

***Capillosclerotium*, a new genus in the order Corticiales causing Stem rot of Cluster Bean (*Cyanopsis tetragonoloba* L.)**

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Received : 10.09.2012

Accepted : 17.02.2013

Published : 29.04.2013

A new genus, *Capillosclerotium*, is proposed and described from the Stem rot of Cluster Bean (*Cyanopsis tetragonoloba*, Fam. Papilionaceae) from India based on morphological and phylogenetic evidences. The new fungus was differentiated from other related genera by having straw coloured, septate, thin and scanty mycelium with clamp connections; absence of monilliod cells; and golden yellow to orange, globose, 1 to 5 mm in diameter, long hairy, soft sclerotia with rind and medulla. The phylogenetic relationship of the basidiomycete genus *Capillosclerotium* with hairy sclerotia was assessed by ribosomal DNA sequences of ITS1 and 2 regions using maximum parsimony and Maximum Composite Likelihood analyses. This new genus formed a distinct monophyletic group from other related genera viz. *Macrophomina*, *Rhizoctonia*, *Sclerotium*, *Sclerotinia* and *Waitea* positioned in the order Corticiales. This fungus is illustrated at the light microscope and compared with other morphologically related taxa and is proposed with *Capillosclerotium indicum* as the type species.

Key words: Anamorphic fungi, Basidiomycete, DNA sequencing, ITS, new taxon, sclerotia

INTRODUCTION

Fungi in the basidiomycete order Corticiales are remarkably diverse nutritionally, including a variety of saprotrophs, plant and fungal pathogens, and lichen-forming fungi. Tracing the origin of this diversity depends on a clearer understanding of the phylogenetic relationships of fungi in the order. A new genus *Capillosclerotium*, which is unusual in its appearance, habit, and geographic provenance infecting the stem of cluster bean (*Cyanopsis tetragonoloba* L., Fam. Papilionaceae) is described in this paper.

The members of order Corticiales often do not produce spores, but produce hyphae and sclerotia (hyphal propagules) in their anamorphic stages (Moore, 1987). The proposed new genus is differentiated from other related genera based on the presence of unusual golden yellow to orange, hairy and soft sclerotia with rind and medulla; absence of monilliod cells; and production of thin and radial mycelium.

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Recent molecular phylogenetic analyses have begun to clarify the composition of the major clades of Agaricomycetes as well as the evolutionary development of its ecological and morphological diversity (Hibbett *et al.*, 2000 ; Hibbett and Thorn, 2001 ; Langer, 2002 ; Hibbett and Binder, 2002 ; Binder *et al.*, 2005 ; Hibbett, 2006 ; Matheny *et al.*, 2006, 2007 ; Larsson 2007). One of the clades of the Agaricomycetes consistently resolved in molecular phylogenies is now recognized formally as the Corticiales K.-H. Larss. (Hibbett *et al.*, 2007), including a single family Corticiaeae Herter (Larsson, 2007). This order is almost entirely composed of resupinate species and characterized by smooth hymenophores, a monomitic hyphal system with clamps, and smooth basidiospores with pink walls, all characteristics of the sexual, basidiospore-producing forms of the fungi (teleomorphs). Asexual forms (anamorphs), named separately by convention in mycology, have only recently been recognized in the Corticiales and assigned with certainty to the group. Members of the order have a wide range of nutritional ecologies, including mutualistic and pathogenic forms as well

as lignicolous saprobes (Diederich *et al.*, 2003; Binder *et al.*, 2005; dePriest *et al.*, 2005; Lawrey *et al.*, 2007).

The new fungus *Capillosclerotium* was investigated phylogenetically using parsimony and likelihood analyses of ITS 1 & 2 sequences with several additional sequences of related genera *viz.* *Waitea*, *Rhizoctonia*, *Laetisaria*, *Corticium*, *Marchandiomyces*, *Polyporus*, *Sclerotium*, *Sclerotinia* and *Macrophomina* and it formed a distinct monophyletic group.

Therefore, *Capillosclerotium indicum* as the type species is described here.

MATERIALS AND METHODS

Collection and isolation of fungal culture

Diseased cluster bean specimens were collected from experimental field of Anand Agricultural University, Anand, Gujarat and brought to laboratory for examination. The fungus was isolated and purified. Single spore isolation was made and deposited in Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi.

