



Microsphaeropsis ononidicola sp. nov. (Microsphaeropsidaceae, Pleosporales) from *Ononis spinosa* L.

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Abstract

A new collection of *Microsphaeropsis* was made from *Ononis spinosa* L. in Italy. Multi-locus phylogenetic analyses of ITS, LSU and β -tubulin gene regions, combined with a detailed morphological analysis confirm its placement in *Microsphaeropsis*, Microsphaeropsidaceae. The novel collection is phylogenetically and morphologically distinct from other *Microsphaeropsis* species and a new species, *Microsphaeropsis ononidicola*, is therefore, introduced in this paper.

Keywords – 1 new species – β -tubulin – ITS – LSU – new species – Italy

Introduction

Microsphaeropsis was established by von Höhnelt (1917), and the genus was originally placed in Didymosphaeriaceae (= Montagnulaceae). Subsequently, *Microsphaeropsis* was assigned to Didymellaceae Gruyter et al. (de Gruyter et al. 2009, Hyde et al. 2013, Wijayawardene et al. 2014, 2016, 2018). Phylogenetic analysis by Chen et al. (2015) clearly showed that *Microsphaeropsis* is basal to Didymellaceae, from which it appears to have a significant evolutionary distance. Therefore, Chen et al. (2015) introduced a new family Microsphaeropsidaceae (Pleosporales, Dothideomycetes) to accommodate *Microsphaeropsis*. *Microsphaeropsis* currently comprises 50 species epithets in Index Fungorum (2018), while Wijayawardene et al. (2017) estimated 37 species, but GenBank has only a few hits for the genus. *Microsphaeropsis*-like species are however, polyphyletic within Dothideomycetes (Hyde et al. 2013, Ariyawansa et al. 2014, Thambugala et al. 2017). Only two species, *M. olivacea* (Bonord.) Höhn. and *M. proteae* (Crous & Denman) Crous & Denman have so far been phylogenetically confirmed in the *Microsphaeropsis*, Microsphaeropsidaceae (Chen et al. 2015).

Ononis spinosa (Fabaceae) is a widespread plant species in Europe and it is commonly known as "Spiny Restharrow". A number of micro-fungi associated with *Ononis spinosa* have been reported in European countries (Wanasinghe et al. 2014, Li et al. 2016, Jayasiri et al. 2017, Farr & Rossman

2018). The aim of the present study is to employ morphology and multi-gene (ITS, LSU and β -tubulin) phylogenetic data to describe a new *Microsphaeropsis* species collected from *Ononis spinosa* L. in Italy.

Materials and Methods

Sample collection, morphological study and isolation

The specimen was collected from dead aerial stems of *Ononis spinosa* L. in the Province of Forlì-Cesena, Italy and isolates were derived via single spore isolation following the method of Phookamsak et al. (2014). Growth rates and colony characteristics were determined from cultures grown on 2 % potato-dextrose agar (PDA) at 25°C in the dark. Morphological observations and photomicrographs were carried out following the method of Thambugala et al. (2015). The living cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) with duplicates in International Collection of Microorganisms from Plants (ICMP) New Zealand. The herbarium materials are deposited in the Herbarium of Mae Fah Luang University (MFLU), Thailand and Guizhou Academy of Agricultural Sciences (GZAAS), China. Facesoffungi (FoF) and Index Fungorum (IF) numbers are registered as explained in Jayasiri et al. (2015) and Index Fungorum (2018), respectively.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh mycelium, following the method of Thambugala et al. (2015). The PCR amplifications were performed in a total volume of 25 μ L of PCR mixtures containing 8.5–9.5 μ L ddH₂O, 12.5 μ L 2 \times PCR Master Mix (TIANGEN Co., China), 1–2 μ L of DNA template, 1 μ L of each primer. The internal transcribed spacer region (ITS), 28S nrDNA (LSU) and β -tubulin gene regions were amplified using appropriate primers following the conditions stipulated in Thambugala et al. (2017). The PCR products were visualized under UV light on 1% agarose electrophoresis gels stained with ethidium bromide. Purification and sequencing of PCR products were carried out at Invitrogen Biotechnology Co., Shanghai, China.

