Tortula and some related genera (Pottiaceae, Musci): phylogenetic relationships based on chloroplast *rps*4 sequences

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Abstract. According to chloroplast *rps*4 sequence data the genus *Syntrichia* forms a monophyletic clade clearly separated from *Tortula*, while *Pottia* is shown to be polyphyletic and the *Rhynchostegiae* species (with rostrate lid) are very similar to *Tortula*. *Crossidium* is probably polyphyletic. The close affinity of *Desmatodon*, *Stegonia*, *Pterygoneurum* and some of the species of *Phascum* with *Tortula* is confirmed by the molecular data.

Key words: Bryophyta, Pottiaceae, *Tortula*, *Crossidium*, *Pottia*, molecular phylogeny, *rps*4.

Introduction

The generic boundaries in the Tribe Pottieae (Limpr.) Dixon of the subfamily Pottioideae (Limpr.) Broth., family Pottiaceae Bruch & Schimp., notably in the series *Pottia-Desmatodon-Tortula-Syntrichia* are particularly difficult to delimit (Blockeel 1990), as the taxonomy of these genera is mainly based on their sporophytic characters, more specifically on the height of the basal membrane of the peristome and on the patterns which the teeth follow when they divide above this membrane (Limpricht 1890, Mönkemeyer 1927, Lawton 1971, Smith 1978, Nyholm 1989). Thus, the genus *Desmatodon* Brid. has been characterised

by a peristome of 16 mainly erect cleft teeth, stretching from near the base or from a low basal membrane up to the apex, becoming divided into two filaments. *Pottia* (Rchb.) Ehrh. ex Fürn. has been separated by the presence of a cleistocarpous or stegocarpous capsule (gymnostomous or with a peristome of 16 imperfect or lanceolate bifid teeth) and *Tortula* Hedw. has been characterised by 32 filiform twisted teeth, united at the base into a tubular membrane of varying length. Also, some authors have considered the genus *Syntrichia* Brid. for including the species of *Tortula* with a twisted peristome from a high spirally tessellated tube (Agnew and Vondráček 1975). However, this separation has not been satisfactory and there is controversy on the taxa that should be included in each genus.

In his new treatment of the Pottiaceae genera, in which the gametophytic characters were emphasized, Zander (1989, 1993) convincingly proposed some characters to segregate genera such as *Chenia* R. H. Zander and *Syntrichia* from *Tortula*. According to this author, *Chenia* differs from *Tortula* in its dentate upper leaf margins, large epapillose laminal cells, thin costa, and red KOH reaction of the upper laminal cells, while *Syntrichia* differs in the crescent shape of the

steroid band in the leaf transverse section, lack of differentiated dorsal epidermal cells, and red KOH reaction. On the other hand, this author included in *Tortula* sect. *Pottia* (Rchb.) Kindb. taxa that had traditionally been placed in other genera, such as *Pottia* (the species with rostrate lid, named by Warnstorf (1916) as *Rhynchostegiae*) or *Phascum* Hedw. (*Phascum cuspidatum* Hedw.), but with gametophytic characters very similar to those of *Tortula*. For the same reason, he included the genus *Desmatodon* in *Tortula* sect. *Tortula* Broth.

There is general agreement concerning some taxonomic changes and phylogenetic considerations provided by Zander (1993) (e.g. the separation of *Syntrichia* from *Tortula*) although other aspects such as the relation of *Pottia*, *Phascum* and *Desmatodon* with *Tortula* are more debatable. Also, the relationships between some genera of Pottieae, whose origin has been supposed to be close to a *Tortula*-like ancestor (Delgadillo 1975, Frey et al. 1990, Cano et al. 1993, Zander 1993) remain unresolved. This is the case with the genera *Crossidium* Jur. and *Pterygoneurum* Jur., which differ from *Tortula* in the presence of supranervial filaments or lamellae, respectively. *Aloina* Kindb. also presents this kind of chlorophyllic structures on the ventral side of the lamina but is excluded from our study because previous DNA assays made by the authors (Werner et al. unpublished) demonstrated that this genus does not belong to the group of genera closely related to *Tortula*. In addition, *Stegonia* Venturi has been supposed to be related to *Pottia* by some authors (Limpricht 1890, Mönkemeyer 1927, Chen 1941, Wareham 1972). However, it has been treated as an independent genus by other recent authors, such as Smith (1978), Nyholm (1989) and Zander (1993).

