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RESEARCH ARTICLE

Proliferosphaera, a new genus from India

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ABSTRACT: *Proliferosphaera*, anamorph gen. nov. and *Proliferosphaera capsici* anamorph sp. nov. are proposed to accommodate a eustromatic fungus collected from the fruits of *Capsicum annuum* L. (Solanaceae) from Gujarat state of India causing die-back and fruit rot disease. The genus is characterized by the presence of dense conidiomata (pycnidia) with distinct zonation and were globose, with one or two ostioles when young, became irregular in shape with successive development of a number of broad and long beaks; attachment of dense mycelium with the matured conidiomata; production of many hyaline, aseptate, thin walled, ellipsoidal to sub-globose conidia with 10 x 2.5-7.5 µm in size having two conspicuous guttules one at each end. *Proliferosphaera* is recognized as a new genus distinct from the closed genera *viz.*, *Capitorostrum, Corniculariella, Cryptosporiopsis, Cytonema, Dinemasporium, Dwiroopa, Endothiella, Monochaetia, Phoma, Phomopsis, Phyllosticta* and *Seimatosporium* in the order Sphaeropsidales both at morphological and molecular (Internal Transcribed Spacer; ITS) analyses. In this article, the type genus *Proliferosphaera* with type species *Proliferosphaera capsici,* is described and illustrated.

Key words: Anamorphic fungus, *Capsicum annuum*, conidiomata, ITS, new taxon

Coelomycetes are known mainly from tropical and temperate region and cause the diseases of vegetative tissues, rot and blemish of growing and harvested fruits (Cook, 1975). A new fungus was found growing on fruits of *Capsicum annuum* L. (Solanaceae) causing fruit rot symptoms. The diseased specimens were collected from Sardar Krushinagar, Gujarat. The fungus produced water soaked lesions on the chilli fruits with black pycnidial bodies at the centre (Fig.1a). At later stages the fruits dried up. Deformed flowers (Fig.1b) were produced thereby affecting the yield. On critical examination based on morphology and ITS region analyses, the fungus was found new and does not match with any of the related genera of the Sphaeropsidales (Sutton, 1980; Aa *et al.*, 2000; De Gruyter *et al.*, 2002; Farr *et al.*, 2003; Boerema *et al.*, 2004; Udayanga *et al.*, 2011; Glienke *et al.*, 2011;). Hence it is described as a new genus with *Proliferosphaera capsici* as type species.

MATERIALS AND METHODS

Collection and isolation of fungal culture

Diseased chilli specimens were collected from experimental field of C. P. College of Agriculture, SDAU, Sardarkrushinagar, Gujarat and brought to laboratory for examination. These cultures were purified and single spore isolations were made on potato dextrose agar (PDA) and deposited in Indian type culture collection (ITCC), Division of Plant Pathology, IARI, New Delhi.

The pathogenicity was proved and symptoms were developed when the pure culture was inoculated on the healthy host plant.

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Phenotype analysis

Phenotypic characterization was performed using various morphological characters *viz.* conidiomata (colour, shape and growth), conidiogenesis, conidiogenous cell and size and shape of conidia and compared with the related genera of the order Sphaeropsidales (Table 1).

Molecular analysis

Genomic DNA of new fungus was isolated by CTAB method (Culling *et al.,* 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 (Table 2) using Clustal-X (Thompson *et al.,* 1997). Phylogenetic analyses were performed in MEGA5 (Tamura *et al.*, 2011)

Taxonomy

Proliferosphaera Prameela gen. nov. (Mycobank: MB800895)

Mycelium tenuis, septatus, brunneus, at conidiomata dense, compactus, in cerculi, brunneus chlamydospora et intercalarius mersus vel subimmersus, fuscum ad melano conidiomata et transeuns, irregularis figurae cum exordine progressus ex ampuls et longus rostrata. Conidiogenous cellae hyalinus, glabra, enteroblastus. Holoblastus ad acro septum ad varius loci supraholoconidiogenous celle. Locus quisque formae mono conidium. Conidia hyalinus, aseptatus ad ellipsoidum.

