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Distribution and phylogeny of *Mycosisymbrium cirrhosum*

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Abstract

The genus *Mycosisymbrium* was described in 1994 from dead leaves of *Vaccinium macrocarpon* and is monotypified by *M. cirrhosum*. There have been no further records of this species. During the study of litter degrading asexual fungi from the Western Ghats of Goa we collected *M. cirrhosum* from leaf litter of *Gnetum ula* and successfully obtained a culture of this fungus through single spore isolation. The multi-gene phylogenetic analysis shows that *Mycosisymbrium* is a well-supported sister genus to *Ochroconis* and *Verruconis* in the family *Sympoventuriaceae* of the order *Venturiales.*

Key words – Asexual morph – *Sympoventuriaceae* – *Venturiales* – Western Ghats

Introduction

It is important to place asexual fungi in families, orders and classes of *Ascomycota* and *Basidiomycota* in order to move towards a natural classification. In this way asexual and sexual fungi can be linked and this reduces redundancy resulting from the dual naming system (Hyde et al. 2011; Wijayawardene et al. 2014). Our work on the phylogeny of asexual fungi (Pratibha & Prabhugaonkar 2015a, 2015b, Pratibha et al. 2014a, 2014b) yielded a rare collection of the monotypic asexual genus *Mycosisymbrium*, with *M. cirrhosum* Carris, originally described from dead leaves of *Vaccinium macrocarpon* from Massachusetts, USA (Carris 1994). The genus is characterized by a discrete aggregates of conidiophores terminating in sterile, filiform appendages and brown, one-septate conidia. There have been no further records of this fungus since it was described. In this study, *M. cirrhosum* was isolated from leaf litter of *Gnetum ula* Brongn. from the forests of Valpoi, Goa, India and was subsequently cultured. Single spore cultures readily sporulated. Multi-gene phylogenetic analysis was carried out to confirm the phylogenetic placement of *Mycosisymbrium* as a well-supported sister genus to *Ochroconis* and *Verruconis* in the family Sympoventuriaceae in the order Venturiales.

Materials and methods

Collection and culturing

Mycosisymbrium cirrhosum was isolated on leaf litter of *Gnetum* from the forests of Sattari, Goa, India. Samples were brought to the laboratory in Zip-lock polythene bags and examined under a stereoscope. The fungus was picked up with a sterile needle, mounted in

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lactophenol and observed under a light microscope.The species was isolated in culture from single conidia as described in Chomnunti et al. (2014). The developing colonies, emerged from individual conidium, were aseptically transferred into fresh plates. After confirming the identity of the culture, molecular sequencing was done at Rajiv Gandhi Centre for Biotechnology, Thiruvananthpuram, Kerala, India. Culture is deposited at Microbial type culture collection (MTCC) at Institute of Microbial Technology, Chandigarh, India. The taxonomic record has been deposited in Faces of Fungi (Jayasiri et al. 2015).

DNA isolation and PCR Analysis

Fresh fungal mycelia (20 mg), scraped from the growing culture incubated at 28°C for 7 days. DNA isolation and PCR analysis was done according to Prabhugaonkar & Bhat (2011). The 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS), 18S nrDNA sequence (SSU), 28S nrDNA sequence (LSU) and RNA polymerase second largest subunit (RBP2) genes were amplified and sequenced using the primer pairs ITS-1F + ITS-4R, NS-1F + NS-4R (White et al. 1990), LR5 + LROR (Crous et al. 2009) and FRPB2-5F + FRPB2-7cR respectively. The sequence quality was checked using Sequence ScannerSoftware v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al. 2010).

Sequence alignment and phylogenetic analysis

The sequences were blasted in GenBank with Blastn. ITS, LSU, SSU and RPB2 data sets were analysed. Based on the blasts and available literature (Samerpitak et al. 2014), further related sequences were assembled. The combined data matrix was aligned using MAFFT v.7 (http://mafft.cbrc.jp/alignment/software) and manually adjusted using MEGA 6.06 to allow maximum alignment and maximum sequence similarity. A phylogenetic analysis was conducted using maximum likelihood (ML) in MEGA 6.06 (Kumar et al. 2008) with 1000 bootstrap replicates. The most suitable substitution models for the respective data sets were selected by using MEGA6.06. Tamura Nei model with Gamma distribution was used in analysis. Gaps were treated as a pairwise deletion and trees were viewed with MEGA6.06. All newly generated sequences used in this study are deposited in GenBank.