The purpose of the present paper is to provide a new and independent dataset (chloroplast *rps*4 sequence) to clarify the circumscription of the genus *Tortula* and the phylogenetic relationships between *Tortula* and some related genera.

Material and methods

Plant material. 36 sequences representing 35 taxa were included in this study. A previous study based on *rps*4 sequences representing a great range of the Pottiaceae (unpublished data) suggests that the clade studied here is monophyletic. *Barbula unguiculata* Hedw. was chosen as outgroup species because it was situated in the sister clade.

Table 1 lists all taxa used in this study, the voucher specimen data or reference, the origin and GenBank accession numbers. Vouchers are deposited at MUB.

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 *rps*4 gene was amplified in a 50 μl final volume with the primers rps5 (Nadot et al. 1994) and trnas (Buck et al. 2000). 1 μl of stock DNA was added as template. The amplification conditions were as follows: 3 min at 94 °C, 35 cycles with 15 sec 94 °C, 30 sec 50 °C and 1 min 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 aligning 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 a ABI Prism 3700 automatic sequencer (Perkin Elmer). The sequences were edited using Bioedit 5.0.9 (Hall 1999) and aligned manually. The data are available from the corresponding author on request. 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

Table 1. Taxa included in the *rps4* sequence analysis. All sequences are submitted to GenBank

Species	Voucher or reference	Origin	GenBank #
Barbula unguiculata Hedw.	MUB 10325	Germany, Baden-Württemberg	AF480952
Crossidium aberrans Holz. & E.B. Bartram	MUB 6089	Spain, Albacete	AF481006
Crossidium crassinerve (De Not.) Jur.	MUB 11622	Morocco, Anti-Atlas	AF481045
Crossidium davidai Catches.	MUB 5349	Spain, Canary Islands, Lanzarote	AF481005
Crossidium laevipilum Thér. & Trab.	MUB 11665	Morocco, Anti-Atlas	AF481046
Crossidium seriatum H.A. Crum & Steere	UNLV, Arizona	U.S.A., Arizona	AF481007
Crossidium seriatum H.A. Crum & Steere	MUB 4354	Spain, Albacete	AF481010
Crossidium squamiferum (Viv.) Jur.	MUB 7982	Spain, Huelva	AF481004
Desmatodon latifolius (Hedw.) Brid.	MUB 11476	Spain, Granada	AF480954
Leptophascum leptophyllum (Müll. Hal.) J. Guerra & M.J. Cano	MUB 10427	Spain, Murcia	AF480960
Microbryum curvicolle (Hedw.) R.H. Zander	MUB 8269	Spain, Almería	AF480971
Microbryum davallianum (Sm.) R.H. Zander	MUB 12537	Spain, Alicante	AF480983
Microbryum rectum (With.) R.H. Zander	MUB 11199	Spain, Valencia	AF480970
Phascum cuspidatum Hedw.	MUB 11344	Spain, Sevilla	AF480972
Pottia intermedia (Turner) Fürnr.	MUB 5430	Germany, Nordrhein Westfalen	AF481013
Pottia lanceolata (Hedw.) Müll. Hal.	MUB 10334	Spain, Murcia	AF480988
Pottia pallida Lindb.	MUB 8768	Spain, Almería	AF480987
Pottia truncata (Hedw.) Bruch & Schimp.	MUB 10336	Spain, Cadiz	AF481044
Pottia wilsonii (Hook.) Bruch & Schimp.	MUB 8740	Spain, Almería	AF481011
Protobryum bryoides (Dicks.) J. Guerra & M.J. Cano	MUB 4936	Spain, Alicante	AF480986
Pterygoneurum lamellatum (Lindb.) Jur.	MUB 11484	Spain, Murcia	AF480959
Stegonia latifolia (Schwägr.) Venturi ex Broth.	La Farge et al. (2000)	-	AF222901
Syntrichia ammonsiana (H.A. Crum & L.E. Anderson) Ochyra	MUB 12225	South Africa, Western Cape Prov.	AF480957
Syntrichia caninervis Mitt.	MUB 10977	Spain, Guadalajara	AF480958
Syntrichia leucostega (Müll. Hal.) R.H. Zander	MUB 12226	South Africa, Western Cape Prov.	AF480982
Syntrichia montana Nees	MUB 10881	Morocco, Rif	AF481031
Syntrichia papillosa (Wils.) Jur.	MUB 12227	South Africa, Western Cape Prov.	AF481032
Syntrichia rigescens (Broth. & Geh.) Ochyra	MUB 11378	Morocco, High-Atlas	AF481037
Syntrichia ruralis (Hedw.) Brid.	MUB 10887	Morocco, Rif	AF480981

