Available online at www.sciencedirect.com
SCIENCE DIRECT®
Organisms, Diversity & Evolution 6 (2006) 33–45
ORGANISMS DIVERSITY & EVOLUTION
www.elsevier.de/ode

Phylogenetic relationships of the moss genus *Pleurochaete* Lindb. (Bryales: Pottiaceae) based on chloroplast and nuclear genomic markers

Michael Grundmann[a,*], Harald Schneider[b], Stephen J. Russell[a], Johannes C. Vogel[a]

[a]*Department of Botany, The Natural History Museum, Cromwell Road, London SW7 5BD, UK*
[b]*Department of Systematic Botany, Albrecht von Haller Institute of Plant Sciences, Georg August University Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany*

Received 9 November 2004; accepted 18 April 2005

Abstract

The phylogenetic relationships of the moss genus *Pleurochaete* was investigated using evidence from chloroplast and nuclear ribosomal DNA sequences (*atpB-abcL* spacer, *rps4*+*rps4-trnS* IGS, *trnL-trnF* region, and ITS1-5.8S-ITS2 region). Monophyly of *Pleurochaete* is confirmed, but the genus is nested within *Tortella*. Five highly supported clades, including *Chionoloma*, *Pseudosymblepharis* and *Trichostomum tenuirostre*, were found, partially corresponding to phytogeographic areas. However, denser sampling is needed to resolve subgeneric relationships. Within *Pleurochaete* three monophyletic clades were recovered: neotropical *Pleurochaete luteola*, European *Pleurochaete squarrosa*, and North American *P. squarrosa*. The relationships between and taxonomic status of these clades are not resolved. Our results point to two hypotheses to explain the current situation: (1) an ancient, wide distribution of *P. squarrosa* on the Laurasian continent, with a subsequent split into two genetically isolated clades and sympatric ecological isolation of *P. luteola*; and (2) a neotropical origin of the genus, followed by long-distance dispersal of *P. squarrosa* into Eurasia. In contrast to previous molecular studies on transatlantic bryophytes, no evidence was found of recent intercontinental gene flow in *P. squarrosa*. Consequently, the two genetically isolated but morphologically indistinguishable clades of *P. squarrosa* may represent a further example for either lineage sorting or cryptic speciation in mosses.

Keywords: Molecular phylogeny; Bryophytes; Cryptic species; Transatlantic distributions; nrITS; cpDNA

Introduction

The systematic position of the small moss genus *Pleurochaete* Lindb. has undergone considerable change over the last 150 years. Morphological characters have been employed to place *Pleurochaete* either as a genus in its own right or as a subgenus within *Tortella*. Bryophytes often display plasticity within recognised taxa and lack distinct morphological characters that facilitate unequivocal systematic treatments. Thus molecular data can help to clarify such long-standing scientific disputes.

Currently four species are recognised in *Pleurochaete* (Zander 1993). *Pleurochaete luteola* (Besch.) Thér. is entirely neotropical (south-eastern United States, around the Gulf of Mexico and scattered throughout South America). *Pleurochaete squarrosa* (Brid.) Lindb. is widely distributed in southern North America (southern United States, Mexico), the Macaronesian Islands,

*Corresponding author
E-mail address: M.Grundmann@nhm.ac.uk (M. Grundmann).

1439-6092/$ - see front matter © 2005 Gesellschaft für Biologische Systematik. Published by Elsevier GmbH. All rights reserved.
doi:10.1016/j.ode.2005.04.005

throughout Mediterranean Europe and North Africa to East Africa (Djibouti, Ethiopia, Kenya, Tanzania), and Asia (Turkey, Iraq, Iran, northern India, China). In Europe, its distribution extends to thermophilous sites as far north as England and Wales, the Netherlands, and northern Germany (Düll 1984). The status of two further species, *Pleurochaete beccarii* Venturi and *P. malacophylla* (Müll. Hal.) Broth., is doubtful. Both species are known from only a few old collections and are not available for molecular study (O'Shea 2003). Morphologically they have been identified as identical or doubtfully distinct from *P. squarrosa*, and already Zander (1993) was not able to investigate type material of *P. beccarii*. The genus *Pleurochaete* is part of the largest family of mosses, the Pottiaceae, which comprises some 1500 species (Zander 1993). *Pleurochaete* is generally placed in the subfamily Trichostomoideae together with species-rich genera such as *Tortella* (Lindb.) Limpr., *Trichostomum* Bruch, and *Weissia* Hedw., and some small and even monotypic genera such as *Calymperastrum* I.G. Stone, *Calyptopogon* (Mitt.) Broth., *Chionoloma* Dixon, *Pseudosymblepharis* Broth., and *Tuerckheimia* Broth. Sollman (2000) transferred all Asian species of *Pseudosymblepharis* to the genus *Chionoloma*. The circumscription of the subfamily is controversial, and the placement of putative relatives, such as *Eucladium* Bruch & Schimp. and *Hyophila* Brid., has been investigated recently (Werner et al. 2004a, b, 2005).

Pleurochaete is morphologically very similar to *Tortella* with which it shares features such as the structurally almost identical sporophyte and the differentiation of sharply separated thin-walled and incrassate cells in the leaf base. Other morphological characters clearly distinguish *Pleurochaete* from *Tortella*. Unique to *Pleurochaete* are the differentiated, thin-walled marginal leaf cells, which extend up from the point of insertion often to above midleaf, whereas in *Tortella* these cells form a coherent basal V-shaped area extending medially to the costa (Crum and Anderson 1981). The status of *Pleurochaete* changed repeatedly in the last 150 years, depending on how authors viewed morphological similarities or dissimilarities in relation to *Tortella*. The type species, *P. squarrosa,* was originally described as *Barbula squarrosa* by Bridel (1827). Lindberg (1864) erected the new genus *Pleurochaete* and highlighted the perichaetia emerging on short lateral branches as the key character for its recognition. A few years later, Limpricht (1888) reduced *Pleurochaete* to a subgenus of *Tortella*. At the end of the 20th century *Pleurochaete* was again treated as a genus distinct from *Tortella*, with special emphasis placed on the position of the perichaetia (Zander 1993; Eckel 1998).

Some recent phylogenetic studies of Pottiaceae placed *P. squarrosa* as sister to *Tortella flavovirens* (Bruch) Broth., but this relationship was only weakly supported in a Bayesian inference (Spagnuolo et al. 1999; Werner et al. 2004b). Such previous studies either were based on insufficient taxon sampling or relied on single molecular markers only, either nuclear ITS or plastidic *rps4*. In this study, we aim to clarify the position of *Pleurochaete* and study the relationship between *P. luteola* and *P. squarrosa* using evidence from three chloroplast genome regions (*atpB-rbcL* spacer, *rps4*+*rps4-trnS* IGS, and *trnL-trnF* region) and from the nuclear ribosomal intergenic spacer regions (nrITS1, 2). We sampled widely within *Tortella*, thus are able to explore two alternative hypotheses: (1) *Pleurochaete* is the sister of *Tortella*; or (2) *Pleurochaete* is nested within *Tortella*.

Material and methods

Taxon sampling

A total of 46 samples were collected, with special emphasis on the genera *Tortella* and *Pleurochaete*. For the latter we included three samples of *P. luteola*, three samples of *P. squarrosa* from North America, and ten samples of *P. squarrosa* from different regions of Europe. East African and East Asian samples have been unavailable, but hopefully will be included in our ongoing studies on phylogeography and population genetics of *Pleurochaete*. In addition to *Tortella* and *Pleurochaete* as the two key genera, representatives of seven other genera of Trichostromoideae were included: *Chionoloma*, *Eucladium*, *Hyophila*, *Pseudosymblepharis*, *Trichostomum*, *Tuerkheimia*, and *Weissia*. Two representatives of Pottioideae, *Didymodon rigidulus* Hedw. and *Triquetrella tristicha* (Müll. Hal.) Müll. Hal., were included as outgroup taxa. Table 1 gives a complete list of taxa used in this study, the corresponding GenBank accession numbers, and the voucher specimen information. Material for DNA extraction was collected in the field or taken from herbaria.

