

# Advances in systematics, taxonomy and host-relationships of neotropical Phyllachorales (Sordariomycetes, Ascomycota)

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Phyllachorales (Sordariomycetes, Ascomycota) is a group of biotrophic, fungi present mainly in tropical regions, considered highly host specific. Thus, species concepts are based partly on the host plants. Species of Phyllachorales are leaf- or stem-inhabiting with shiny black stromata, known as “tropical tar spot fungi”. Molecular phylogenetic studies of the Phyllachorales are limited. We have used molecular data from Neotropical collections to elucidate their relationships within Sordariomycetes as well as their family classification. Five partial nuclear gene regions were amplified and sequenced: nrLSU, nrSSU, ITS1-5.8S-ITS2, *RPB2*, and *TEF1* (EF-1 $\alpha$ ), to obtain a high resolution tree of Phyllachorales, using Maximum Likelihood and Bayesian Inference analyses. The position of Phyllachorales in the subclass Sordariomycetidae (Sordariomycetes) is confirmed, as well as the monophyly of the order, traditionally with two families, Phyllachoraceae and Phaeochoraceae. However, the traditional family and generic classification based on morphological characteristics often was not reflected in our molecular phylogenies. The family Phaeochoraceae is supported as a monophyletic group, while Phyllachoraceae and the genus *Phyllachora* are polyphyletic. Therefore, the Phyllachoraceae and *Phyllachora* were emended, requiring the recognition of the new family Telimenaceae and the emendation of *Telimena*. Phaeochoraceae include species characterised by immersed to erumpent stromata without a clypeus, occurring exclusively on Areaceae. Phyllachoraceae include species with immersed stromata and reduced pseudostroma growing on Poaceae and species with erumpent to superficial stromata occurring in monocot and eudicot host plants. Telimenaceae include species with immersed perithecia, a more developed pseudostroma, infecting species of different families, but not Poaceae.

# Status of *Phyllachora sensu stricto* and other *Phyllachora*-like species in Asia

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The order Phyllachorales is currently placed in the subclass Sordariomycetidae of Sordariomycetes and comprises three families, Phaeochoraceae, Phyllachoraceae and Telimenaceae which are supported by phylogenetic data. The family Phyllachoraceae currently comprises 60 genera, their species have been documented from a variety of plant host families and most taxa have been presumed to be host-specific. Most members of this family are obligate biotrophs which produce black "tar spots" on leaves and stems of their host plants. *Phyllachora* is the type genus of the order Phyllachorales and has more than 1500 names listed in Index Fungorum (January 2018). Those species were named using the concept of host-specificity, as the genus has been accepted as obligate biotrophic parasites. Most taxa have overlapping characteristics and lack phylogenetic support as they are generally unculturable. To date, there is still taxonomic confusion concerning the taxonomy of *Phyllachora* as species of genera have been reported to be polyphyletic. Our recent work involves taxonomic studies using morpho-molecular evaluation of tropical members of the genus, mainly from Asian countries to provide a basis for identification of the genus *Phyllachora* and allied taxa. DNA was successfully extracted from phyllachora-like taxa on various host plants, including mono- and di-cotyledons. Analyses of ITS, LSU and SSU sequence data were used to generate phylogenies, which revealed their diverse placements in Sordariomycetes. Host plant families were suitable for delimitating genera, which were highly specific. The research, based on the phylogenetic results will lead to a narrower circumscription of *Phyllachora* and phyllachora-like taxa.

# Phylogeny of *Apiosphaeria* (Phyllachoraceae)

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*Apiosphaeria guaranitica*, the causal agent of brown crust disease of several bignoniaceous hosts, among them *Handroanthus* and *Tabebuia* species, has been traditionally placed in Phyllachoraceae, based exclusively on morphological studies, without any supporting molecular evidence. We provided molecular data linking sexual and asexual forms of the fungus, and properly allocated *A. guaranitica* through phylogenetic analysis based on multilocus analyses using nuclear small subunit ribosomal DNA (*nrSSU*), nuclear large subunit ribosomal DNA (*nrLSU*), nuclear internal transcribed spacer ribosomal DNA (*nrITS*), second largest subunit of RNA polymerase II (*RPB2*) and translation elongation factor 1 (*TEF1*) genome loci. Furthermore, a divergence time estimate of this lineage was performed on the basis of both fossil-calibration (FC) and secondary calibration (SC) strategies. Our results showed a natural phylogenetic placement of *Apiosphaeria* within Diaporthaceae (Diaporthales), where it represents an ancient lineage of the crown-group of Diaporthaceae, diverging during the late Paleocene at 61.15 (FC) and 60.63 (SC) MYA. This is a pioneering study on the divergence time within the Diaporthales based on the fossil *Spataporthe taylori*.

# An overview of Phyllachorales from the Brazilian Cerrado

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Phyllachorales M.E. Barr (Ascomycota, Pezizomycotina, Sordariomycetes, Sordariomycetidae) comprise mostly Tropical, seldom Subtropical fungi, forming “tar spots” on leaves and green twigs, infecting hosts belonging in several botanical families. Currently, the families Phaeochoraceae, Telimenaceae e Phyllachoraceae are recognized. The latter with 51 described genera, 1246 accepted species names, with 942 belonging to the genus *Phyllachora*. Phylogenetic studies using molecular markers have shown that Phyllachoraceae and the genus *Phyllachora* are polyphyletic. Mycological inventories of the Cerrado domain pointed out that Phyllachorales represents the third group of fungi most frequently found, but its phylogenetic relationships within Phyllachorales and Sordariomycetes are poorly understood due to the shortage of molecular data of local specimens. We investigate the phylogenetic relationships of phyllachoroid fungi based on partial sequences of *nrITS*, *nrSSU* and *nrLSU* of ribosomal DNA, *RPB2* and *TEF1- $\alpha$* . Additionally, based on morphological and molecular data phyllachoroid species belonging in genera *Apiosphaeria*, *Camarotella*, *Cocodiella*, *Ophiodothella*, *Phaeochorella*, *Phyllachora*, and *Telimena* from Cerrado were taxonomically revised. The results revealed that the phyllachoroid from Cerrado form a polyphyletic group, distributed between Sordariomycetes of two orders: Diaporthales and Phyllachorales.