Phylogenetic analyses

Newly generated sequences were subjected to standard BLAST searches of GenBank to determine the primary identity of the isolate. Multi-gene phylogenetic analyses based on selected ITS, LSU and β -tubulin, sequence data in Didymellaceae and Microsphaeropsidaceae were used to establish the phylogenetic placement of the new isolate. *Leptosphaeria doliolum* was used as the outgroup taxon. All sequences used in this study were downloaded from GenBank, following Chen et al. (2015). GenBank accession numbers and culture collection numbers of gene sequences used to construct the phylogenetic tree are listed in Table 1. Single gene data sets were aligned with Bioedit 7.1.3.0 (Hall 1999) and the consensus sequences were further improved with MUSCLE implemented in MEGA 5v (Tamura et al. 2011). Alignments were checked and optimized manually when necessary. Phylogenetic analyses were based on maximum likelihood (ML) criterion using RAxMLHPC BlackBox (8.2.10) (Stamatakis 2006, Stamatakis et al. 2008) in the CIPRES portal (Miller et al. 2010). The general time reversible model of evolution including estimation of invariable sites (GTRGAMMA + I) and assuming a discrete gamma distribution with four rate categories was used for the ML analysis. The best scoring tree was selected and visualized with MEGA v. 5 (Tamura et al. 2011) and Adobe Illustrator CS3 software. ML Bootstrap supports (BS) (greater than 70 %) are shown above or below each node. The alignment was deposited in TreeBASE (<http://www.treebase.org/>), as study ID: 22331. All the newly generated sequences in this study were deposited in GenBank (Table 1).

Table 1 Culture collection and GenBank accession numbers used in the multi-gene phylogenetic analysis. Newly generated sequences/isolates are shown in bold.

Name	Culture Collection no. *	ITS	LSU	β -tubulin
<i>Didymella calidophila</i>	CBS 448.83	FJ427059	GU238052	FJ427168
<i>Didymella exigua</i>	CBS 183.55	GU237794	EU754155	GU237525
<i>Didymella rumicicola</i>	CBS 683.79	KT389503	KT389721	KT389800
<i>Leptosphaeria doliolum</i>	CBS 505.75	JF740205	GQ387576	JF740144
<i>Microsphaeropsis olivacea</i>	CBS 432.71	GU237863	GU237987	GU237548
	CBS 442.83	GU237865	EU754171	GU237547
	CBS 233.77	GU237803	GU237988	GU237549
<i>Microsphaeropsis ononidicola</i>	MFLUCC 15–0459, ICMP 21575	MG967670	MG967668	MG973087
<i>Microsphaeropsis proteae</i>	CBS 111303	JN712495	JN712561	–
	CBS 111320	JN712496	JN712562	JN712649
	CBS 111319	JN712497	JN712563	JN712650

*CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; ICMP: International Collection of Microorganisms from Plants; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand

Results

Phylogenetic analysis

The resulting phylogenetic tree obtained from the concatenated analysis of an ITS, LSU and β -tubulin sequence dataset included ten strains, representing six species in Didymellaceae and Microsphaeropsidaceae with *Leptosphaeria doliolum* as the out group taxon (Fig. 1). The family Microsphaeropsidaceae is represented by only one genus, *Microsphaeropsis* and it contains *M. olivacea*, *M. proteae* and the strain MFLUCC 15–0459 which is grouped in Microsphaeropsidaceae as a basal clade with high bootstrap support (98%, Fig. 1).

Taxonomy

Microsphaeropsidaceae Q. Chen, L. Cai & Crous, Studies in Mycology 82: 213 (2015)

Microsphaeropsis Höhn., Hedwigia 59: 267. 1917.

Notes – The genus *Microsphaeropsis* is characterised by pycnidial, immersed or erumpent, solitary or confluent, ostiolate conidiomata, phialidic, conidiogenous cells and pale brown to yellowish

or greenish brown, globose, cylindrical to bacilliform, ellipsoidal to oblong, 0–1-septate conidia (Chen et al. 2015). There are no records of the sexual morph for this genus (Wijayawardene et al. 2016, 2017).