DNA extraction and sequencing

Using a modified CTAB method (Doyle and Doyle 1987), total genomic DNA was extracted from single shoots in *Pleurochaete* and other larger species, from several shoots each in small species. Samples were ground using pestle and mortar with acid-washed sand. Extractions used 500 µl CTAB buffer, 50 µl sarkosyl buffer and 5 µl β-mercaptoethanol, and were incubated at 60 °C for 1 h. During incubation the samples were vortexed occasionally. An equal volume of SEVAC (chloroform:isoamylalcohol, 24:1) was added, the mixture vortexed and centrifuged at 13,000 rpm for 3 min. Clear supernatants were transferred to fresh tubes without disturbing the white interface. After repeating the SEVAC procedure, the supernatants were combined with a 2/3 volume of ice-cold isopropanol, and incubated for 1–2 h on crushed ice. The isopropanol

Table 1. Taxa sampled, voucher details, sample origins, collectors, and GenBank accession numbers

No.	Taxon	Voucher no. /BM barcode	Herbarium	Origin	Collector	GenBank accession number			
						ITS	*atpb-rbcL*	*rps4*	*trnL-F*
1	*Chionoloma bombayense* (C. Muell.) Sollman	R.E Magill & T. Pocs 13189	BM	Comoros, Mayotte	R.E Magill & T. Pocs	AY854389	AY950308	AY950353	AY950399
2	*Chionoloma bombayense* (C. Muell.) Sollman	D.G. Long 12608	BM	Malawi	D.G. Long	AY854390	AY950309	AY950354	AY950400
3	*Didymodon rigidulus* Hedw.	FJR15	BM	United Kingdom		AY854391	AY950310	AY950355	AY950401
4	*Eucladium verticillatum* (Hedw.) Bruch & Schimp.	BM000824490	BM	Slovenia, Triglav Nature Reserve	MG & JCV	AY854392	AY950311	AY950356	AY950402
5	*Hyophila involuta* (Hook.) A. Jaeger	BM000824492	BM	Belize	F.J. Rumsey	—	AY950312	AY950357	AY950403
6	*Pleurochaete luteola* (Besch.) Thér.	W.S. Judd & J.D. Skean Jr. 6812	GOET	Haiti	W.S. Judd & J.D. Skean Jr.	AY854393	AY950313	AY950358	AY950404
7	*Pleurochaete luteola* (Besch.) Thér.	BM000824501	BM	Mexico, Veracruz	C.J. Cox	AY854394	AY950314	AY950359	AY950405
8	*Pleurochaete luteola* (Besch.) Thér.	F.D. Bowers 15236	SMS	USA, Alabama	F.D. Bowers	AY854395	AY950315	AY950360	AY950406
9	*Pleurochaete squarrosa* (Brid.) Lindb.	BM000824506	BM	Germany, Bavaria	MG	AY854396	AY950316	AY950361	AY950407
10	*Pleurochaete squarrosa* (Brid.) Lindb.	BM000824511	BM	Croatia, Dubrovnik	MG & JCV	AY854397	AY950317	AY950362	AY950408
11	*Pleurochaete squarrosa* (Brid.) Lindb.	BM000824502	BM	Hungary, Baranya Megye	B. Papp	AY854398	AY950318	AY950363	AY950409
12	*Pleurochaete squarrosa* (Brid.) Lindb.	BM000824508	BM	Italy, Sardinia	MG	AY854399	AY950319	AY950364	AY950410
13	*Pleurochaete squarrosa* (Brid.) Lindb.	BM000824505	BM	Italy, Calabria	MG & JCV	AY854400	AY950320	AY950365	AY950411
14	*Pleurochaete squarrosa* (Brid.) Lindb.	BM000824504	BM	Germany, Lower Saxony	MG	AY854401	AY950321	AY950366	AY950412
15	*Pleurochaete squarrosa* (Brid.) Lindb.	BM000824507	BM	Portugal, Coimbra	C. Durães, H. Hunt & JCV	AY854402	AY950322	AY950367	AY950413
16	*Pleurochaete squarrosa* (Brid.) Lindb.	BM000824509	BM	Spain, Mallorca	JCV	AY854403	AY950323	AY950368	AY950414
17	*Pleurochaete squarrosa* (Brid.) Lindb.	BM000824510	BM	Spain, Mallorca	JCV	AY854404	AY950324	AY950369	AY950415
18	*Pleurochaete squarrosa* (Brid.) Lindb.	P.L. Redfearn 40385	SMS	USA, Missouri	P.L. Redfearn	AY854405	AY950325	AY950370	AY950416
19	*Pleurochaete squarrosa* (Brid.) Lindb.	B. Allen 22346	GOET	USA, Arkansas	B. Allen	AY854406	AY950326	AY950371	AY950417
20	*Pleurochaete squarrosa* (Brid.) Lindb.	S.L. Timme 15657	SMS	USA, Oklahoma	S.L. Timme	AY854407	AY950327	AY950372	AY950418
21	*Pleurochaete squarrosa* (Brid.) Lindb.	BM000824503	BM	Germany, Thuringia	MG	AY854408	AY950328	AY950373	AY950419
22	*Pseudosymblepharis richardsii* (E.B. Bartram) B.H. Allen	BM000824489	BM	Belize	F. J. Rumsey	AY854409	AY950329	AY950374	AY950420
23	*Tortella arctica* (Arnold) Crundw. & Nyholm	O.M. Afonina 22.07.85	BM	Arctic Russia, Wrangel Island	O.M. Afonina	AY854410	AY950330	AY950375	AY950421
24	*Tortella arctica* (Arnold) Crundw. & Nyholm	0795-867.1	MSUN	Greenland	F.J.A. Daniels	AY854411	AY950331	AY950376	AY950422
25	*Tortella densa* (Lorentz & Molendo) Crundw. & Nyholm	E. Wiltshire 30.11.92	BM	Ireland, Claire	Elinor Wiltshire	AY854412	AY950332	AY950377	AY950423
26	*Tortella flavovirens* (Bruch) Broth.	H. Streimann 54900	BM	South Australia	H. Streimann	AY854413	AY950333	AY950378	AY950424
27	*Tortella flavovirens* var. *flavovirens* (Bruch) Broth.	BM000824500	BM	Spain, Mallorca	MG & JCV	AY854414	AY950334	AY950379	AY950425
28	*Tortella flavovirens* var. *glareicola* (T.A. Chr.) Crundw. & Nyholm	BM000824499	BM	The Netherlands, Zuid-Holland	J. Kortselius & MG	AY854415	AY950335	AY950380	AY950426
29	*Tortella fragilis* (Hook. & Wilson) Limpr.	M. Ignatov 23.07.91	BM	Russia, Gorno-Altai	M. Ignatov	AY854416	AY950336	AY950381	AY950427
30	*Tortella fragilis* (Hook. & Wilson) Limpr.	0795-869.5	MSUN	Greenland	F.J.A. Daniels	AY854417	AY950337	AY950382	AY950428
31	*Tortella humilis* (Hedw.) Jenn.	P.L. Redfearn Jr. 20.05.92	BM	USA, Arkansas	P.L. Redfearn Jr.	AY854418	AY950338	AY950383	—

Table 1. (*continued*)