Results

Phylogenetic analyses

Thirty taxa are included in the phylogenetic analysis (Table 1, Fig. 1). Preliminary phylogenetic analysis showed that *Mycosisymbrium* has affinities with *Ochroconis* and *Verruconis* (Sympoventuriaceae, Venturiales, Zhang et al. 2011, Machouart et al. 2014, Samerpitak et al. 2014). A dataset of Sympoventuriaceae and Venturiaceae from the order Venturiales was assembled. *Pleospora herbarum* (Pleosporales) was selected as the out-group taxon. With multi-gene phylogenetic analysis it is observed that genus forms a well-supported clade within Sympoventuriaceae (Venturiales, Dothideomycetes) with *Ochroconis* and *Verruconis* as sister genera, confirming the distinctiveness of the genus (Fig. 1).

Taxonomy

Mycosisymbrium cirrhosum Carris Fig. 2

Facesoffungi number: FoF 01834, FOF 01835

Colonies on natural substrate effuse, brown; mycelium immersed. *Colonies* on MEA woolly, brown, reverse black, margin serrated, attaining a diam. of 1.9–2.2 cm in 10 days. Sexual morph: Undetermined. Asexual morph: *Conidiophores* macronematous, clustered, discrete, mononematous, branched, light brown, smooth-walled, 30–52 × 2–3 µm, each branch terminating in a filiform appendage. Appendages hyaline, flexuous, 22–35 × 1 µm. *Conidiogenous cells* mono

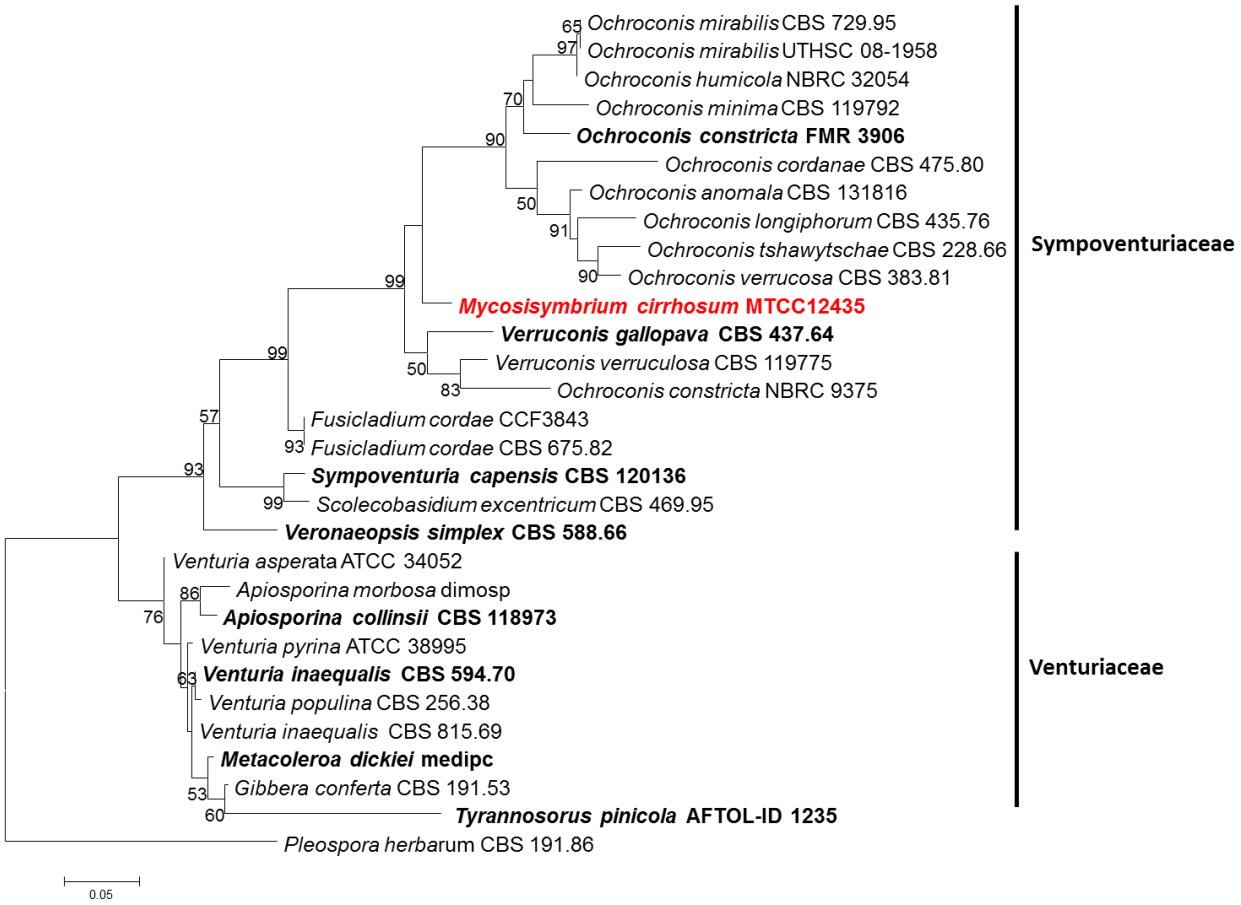

Fig. 1 – Maximum likelihood (ML) tree inferred from SSU, ITS, LSU and RPB2 showing the relationship of *Mycosisymbrium cirrhosum* with *Ochroconis* in Sympoventuriaceae (Venturiales). New sequence data is in red. Ex-type strains are in bold.

to polyblastic, light brown, smooth walled, denticulate, 6–10.5 × 2– 3 µm. *Conidia* solitary, oblong, rounded at the apex, brown, smooth-walled, 1-septate, slightly constricted at septum, 7.5–9 × 2.5–4 µm.

