Apothecial ancestry, evolution, and re-evolution in *Thelebolales* (*Leotiomycetes, Fungi*)

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Abstract

Closed cleistothecia-like ascomata have repeatedly evolved in non-related perithecioid and apothecioid lineages of lichenized and non-lichenized *Ascomycota*. The evolution of a closed darkly pigmented ascoma to protect asci and ascospores is conceived as either an adaptation to harsh environmental conditions or specialized dispersal strategies. Species with closed ascomata have mostly lost sterile hymenial elements (paraphyses) and the capacity to actively discharge ascospores. The class *Leotiomycetes*, one of the most speciose classes of *Ascomycota*, is mainly apothecioid, paraphysate, and possess active ascospore discharge. Lineages with closed ascomata, and their morphological variants, have evolved independently in several families, such as *Erysiphaceae*, *Myxotrichaceae*, *Rutstroemiaceae*, etc. *Thelebolales* is a distinctive order in the *Leotiomycetes*. It has two widespread families (*Thelebolaceae*, *Pseudeurotiaceae*) with mostly closed ascomata, evanescent asci, and thus passively dispersed ascospores. Within the order, closed ascomata dominate and a great diversity of peridia have evolved as adaptations to different dispersal strategies. The type genus, *Thelebolus*, is an exceptional case of ascomatal evolution within the order. Its species are the most diverse in functional traits, encompassing species with closed ascomata and evanescent asci, and species with open ascomata, active ascospore discharge and paraphyses. Open ascomata were previously suggested as the ancestral state in the genus which evolved mammals and birds as dispersal agents. In this scheme, species with closed ascomata, lack of paraphyses, and passive ascospore discharge exhibit derived traits that evolved in adaptation to cold ecosystems. Here, we used morphological and phylogenetic methods, as well as reconstruction of ancestral traits for ascomatal type, asci dehiscence, presence or absence of paraphyses and ascospore features to explore evolution within *Thelebolales*. We demonstrate the apothecial ancestry in *Thelebolales* and propose a new hypothesis about the evolution of the open ascomata in *Thelebolus* involving a process of re-evolution where active dispersal of ascospores appears independently twice within the order. We propose a new family, *Holwayaceae*, within *Thelebolales* that retains phenotypic features exhibited by species of *Thelebolus*, i.e. pigmented capitate paraphyses and active asci discharge with an opening limitation ring.