Type species – *Microsphaeropsis olivacea* (Bonord.) Höhn., Hedwigia 59: 267. 1917.

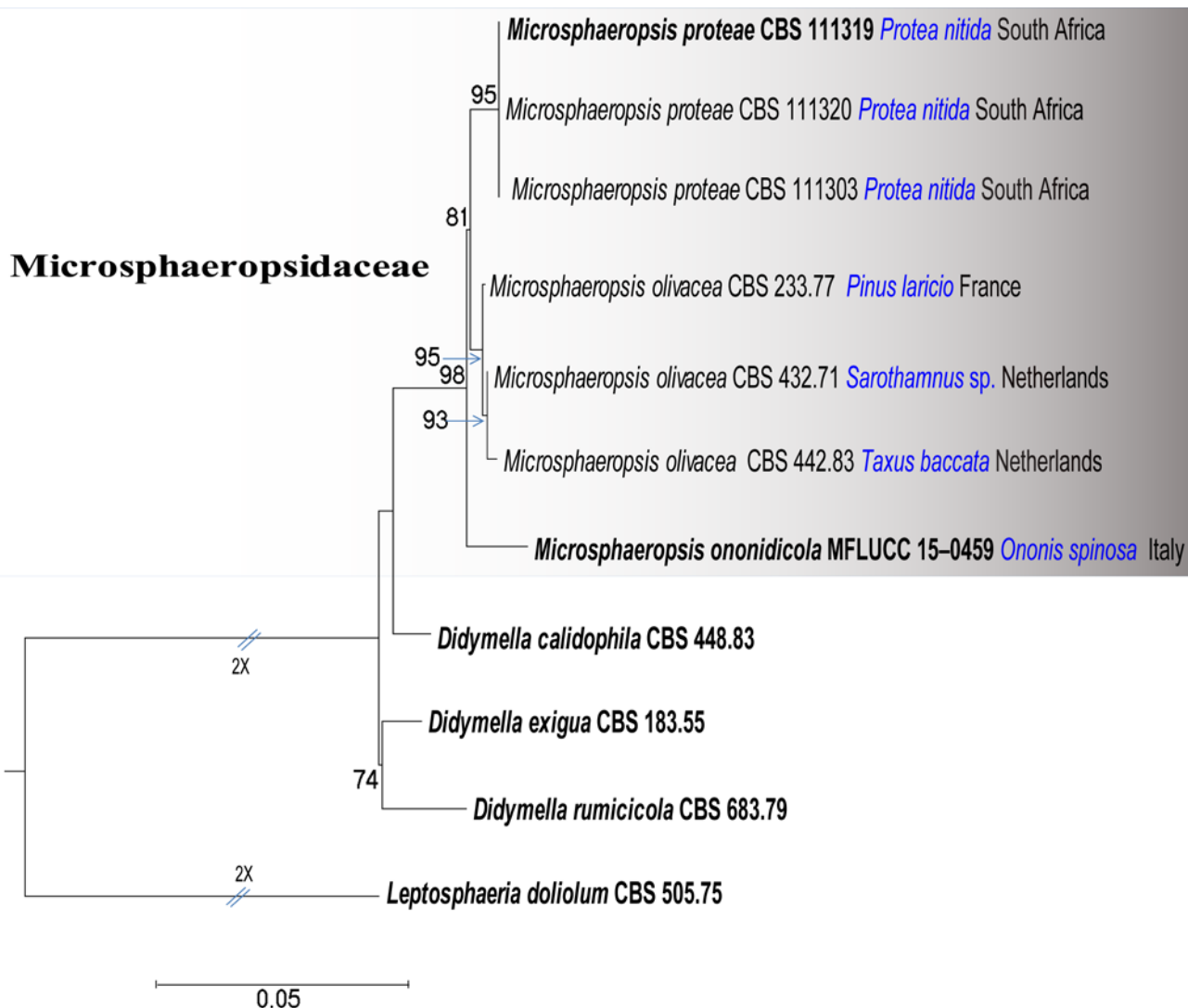


Fig. 1 – Maximum likelihood tree from analysis of combined ITS, LSU and β -tubulin sequence data of species in Didymellaceae and Microsphaeropsidaceae. Bootstrap support values greater than 70% are given above or below the nodes. Culture accession numbers are mentioned along with the species name, while hosts and the reported county for *Microsphaeropsis* species are given after culture accession numbers. The tree is rooted to *Leptosphaeria doliolum* and ex-type strains are in black bold.

