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Molecular phylogenetic analysis reveals two new species of *Discosia* from Italy

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Abstract

Two fresh collections of *Discosia* were made from dead leaves of *Fagus sylvatica* in Italy. As these collections could not be cultured, the fruiting bodies were directly used for sequencing using a Forensic DNA Extraction Kit. Based on analyses of the concatenated internal transcribed spacer regions of the nrDNA operon (ITS) and large subunit rDNA (LSU) gene sequences, as well as morphological characters, the fresh collections are introduced as two new species, namely *D. italica* and *D. fagi*. Phylogenetically, these two species are distinct from all other *Discosia* species. Morphologically, *D. italica* is somewhat similar with *D. fagi*, but can be distinguished using dimension of conidiomata and conidiogenous cells. Descriptions and illustrations of the new taxa are provided herein.

Key words: Asexual morphs, Amphisphaeriaceae, Phylogeny, Taxonomy

Introduction

Discosia (Amphisphaeriaceae) is a widely distributed genus of coelomycetes (Sutton 1980, Vanev 1992b, Nag Raj 1993, Wołczańska *et al.* 2004). Most species are endophytes or saprobes on various vascular plants in the tropical and temperate regions (Subramanian & Reddy 1974, Sutton 1980, Vanev 1992b, 1996, Nag Raj 1993, Okane *et al.* 1998). Some species in this genus are pathogens, such as *Adisciso yakushimense* Kaz. Tanaka, Okane & Hosoya on *Symplocos prunifolia* (Tanaka *et al.* 2011), as well as *A. kaki* Kaz. Tanaka, J. Yamam. & Toy. Sato on *Diospyros kaki* (Yamamoto *et al.* 2012), forming leaf spots on terrestrial plants from June to September.

The genus *Discosia* is characterized by uni- to multilocular conidiomata with multi-layered walls, occurring singly or in clusters. Conidiogenous cells are monoblastic, phialidic to annellidic. Conidia are almost hyaline to pale brown, and with polar or subpolar appendages inserted in the median part of the end cells (Sutton 1980, Nag Raj 1993).

Traditionally, morphological characters and host-association have been used to classify *Discosia* to species (Subramanian & Reddy 1974, Sutton 1980, Vanev 1991, 1992a, 1996, Nag Raj 1993). However, most species in this genus have overlapping characters, such as the location of the conidial septa and appendages, varying proportional lengths of conidial cells, and overall conidium size (Sutton 1977, 1980, Nag Raj 1993, Jeewon *et al*. 2002, Barber *et al.* 2011, Tanaka *et al.* 2011). The classification, validity, and delimitation of this genus have been problematic (Subramanian & Reddy 1974, Vanev 1991, 1992b, Sotton 1980, Nag Raj 1993, Jeewon *et al*. 2002). Subramanian & Reddy (1974) divided the taxa under *Discosia* into four sections based on the conidial morphology (size, septation and pigmentation). Vanev (1991) expanded this concept and grouped the species of *Discosia* into six sections, viz., *D*. sect. *Discosia*, *D*. sect. *Laurina* Vanev, *D*. sect. *Clypeata* Vanev, *D*. sect. *Libertia* Vanev, *D*. sect. *Strobilina* Vanev and *D*. sect. *Poikilo-*

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D. aff. *pleurochaeta* (GenBank, AB593713, Identities = 806/808 (99%), no gaps) and *Discosia* sp. 1' (GenBank, AB593710, Identities = 806/808 (99%), no gaps). The closest hits using the ITS sequence had similarity to *Discosia* sp. (GenBank, AF405303, Identities = 549/560 (98%), Gaps = 2/560 (0%)), *D.* aff. *artocreas* (GenBank, AB594772, Identities = 525/536 (98%), Gaps = 3/536 (0%)) and *D. pseudoartocreas* (GenBank, KF777161, Identities = 557/569 (98%), Gaps = 4/569 (0%)). However, phylogenetic analyses derived from combined ITS and LSU sequences show that *D. fagi* is clearly distinct from all other *Discosia* species (Figure 1). Morphologically, *D. fagi* resembles *D. italica*, but can be distinguished using dimension of conidiomata, being slightly larger than *D. italica* (100–250 µm diam., and 20–45 µm high). Furthermore, the conidiogenous cells of *D. fagi* also longer than *D. italica* 2–5 ($\bar{x}$ = 3) µm. Based on the substantive evidence provided above, *D. fagi* is introduced as novel species of *Discosia*.

In this paper, it has not been possible to apply LSU alone in resolving the species within *Discosia*, especially for species where very few morphological differences exist. Therefore, analyses of the combined sequences of ITS and LSU genes are used to delimit the species within *Discosia*. They also provide a significant evidence to justify that *D. italica* and *D. fagi* are distinct from each other. Nevertheless, the identification of interspecific relationship is problematic within the genus *Discosia*. *D. pini*, *D.* aff. *brasiliensis* and the type species *D. artocreas* clustered together with high bootstrap support, and this is in congruence with the observations of Tanaka *et al*. (2011). Thus, the interspecific relationship within *Discosia* should be studied further using multi-gene data. Moreover, on the bases of molecular data (ITS, LSU), the dimension of conidiomata and morphology of the conidiogenous cells and conidia, interspecies relationship can be distinguished. It would be premature to discuss the phylogenetic significance of various species, given that sequence data is available for only a small number of *Discosia* species. Future studies should focus on larger sampling of *Discosia* species and evaluation by molecular sequence data.

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