No.	Taxon	Voucher no. /BM barcode	Herbarium	Origin	Collector	GenBank accession number			
						ITS	*atpb-rbcL*	*rps4*	*trnL-F*
32	*Tortella humilis* (Hedw.) Jenn.	E. Zardini & P. Aquino 32386	BM	Paraguay, Guaira	E. Zardini & P. Aquino	AY854419	AY950339	AY950384	AY950429
33	*Tortella inclinata* (R. Hedw.) Limpr.	BM000824494	BM	Germany, Northrhine-Westphalia	C. Schmidt	AY854420	AY950340	AY950385	AY950430
34	*Tortella nitida* (Lindb.) Broth.	BM000824493	BM	Spain, Mallorca	MG & JCV	AY854421	AY950341	AY950386	AY950431
35	*Tortella tortuosa* (Hedw.) Limpr.	T.A.J. Hedderson 5548	BM	Canada, Newfoundland	T.A.J. Hedderson	AY854422	AY950342	AY950387	AY950432
36	*Tortella tortuosa* (Hedw.) Limpr.	MG 010115		Italy, Trentino-Alto Adige	JCV	AY854423	AY950343	AY950388	AY950433
37	*Tortella tortuosa* (Hedw.) Limpr.	BM000824495	BM	Canada, British Columbia	JCV	AY854424	AY950344	AY950389	AY950434
38	*Trichostomum brachydontium* var. *cuspidatum* (Braithw.) L.I. Savicz	BM000824497	BM	Germany, Northrhine-Westphalia	C. Schmidt	AY854425	AY950345	AY950390	AY950435
39	*Trichostomum crispulum* var. *angustifolium* Bruch & Schimp.	BM000824498	BM	Germany, Northrhine-Westphalia	C. Schmidt	AY854426	AY950346	AY950391	AY950436
40	*Trichostomum pallidisetum* H. Müll	BM000824496	BM	Germany, Thuringia	C. Schmidt	AY854427	—	AY950392	AY950437
41	*Trichostomum tenuirostre* (Hook. & Taylor) Lindb.	BM000824487	BM	Germany, Northrhine-Westphalia, Plettenberg	C. Schmidt	AY854428	AY950347	AY950393	AY950438
42	*Trichostomum tenuirostre* (Hook. & Taylor) Lindb.	BM000824488	BM	Germany, Northrhine-Westphalia, Hagen	C. Schmidt	AY854429	AY950348	AY950394	AY950439
43	*Triquetrella tristicha* (Müll. Hal.) Müll. Hal.	T.A.J. Hedderson 11737	BM	South Africa, Western Cape	T.A.J. Hedderson	AY854430	AY950349	AY950395	AY950440
44	*Tuerckheimia valeriana* (E.B. Bartram) R.H. Zander	Bryotheca Gotting., Fasc. 9 (2001), No. 38	GOET	Costa Rica	I. Holz & A. Schaefer	AY854431	AY950350	AY950396	AY950441
45	*Weissia controversa* Hedw.	B. Allen 29.03.95	BM	USA, Missouri	B. Allen	AY854432	AY950351	AY950397	AY950442
46	*Weissia controversa* var. *densifoli*a (Bruch & Schimp.) Wilson	BM000824491	BM	UK, S.E. Yorkshire	R. D. Porley	AY854433	AY950352	AY950398	AY950443

was removed after 4 min centrifugation at 13,000 rpm, and the DNA pellet washed with 500 µl 70% ethanol, dried and dissolved in 30 µl of molecular-grade water.

The nuclear ITS1-5.8S-ITS2 region and three chloroplast regions (*trnL-trnF*, *rps4* + *rps4*−*trnS* IGS, and the *atpB-rbcL* spacer) were amplified using polymerase chain reaction (PCR) in 20 µl volumes containing 1 × NH_4 reaction buffer, 3 mM $MgCl_2$, 0.4 mM of each primer, 0.5 mM dNTPs in equimolar ratio, two units BIOTAQ^{TM} DNA polymerase (Bioline), and 0.5 µl of template DNA. ITS reactions were performed additionally in 1 M betaine to prevent the formation of secondary structures (Chakrabarti and Schutt 2001). Primer sequences and PCR cycling conditions are given in Tables 2 and 3, respectively. PCR products were cleaned using GFX^{TM} PCR and gel band purification kit (Amersham Biosciences) following the manufacturer's protocol. Cycle sequencing products were generated using aliquots of 1–5 µl of PCR product in 10 µl reactions, using Big Dye version 3.1 sequencing buffer (Applied Biosystems Inc.) and the PCR primers. Reactions were performed on an ABI 377 Automated Sequencer (PE Biosystems) or a 3730 DNA Analyser (Applied Biosystems Inc.). In order to get all sequences of the four DNA regions used in this work from a single extraction, all sequences were prepared by ourselves. No data could be obtained for the *atpB-rbcL* spacer of *Trichostomum pallidisetum* H. Müll., the *trnL-trnF* region of *Tortella humilis* (Hedw.) Jenn. from the US, and nrITS of *Hyophila involuta* (Hook.) A. Jaeger. These missing sequences were treated as 'missing data'. NrITS sequences were checked for the occurrence of polymorphisms, and their identity was checked via BLAST searches.

Sequence editing and alignment

For each accession, forward and reverse sequence strands were assembled in SeqMan II (LaserGene Systems Software; DNAStar, Inc.). Consensus sequences were aligned manually using MegAlign (LaserGene Systems Software; DNAStar, Inc.) and MacClade 4.0 (Maddison and Maddison 2000). Ambiguously aligned regions and incomplete data (i.e. at the beginnings and ends of sequences) were excluded from subsequent analyses.

We generated three sequence data sets on all accessions: (1) chloroplast DNA regions (*trnL-trnF*∼510 nucleotides, *rps4*∼620 nucleotides, *atpB-rbcL* spacer∼620 nucleotides; in total∼1750 nucleotides); (2) nrITS region (∼950 nucleotides); and (3) the combined data set with the four sequenced regions, comprising ∼2700 nucleotides for each specimen. Gaps were coded as question marks instead of using a fifth-state coding. In order to increase resolution of the trees within the genus *Pleurochaete*, a second, smaller set of accessions was created, containing all *Pleurochaete* samples and closer relatives of *Tortella*, with *T. humilis* serving as outgroup taxon. In this smaller dataset we: (1) included several

Table 2. Primers used to amplify and sequence the genomic regions

Gene	Primer	Sequence 5′–3′	Direction	References
atpb-rbcL	ATPB-1	ACA TCK ART ACK GGA CCA ATA A	Forward	Chiang et al. (1998)
	RBCL-1	AAC ACC AGC TTT RAA TCC AA	Reverse	Chiang et al. (1998)
rps4	RPS5	ATG TCC CGT TAT CGA GGA CCT	Forward	Nadot et al. (1995)
	TRNAS	TAC CGA GGG TTC GAA TC	Reverse	Nadot et al. (1995)
trnL-trnF	TRNC	CGA AAT CGG TAG ACG CTA CG	Forward	Taberlet et al. (1991)
	TRNF	ATT TGA ACT GGT GAC ACG AG	Reverse	Taberlet et al. (1991)
nrITS	17SE	ACG AAT TCA TGG TCC GGT GAA GTG TTC G	Forward	Sun et al. (1994)
	26SE	TAG AAT TCC CCG GTT CGC TCG CCG TTA C	Reverse	Sun et al. (1994)
	ITSP1A	GGA AGG AGA AGT CGT AAC AAG G	Forward	Barkman (1998)
	ITS-4	TCC TCC GCT TAT TGA TAT GC	Reverse	Baldwin (1992)

Table 3. Cycling conditions

Gene	Initial denaturation	Number of cycles	Denaturation	Annealing	Extension	Final extension
atpb-rbcL	94 °C for 5 min	30	94 °C for 45 s	57 °C for 75 s	72 °C for 75 s	72 °C for 10 min
rps4	94 °C for 4 min	35	94 °C for 30 s	50 °C for 30 s	72 °C for 90 s	72 °C for 7 min
trnL-trnF	94 °C for 2 min	30	94 °C for 30 s	50 °C for 30 s	72 °C for 60 s	72 °C for 5 min
nrITS	94 °C for 2 min	30	94 °C for 30 s	50 °C for 30 s	72 °C for 2 min	72 °C for 5 min

parts of the alignment which had been omitted from the larger dataset due to too many ambiguities, and (2) were able to conduct a simple indel coding (Simmons and Ochoterena 2000; Simmons et al. 2001). All of this yielded 46 additional, parsimony informative characters (*atpB-rbcL* IGS with 6, *trnL-trnF* with 2, nrITS with 38 indels). We chose a conservative approach, using only unambiguous alignments of indels for this dataset. They represent a considerable portion of the potential phylogenetic information in sequence-based matrices.