***Proliferosphaera* Prameela gen. nov. (Mycobank: MB800895)**

The fungus produce thin, septate, pale brown mycelium and dense conidiomata (pycnidia) arranged in circles (Fig.1c). Chlamydospores are brown and intercalary. Conidiomata immersed and semi-immersed, dark brown to black and become irregular in shape with successive development of broad and long beak (Fig.1d, e). Conidiogenous cells hyaline, smooth, enteroblastic. Conidiogenisis holoblastic with localized apical wall building, simultaneously at different loci over the whole conidiogenous cell. Each locus is forming one conidium (Fig.1i,j). Conidia hyaline, aseptate and ellipsoida (Fig.1k).

Type species: *Proliferosphaera capsici* sp. nov.

***Proliferosphaera capsici* Prameela sp. nov. (Mycobank: MB800896)**

Mycelium leptodermus, septatus, brunneus, multiguttatus. Intercalarius, brunneus chlamydospora vel solitarius vel catenuliformis evolvo ad posterius status. Conidiomata mersus vel subimmersus, fuscus cum inmaturus, melanoscere co-etas. Conidiomata inaequaliscere figurae cum exordine progressus numerus cressus et longus rostrata et densus mycelium afficionis maturus conidiomata. Pycnidium globosus, cum mono vel bi ostioli, leptodermus pseudoparanchyma, in maturus pachypleurus. Conidiogenous cellae hyalinus, glabra, enteroblastus, discrete, ampulliforme ad doliiforme. Holoblastus ad various loci. Locus quisque mono conidium. Conidia hyalinus, aseptatus, ellipsoidum ad subglobosum, 5-10 x 2.5-7.5 mm in magnitudinis cum biguttulae mono ad uterque terrninatum.

***Proliferosphaera capsici* Prameela sp. nov (Mycobank: MB800896)**

The growth of the new fungus on PDA is characterized by the presence of sparse thin mycelium and dense conidiomata (pycnidia) with distinct zonations. The mycelium was thin, septate, pale brown with dense oil globules. Intercalary and brown chlamydospores either solitary or in chains were developed at later stages. Conidiomata immersed and semi-immersed, dark brown when young became black with age. The conidiomata become irregular in shape with successive development of a number of broad and long beaks and dense mycelium is attached with the matured conidiomata. Pycnidia globose, thin pseudo parenchymatous walled, with one or two ostioles, on maturity becoming thick walled. Conidiogenous cells hyaline, smooth, enteroblastic, descrete, ampulliform to doliiform. Conidiogenisis holoblastic with localized apical wall building, simultaneously at different loci over the whole conidiogenous cell. Each locus is forming one conidium. Conidia hyaline, aseptate, ellipsoidal to sub-globose, 5-10 x 2.5-7.5 mm in size with two conspicuous guttules one at each end.

Host: *Capsicum annuum* L. (Solanaceae) causing die back and fruit rot disease

Distribution: Western India (Gujarat state)

Etymology: *Proliferosphaera*, derived from English, meaning "rapid growth and reproduction of new parts of conidiomata"

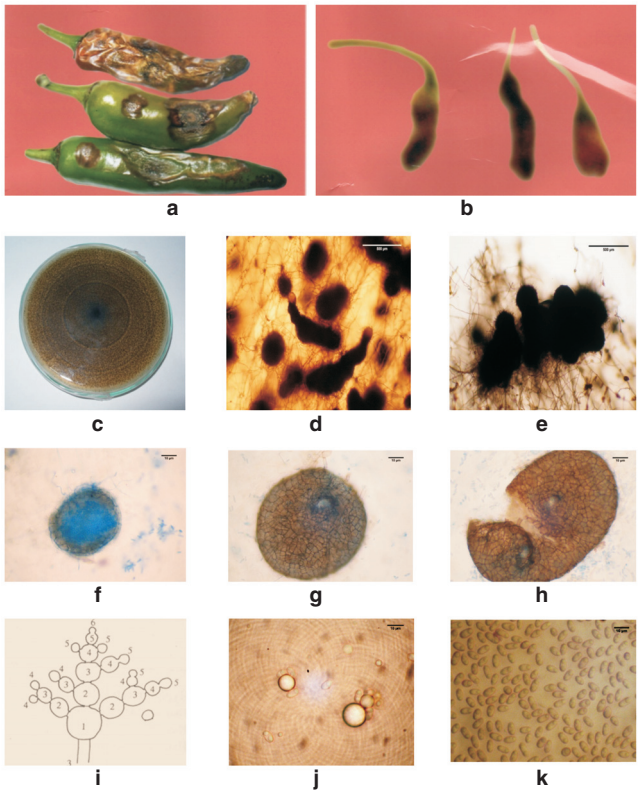

Fig. 1. a. Symptoms on chilli fruit, b. Symptoms on flower, c. Colony on Potato Dextrose Agar, d, e. Conidiomata, f,g,h. young Pycnidia, i,j. Conidial Ontogeny, k. Conidia

with type species, *Proliferosphaera capsici* named after the host, *Capsicum annuum* L.