Material examined – INDIA, Goa, Valpoi, on leaf litter of *Gnetum ula* (Gnetaceae),26 January, *J. Pratibha* (Herb. No. VTL-5); living culture. MTCC12435

Notes – The new collection is identical to the original protologue in all morphological characters, except for the size of appendages, conidiogenous cells and conidia, with a maximum variation of only 2 µm.

Discussion

This second report of poorly known taxon extends its distribution to Western Ghats, India from its original locality in USA (Carris 1994) with a new host record. This also provides a natural phylogenetic placement for the genus. In this study we show using molecular data that *Mycosisymbrium* is well-supported sister genus to *Ochroconis* and *Verruconis* in the family Sympoventuriaceae, in the order Venturiales (Samerpitak et al. 2014, Machouart et al. 2014, Hyde et al. 2013, Zhang et al. 2011). This relation is further supported by morphological similarity to genus *Ochroconis* in having two celled spores, slightly constricted at the septa and polyblastic conidiogenous cells. However *Mycosisymbrium* differs from *Ochroconis* in having discrete aggregates of conidiophores terminating in sterile, filiform appendages. *Ochroconis* species are oligotrophic saprobes and some opportunistic species cause infections in vertebrates, its taxonomic status was recently reviewed by Machouart et al. (2014) using multi-gene phylogenetic analysis.

