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

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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,

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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