Table 1 (continued)

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Tortula atrovirens (Sm.) Lindb.	MUB 11352	Spain, Seville	AF480990
Tortula brevissima Schiffn.	MUB 4933	Spain, Granada	AF481009
Tortula canescens Mont.	MUB 11381	Spain, Albacete	AF481001
Tortula muralis Hedw.	MUB 12540	Spain, Canary Islands, La Palma	AF481016
Tortula revolvens (Schimp.) G. Roth	MUB 11347	Spain, Malaga	AF481030
Tortula subulata Hedw.	MUB 10951	Morocco, Rif	AF481000
Tortula vahliana (K.F. Schultz) Mont.	MUB 2513	Spain, Alicante	AF481042

descent = no, COLLAPSE = yes. Parsimony analysis was not expected to swap to completion (see Soltis et al. 1998). Maxtrees was set to 100000. Parsimony analysis were performed with equal weighting of characters and transformations. A bootstrap analysis with 100 replicates was performed with the settings as mentioned before, but with MAXTREES set to 10000.

The Bayesian approach (Rannala and Yang 1996, Mau and Newton 1997, Mau et al. 1999) is similar to Maximum Likelihood (ML) 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 re-scales likelihoods to true probabilities in that the sum of the probabilities over all trees is 1.0 with the Bayesian approach, which means that ordinary probability theory can be used to analyze the data (Hall 2001). The program MrBayes (Huelsenbeck and Ronquist, in press) was used to obtain an estimation of the phylogenetic relationships under the Bayesian approach. Four charsets were defined representing the codon position of the coding region and the noncoding spacer. A partition (bycodon) in these four charsets was defined. The following settings were used: lset = nst6, rates = sitespec, sitepartition = bycodon. The Markow chain settings were: ngen = 400 000, printfreq = 1000, samplefreq = 100, nchains = 4. The first 100 000 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.

Results

The aligned sequences have a length of 661 bp. All *Tortula* s.l. species share a 4 bp deletion compared with *Barbula unguiculata*. 129 bp of the aligned sequences were variable and 61 of them parsimony informative.