Material examined: Diseased specimens were collected from Sardar Krushinagar, Gujarat. R. L. Patel 23.01.2009, ITCC No. 6494 (Culturotype), Gene Bank Accession No. HQ689653.

ITS region analysis

The ITS dataset of 9 taxa (Table 2) resulted in data matrix of 370 sites. The most parsimonious tree with length = 553 is shown (Fig. 2). The composite index is 0.310189 for all sites and the consistency index (0.635575), the retention index (0.445545) and composite index (0.283177) 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. 2) in which *Proliferosphaera* did not cluster together with any other genera.

The ITS sequence obtained from *Proliferosphaera capsici*, consisting of the two spacer, have size of 526 bp. The results of the ITS region sequence similarity comparisons showed that *Proliferosphaera capsici* had similarities with all the eight related genera only up to 66.1% which supports *Proliferosphaera* as a new genus.

The fungus examined and described, showed some peculiar characters in the structure of conidiomata,

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

S. No.	Genera	Conidiomata	Ostiole	Conidiophore	Conidiogenous Cell	Conidia
1.	*Capitorostrum* Batista Sutton (1980)	Superficial, solitary, separate with long neck	single	absent	ampulliform, smooth	Pale brown, eguttulate, 3-4x 1.5-2mm
2.	*Cornicullariella* Karst. Sutton (1980)	Eustromatic, single or in groups, conical,	single	present	Phialidic, determinate, integrated, subulate	Single septate, eguttulate, falcate, 45-75x3.5-4mm
3.	*Cryptosporiopsis* Bub.&Kabat Sutton (1980)	Acervular stromatic	single	present	phialidic, discrete, cylindrical, with proliferations	Straight or ellipsoidal, apex obtuse, truncate scar at the base, 25- 33.5x9.5-l0mm
4.	*Cytonema* Hohn., Sutton (1980)	Eustromatic,separate, globose, glabrous, with a rostrate apex	single	present	Phialidic determinate, integrated, apical or on small or long branches	Acropleurogenous, allantoid, eguttulate, 2.5-3.5xlmm
5.	*Dinemasporium* Lev. *(Pycnidiochaeta Camara*) Nag raj (1978)	Superficial, black, cupulate, setae present	nil	present	Enteroblastic with a minute channel	Pale brown, allantoids, fusiform, with a single guttule each end,3.5-11 x 1-2.5mm
6.	*Dwiroopa* Subram. & Muthumary Farr *et al.* (2003)	solitary, uni- to multiloculate, initiallyimmersed in the substratum, eventually becoming erumpent	nil	absent	holoblastic. rectangular, ampulliform,not branched.	Macroconidia obovoid to ovoid; with longitudinal slits, base truncate, 21.2–26. x 12.0–17.2µm, Mesoconidia ellipsoidal, 7.6–12.7µm 3.4–5.9µm
7.	*Endothiella* Sacc. Sutton (1980)	Pseudostromatic, reddish, pruinose, uni or multilocular	1 or more	present	Phialidic, determinate, integrated, apical or on small branches	Hyaline, ellipsoidal, eguttulate or guttulate, 3.5-4.5 x 1.5-2µm
8.	*Monochaetia* Sacc. Sutton (1980)	Acervular, composed of brown,thin walled textura angularis	nil	present	Holoblastic, indeterminate, cylindrical	4-euseptate,apical and basal cell hyaline with short, central appendage, at the apex filiform appendage,15-37 x 5-8.5µm
9.	*Phoma* Sacc. Boerema *et al.* (2004)	Immersed or semi-immersed, ob-pyriform, unilocular, brown, globose	1 or more, central	Occasionally present	Enteroblastic, phialidic, integrated or discrete	Hyaline, aseptate or one septate, eguttulate, ellipsoidal, cylindrical, fussiform, pyriform or globose,3-10 x 1.5- 4.5µm
10.	*Phomopsis* Sacc. Sacc Udayanga *et al.* (2011)	Eustromatic, immersed, separate or aggregated, uni or multilocular, globose, brown	One or more, circular, papillate	present	Enteroblastic, phialidic,determinate, integrated, cylindrical, rarely discrete	Two types. α-conidia hyaline, fusiform, biguttulate, aseptate,7-10 x 2.5mm; β conidia filiform, straight or hamate, eguttulate, aseptate, 20-30µm long
11.	*Phyllosticta* Pers. Glienke *et al.* (2011)	Black, aggregated, superficial to erumpent, globose to ampulliform,	single	present	terminal, subcylindrical to doliiform, hyaline, smooth	8–12.5X5–7 µm, solitary, hyaline, aseptate, coarsely guttulate, ellipsoid to obovoid, truncate base with apical appendage
12.	*Seimatosporium* Cdain Sturm (*Allelochaeta,* Petrak) Nag raj (1978)	Acervular, immerse, brown	nil	present	holoblastic, integrated or discrete, 0-many simple annellations	Cylindrical, fusiform, clavate, 2-5 euseptate sometimes with appendages, 9.5-37.5 x 3-10.5µm
13.	*Proliferosphaera* gen. nov.	Immersed or semi - immersed, black, irregular, proliferating with no. of broad and long beaks	1 or more	absent	Enteroblastic, discrete, ampulliform to doliiform	Aseptate, Sub globose to ellipsoidal, eguttulate, 5-10x 2.5-7.5µm