Phylogenetic analyses

Maximum parsimony analyses were performed using PAUP 4.0 (Swofford 2000), with the following options: heuristic search, 100 random-addition-sequences, tree bisection-reconnection (TBR) branch swapping, MULTrees option on, collapse zero-length branches on, saving all most parsimonious trees, gaps treated as missing data. Branch support was estimated by bootstrap analysis (BS; Felsenstein 1985) with full heuristic searches, 1000 bootstrap replicates, 10 random-addition-sequence replicates per bootstrap replicate, and TBR branch swapping.

Comparing the bootstrap consensus trees by eye tested the compatibility among the four single gene data sets. Data sets were accepted as compatible if non-corresponding clades lacked a BS value >50% for each of the two data sets (Johnson and Soltis 1998). The ILD test as implemented in PAUP* 4.0 was also employed to assess heterogeneity among data sets, despite recent critiques rejecting this test as a measure for compatibility (Johnson and Soltis 1998; Yoder et al. 2001; Barker and Lutzoni 2002; Darlu and Lecointre 2002).

A hierarchical likelihood-ratio test as implemented in Modeltest 3.04 (Posada and Crandall 1998) was used to select the model of nucleotide substitution and corresponding parameters that best fit the existing data. This model and parameters were implemented in maximum-likelihood analyses that were carried out with PAUP 4.0 (Swofford 2000) by employing heuristic searches with 100 random-addition-sequence replicates, TBR branch swapping, MULTrees option on, collapsing zero-length branches, and saving all trees. Branch support was estimated by BS (Felsenstein 1985) with full heuristic searches, 1000 bootstrap replicates, 10 random-addition-sequence replicates per bootstrap replicate, and TBR branch swapping.

Results

Large data set, nuclear and chloroplast data

The large data set—including 46 accessions for *atpB-rbcL* IGS, *trnL-trnF* region, *rps4*+*rps4-trnS* IGS, and nrITS1-5.8S-ITS2—resulted in 3226 nucleotide sites, of which 675 were excluded as ambiguously aligned or forming incomplete ends. Of the remaining 2551 nucleotide sites 446 are variable, but only 223 parsimony-informative characters were included in the analyses. The results of the ILD test are not suggesting any heterogeneity between cpDNA and nuclear genomic information.

The maximum-parsimony analysis yielded three equally optimal trees with tree length of 717, a consistency index (CI) of 0.739, retention index (RI) of 0.777, and rescaled retention index (RC) of 0.574. The strict consensus tree of all most-parsimonious trees is presented in Fig. 1, with MP bootstrap confidence values >50% given above the branches.

The first two nodes of the ingroup separate *Hyophila* and *Tuerckheimia* from the remaining taxa (BS = 100%). The following node gives a polytomy with only one clade (II) containing all taxa of the genera *Pleurochaete*, *Tortella*, *Chionoloma* and *Pseudosymblepharis* and one species of the genus *Trichostomum* fairly well supported (BS = 88%). This clade II is poorly resolved, and the strict consensus tree shows a polytomy bearing three subclades. The first subclade (IIa, BS = 99%) consists of the pantropical *Chionoloma bombayense* (C. Müll.) Sollman, the cosmopolitan *Trichostomum tenuirostre* (Hook. & Taylor) Lindb., and *Pseudosymblepharis richardsii* (E.B. Bartram) B.H. Allen, endemic to Central America and the Caribbean. The second subclade (IIb, BS = 52%) contains *Pleurochaete* and all taxa of *Tortella*, except for both accessions of *T. humilis* which are joined in the third subclade (IIc, BS = 100%).

The large *Pleurochaete*/*Tortella* clade (IIb) is composed of three multi-accession clades with a BS of 100%. Relationships of *Tortella nitida* (Lindb.) Broth. are not resolved as well as the relationships among the three clades. In the first group all accessions of *Pleurochaete* are incorporated, the second group consists of temperate to arctic species of *Tortella*, namely *T. arctica*, *T. fragilis*, *T. densa*, *T. inclinata*, and *T. tortuosa*. The remaining 'groups' of Mediterranean species are *T. flavovirens*, with both var. *flavovirens* and var. *glareicola*, and *T. nitida*, respectively.

P. luteola is a highly supported clade (BS = 95%). *Pleurochaete* samples of European origin group together as two subclades but lack support (BS = 64%). The relationships of the North American accessions of *P. squarrosa* could not be resolved in this analysis. On the first node in the circumpolar *Tortella* clade, *T. tortuosa* branches from the remaining taxa (BS = 95%). The next dichotomy separates *T. inclinata* plus *T. densa* (BS = 92%) from a group containing *T. arctica* and *T. fragilis* (BS = 96%).

The phylogram of the maximum-likelihood analysis, using the same large data set used for maximum-parsimony analysis (see above), found a single tree having the highest likelihood, with a likelihood score

