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

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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 (\textit{atpB}-\textit{abcL} spacer, \textit{rps}4 + \textit{rps}4-\textit{trn}S IGS, \textit{trnL}-\textit{trn}F region, and \textit{ITS}1-5.8\textit{S}-\textit{ITS}2 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.

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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,
northern Germany (Düll 1984). The status of two further

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 unavail-

able, but hopefully will be included in our ongoing studies

on phyleogeography and population genetics of Pleuro-

chaete. In addition to Tortella and Pleurochaete as the
two key genera, representatives of seven other genera of

Trichostomoidae were included: Chionoloma, Eucladium,

Hyphila, Pseudosymblepharis, Trichostomum, Tuerkheimia,

and Weissia. Two representatives of Pottioideae,

Didymodon rigidulus Hedw. and Tricentrella 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 extrac-
tion 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 mix-
ture 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
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</table>
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 × NH4 reaction buffer, 3 mM MgCl2, 0.4 mM of each primer, 0.5 mM dNTPs in equimolar ratio, two units BIOTAQ™ 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™ PCR and gel band purification kit (Amersham Biosciences) following the manufacturer’s protocol. Cycle sequencing products were generated 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

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

### Table 3. Cycling conditions

<table>
<thead>
<tr>
<th>Gene</th>
<th>Initial denaturation</th>
<th>Number of cycles</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Final extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>atpB-rbcL</td>
<td>94 °C for 5 min</td>
<td>30</td>
<td>94 °C for 45s</td>
<td>57 °C for 75s</td>
<td>72 °C for 75s</td>
<td>72 °C for 10 min</td>
</tr>
<tr>
<td>rps4</td>
<td>94 °C for 4 min</td>
<td>35</td>
<td>94 °C for 30s</td>
<td>50 °C for 30s</td>
<td>72 °C for 90s</td>
<td>72 °C for 7 min</td>
</tr>
<tr>
<td>trnL-trnF</td>
<td>94 °C for 2 min</td>
<td>30</td>
<td>94 °C for 30s</td>
<td>50 °C for 30s</td>
<td>72 °C for 60s</td>
<td>72 °C for 5 min</td>
</tr>
<tr>
<td>nrITS</td>
<td>94 °C for 2 min</td>
<td>30</td>
<td>94 °C for 30s</td>
<td>50 °C for 30s</td>
<td>72 °C for 2 min</td>
<td>72 °C for 5 min</td>
</tr>
</tbody>
</table>
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+rrps4-trnS IGS, and nrITS1-5.8S-nrITS2—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
of $-\text{Ln} = 7795.05$ 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, $\text{CI} = 0.745$, $\text{RI} = 0.761$, and $\text{RC} = 0.566$. 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, $\text{CI} = 0.756$, $\text{RI} = 0.811$, and $\text{RC} = 0.613$.

Both nuclear and chloroplast data sets retrieved nearly identical topologies, with some noteworthy differences. The nrITS data set confirms a $P. \text{luteola}$ clade, whereas the cpDNA data set weakly supports a European $P. \text{squarrosa}$ 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 flavivirens 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 flavivirens 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 \((-\ln \text{likelihood} = 4717.50, \text{GTR} + I\) 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 above. 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 *Pseudosymbalepharis*, 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.

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**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 sinusus (Mitt.) Hilp. in their analysis of \( rps4 \) sequences in Pottiaceae and found it to be closely related to Didymodon Hedw. in the Pottioidae subfamily. Smith (2004) transferred it back to the genus Trichostomum, but our results place \( T. tenuirostre \) next to Chionoloma/Pseudosyblepharis in the Trichostomoideae subfamily. 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 Pseudosyblepharis; genus 2 with five species of Tortella (\( T. arctica \), \( T. fragilis \), \( T. densa \), \( T. inclinata \), 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 relics 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;
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 and Allen 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, Cecilia Durães, Jochen Heinrichs, Joop Kortsellus, 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


