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Phylogenetic relationships of the Gomphales based on nuc-25S-rDNA, mit-12S-rDNA, and mit-*atp*6-DNA combined sequences

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ABSTRACT

Phylogenetic relationships among Geastrales, Gomphales, Hysterangiales, and Phallales were estimated *via* combined sequences: nuclear large subunit ribosomal DNA (nuc-25S-rDNA), mitochondrial small subunit ribosomal DNA (mit-12S-rDNA), and mitochondrial *atp*6 DNA (mit-*atp*6-DNA). Eighty-one taxa comprising 19 genera and 58 species were investigated, including members of the Clathraceae, Gautieriaceae, Geastraceae, Gomphaceae, Hysterangiaceae, Phallaceae, Protophallaceae, and Sphaerobolaceae. Although some nodes deep in the tree could not be fully resolved, some well-supported lineages were recovered, and the interrelationships among *Gloeocantharellus*, *Gomphus*, *Phaeoclavulina*, and *Turbinellus*, and the placement of *Ramaria* are better understood. Both *Gomphus sensu lato* and *Ramaria sensu lato* comprise paraphyletic lineages within the Gomphaceae. Relationships of the subgenera of *Ramaria sensu lato* to each other and to other members of the Gomphales were clarified. Within *Gomphus sensu lato*, *Gomphus sensu stricto*, *Turbinellus*, *Gloeocantharellus* and *Phaeoclavulina* are separated by the presence/absence of clamp connections, spore ornamentation (echinulate, verrucose, subreticulate or reticulate), and basidiomal morphology (fan-shaped, funnel-shaped or ramarioid). *Gautieria*, a sequestrate genus in the Gautieriaceae, was recovered as monophyletic and nested with members of *Ramaria* subgenus *Ramaria*. This agrees with previous observations of traits shared by these two ectomycorrhizal taxa, such as the presence of fungal mats in the soil. *Clavariadelphus* was recovered as a sister group to *Beenakia*, *Kavinia*, and *Lentaria*. The results reaffirm relationships between the Geastrales, Gomphales, Hysterangiales, and the Phallales, suggesting extensive convergence in basidiomal morphology among members of these groups. A more extensive sampling that focuses on other loci (protein-coding genes have been shown to be phylogenetically informative) may be useful to answer questions about evolutionary relationships among these fungal groups.

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Introduction

The gomphoid fungi occupy a unique position in the phylogeny of higher Basidiomycetes (Bruns *et al.* 1998; Pine *et al.* 1999; Hibbett & Thorn 2001; Hosaka *et al.* 2006). They are prominent in most forest ecosystems as saprotrophs and mutualists. The fungi in this group are also characterized by a wide range of basidiomal morphologies, from stalked ramarioid/clavarioid to cantharelloid-gomphoid, clavate, resupinate–odontoid, to sequestrate. Molecular studies reveal that gomphoid fungi are closely related to taxa in the Geastrales, Hysterangiales, and Phallales (Colgan *et al.* 1997; Hibbett *et al.* 1997; Pine *et al.* 1999; Humpert *et al.* 2001; Hosaka *et al.* 2006).

Taxonomy of the Gomphales has traditionally relied upon morphological characters now known to be subject to parallel evolution and phenotypic plasticity (Moncalvo *et al.* 2000). Consequently, many current genera and families are artificial, and taxonomic limits and identity of natural groups in the orders Geastrales, Gomphales, Hysterangiales, and Phallales are being re-examined.

Past workers (Maire 1902, 1914; Eriksson 1954; Heim 1954) recognized the relatedness of diverse morphologies within the Gomphales from microscopic and macrochemical characters, including cyanophilic spore ornamentation, chiastic basidia, hyphal construction, and positive hymenial reaction to ferric sulfate (Eriksson 1954; Donk 1961, 1964; Petersen 1971a; Villegas *et al.* 1999). Donk (1961, 1964) proposed the family Gomphaceae to include the resupinate–odontoid genera *Kavinia* and *Ramaricium*, the stalked clavarioid–ramarioid genera *Lentaria* and *Ramaria*, the stalked hydnoid genus *Beenakia*, the stipitate agaricoid genus *Gloeocantharellus*, and the pileate genera *Chloroneuron* and *Gomphus*. Corner (1970) proposed Ramariaceae to include *Delentaria*, *Kavinia*, *Lentaria*, and *Ramaria*. He excluded the pileate genera because no intermediate species linked the gomphoid and ramarioid morphologies. Petersen (1971a) suggested a gomphoid ancestral morphology for the family and later revised Donk's and Corner's familial classifications to include *Beenakia*, *Gomphus*, *Kavinia*, *Ramaricium*, *Ramariopsis*, and *Ramaria* (Petersen 1973, 1988).