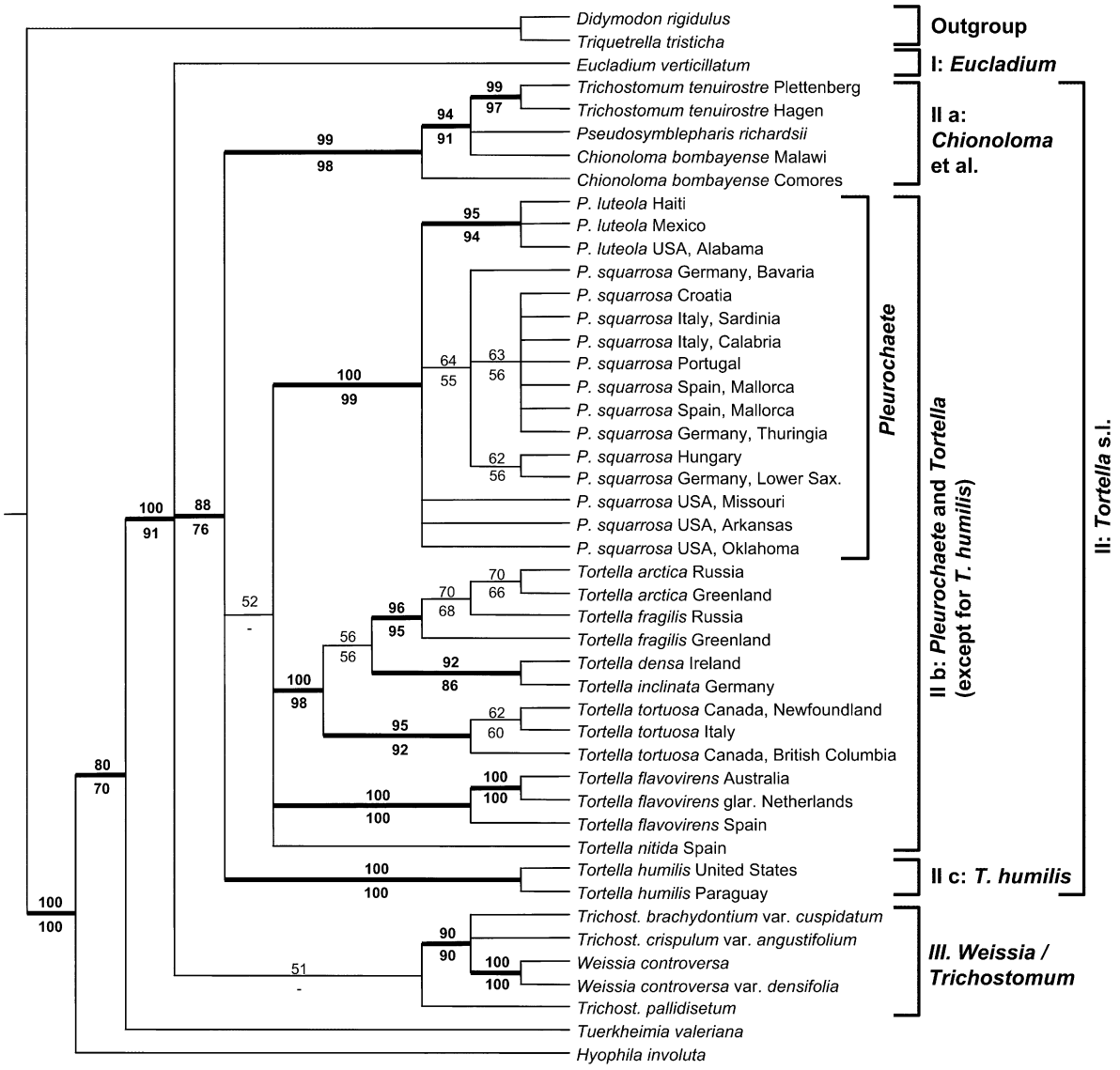

Fig. 1. Rooted strict-consensus tree of three equally parsimonious trees of the combined nrITS and cpDNA data of all 46 accessions. Bootstrap confidence values >50% are given above branches. Numbers below branches indicate bootstrap values of the corresponding ML analysis.

of $-\mathrm{Ln} = 7795.05$ under a GTR+G+I model of evolution. The overall topology is very similar. Bootstrap confidence values are given in Fig. 1. The 'backbone' of the *Tortella*/*Pleurochaete* clade suffers again from an insufficient number of substitutions. All subclades found in maximum-parsimony analysis can be found here as well.

Large data set, comparison of nuclear vs. chloroplast data (trees not shown)

The nuclear ITS data set of all taxa includes 808 nucleotide sites, of which 607 are constant. Ninety-five out of 201 variable characters are parsimony-informative. The analysis yielded 30 equally parsimonious trees. These trees were of length 333, CI = 0.745, RI = 0.761, and RC = 0.566. The chloroplast data set of all taxa includes 1743 nucleotide sites, of which 1498 are constant. One hundred and twenty-eight out of 245 variable characters are parsimony-informative. The analysis yielded four equally parsimonious trees. These trees were of length 373, CI = 0.756, RI = 0.811, and RC = 0.613.

Both nuclear and chloroplast data sets retrieved nearly identical topologies, with some noteworthy differences. The nrITS data set confirms a *P. luteola* clade, whereas the cpDNA data set weakly supports a European *P. squarrosa* clade (BS = 61%). The Mediterranean species *Tortella flavovirens* and *T. nitida* build a common clade as sister taxon to *Pleurochaete* in the

tree on nrITS data. The sister-group relationships of *Pleurochaete* are not resolved by the chloroplast DNA data. Possibly, the limited resolution in the chloroplast DNA data set is due in part to the *trnL−trnF* sequence of *Tortella nitida* being identical to the sequence of European *P. squarrosa*.

Small data set, nuclear and chloroplast data including indel coding

The small data set, including 30 accessions for all genes, resulted in 2937 nucleotide sites, of which 306 were excluded as ambiguously aligned or forming incomplete ends. Of the remaining 2631 nucleotide sites 244 are variable. One hundred and nineteen parsimony-informative characters are included in the analyses. Indel coding yielded 38 parsimony-informative characters in the nrITS data set; in *trnL-trnF* and *atpB-rbcL* two and six indels, respectively, could be scored unambiguously.

The maximum-parsimony analysis yielded eight equally optimal trees. Most parsimonious trees were of length 296, CI = 0.861, RI = 0.929, and RC = 0.801. The strict consensus tree of all most-parsimonious trees is presented in Fig. 2, with bootstrap support values >50% given above the branches.

On the first node of the ingroup two well-supported clades are established, the first (BS = 80%) comprising all accessions of *Pleurochaete* and the Mediterranean species *Tortella flavovirens* and *T. nitida*, the second clade (BS = 100%) comprising the temperate to arctic species of *Tortella*. The first clade is divided into two subclades, one including all *Pleurochaete* accessions (BS = 100%), the other including *Tortella flavovirens* and *T. nitida* (BS = 85%).

The *Pleurochaete* subclade exhibits a polytomy consisting of three clades: (1) *P. luteola* (BS = 89%), (2) European *P. squarrosa* (BS = 80%), and (3) American *P. squarrosa* (BS = 80%). This improves the resolution of the large data set significantly, merging the American *P. squarrosa* samples in a relatively well-supported clade. In the *P. luteola* clade the Caribbean sample (Haiti) is separated from samples from Mexico and the US (Alabama) (BS = 66%). The European *P. squarrosa* clade is divided in a clade containing three Central European samples and another containing the remaining samples, originating mainly from Mediterranean Europe.

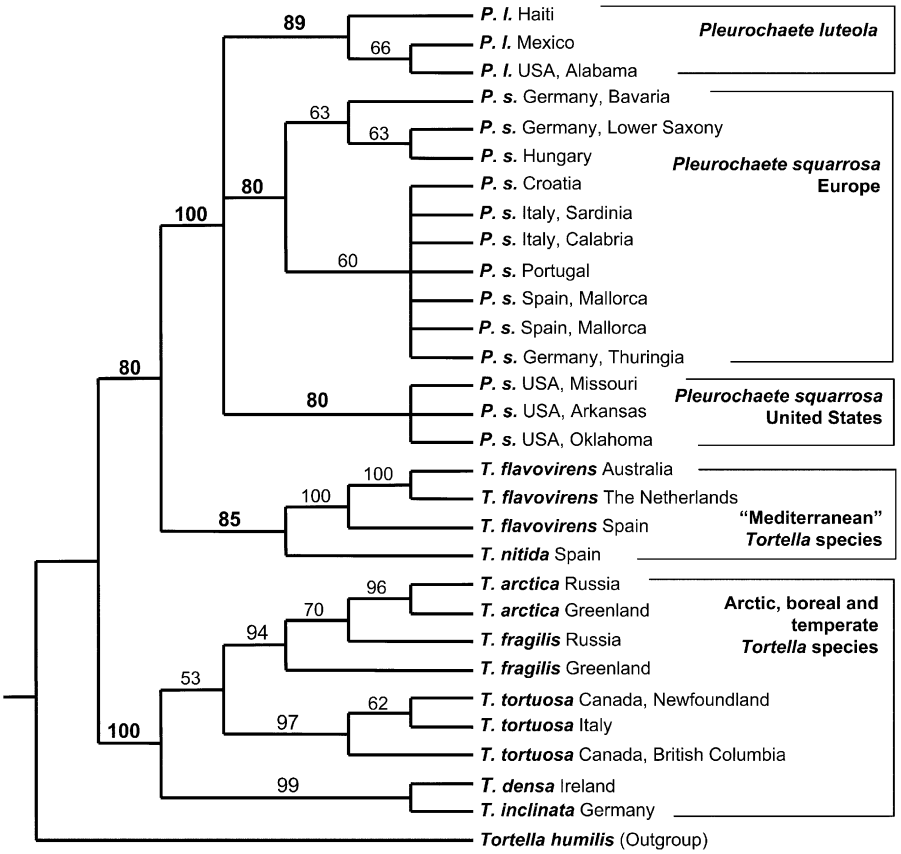

Fig. 2. Rooted strict-consensus tree of eight equally parsimonious trees of the combined nrITS and cpDNA data including indel coding, comprising all *Pleurochaete* accessions and closely related *Tortella* species. Bootstrap confidence values >50% are given above branches.

