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Ascus types are phylogenetically misleading in *Trapeliaceae* and *Agyriaceae* (*Ostropomycetidae*, *Ascomycota*)

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ABSTRACT

The phylogeny of *Agyriaceae* was investigated using MP and Bayesian approaches based on a combined dataset of nuLSU rDNA, mtSSU rDNA, and *RPB1* sequences of 78 ascomycetes. The type genus of the family is shown to be a strongly supported sister to *Coccotremataceae* + *Pertusariaceae*, whereas the remaining species currently classified in *Agyriaceae* have a well-supported sister-group relationship with *Baeomycetales*. Monophyly of *Agyriaceae* is significantly rejected using two independent alternative topology tests. The micromorphology in *Agyriaceae s. lat.*, *Coccotremataceae*, and *Pertusariaceae* is restudied. It is confirmed that the ascus type of *Agyrium* agrees with that of other taxa currently placed in *Agyriaceae*, and hence the ascus type is interpreted as homoplasious and phylogenetically misleading in this group of fungi. Consequently, we suggest that *Trapeliaceae* be resurrected for the lichenized taxa currently classified in *Agyriaceae*, and that this family be placed in *Baeomycetales*. The ordinal classification in *Ostropomycetidae*, especially the circumscription of *Pertusariales* and its distinction from *Agyriales*, requires additional studies.

Introduction

The ascus type is one of the main morphological characters for the classification of ascomycetes and is widely used for the circumscription of taxa ever since the seminal work of Luttrell (1951, 1955), who distinguished major types of asci and used them for distinction of major clades in ascomycetes. Within *Lecanoromycetes* several types of functionally unitunicate asci are distinguished based on amyloid reactions in the ascal walls, in particular, of the apical structures (e.g. Bellemère & Letrouit-Galinou 1987; Chadefaud *et al.* 1963; Chadefaud 1973; Honegger 1982). Hafellner (1984) was the first to use these ascus types in the classification of *Lecanoromycetes*, and since then, this character has rapidly become a popular taxonomic character. However, some pre-molecular studies have already indicated that different ascus types might occur in natural groups (e.g. Lutzoni & Brodo 1995; Rambold *et al.* 1994) and intermediate forms between some ascus types were found (e.g. Hertel & Rambold 1985). Hence, the schematic use of ascus types in the classification was criticized (Hawksworth 1994: 379–387; Tibell 1998). Early molecular studies had already showed that the taxonomic importance of the general ascus types in filamentous ascomycetes, such as proto-, uni-, or bitunicate asci, has been overestimated. For example, Berbee (1996) demonstrated that bitunicate asci occur in unrelated clades, while Wedin and colleagues (Wedin & Tibell 1997; Wedin *et al.* 1998) demonstrated homoplasy of prototunicate asci. Within *Lecanoromycetes*, several studies showed that ascus types might vary within monophyletic clades (e.g. Buschbom & Mueller 2004; Ekman & Wedin 2000; Lumbsch *et al.* 2001b; Schmitt & Lumbsch 2004; Reeb *et al.* 2004; Wedin *et al.* 2005). The current study on the circumscription of

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Agyriaceae provides another example in which the ascus type is phylogenetically misleading.

Agyriaceae is a medium-sized family incorporating about 15 genera (Eriksson 2006), which includes non-lichenized and lichenized species with crustose to squamulose or placodioid thalli, and ascomata formed by a weakly hemiangiocarpous development (Lumbsch 1997). Together with *Anamylopsoraceae* (Lumbsch *et al.* 1995), they are classified in the order *Agyriales* (Lumbsch *et al.* 2001a). Previously, the taxa in this order had been placed in different families in *Lecanorales* (Poelt 1974), close to *Pertusariaceae* (Henssen & Jahns 1973) or in a separate suborder *Agyriineae* within *Lecanorales* (Hafellner *et al.* 1994; Lumbsch 1997). The non-lichenized and lichenized taxa in *Agyriaceae* were previously placed in separate families, before Rambold & Triebel (1990) merged the families. Before that, the majority of lichenized species currently classified in *Agyriaceae* were placed in *Trapeliaceae*. This family was described by Hertel (1970) including *Orceolina*, *Placopsis*, *Trapelia* and *Trapeliopsis*. Although Henssen & Jahns (1973) restricted the family to the type genus and placed *Placopsis* in *Pertusariaceae*, Hafellner (1984) followed the original circumscription of Hertel, but accepted section *Aspiciliopsis* of *Placopsis* as a separate genus, which was supported by Schmitt *et al.* (2003). Hafellner (1984) placed *Placynthiella* (syn. *Saccomorpha*) in a monotypic family *Saccomorphaceae*, whereas Coppins & James (1984) placed the genus in *Trapeliaceae*, which was supported by molecular studies (e.g. Lumbsch *et al.* 2001a; Schmitt *et al.* 2003). *Rimulariaceae* was described as a monotypic family by Hafellner (1984), but was shown to be close to *Agyriaceae* and *Trapeliaceae* (Hertel & Rambold 1990). Consequently, Lumbsch (1997) synonymized *Rimulariaceae* with *Agyriaceae*, which was supported by molecular data (Lumbsch *et al.* 2007). Other families, placed in *Agyriales* by Lumbsch *et al.* (2001a) and Lumbsch (1997) (as *Agyriineae*), were found to belong elsewhere in subsequent molecular studies. This includes *Elixiaceae*, which was found to belong to *Umbilicariales* (Hibbett *et al.* 2007; Lumbsch *et al.* 2004; Wedin *et al.* 2005) and *Schaereriaceae* (Lumbsch *et al.* 2004; Wedin *et al.* 2005; Lumbsch *et al.* 2007) with uncertain relationships in *Ostropomycetidae*.