Microsphaeropsis ononidicola Thambug., Camporesi & K.D. Hyde, sp. nov. Fig. 2

Index Fungorum number: IF554281; Facesoffungi number: FoF04186

Etymology – The species epithet “*ononidicola*” refers to the host genus *Ononis* on which the holotype occurs

Holotype – MFLU 16–2601

Saprobic on stems of *Ononis spinosa* L. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 100–190 μm high \times 100–230 μm diam. (\bar{x} = 135 \times 155 μm , n = 10), pycnidial, scattered, solitary, aggregated or gregarious, immersed, slightly erumpent, black, globose to subglobose, uni- to

bi-loculate, ostiolate. *Conidiomatal wall* 12–24 μm wide consisting of light to dark brown, thick-walled cells of *textura angularis*, becoming hyaline to lightly pigmented towards the conidiogenous region. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2.5–4.2 \times 3.5–4 μm (\bar{x} = 3.5 \times 3.8 μm , n = 15), enteroblastic, phialidic, hyaline, cylindrical, discrete or integrated, smooth. *Conidia* 4–6 \times 2–2.8 μm (\bar{x} = 5 \times 2.3 μm , n = 50), hyaline to yellowish brown, aseptate, obovoid to ellipsoidal, straight, smooth-walled, sometimes minutely guttulate.

Culture characteristics – Colonies growing on PDA, reaching a diameter of 30 mm after 10 d at 25 °C, circular to irregular, flat to slightly raised, moderately dense, surface initially white, becoming pale saffron to pale white; saffron to dark brown, smooth surface with entire to slightly filamentous edge.

Material examined – ITALY, Province of Forlì-Cesena [FC], Valbura - Premilcuore, dead aerial stems of *Ononis spinosa* L. (Fabaceae), 13 June 2014, Erio Camporesi IT 1237 (MFLU 16–2601, holotype), *ibid.* (GZAAS 16–0129, isotype), ex-type living culture MFLUCC 15–0459, ICMP 21575

Notes – *Microsphaeropsis ononidicola* can be distinguished from *M. olivacea* and *M. proteae* by its uni- to bi-loculate conidiomata with wider conidiomatal wall and smaller conidia (*Microsphaeropsis olivacea* (5–)6–7(–8.5) \times (3–)3.5–4 μm and *M. proteae* 5–8 \times 3.5–4 μm ; Crous et al. 2011, Chen et al. 2015). *Microsphaeropsis proteae* has only been reported from *Protea nitida* Mill. (Proteaceae) in South Africa, while *M. olivacea* has been reported on many different hosts worldwide (Sutton 1980, Chen et al. 2015). This is the first record of *Microsphaeropsis* species on *Ononis spinosa* L. in Italy.