Optimization of culture medium

The fungus was grown at 25°C for 20 days in three different media *viz.* Potato dextrose agar (PDA), Carrot potato agar (CPA) and Oat meal agar (OMA) for standardization of suitable culture medium for the maximum growth and sclerotial production.

Phenotype analysis

Phenotypic characterization was performed using morphological characters of Sclerotia (colour, shape and growth) and mycelium under stereo and compound microscopes and compared with the related genera (Table 1).

Molecular analysis

Genomic DNA of new fungus was isolated by CTAB method (Culling, 1992). Approximately 650 bp of ITS region in the rDNA fragments were amplified using universal primers ITS1 (5'GGAAGTAAAAGTCGTAACAAGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') (White *et al.*, 1990). Purified PCR products were sequenced

using ITS universal primers by Bangalore Genei (Bangalore, India). DNA sequences were aligned with ITS sequences of related fungal genera using Clustal-X (Thompson *et al.*, 1997). Phylogenetic and pairwise distance analyses were performed in MEGA5 (Tamura *et al.*, 2011).

RESULTS

Optimization of culture medium

Maximum growth and sclerotial production was observed in Oat meal agar medium at 25°C after 20 days of inoculation. Many numbers of single and aggregated sclerotia of different sizes were distributed throughout the Petriplate (Fig 1).

Taxonomy

Capillosclerotium Prameela & Deeba gen. nov.
Mycobank No.: MB801129

Incrementa novum fungus in avenae farinam agar est proprium coram paleas coloris, sparse, tenues, radiales et septatae mycelium cum Fibulae; constructione ad punctum originis lateralis rami a principalis hypha; septum in germen iuxta commissuram cum principalis axis; globosis, luteo coloris crus sclerotia cum insolita tenuis alba capillos in eius superficie succrevit ad post gradus et cum distincta cortices et medulla et absentia monillioideis cellulis. (Fig. 2a-h).

Type species: *Capillosclerotium indicum* sp. nov.
Capillosclerotium Prameela & Deeba gen. nov.
Mycobank No.: MB801129

The growth of the new fungus on Oat meal agar (Fig. 2 a) is characterized by the presence of straw coloured, sparse, thin, radial and septate mycelium with clamp connections (Fig. 2 b & c); construction at the point of origin of lateral branches from the main hypha (Fig. 2 d); septum in the branch near the junction with the main axis (Fig. 2 d); Globose, golden yellow to orange coloured shiny sclerotia with unusual thin white hairs on its surface (Fig. 2 e, f & g); sclerotia made with distinct rind and medulla (Fig. 2h); and absence of monillioideis cells.

Type species: *Capillosclerotium indicum* sp. nov.
Capillosclerotium indicum Prameela & Deeba sp. nov.

Table 1. : Comparative account of *Capillosclerotium* gen. nov. with related genera

S. No.	Genera	Mycelium	Sclerotia			Monillioid Cells
			Structure	Shape	Size	
1.	<i>Macrophomina</i> (Tassi) Goid. (Goidanich 1947)	Hyaline to brown, septate, branched	Black, hard, smooth	Round to oblong or irregular	50-300µm	Absent
2.	<i>Sclerotium</i> Sacc. (Saccardo 1911)	White, septate, highly branched	Brown to black, hard, differentiated into rind and medulla	Elongate or globose	230-270 µm	Absent
3.	<i>Sclerotinia</i> (Lib.) de Bary, (de Bary 1884)	Hyaline, closely septate, much branched	Black, hard, smooth differentiated into rind and medulla	Irregular	2-12mm	Absent
4.	<i>Rhizoctonia</i> Kühn (Kühn 1858)	Brown, septate, Short celled, abundantly branched, stout, without clamp connections	Light brown to black, soft, smooth, not differentiated into rind and medulla	Irregular	300-710 µm	Present
5.	<i>Waitea</i> Warcup & P.H.B. Talbot (Warcup and Talbot 1962)	Hyaline, septate, irregularly branched, without clamp connections	Pinkish orange to brown, soft, smooth, not differentiated into rind and medulla	Irregular	0.5-3 mm	Present
6.	<i>Capillosclerotium</i> gen. nov. Prameela & Deeba	Straw coloured, septate, less branched, thin, with clamp connections	Golden yellow to orange, soft, shiny with unusual long, thin, white hairs on its surface, differentiated into rind and medulla	Globose	1-5 mm	Absent