Figures 1 and 2 show the phylograms obtained under the Bayesian approach and
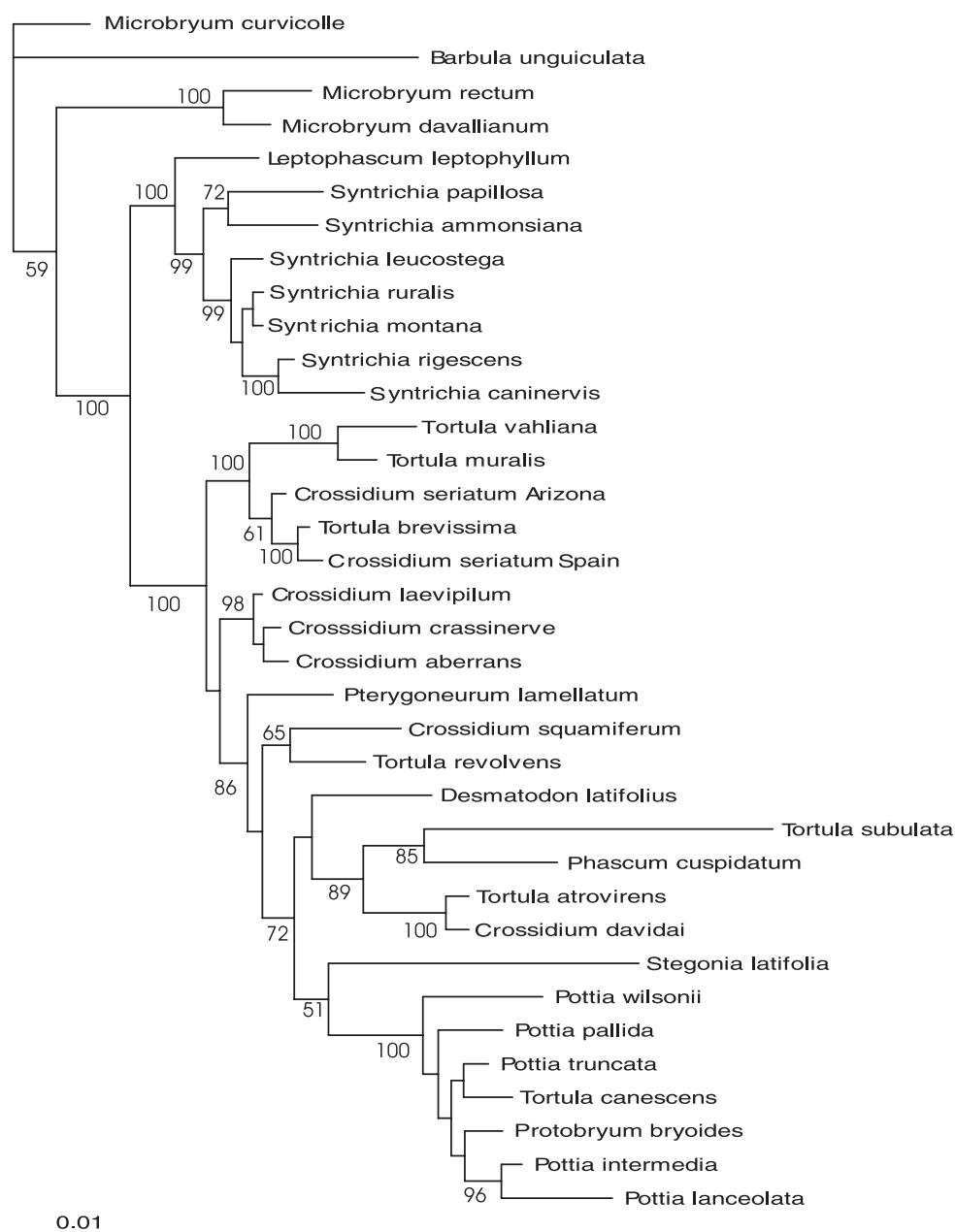
Fig. 1. Bayesian tree for 36 Pottieae *rps*4 sequences. The general time-reversible model was assumed. Site specific rate variations were assigned for the three codon positions and the noncoding spacer. The tree is rooted with the sequence of *Barbula unguiculata*. Numbers indicate the clade credibility values of the nodes. Values below 50% are not shown

using the Neighbor-Joining method. In addition, Fig. 2 gives the bootstrap values obtained by Maximum Parsimony.

Microbryum curvicolle (Hedw.) R. H. Zander, *M. davallianum* (Sm.) R. H. Zander and *M. rectum* (With.) R. H. Zander were

clearly separated from all the taxa supposedly closely related to *Tortula*.