The synonymization of *Trapeliaceae* under *Agyriaceae* was mainly based on the similar ascus type (Rambold & Triebel 1990). Given the variability of the ascus types in *Agyriaceae* and the number of taxa in this group misplaced on the basis of morphological characters, this classification needed re-examination. This is especially important as the order and family names (*Agyriales*, *Agyriaceae*) of the clade is based on the generic name *Agyrium*. Hence, we gathered sequence data of *Agyrium rufum*, the type species of the genus, to evaluate the phylogenetic placement of the genus, based on MP and Bayesian analyses of a dataset comprising partial sequence data of three independent genetic markers.

Materials and methods

Materials

Data matrices of 78 ascomycetes were assembled using sequences of nuLSU, mtSSU rDNA, and *RPB1* sequences. Specimens and sequences used for the molecular analyses are compiled in Table 1. We included *Hypocenomyce scalaris* and two *Umbilicaria* species as outgroup, as these taxa appeared basal to *Lecanoromycetidae* and *Ostropomycetidae* in recent phylogenetic studies (Lumbsch *et al.* 2007; Miadlikowska *et al.* 2007). With the exception of *Agyrium rufum*, all sequences used in this study have been published previously (Lumbsch *et al.* 2007). The following two *Agyrium rufum* samples were used for the molecular and morphological studies: Sweden, Västerbotten, Hörnefors par., Norrmjöle, Kläppuden, Wedin 7931 (UPS). USA, Missouri, Greene Co., Rocky Barrens Conservation Area, 0.6 miles E of Greene Co. Farm Road 105, Buck 48698 (F). The following specimens were used for anatomical comparison of the ascus type in *Ostropomycetidae*: *Coccotrema cucurbitula*: Australia, NSW, Barrington Tops NP, Lumbsch 8666j & Filson (F); *Coccotrema maritimum*: Canada, British Columbia, Vancouver Island, Hot Springs Cove, 13 Jun 2004, Schmitt (F); *Pertusaria flavicunda*: Mexico, Baja California [Nash, Lich. Exs. 239] (F); *Pertusaria pertusa*: Turkey, Prov. Izmir [John, Lich. Anatol. Exs. 66] (F); *Trapelia coarctata*: Germany, Baden-Württemberg, Hochfirst, Lumbsch 84 (F).

Molecular methods and sequence alignments

DNA isolation, PCR, and sequencing conditions followed procedures described in Lumbsch *et al.* (2007). The new sequences of *Agyrium rufum* were added into the alignment used in Lumbsch *et al.* (2007) and manually aligned.

Phylogenetic analysis

The alignments were analysed by MP and a Bayesian approach (B/MCMC) (Huelsenbeck *et al.* 2000; Larget & Simon 1999). To test for potential conflict, parsimony BS analyses were performed on each individual dataset, and 75 % BS consensus trees were examined for conflict (Lutzoni *et al.* 2004).

MP analyses were performed using the program PAUP* (Swofford 2003). Heuristic searches with 200 random taxon addition replicates were conducted with tree bisection–reconnection (TBR) branch swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. Bootstrapping (Felsenstein 1985) was performed based on 2K replicates with random sequence additions.

The B/MCMC analyses were conducted using the MrBayes 3.1.1 program (Huelsenbeck & Ronquist 2001). The analyses were performed assuming the general time reversible model of nucleotide substitution (Rodriguez *et al.* 1990), including an estimation of invariant sites, and assuming a discrete gamma distribution with six rate categories (GTR + I + G). The dataset was portioned into five parts (nuLSU, mtSSU, 1st, 2nd, 3rd codon positions of *RPB1*). Each partition was allowed to have its own parameters (Nylander *et al.* 2004). No molecular clock was assumed. A run with 4M generations starting with a random tree and employing 12 simultaneous chains was executed. Every 100th tree was saved into a file. The first 200K generations (i.e. the first 2K trees) were deleted as the 'burn in'. We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=tracer) to ensure that stationarity was achieved after the first 200K generations by