Mycobank No. : MB801130

Incrementum mycelium in cultura medium modica sed sclerotial formationem tardius. Mycelium est septatis, albis initialis augmento, decet paleas coloris cum saeculi. Lateralibus ramis a principalis hypha sunt construi in puncto originis et septum occurrit in germen prope commissuram cum principalis axis. Et erat, tenues et exiguum, laevibus, velutinae cum minus ramosa. An asexual multicellulare sclerotia sunt formatur botrus coit vegetabilis hypha cellulis quod factus intertextis et aggregatum simul. In sclerotia saepe in una forma globosa, lucidis colore luteo rhoncus ut, mollis et V ad I mm diam. In sclerotia erant adfecti involutum per mollis et album tenues capillos. Sclerotia sunt ostendens distincta cortices et medulla in opprimendi sub lucem microscopio. Monilioid cellulis fuerunt.

Capillosclerotium indicum Prameela & Deeba sp. nov. (Fig. 2.a-h)

Mycobank No. : MB801130

The growth of mycelium in the culture medium was moderate but the sclerotial formation was slow.

Mycelium is septate, white at initial stage of growth, becoming straw coloured with the age. Lateral branches from the main hypha were constructed at the point of origin and septum occurs in the branch near the junction with the main axis. And it was, thin and scanty, effused, velvety with less branching. An asexual multicellular sclerotia were formed by a cluster of condense vegetative hypha cells which become interwoven and aggregate together. The sclerotia were often single, globose in shape, shiny, golden yellow to orange in colour, soft and 1 to 5 mm in diameter. The sclerotia were unusually enveloped by soft and white thin hairs. Sclerotia were showing distinct rind and medulla on crushing under light microscope. Monilioid cells were absent.

Host: Cluster Bean (*Cyanopsis tetragonoloba*; Papilionaceae) showing stem rot disease

Distribution: Western India (Gujarat state)

Etymology: *Capillosclerotium*, derived from English, meaning "hairy sclerotia" with type species, *Capillosclerotium indicum* named after its origin.

Material examined: Diseased specimens were collected from experimental field of Anand Agricul-

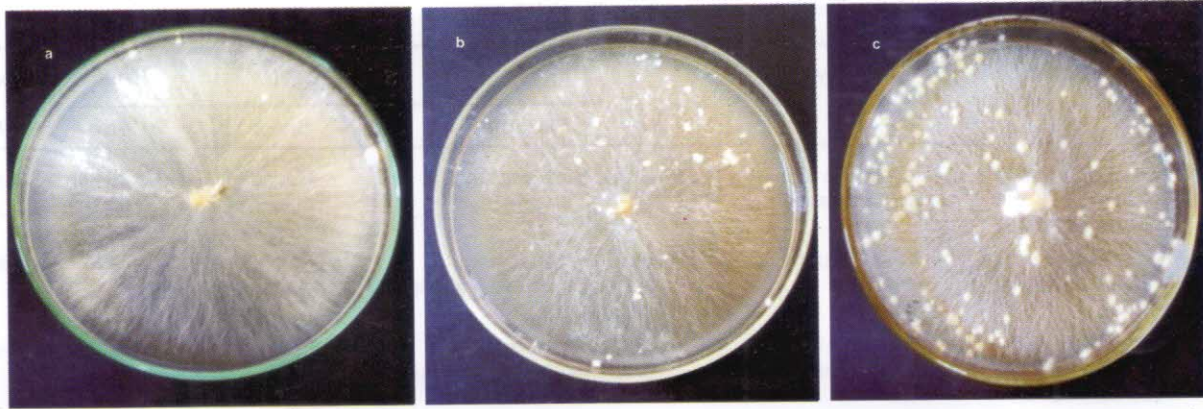


Fig. 1: Growth of *Capillosclerotium indicum* at 20 days after inoculation on a) Potato dextrose agar (PDA), b) Carrot potato agar (CPA), c) Oat meal agar (OMA)