Sister-group relationships between the three *Pleurochaete* clades are unresolved.

The phylogram of the maximum-likelihood analysis, using the same small data set used for maximum parsimony analysis (see above), is shown in Fig. 3 (−ln likelihood = 4717.50, GTR + I model of evolution). The topologies of the trees of both small combined data sets are almost identical, showing only one difference. The clade of *Tortella inclinata* and *T. densa* is sister clade to all other circumpolar species in maximum-parsimony analysis, whereas in the maximum-likelihood phylogram it forms a clade with *T. arctica* and *T. fragilis* being sister to *T. tortuosa*. The weak bootstrap value of 53% in maximum parsimony indicates the degree of uncertainty in this case.

Small data set, comparison of nuclear vs. chloroplast data (trees not shown)

Within *Pleurochaete* the clades of *P. luteola* and American *P. squarrosa* (BS = 76% and 80%, respectively) are entirely based on nuclear data. The chloroplast-data tree is poorly resolved, looking identical to the tree in the large data set and yielding very similar bootstrap values. On the other hand, the monophyly of European *Pleurochaete* is lost on nrITS data alone. Only the three Central European samples mentioned above are still combined. The clade connecting *Pleurochaete* and the Mediterranean *Tortella* species (BS = 87%) is realised in the tree on nuclear data only, the chloroplast tree showing the same trichotomy found in the trees described above. A considerable proportion of resolution at the lower taxonomic level in *Pleurochaete* and *Tortella* results from indel coding in the nrITS alignment. Indel coding is not available at the higher taxonomic level due to the presence of too many ambiguities.

Discussion

Generic circumscriptions

Our results give clear support for the monophyly of *Pleurochaete*, which in turn nests within a clade corresponding to *Tortella* in a broad sense. This *Tortella* s.l. clade includes *Pleurochaete*, two further small genera, *Chionoloma* and *Pseudosymblepharis*, and *T. tenuirostre*. In respect to these small genera, the species-rich genus *Tortella* appears to be paraphyletic. *T. tenuirostre* has been treated as a member of the genus *Oxystegus* (Limpr.) Hilp. (Smith 1978). However, Werner et al.

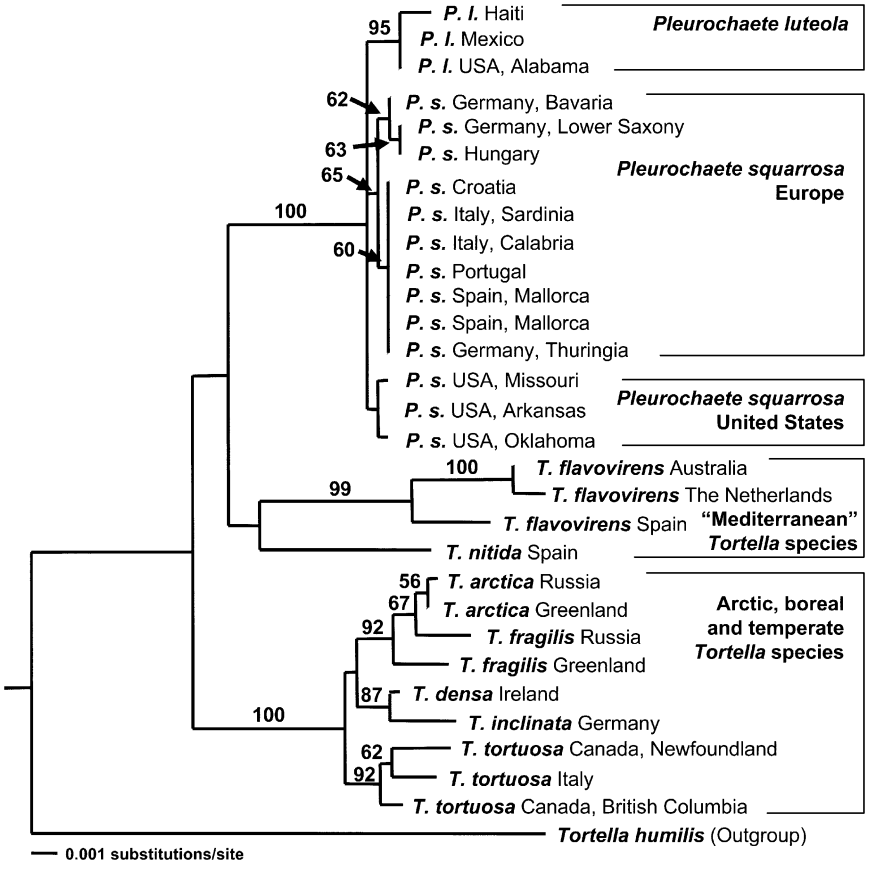

Fig. 3. Molecular phylogeny comprising all *Pleurochaete* accessions and closely related *Tortella* species, based on ML analysis of nrITS and cpDNA data sets. Bootstrap confidence values >50% are given above branches.

(2004b) included *Oxystegus sinuosus* (Mitt.) Hilp. in their analysis of *rps4* sequences in Pottiaceae and found it to be closely related to *Didymodon* Hedw. in the Pottioideae subfamily. Smith (2004) transferred it back to the genus *Trichostomum*, but our results place *T. tenuirostre* next to *Chionoloma*/*Pseudosymblepharis* in the Trichostomoideae subfamiliy. Similar discrepancies between traditional classification and phylogenetic relationships have been reported for other genera of Pottiaceae (Werner et al. 2004a, b). The current sampling of *Tortella* and putative relatives appears to be insufficient to draw final conclusions or to erect a new classification. However, some suggestions for future treatment of these genera can be made based on our results. On the one hand, species of small genera such as *Pleurochaete* could be transferred to *Tortella* to avoid a paraphyletic genus. On the other hand, the genus *Tortella* could be restricted to a small subset of species, and most species currently placed in *Tortella* transferred to newly defined genera. By doing so, the genus *Pleurochaete* could be conserved. Assuming that our results reflect the true phylogeny of *Tortella*, the minimum number of genera could be estimated based on the phylogeny obtained with three chloroplast markers and nrITS sequence data. However, under these conditions at least five genera would have to be distinguished. Besides *Pleurochaete*, the other four genera would include the following species groups: genus 1 with *T. tenuirostre*, *Chionoloma* and *Pseudosymblepharis*; genus 2 with five species of *Tortella* (*T. arctica*, *T. fragi*lis, *T. densa*, *T. inclinata*, and *T. tortuosa*); genus 3 with *T. flavovirens* and *T. nitida*; and one monotypic genus including *T. humilis*. The distinction of these genera appears to be problematic, because no obvious synapomorphic morphological character states are currently known to identify most of these genera. In addition, the introduction of many new combinations would result in confusion rather than easy comparability with existing treatments of these taxa in floristic accounts. In contrast, the acceptance of a large genus *Tortella* including *Pleurochaete* would result in a single new combination in the first instance. We favour this latter solution and thus propose the treatment of *P. squarrosa* and *P. luteola* as members of the genus *Tortella*. In this classification *Tortella squarrosa* Limpricht is used instead of *P. squarrosa*. However, a recombination is needed for *P. luteola*. From our results no final decision regarding the correct placement of the *Chionoloma* clade can be made, thus leaving open whether it should become a genus in its own right or also be included in the genus *Tortella*.