All species of *Syntrichia* form a monophyletic lineage and are a sister clade to *Leptophascum leptophyllum* (Müll. Hal.) J. Guerra & M. J. Cano.
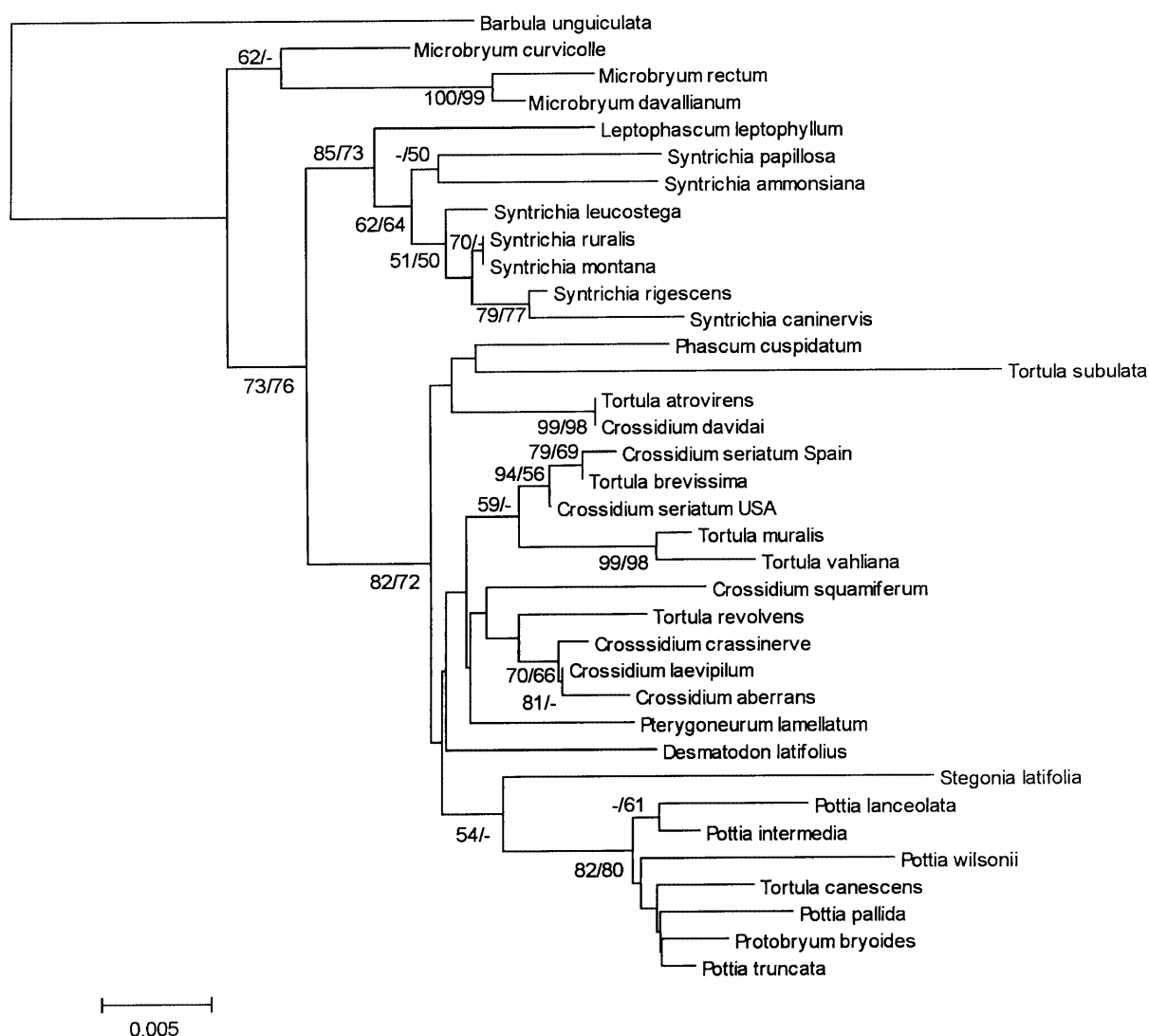
Fig. 2. Neighbor-Joining tree of the 36 *rps*4 sequences using Kimuras two parameter distances. Numbers on the branches indicate percentage support in 1000 bootstrap replications/percentage support in 100 bootstrap replications using unweighted Maximum Parsimony. Bootstrap values below 50% are not shown

Crossidium laevipilum Thér. & Trab., *C. crassinerve* (De Not.) Jur. and *C. aberrans* Holz. & E. B. Bartram are in the same clade, but *C. squamiferum* (Viv.) Jur. is included in the clade with *Tortula revolvens* (Schimp.) G. Roth and other species of the same and other genera. *Crossidium davidai* Catches. is most closely related to *Tortula atrovirens* (Sm.) Lindb. and *Crossidium seriatum* (H.A. Crum) Steere is found near *Tortula brevissima* Schiffn., *T. muralis* Hedw. and *T. vahliana* (Schultz.) Mont.

Pterygoneurum lamellatum (Lindb.) Jur., *Desmatodon latifolius* (Hedw.) Brid., and *Phascum cuspidatum* are situated within the *Tortula*-complex, although their exact placement within this complex remains ambiguous.