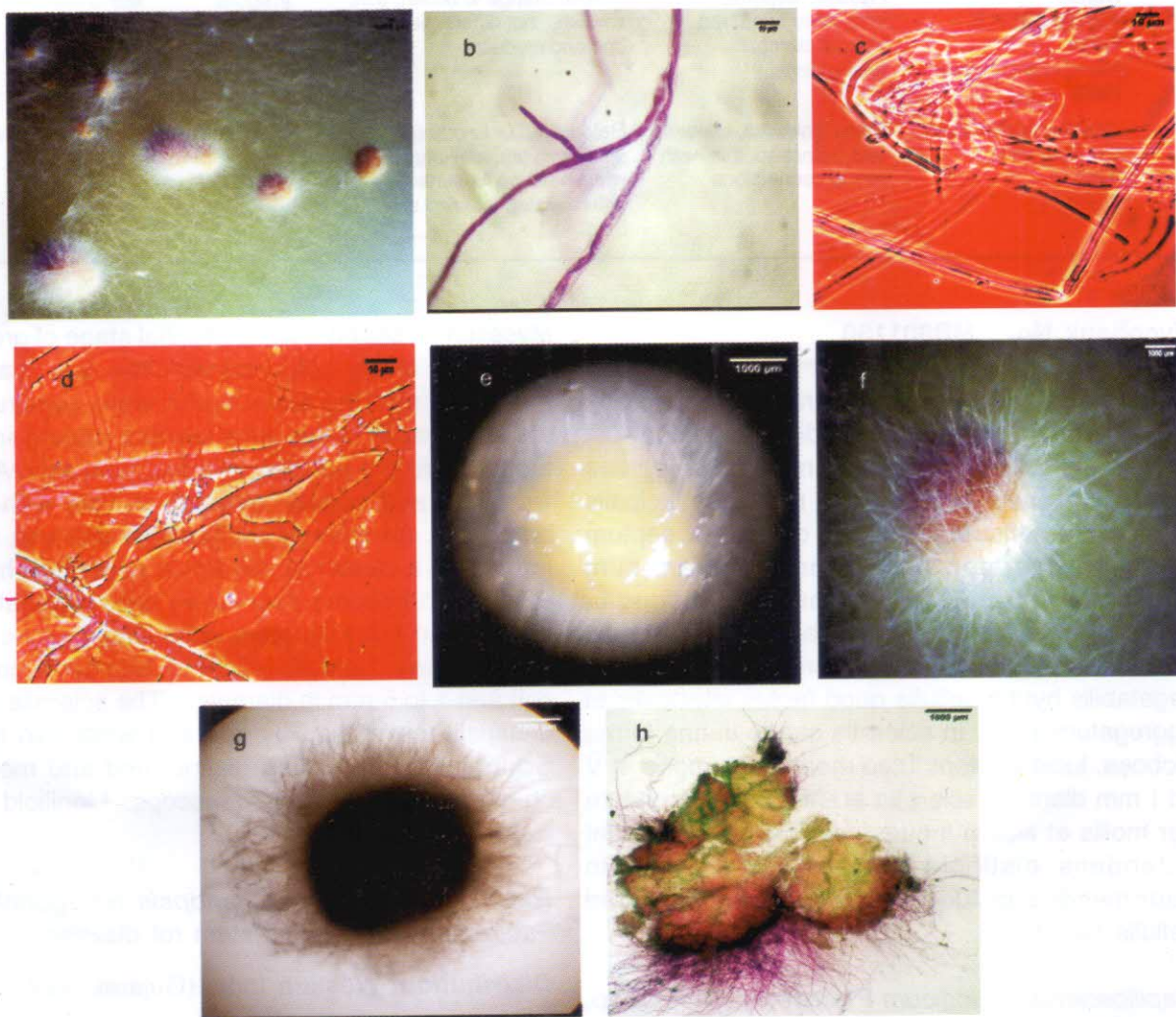


Fig. 2 : *Capillosclerotium indicum*: a) sclerotia in the culture, b&c) scanty and septate (arrow) mycelium with clamp connections (arrows), d) constriction and septum formation near points of hyphal origin e) golden yellow, shiny young sclerotium, f) orange coloured, round matured sclerotium with long white soft hairs, g) sclerotium under compound microscope, h) crushed sclerotium with rind and medulla