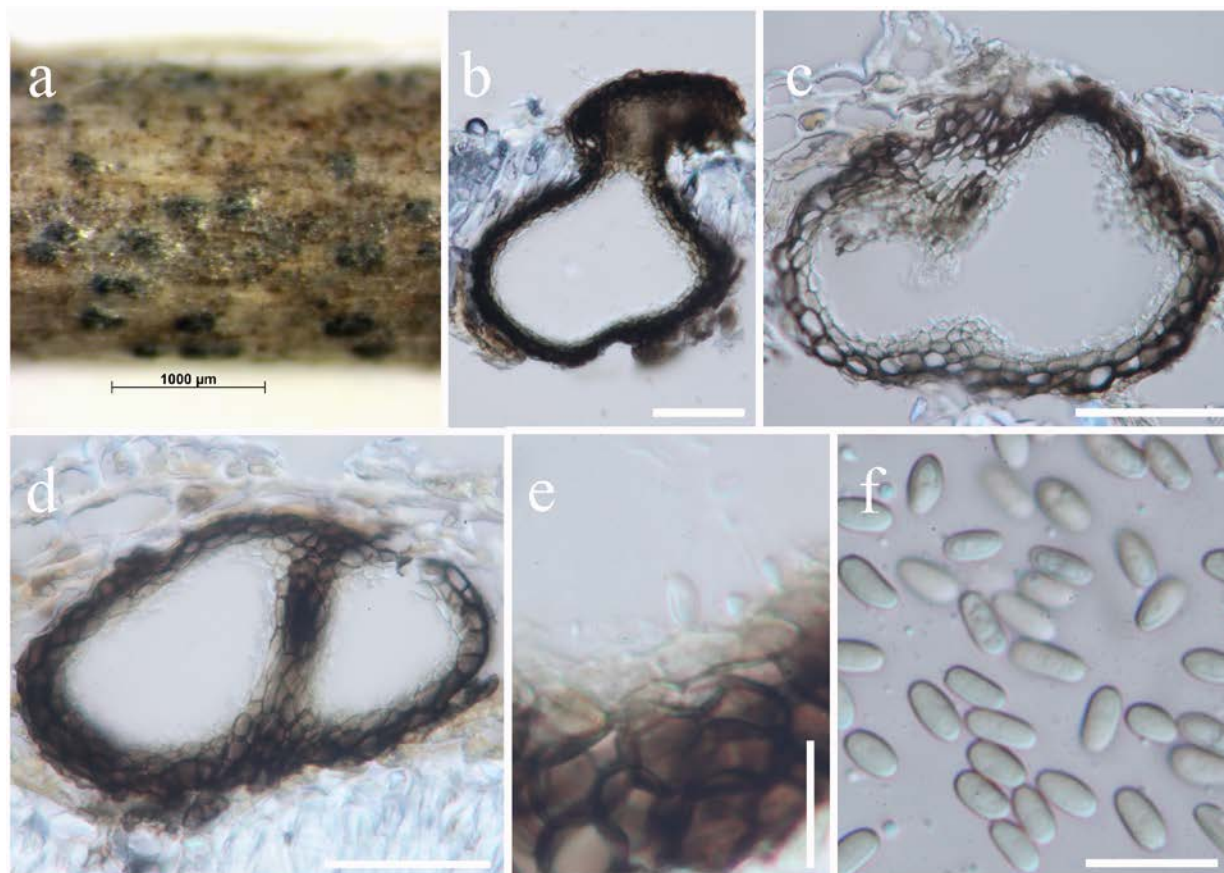


Fig. 2 – *Microsphaeropsis ononidicola* (holotype). a Appearance of conidiomata on host surface. b–d Vertical sections through conidiomata. e Conidiogenous cells and developing conidia. f conidia. Scale bars = b–d = 50 μm ; e, f = 10 μm .

Discussion

Microsphaeropsis-like taxa (such as *Aaosphaeria* Aptroot, *Coniothyrium* Corda, *Neomicrosphaeropsis* Thambugala et al., *Paraconiothyrium* Verkley, *Phaeosphaeriopsis* Câmara et al.) are polyphyletic within Dothideomycetes and they have been reported in several families including Coniothyriaceae, Didymellaceae, Didymosphaeriaceae, Phaeosphaeriaceae and Roussoellaceae (Hyde et al. 2013, Ariyawansa et al. 2014, Phookamsak et al. 2014, Wijayawardene et al. 2016, Thambugala et al. 2017). In this paper *Microsphaeropsis ononidicola* is introduced as a new species and phylogenetically its placement is established in Microsphaeropsidaceae. *Microsphaeropsis* species have been reported from a wide range of hosts worldwide and *M. ononidicola* is the first record of *Microsphaeropsis* species on *Ononis spinosa* L. (Sutton 1980, de Gruyter et al. 2009, Chen et al. 2015, Farr & Rossman 2018). With the exception of *Microsphaeropsis ononidicola*, only *M. olivacea* and *M. proteae* so far have been phylogenetically confirmed in the genus *Microsphaeropsis*, Microsphaeropsidaceae. Therefore, molecular analyses of other *Microsphaeropsis* and microsphaeropsis-like species are necessary to confirm their taxonomic placements.

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