Morphological and recent molecular data (mitochondrial and nuclear rDNA) have been used to infer inter- and intraspecific relationships among genera in this group of fungi. According to Pine *et al.* (1999), Villegas *et al.* (1999), Humpert *et al.* (2001), and Hosaka *et al.* (2006), the Gomphales includes the genera *Beenakia*, *Clavariadelphus*, *Gautieria*, *Gloeocantharellus*, *Gomphus*, *Kavinia*, *Lentaria*, *Phaeoclavulina*, *Ramaria*, *Ramaricium*, and *Turbinellus*. Hosaka *et al.* (2006) demonstrated the Gomphales to be a sister group to the Phallales, represented by the families Clathraceae (*sensu* Chevallier), Phallaceae (*sensu* Corda), Lysuraceae, Protophallaceae (*sensu* Zeller), Claustulaceae, and Trappeaceae. Both Gomphales and Phallales are closely related to the Hysterangiales (*sensu* Hosaka & Castellano) and the Geastrales.

Villegas *et al.* (1999), using morphological traits, proposed the Gomphales to be monophyletic and delimited by the presence of mycelial cords or rhizomorphs. It included the families Beenakiaceae (*Beenakia*, *Kavinia*, and *Ramaricium*), Gomphaceae (*Gomphus* and *Gloeocantharellus*), Lentariaceae (*Lentaria*), and Ramariaceae (*Ramaria*). According to Singer (1949), Heim (1954), Heinemann (1958), Donk (1964), Giachini (2004) and Hosaka *et al.* (2006), *Clavariadelphus* is a member of the Gomphales. The results of Villegas *et al.* (1999), however, disagree with the premises that *Clavariadelphus*, *Gomphus*, and *Ramaria* are members of the same order. According to those authors *Clavariadelphus* is not grouped within but rather a sister group to the Gomphales.

Pine *et al.* (1999) and Humpert *et al.* (2001), on the other hand, using sequences of both mitochondrial (mit-12S-rDNA) and nuclear (nuc-25S-rDNA) loci, showed that a gomphoid–phalloid clade including *Clavariadelphus*, *Geastrum*, *Gloeocantharellus*, *Gomphus* (monophyletic), *Lentaria*, *Ramaria* (paraphyletic), *Pseudocolus*, and *Sphaerobolus* was recovered in all analyses performed. Pine *et al.* (1999) showed that *Gomphus sensu lato* represented a terminal monophyletic group in the gomphoid–phalloid clade (although just two taxa were sampled), having *Ramaria* as sister group (Figs 1–3 in Pine *et al.* 1999). Based on morphological as well as molecular characters, Giachini (2004) revisited the generic concepts in the family Gomphaceae and recombined the species of *Gomphus sensu lato* into *Gloeocantharellus*, *Gomphus sensu stricto*, and the resurrected genera *Phaeoclavulina* and *Turbinellus*.

Cantharelloid/gomphoid and clavarioid fungi have historically been prominent in hypotheses about the origin of fleshy basidiomycetes (Singer 1947, 1986; Heim 1954; Corner 1966; Harrison 1971; Petersen 1971a; Corner 1972; Jülich 1981; Miller & Watling 1987). Their fruiting forms can be arranged in a transformation series, from clavate at one end, cantharelloid/gomphoid intermediately, and agaricoid at the other extreme. Corner (1972) proposed the ''*Clavaria* theory'' of basidiomycete evolution in which cantharelloid and clavarioid fungi were to be regarded as ancestral, and from which all other Homobasidiomycetes have been derived. He suggested that simple clavate morphologies (e.g. *Clavaria*) with smooth hymenia gave rise to intermediate cantharelloid species (e.g. *Cantharellus*, *Craterellus*), and from those were derived the wrinkled or folded hymenial gomphoid species (e.g. *Gomphus*, *Turbinellus*). Other authors agree on transformations among ramarioid, cantharelloid, and agaricoid forms but propose the opposite polarity, suggesting that lineages containing cantharelloid, ramarioid, and club-like fungi have been derived from agaricoid ancestors (Fiasson *et al.* 1970; Arpin & Fiasson 1971; Petersen 1971a; Singer 1986).

In this paper we analyze phylogenetic relationships among major evolutionary lineages of gomphoid fungi using combined sequence data from nuclear (nuc-25S-rDNA) and mitochondrial-encoded ribosomal and non-ribosomal RNA genes (mit-12S-rDNA, mit-*atp*6-DNA). Our taxonomic sampling focused on the Gomphales *sensu* Jülich (1981). Major questions tested in this study were:

1) Is *Gomphus sensu lato* monophyletic?
2) Are genera within *Gomphus sensu lato* monophyletic?
3) Are the Gomphales, Hysterangiales, Phallales, and Geastrales closely related?
4) How have basidiomatal morphology, presence or absence of clamp connections, and substrate affinity evolved within the Gomphales?