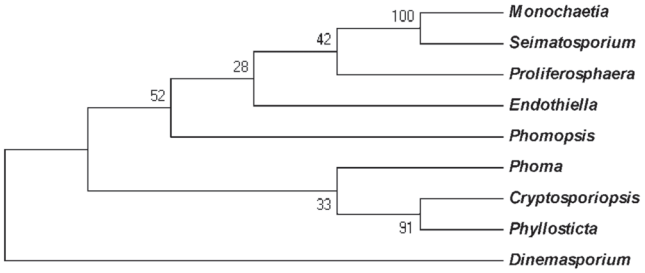

Fig. 2. Phylogenetic relationships of different related genera by analysis of ITS sequences. The tree was obtained from analysis by the Maximum Parsimony method using 1000 replicates.

Table 2. Different related genera used for Molecular phylogeny

S. No.	Name of the Genera	GenBank Accession No.
1	*Cryptosporiopsis* sp.	JF288555.1*
2	*Dinemasporium strigosum*	JQ889285*
3	*Endothiella gyrosa*	AF452117*
4	*Monochaetia camelliae*	AF377286*
5	*Phoma fimeti*	JN003227*
6	*Phomopsis* sp.	AB640843*
7	*Phyllosticta ericarum*	JX069865*
8	*Seimatosporium eucalypti*	JQ966946*
9	*Proliferosphaera capsici*	HQ689653

*taken from NCBI

conidiogenous cells and size of conidia. The most distinctive aspect of *Proliferosphaera capsici* is the presence of immersed or semi-immersed, black, irregular, proliferating conidiomata with no. of broad and long beaks; enteroblastic, discrete, ampulliform to doliiform conidiogenous cells; subglobose to ellipsoidal conidia with 5-10 x 2.5-7.5 mm in size having 2 guttules. Such characters in Sphaeropsidales are unusual and provide justification for creation of this new taxon (Sutton, 1980; Aa *et al.,* 1990; Aa *et al.,* 2000; De Gruyter *et al.,* 2002; Aa and Vanev*,* 2002; Farr *et al.,* 2003; Boerema *et al*., 2004; Udayanga *et al.,* 2011; Glienke *et al.,* 2011).

Even though *Proliferosphaera* resembles *Capitorostrum*, *Cryptosporiopsis* and *Dwiroopa* for the absence of conidiophores and with *Phoma, Phomopsis* and *Endothiella* for the presence of more than one ostiole, all the characters of this new fungus both at morphological and molecular level are not found (Table 1, Fig. 2) in any related genera of Coelomycetes (Nagraj, 1978; Sutton, 1980; Farr *et al.,* 2003; Boerema *et al.,* 2004; Kirk *et al.,* 2008; Udayanga *et al*., 2011; Glienke *et al.,* 2011). Hence this taxon is described as a new genus, *Proliferosphaera*, named after the presence of proliferating conidiomata with type species, *Proliferosphaera capsici* named after the host.

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