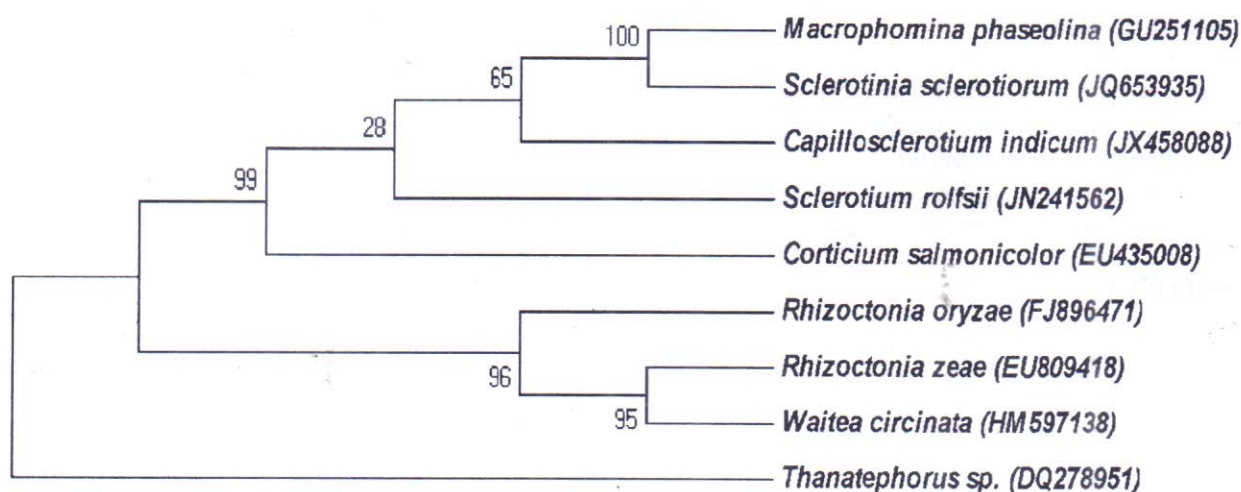


Fig. 3 : Phylogenetic relationships of different related genera by analysis of ITS sequences. The tree was obtained from analysis by the Maximum Parsimony method using 1000 replicates.

tural University, Anand, Gujarat, R.N. Pandey, 05.08.2010, ITCC No. 7099 (Culturotype), Gene Bank Accession No. JX458088

ITS region analysis

The ITS dataset of 9 taxa resulted in data matrix of 327 sites. The most parsimonious tree with length = 483 is shown (Fig.3). The composite index is 0.484702 for all sites and the consistency index (0.720930), the retention index (0.604938) and composite index (0.436118) are for parsimony-informative sites. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The strict consensus tree was constructed (Fig.3) in which *Capillosclerotium* did not cluster together with any other genera.

The ITS sequence obtained from *Capillosclerotium indicum*, consisting of the two spacer, have size of 420 bp. Analyses were conducted using the Maximum Composite Likelihood. Evolutionary Divergence between Sequences was estimated between 60.8-93.3%.

DISCUSSION

The fungus examined and described showed some unusual characters in the structure of mycelium and sclerotia. *Capillosclerotium indicum* produced distinctive septate, radial, straw coloured, thin and less branched mycelium with clamp connections. Sclerotia are globose, golden yellow to orange,

shiny and produced thin long white hairs on its surface which is a peculiar character and distinct from any other sclerotia producing fungi. This new fungus is showing some similar characters with the genus *Rhizoctonia* i.e. absence of asexual spores; septum formation in hyphal branches near points of hyphal origin. But it differs in the clamp connections, size and colour of the sclerotia, hyphal branching and monilliod cells (Baker, 1970; Parmeter and Whitney, 1970; Brown and McCarter, 1976; Anderson, 1982). 2004). *Capillosclerotium indicum* also differs with other sclerotial fungi viz., *Macrophomina*, *Sclerotium*, *Sclerotinia* and *Waitea* in many ways (Goidanich, 1947; Saccardo, 1911; de Bary, 1884; Kühn, 1858; Warcup and Talbot, 1962).

Recent molecular studies have established that corticiales species are distributed throughout the Agaricomycetes (Hibbett *et al.*, 2000; Hibbett and Thorn, 2001; Langer, 2002; Hibbett and Binder, 2002; Binder *et al.*, 2005; Hibbett, 2006; Matheny *et al.*, 2006, 2007; Larsson, 2007; Hibbett *et al.*, 2007). The results of our study confirm and expand on these prior observations. A new genus *Capillosclerotium* is proposed in the order Corticiales due to its closeness to the corticiales fungi but differing 62.3% based on internal transcribed spacer region analysis. This new sclerotial fungus is also differing with the other sclerotial fungi with 60.3-93% evolutionary divergence. Our results confirm that *Capillosclerotium* is monophyletic, but *Macrophomina*, *Rhizoctonia*, *Sclerotium*, *Sclerotinia* and *Waitea* were in a sister clade.

ACKNOWLEDGMENTS

The authors thank the Head, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi for the facilities provided. Financial support from Indian Council of Agricultural Research, Govt. of India, New Delhi, is gratefully acknowledged.

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