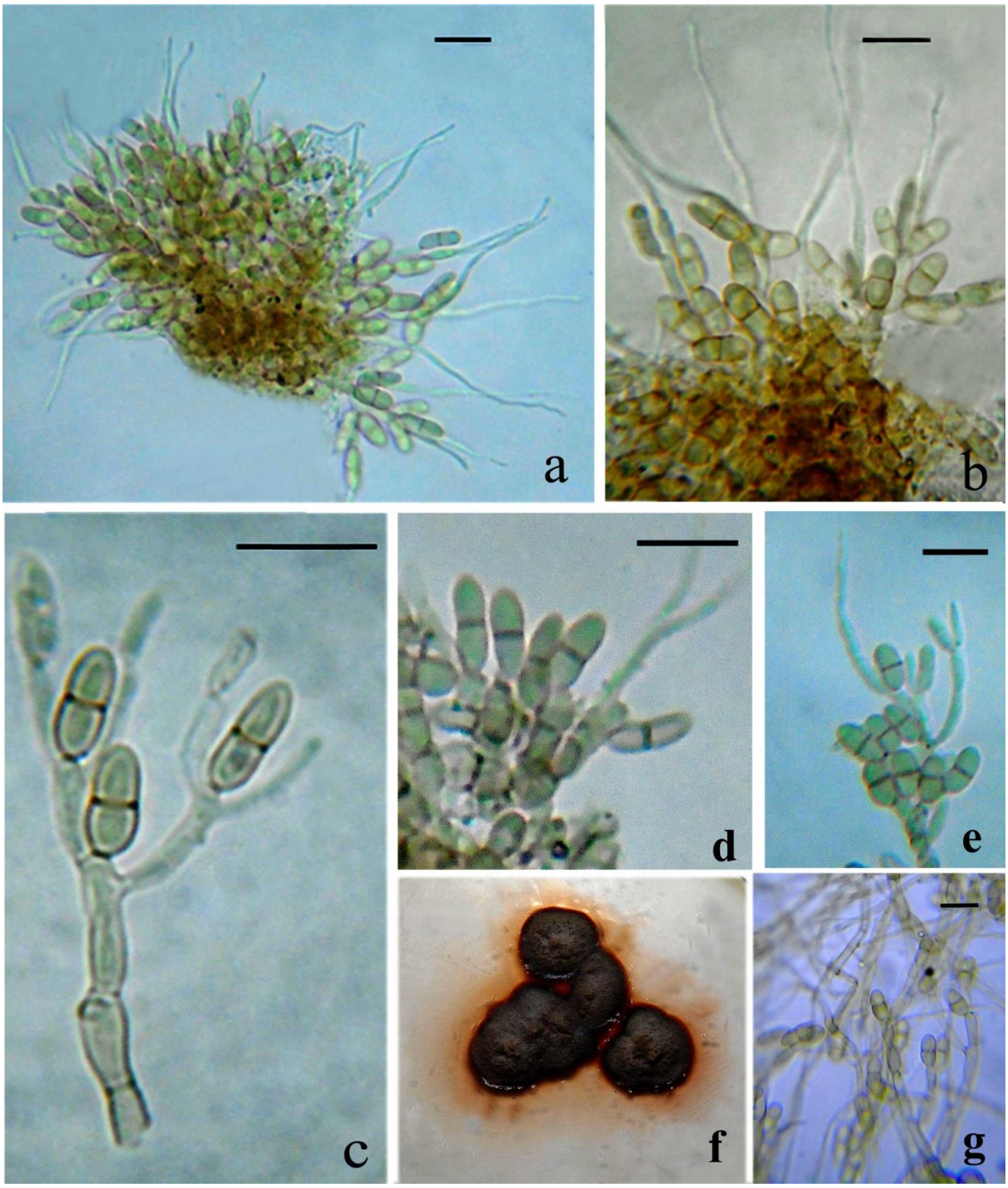

Fig. 2 – *Mycosisymbrium cirrhosum*.a, Conidiophore aggregates with conidia, b, Filiform appendages terminating from conidiophores. c- e, Conidiogenous cells and conidia. f, Culture. g, Conidiophores in culture. Scale bars = 10 µm.

The current study did not reveal the sexual morph of *Mycosisymbrium*. Furthermore, the study strengthens the generic concepts of *Mycosisymbrium* by providing molecular evidence, in addition to morphological evidence provided by Carris (1994). Index Fungorum/ MycoBank (MB 27274) mentions that *Mycosisymbrium*is a synonym of *Scolecobasidiella,* while listing the name *Mycosisymbrium cirrhosum* as legitimate, hence creating confusion. The current collection supports the distinctiveness of *Mycosisymbrium* from *Scolecobasidiella* and other sister genera in Sympoventuriaceae in having mononematous conidiophores, having distinct branching, in producing an aggregation of conidiophores and having each branch terminating in a filiform appendage.

Table 1 Sequence data used in combined ITS, SSU, LSU and RBP2 analyses. Newly deposited sequences are in bold.

Taxon	Accession no.	ITS	SSU	LSU	RPB2
Apiosporina collinsii	CBS 118973	--	GU296135	GU301798	GU357778
Apiosporina morbosa	dimosp	--	EF114718	EF114694	--
Fusicladium cordae	CCF3843	FN549910	--	FN377748	--
Fusicladium cordae	CBS 675.82	FN549913	--	FN398149	--
Gibbera conferta	CBS 191.53	--	GU296150	GU301814	GU357758
Metacoleroa dickiei	medipc	--	EF114719	EF114695	--
Mycosisymbrium cirrhosum	**MTCC12435**	**KR259883**	**KR259885**	**KR259884**	**KR349124**
Ochroconis anomala	CBS 131816	--	KF156065	KF156137	--
Ochroconis constricta	FMR 3906	LM644509	--	LM644552	--
Ochroconis constricta	NBRC 9375	DQ307327	AB564608	AB564619	DQ415431
Ochroconis cordanae	CBS 475.80	KF156022	KF156058	KF156122	--
Ochroconis humicola	NBRC 32054	--	AB564607	AB564618	AB564629
Ochroconis longiphorum	CBS 435.76	KF156038	KF156060	KF156135	--
Ochroconis minima	CBS 119792	KF156027	KF156086	KF156133	--
Ochroconis mirabilis	CBS 729.95	KF156029	KF156082	KF156144	--
Ochroconis mirabilis	UTHSC 08-1958	LM644517	--	LM644561	--
Ochroconis tshawytschae	CBS 228.66	KF156016	KF156064	KF156128	--
Ochroconis verrucosa	CBS 383.81	KF156015	KF156067	KF156129	--
Pleospora herbarum	CBS 191.86	KC584239	GU238232	GU238160	KC584471
Scolecobasidium excentricum	CBS 469.95	--	KF282683	KF282669	--
Sympoventuria capensis	CBS 120136	KF156039	KF156094	KF156104	--
Tyrannosorus pinicola	AFTOL-ID 1235	--	DQ471025	DQ470974	DQ470928
Venturia asperata	ATCC 34052	--	EF114736	EF114711	--
Venturia inaequalis	CBS 594.70	KF156040	GU296205	GU301879	GU357757
Venturia inaequalis	CBS 815.69	--	GU296204	GU301878	GU357756
Venturia populina	CBS 256.38	EU035467	GU296206	GU323212	GU357769
Venturia pyrina	ATCC 38995	--	EF114739	EF114714	--
Veronaeopsis simplex	CBS 588.66	KF156041	KF156095	KF156103	--
Verruconis gallopava	CBS 437.64	HQ667553	KF156053	KF156112	--
Verruconis verruculosa	CBS 119775	KF156014	KF156055	KF156106	--

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