Relationships within *Pleurochaete*

Within the *Pleurochaete* clade, we recovered three putative monophyletic lineages. The first one corresponds to *P. luteola* and was supported by both the cpDNA and nrITS data sets and highly supported by the combined data sets, with bootstrap support of 95% (MP of large data set with cpDNA + nrITS) or 89% (MP of small data set with cpDNA + nrITS + indel coding). The second monophyletic lineage comprises all European accessions of *P. squarrosa*, whereas the three accessions of *P. squarrosa* from the USA formed a third lineage. The latter two clades were not recovered with the nrITS data set alone, that instead supported a large polytomy including the *P. luteola* clade. The nrITS and cpDNA data sets combined recovered a European *P. squarrosa* clade. The North American *P. squarrosa* clade was supported in the small data set only. Both the North American and European clades had a BS support of 80% in the MP analyses of the small data set with cpDNA, nrITS + indel coding (Fig. 2). However, the relationships among these three clades were unresolved, and the ML analyses resulted in a polytomy for the three lineages. It is therefore unclear if these three lineages should be treated as three distinct species or as one species with three subspecies. Thus, further information is required, such as from additional genomic markers and/or an expanded taxon sampling covering African and Asiatic diversity of *Pleurochaete*.

Five species of *Tortella* were sampled from more than a single specimen in this study. Three of these five, *T. flavovirens*, *T. humilis*, and *T. tortuosa*, were recovered as monophyletic. In contrast, *T. arctica* appears to be nested within *T. fragilis*, and this species pair presents interesting future challenges. We also always recovered *T. densa* and *T. inclinata* as sister taxa, but the global relationships of *Tortella* are still controversial. A conclusive study would require much denser taxon sampling.

In light of our results some aspects of the geographical distribution of *Pleurochaete* are worth discussing. We found three monophyletic clades within *Pleurochaete* in two continents of the northern hemisphere. The morphologically indistinguishable clades of *P. squarrosa* show a disjunction between Mediterranean Europe and North America, whereas in the Americas the two morphologically distinct species, *P. squarrosa* and *P. luteola*, have overlapping distribution ranges, although they differ in habitat preferences. *P. luteola* is a highland species, whereas *P. squarrosa* occurs preferably in lowland habitats of northern America (Eckel 1998). In contrast, the European *P. squarrosa* lineage occurs from sea level to mountain habitats (at least up to 1600 m in eastern Spain; MG personal observation). Disjunct distributions between Europe and North America are well documented for bryophytes, ferns and flowering plants (Schofield 1988). Such biogeographic patterns have been interpreted as evidence of repeated intercontinental dispersal or as relicts of an ancient, probably continuous distribution, at the same time invoking morphological stasis over long time (Quandt et al. 2001; Shaw 2001; Shaw et al. 2002;

Groth et al. 2003; McDaniel and Shaw 2003; Pfeiffer et al. 2004; Stech and Dohrmann 2004). These interpretations are two extreme hypotheses in the long-lasting controversy concerning the origin of bryophyte diversity shared between continents (Shaw 2001).

From our results on *Pleurochaete*, one scenario would have to assume an ancient distribution of *P. squarrosa* across the Laurasian continent, which has been separated by continental drift. Under this scenario all three lineages within *Pleurochaete* are the result of geographic separation and interruption of gene flow between Europe and northern America, and of ecological separation between *P. luteola* and *P. squarrosa* in the New World. Here, we would not have to infer whether the ecological split predates or postdates the separation of European and northern American lineages of *P. squarrosa*.

The other scenario, favouring more recent, even if rare, long-distance dispersal events, assumes a neo-(sub)tropical origin of the genus and a subsequent transcontinental dispersal and spreading over the European area of *P. squarrosa*. In this case the European and American *P. squarrosa* would be conspecific, whereas in the first scenario the vicariance in *P. squarrosa* would lead to reciprocal monophyly of the two clades and finally cryptic speciation through genetic drift, assuming ongoing genetic isolation and the lack of selection of different morphotypes in the Old and New Worlds, respectively. However, the speed of achieving reciprocal monophyly depends on a number of factors including population size, generation time, mutation rate, and sex ratios (Shaw et al. 2002).

In the last decade, molecular techniques uncovered several cases of cryptic species of both liverworts and mosses that are morphologically indistinguishable or with subtle differences, but genetically separate, even if they show primary or secondary sympatry (for review see Shaw 2001). Investigation of nrITS variation in 70 populations of the two northern hemispherical species of *Mielichhoferia* Nees & Hornsch. revealed two cryptic species within *M. elongata* (Hoppe & Hornsch.) Nees & Hornsch., one of them restricted to North America, the other in both North American and Europe (Shaw 2000). Shaw and Allen (2000) demonstrated that phylogenetic patterns are consistent with the hypothesis of *Fontinalis antipyretica* Hedw. as an ancient northern hemisphere species distributed across North America and Europe that subsequently differentiated into geographical cryptic species. As for *Pleurochaete*, relationships in *Fontinalis* Hedw. are resolved best by nuclear ribosomal ITS DNA. Recently Shaw et al. (2003) studied three species of moss with a North American–European disjunctive distribution pattern in Mediterranean climates, to some extent comparable to our observations on *P. squarrosa*. *Claopodium whippleanum* (Sull.) Renault & Cardot and *Scleropodium touretii* (Brid.) L.F. Koch, both pleurocarpous mosses with rare or occasional production of sporophytes, showed more or less uniformity in nrITS sequences in both European and American populations. Only *C. whippleanum* had genetic diversity and several haplotypes in the American area. The monomorphic European clade of *C. whippleanum* was separated from the American populations by a single mutation. In *Scleropodium touretii* the American clade was nested within a European/Macaronesian clade. In contrast, *Dicranoweisia cirrata* (Hedw.) Lindb., an acrocarpous moss producing abundant spores and asexual gemmae, shows no geographical structure, and neither molecular nor morphological data resolve European and North American populations as distinct groups.

Pleurochaete species spread mainly through undefined fragments of single plants or parts of the characteristically loose cushions. Small spores, released from erect, long-stemmed capsules with a peristome consisting of long filaments are rarely produced but are capable of long-range dispersal (During 1992). In contrast to *Dicranoweisia cirrata*, for which the question whether the shared haplotypes are an outcome of recent intercontinental dispersal or retention of ancestral polymorphism could not be answered, there is no evidence of recent intercontinental gene flow in nrITS sequences in *Pleurochaete*. Consequently, the two genetically isolated, but morphologically indistinguishable (or at least very similar), clades of *P. squarrosa* might represent an additional example for cryptic or nearly cryptic speciation (Shaw 2001) in mosses. A detailed analysis of morphological variation within and between Old and New World populations, including the East African and Asian area, could potentially provide deeper insights into the intraspecific structure and phylogeography of *P. squarrosa* on a global scale.

Acknowledgements

We are grateful to Matthias Ahrens, Cymon Cox, Cecília Durães, Jochen Heinrichs, Joop Kortselius, Fred Rumsey and Carsten Schmidt for providing material for sequencing, and especially to Paul L. Redfearn (SMS) for the loan of specimens and the collecting of fresh material, and to Howard Matcham, Brian O'Shea and Rosa M. Ros for revising some herbarium specimens. We thank Bob Press for corrections and comments on this paper. Financial support from NERC Grant GR/12073 and the Natural History Museum Research Fund are gratefully acknowledged.

References

Baldwin, B.G., 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. Mol. Phylog. Evol. 1, 3–16.

Barker, F.K., Lutzoni, F.M., 2002. Utility of the incongruence length difference test. Syst. Biol. 51, 625–637.