The *Pottia* species belonging to the *Rhynchostegiae* group (according to Warnstorf 1916) and *Protobryum bryoides* (Dicks.) J. Guerra & M. J. Cano, both included by Zander within *Tortula*, are situated together with *Tortula canescens* Mont. on a clade with high bootstrap support (82% with NJ,

80% with MP, 100% clade credibility value under Bayesian inference) with *Stegonia latifolia* (Schwägr.) Venturi ex Broth. as sister group.

Our data also support the separation of *Protobryum bryoides* from the cleistocarpous species of the genus *Microbryum*.

Discussion

From our molecular analysis it is clear that the species placed in *Microbryum* Schimp. by Zander (1993) are not closely related with the species considered by this author in *Tortula* sect. *Pottia*. In this clade are included the cleistocarpous *M. curvicolle* which was previously considered by other authors (e.g. Mönkemeyer 1927, Smith 1978, Nyholm 1989) to belong to the genus *Phascum*, the stegocarpous *M. davallianum* with a conic lid (placed in *Pottia* by Warnstorf (1916) as *Conostegiae*), and the cleistocarpous *M. rectum*, which has a slightly differentiated lid and considered by other authors to belong to the genera *Pottia* (Mönkemeyer 1927, Smith 1978, Nyholm 1989) or *Phascum* (Limpricht 1890). Guerra and Cano (2000) suggested that the *Conostegiae* species of *Pottia* (including the stegocarpous *P. starckeana* (Hedw.) Müll. Hal. and *P. davalliana* (Sm.) C.E.O. Jensen) are phylogenetically closely related with the cleistocarpous *Microbryum*, due to the great similarity of the morphological features of their gametophytes. On the other hand, the *rps*4 data indicate that the *Conostegiae* group of *Pottia* and the other group of species with rostrate operculum (*Rhynchostegiae*), such as *P. intermedia* (Turner) Fürnr., *P. lanceolata* (Hedw.) Müll. Hal., *P. pallida* Lindb. and *P. truncata* (Hedw.) Bruch. & Schimp., belong to two different evolutionary lineages. Thus, both the data presented here and by Zander (1993), imply that the old concept of the genus *Pottia* section or subgenus *Eupottia* (*Conostegiae* + *Rhynchostegiae*) cannot be upheld. Zander (1993) included the *Rhynchostegiae* species of *Pottia* as well as *Pottia bryoides* within *Tortula*. The last species has been considered by some

authors (e.g. Brotherus 1924, Mönkemeyer 1927, Nyholm 1989) to form its own section or subgenus (*Mildeella*) within *Pottia* or as an independent genus, *Mildeella bryoides* (Dicks.) Limpr. (genus *Mildeella* Limpr. nom. illeg.) or *Protobryum bryoides*, due to the presence of a cleistocarpous capsule but with a slightly differentiated and non-functional peristome. The *Rhynchostegiae* species of *Pottia*, *Protobryum bryoides* and *Tortula canescens* form a relatively well supported clade, with *Stegonia latifolia* as a possible sister.

Stegonia latifolia has been considered as a member of *Pottia* despite its different gametophyte, such as the bulbiform plants, very concave, nearly circular to very broadly ovate or elliptical leaves and the weak costa ending below the leaf apex. The well developed peristome is similar to that of some *Pottia* species. Zander (1993) also included in *Stegonia* a cleistocarpous species, (*S. hyalinotricha* ["*um*"] Cardot & Thér.) R. H. Zander, stating that "this genus, like *Tortula*, shows a reduction series in sporophyte characters". Our present data suggest a phylogenetic proximity of *Stegonia latifolia* to the *Rhynchostegiae* species of *Pottia*, but the relatively poor bootstrap support (54% with NJ, below 50% with MP, 51% clade credibility value using Bayesian inference) preclude a definitive conclusion.

The *Pottia* clade might be interpreted as a group in which reduced peristomes evolved. The only *Pottia*-like species of this clade with a well developed peristome is *Pottia lanceolata*, with long, lanceolate erect teeth; *Protobryum bryoides* being the only cleistocarpous species with some non-functional peristomial processes. *Tortula canescens*, with its long twisted peristome and developed basal membrane, might represent a model of the common ancestor in this clade. This species appears within the *Rhynchostegiae* clade of *Pottia* but, since the support values within this clade are low, one cannot be very confident about the exact topology within this clade. This is also demonstrated by conflicting topologies obtained by different methods of data analysis (Figs. 1 and 2).

