefolia* (E. B. Bartram) Grout, Colombia, Departamento del Valle, NY 00607640, *Churchill & Holloender 15280*, AY796229; *Weissia latiuscula* Müll. Hal., South Africa, Northern Province, NY, *Perold 444*, AY796248; *Weissia levieri* (Limpr.) Kindb., Spain, Granada, MUB 14226, *Ros & Rams s.n.*, AY796232; *Weissia longidens* Cardot, China, Guizhou Province, DUKE, *Tan 91-709*, AY796224; *Weissia longifolia*, Morocco, Central Atlas, MUB 13799, *Muñoz s.n.*, AY796234; *Weissia longifolia*, U.K., Shropshire, BBSUK C.2001.019.434, *Sleath s.n.*, AY796235; *Weissia ludoviciana* (Sull.) W. D. Reese & B. A. E. Lemmon, U.S.A., Alabama, DUKE, *Bowers, 15985*, AY796244; *Weissia muehlenbergiana* (Sw.) W. D. Reese & B. A. E. Lemmon, U.S.A., South Carolina, DUKE, *Nelson 13788, Moore & Edwards*, AY796231; *Weissia multicapsularis* (Sm.) Mitt., U.K., East Cornwall, BBSUK C.2001.022.3062, *Holyoak DTH 98-50*, AY796233; *Weissia personii* Kindb., Spain, La Coruña, MUB 12731, *Cano s.n.*, AY796238; *Weissia rostellata* (Brid.) Lindb., U.K., North Devon, BBSUK C.2002.019.381, *Holyoak s.n.*, AY796236; *Weissia rutilans* (Hedw.) Lindb., Germany, Baden-Württemberg, MUB 15003, *Ros & Werner s.n.*, AY796239; *Weissia sharpii* L. E. Anderson & B. A. E. Lemmon, U.S.A., Oklahoma, DUKE, *Allen 6214*, AY796237; *Weissia squarrosa* (Nees & Hornsch.) Müll. Hal., Italy, Trentino-Alto Adige, MUB 14996, *Ros & Werner s.n.*, AY796247; *Weissia triumphans* (De Not.) M. O. Hill, Spain, Almería, MUB 14993, *Werner & Ros s.n.*, AY796272; *Weissia wimmeriana* (Sendt.) Bruch & Schimp., Italy, Trentino-Alto Adige, MUB 14995, *Ros & Werner s.n.*, AY796220.