Barkman, T.J., 1998. Evolution of *Dendrochilum* subgenus *Platyclinis* section *Eurybrachium* investigated in a phylogenetic context. Ph.D. dissertation, University of Texas, Austin, TX (cited after: Barkman, T.J., Simpson, B.B., 2001. Origin of high-elevation *Dendrochilum* species (Orchidaceae) endemic to Mount Kinabalu, Sabah, Malaysia. Syst. Bot. 26, 658–669).

Bridel, S., 1827. Bryologia Universa, vol. 1. Barth, Leipzig.

Chakrabarti, R., Schutt, C.E., 2001. The enhancement of PCR amplification by low molecular weight amides. Nucleic Acids Res. 29, 2377–2381.

Chiang, T.Y., Schaal, B.A., Peng, C.-I., 1998. Universal primers for amplification and sequencing a noncoding spacer between the *atpB* and *rbcL* genes of chloroplast DNA. Bull. Bot. Acad. Sin. 39, 245–250.

Crum, H.A., Anderson, A., 1981. Mosses of Eastern North America, vol. I. Columbia University Press, New York.

Darlu, P., Lecointre, G., 2002. When does the incongruence length difference test fail? Mol. Biol. Evol. 19, 432–437.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Düll, R., 1984. Distribution of the European and Macaronesian mosses (Bryophytina). Part 1. Bryol. Beitr. 4, 1–113.

During, H.J., 1992. Ecological classifications of bryophytes and lichens. In: Bates, J.W., Farmer, A.M. (Eds.), Bryophytes and Lichens in a Changing Environment. Clarendon Press, Oxford, pp. 1–31.

Eckel, P.M., 1998. Re-evaluation of *Tortella* (Musci, Pottiaceae) in conterminous USA and Canada with a treatment of the European species *Tortella nitida*. Bull. Buffalo Soc. Nat. Sci. 36, 117–191.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Groth, H., Lindner, M., Wilson, R., Hartmann, F.A., Schmull, M., Gradstein, S.R., Heinrichs, J., 2003. Biogeography of *Plagiochila* (Hepaticae): natural species groups span several floristic kingdoms. J. Biogeogr. 30, 965–978.

Johnson, L.A., Soltis, D.E., 1998. Assessing congruence: empirical examples from molecular data. In: Soltis, D.E., Soltis, P.S., Doyle, J.J. (Eds.), Molecular Systematics of Plants II. DNA Sequencing. Kluwer Academic Publishers, Boston, pp. 297–348.

Limpricht, K.G., 1888. *Tortella*. In: Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. 2 Aufl., 4 Band: Die Laubmoose Deutschlands, Oesterreichs und der Schweiz, 1. Abtheilung. Eduard Kummer, Leipzig, pp. 599–610.

Lindberg, S.O., 1864. De Tortulis et ceteris Trichostomeis europæis. Öfvers. Kongl. Vetensk.-Akad. Förh. 21, 213–254.

Maddison, D.R., Maddison, E.P., 2000. MacClade 4.0. Sinauer Associates Inc., Sunderland.

McDaniel, S.F., Shaw, A.J., 2003. Phylogeographic structure and cryptic speciation in the trans-antarctic moss *Pyrrhobryum mnioides*. Evolution 57, 205–215.

Nadot, S., Bittar, G., Carter, L., Lacroix, R., Lejeune, B., 1995. A phylogenetic analysis of monocotyledons based on the chloroplast gene *rps4*, using parsimony and a new numerical phenetics method. Mol. Phylog. Evol. 4, 257–282.

O'Shea, B.J., 2003. Checklist of the mosses of sub-Saharan Africa (version 4, 12/03). Trop. Bryol. Res. Rep. 4, 1–182.

Pfeiffer, T., Schaumann, F., Hässel de Menéndez, G.G., Frey, W., 2004. Inter- and infraspecific relationships in the Gondwanan liverwort genus *Hymonophyton* (Hymenophytaceae, Hepaticophytina). Studies in austral temperate rain forest bryophytes 23. Aust. J. Bot. 17, 407–421.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Quandt, D., Frahm, J.-P., Frey, W., 2001. Patterns of molecular divergence within the palaeoaustral genus *Weymouthia* Broth. (Lembophyllaceae, Bryopsida). J. Bryol. 23, 305–311.

Schofield, W.B., 1988. Bryophyte disjunctions in the Northern Hemisphere: Europe and North America. Bot. J. Linn. Soc. 98, 211–224.

Shaw, A.J., 2000. Molecular phylogeography and cryptic speciation in the mosses, *Mielichhoferia elongata* and *M. mielichhoferiana* (Bryaceae). Mol. Ecol. 9, 595–608.

Shaw, A.J., 2001. Biogeographic patterns and cryptic speciation in bryophytes. J. Biogeogr. 28, 253–261.

Shaw, A.J., Allen, B., 2000. Phylogenetic relationships, morphological incongruence, and geographic speciation in the Fontinalaceae (Bryophyta). Mol. Phylog. Evol. 16, 225–237.

Shaw, A.J., McDaniel, S.F., Werner, O., Ros, R.M., 2002. Phylogeography and phylodemography. Bryologist 105, 373–383.

Shaw, A.J., Werner, O., Ros, R.M., 2003. Intercontinental Mediterranean disjunct mosses: morphological and molecular patterns. Am. J. Bot. 90, 540–550.

Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49, 369–381.

Simmons, M.P., Ochoterena, H., Carr, T.G., 2001. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses. Syst. Biol. 50, 454–462.

Smith, A.J.E., 1978. The Moss Flora of Britain and Ireland. Cambridge University Press, Cambridge, UK.

Smith, A.J.E., 2004. The Moss Flora of Britain and Ireland, second ed. Cambridge University Press, Cambridge, UK.

Sollman, P., 2000. A taxonomic revision of *Pseudosymblepharis* Broth. (Musci: Pottiaceae) in Asia. Trop. Bryol. 18, 129–145.

Spagnuolo, V., Caputo, P., Cozzolino, S., Castaldo, R., de Luca, P., 1999. Patterns of relationships in Trichostomoideae (Pottiaceae, Musci). Plant Syst. Evol. 216, 69–79.

Stech, M., Dohrmann, J., 2004. Molecular relationships and biogeography of two Gondwanan *Campylopus* species, *C. pilifer* and *C. introflexus* (Dicranaceae). Monogr. Syst. Bot. Missouri Bot. Gard. 98, 415–431.

Sun, Y., Skinner, D.Z., Liang, G.H., Hulbert, S.H., 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theor. Appl. Genet. 89, 26–32.

Swofford, D.L., 2000. PAUP*: Phylogenetic Analysis Using Parsimony (*and other Methods), version 4. Sinauer Associatiates Inc., Sunderland, MA.

Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for the amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17, 1105–1109.

Werner, O., Jiménez, J.A., Ros, R.M., 2004a. The systematic position of the moss *Kingiobryum paramicola* (Pottiaceae) based on molecular and morphological data. Bryologist 107, 215–221.

Werner, O., Ros., R.M., Cano, M.J., Guerra, J., 2004b. Molecular phylogeny of Pottiaceae (Musci) based on chloroplast *rps4* sequence data. Plant Syst. Evol. 243, 147–164.

Werner, O., Ros, R.M., Grundmann, M., 2005. Molecular phylogeny of Trichostomoideae (Pottiaceae, Bryophyta) based on nrITS sequence data. Taxon 54, 361–368.

Yoder, A.D., Irwin, J.A., Payseur, B.A., 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. Syst. Biol. 50, 408–424.

Zander, R.H., 1993. Genera of the Pottiaceae: mosses of harsh environments. Bull. Buffalo Soc. Nat. Sci. 32 (i–vi), 1–378.