The analysis supports the recognition at genus level of *Syntrichia*. The genus *Syntrichia* was established in Bridel (1801) but later its species were generally considered to belong to *Tortula* (De Notaris 1838, Lawton 1971, Smith 1978, Crum and Anderson 1981, Noguchi 1988, Mishler 1994), probably due to difficulties in separating some species of the two genera based on sporophytic characters. Subsequently Zander (1989, 1993) separated the two genera based on differences in nerve anatomy. Many authors agreed with Zander in reestablishing *Syntrichia* as a genus (Düll 1992; Ochyra 1992, 1994; Anderson 1997; Gallego et al. 2003, in press). ITS 1 sequence data showed that *Tortula muralis* and *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr are only distantly related (Spagnuolo et al. 1999), but this dataset did not include more species of these two genera.

Leptophascum leptophyllum is a species with an unclear taxonomic position. For many years it has been considered as a member of *Tortula* (*T. rhizophylla* (Sakurai) Z. Iwats. & K. Saito), although Corley et al. (1981) mentioned that it "is completely anomalous in *Tortula*". Zander (1989) included it in the newly created genus *Chenia* R. H. Zander. Arts and Sollmann (1991) discovered that *Phascum leptophyllum* Müll. Hal. is the earliest name for this species. Finally Guerra and Cano (2000) created the monotypic genus *Leptophascum* J. Guerra & M. J. Cano. Based on a cladistic analysis of morphological data, Zander (1993) came to the conclusion that it is related to *Syntrichia*, a point of view supported by our data in spite of the few shared morphological features.

The distinctive character of the genus *Crossidium* is the presence of supracostal filaments, 1–12 cells in length, in the upper part of the leaf and forming a pad. This character seems to be an adaption to arid climates (Frey and Kürschner 1988, Guerra et al. 1992). Similar morphological adaptions are found in other Pottiaceae, such as *Aloina*, *Aloinella* Cardot and some species of *Pseudocrossidium* R.S. Williams. The morphological

traits of *Crossidium* are profoundly discussed by Delgadillo (1975) and Cano et al. (1993). Zander (1993) suggested that *Crossidium* sect. *Pseudocrossidium* Holz. & E.B. Bartram (*C. aberrans* Holz. & E.B. Bartram and *C. seriatum* included in this study) may be more closely related with *Tortula* sect. *Pottia* than to *Crossidium* s. str., through such species as *T. atherodes* R.H. Zander (= *Phascum cuspidatum*) and *T. protobryoides* R.H. Zander (= *Protobryum bryoides*) with their flask-shaped protuberant ventral costal cells, or *Tortula* sect. *Tortula* (forms like *T. atrovirens* or *T. revolvens* included here) with their ventral costal pads of vertically elongate cells.

Crossidium davidai is the only studied *Crossidium* species lacking a hyaline hairpoint on the leaves. *Crossidium seriatum* possesses, like *C. davidai*, 4–6 papillae per cell and short filaments, while *Crossidium aberrans* has 1–2 papillae and also short filaments. In the other species included here (*C. crassinerve* and *C. laevipilum*) the papillae are very rare and the filaments are longer. Finally, *Crossidium squamiferum* is characterised by smooth cells, up to 10–11 cells long filaments, and thickened cell walls in leaves and filaments. Interestingly, *Tortula revolvens*, the closest neighbour of *Crossidium squamiferum*, differs from other *Tortula* s.l. species in its reduced papillosity, while *T. brevissima* and *T. muralis* show a papillosity similar to *Crossidium seriatum*. The *rps*4 data suggest that *Tortula atrovirens* is closely related to *Crossidium davidai* (99%, 98% and 100% support with NJ, MP and Bayesian inference), while *Tortula brevissima*, *T. muralis* and *T. vahliana* are closely related to *Crossidium seriatum* (59% and 100% support with NJ and Bayesian inference, below 50% with MP), and *T. revolvens* might form a monophyletic clade with *Crossidium squamiferum*, although this is only supported by Bayesian inference. Our study therefore suggests that *Crossidium* is polyphyletic. *Tortula brevissima* is morphologically very close to *Crossidium seriatum* and, especially in Europe, it is sometimes difficult to distinguish between these species. The same is true for the pair of

