

Minutisphaerales (Dothideomycetes, Ascomycota): a New Order of Freshwater Ascomycetes Including a New Family, Minutisphaeraceae, and Two New Species from

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Abstract: Minutisphaera is a recently established genus of freshwater Dothideomycetes characterized by small, globose to subglobose or apothecioid, erumpent to superficial, brown ascomata; fissitunicate, eight-spored, ovoid to obclavate asci; and 1-2-septate, clavate to broadly fusiform, hyaline to pale brown ascospores with or without a gelatinous sheath and filamentous appendages. The genus currently contains two species: *M. fimbriatispora*, the type species, and *M. japonica*. The higher-level phylogenetic relationship of Minutisphaera within the Dothideomycetes currently is unresolved. To establish the phylogenetic position of Minutisphaera within the Dothideomycetes and evaluate the phylogenetic affinities of newly collected Minutisphaera-like taxa, we sequenced three rDNA regions-18S, ITS1-5.8SITS2 (ITS) and 28S nuc rDNA, and a protein-coding gene, MCM7, for newly collected strains of Minutisphaera. Based on maximum likelihood and Bayesian analyses of a combined dataset (18S and 28S) composed of 167 taxa, a more refined dataset (28S and MCM7) comprising 52 taxa and a separate ITS dataset, and an examination of morphology, we describe and illustrate two new species of Minutisphaera. The Minutisphaera clade was strongly supported within the Dothideomycetes with likelihood and Bayesian statistics but did not share phylogenetic affinities with any existing taxonomic group within the Dothideomycetes. We therefore establish a new order, Minutisphaerales, and new family, Minutisphaeraceae, for this monophyletic clade of freshwater ascomycetes. Chemical analysis of the organic extract *M. aspera* (G427) resulted in isolation and characterization of five known secondary metabolites, of which four were dipeptides (1-4) and one an aromatic polyketide (5). Conversely, two aromatic polyketides (5, 6) were isolated and identified from the organic extract of *M. parafimbriatispora* (G156-4). The isolated compounds were tested for their antimicrobial activity against an array of bacteria and