species formed by *Tortula atrovirens* and *Crossidium davidai*, although it should be borne in mind that the Canarian specimens of the latter are morphologically closer to *Tortula atrovirens* than the Australian specimens. Thus samples from Northern Africa with their supracostal filaments 2(3) cells high have been considered to be xerophytic modifications of *Tortula atrovirens* growing in very dry conditions (Cano et al., 2002). In these two cases, a more detailed investigation will be necessary to delimit the species boundaries. Our data suggest that the filaments on the ventral side of the costa, which are typical of species placed in the genus *Crossidium*, have evolved from ancestors close to the genus *Tortula*, as proposed by Chen (1941), Delgadillo (1975) and Cano et al. (1993), however not once, but several times. According to the *rps*4 data, *Crossidium aberrans* is placed near *C. crassinerve* and *C. laevipilum*, although *C. aberrans* is more similar to some species of *Tortula* because of the short supracostal filaments. Therefore this species might represent a model for the transition between *Tortula* and the species of *Crossidium* with long supracostal filaments and smooth or slightly papillose laminal cells.

Similar adaptions to those of *Crossidium* are present in the genus *Pterygoneurum*, consisting of lamellae on the ventral surface of the costa. Nevertheless sporophytic characters are very heterogeneous within the genus. For instance, *P. ovatum* (Hedw.) Dix., *P. sampaianum* (A. M. L. Guim.) A. M. L. Guim. and *P. subsessile* (Brid.) Jur. have gymnostomous capsules and large spores (25–57 (60) μm). The last species has an immersed capsule, while *P. lamellatum* has an exerted one, a peristome with a basal, sometimes fragile membrane, and peristome teeth ranging from short to moderately long and slightly twisted; furthermore, this species possesses smaller spores (15–25(30) μm) than the rest of the species of *Pterygoneurum*. The spores of *P. lamellatum* are more similar in size and morphology to those present in the genera *Crossidium* and *Tortula* (Carrión et al. 1995). Also, some taxa

with a cleistocarpous capsule are included in the genus (*P. kozlovii* Laz. and *P. subsessile* var. *kieneri* Habeeb). Frey et al. (1990), Delgadillo (1975) and Zander (1993) discussed the possibility that *Pterygoneurum* might be derived from (ancestors of) *Crossidium*. In our analysis, the position of *P. lamellatum* is not clearly resolved. With NJ it seems to be closely related with *Tortula revolvens* and some species of *Crossidium*, but the bootstrap support is below 50% and the data are conflicting with the Bayesian tree. Data on the species with large spores and a gymnostomous capsule and on those with a cleistocarpous capsule are not available (the assays with *P. ovatum* were not successful). Therefore, a final conclusion cannot be drawn with the present data. Whatever the case, it seems that morphological adaptions such as filaments or lamellae on the ventral surface of the costa, developed independently at various times within the Pottioideae.

The *rps*4 sequence data clearly place *Desmatodon latifolius* and *Phascum cuspidatum* within *Tortula* s.l. excluding *Syntrichia*. Both are situated in somewhat isolated clades. *Phascum cuspidatum* might be related with *Tortula subulata*, but the support is below 50% with both NJ and MP, while Bayesian inference gives a clade credibility value of 85%.

Definition of the genus *Tortula* remains unresolved. At present it comprises a high number of species with a heterogeneous combination of morphological characters in the gametophyte, and only a deeper study including as many species as possible and using additional molecular markers will help resolve the problem.

The molecular analysis carried out suggests that the evolution within Pottiaceae is characterised by a high degree of parallel and convergent evolution. Characters such as cleistocarpous capsules, hairy filaments on leaves or costa, probably have evolved various times and independently as a response to environmental pressures. This leads to a situation where it is extremely difficult to reconstruct phylogenetic relationships within this family

based on morphological data alone. Due to the theoretically almost unlimited number of characters, molecular analysis combined with classical morphological technique should contribute to a better understanding of the phylogeny of this important family of mosses.

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