Three new lineages in Mycosphaerellaceae: Neoacervuloseptoria gen. nov., Neocercosporella gen. nov., and Neoramulariopsis gen. nov. based on the new species Neocercosporella peristrophes

Sanjay Yadav
Banaras Hindu University Faculty of Science
Raghvendra Singh (drsinghtaxon@gmail.com)
Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, U,P, India 221005 https://orcid.org/0000-0002-8672-6149
Sanjeet Kumar Verma
Banaras Hindu University Faculty of Science
Gargee Singh
DDU Gorakhpur University: Deen Dayal Upadhyaya Gorakhpur University
Prakash Kushwaha
Madhav Shiksha Mahavidyalaya

Research Article

Keywords: Anamorph, Mycosphaerellales, Dothideomycetes, Multigene-phylogeny, Nomenclature, New taxon

Posted Date: July 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1626095/v1

Version of Record: A version of this preprint was published at Mycological Progress on March 16th, 2023. See the published version at https://doi.org/10.1007/s11557-023-01871-y.

Abstract

A colourless hyphomycetous fungus was found on living leaves of *Peristrophe bicalyculata* in India. A multigene phylogenetic analysis (LSU-*RPB2*-ITS) of this strain was performed represents no other known lineage with similar morphology in *Mycosphaerellaceae*, hence the new genus *Neocercosporella* is proposed with *N. peristrophes* comb. nov., based on *Cercosporella peristrophes* (≡ *Pseudocercosporella andrographidis*), as type species. Phylogenetic examinations and ultrastructure of conidiogenous loci and hila of conidia of both the type materials from *N. peristrophes* and *P. andrographidis* confirm identical strains. The conidiogenous loci are conical, having very small rim-like depression on the top encircling a small flat protuberant like structure that make this novel strain differs from other closely related members of the ramularioid complex. Superficially, the colourless nature with thickened and darkened loci and hila make it closer to *Cercosporella*, but differs in having terminal and intercalary conidiogenous cells and weak catenation in conidia. Phylogenetically, *Neocercosporella* is distant from the *Cercosporella s. str.* clade (type species *C. virgaureae*). The addition of this novel strain, segregated closely related species in *Mycosphaerellaceae* therefore, three new genera and four new combinations are introduced in this study. New genera are: *Neoacervuloseptoria* gen. nov. *Neocercosporella* gen. nov. and *Neoramulariopsis* gen. nov. New combinations are: *Neoacervuloseptoria fraxini* comb. nov., *Neocercosporella peristrophes* comb. nov., *Neoramulariopsis catenulata* comb. nov. and *Neoramulariopsis dolichandrae* comb. nov.

Introduction

Based on phylogenetic data, most of the cercosporoid genera with and without connection with mycosphaerella-like sexual morphs belong to *Mycosphaerellaceae* (*Mycosphaerellales, Dothideomycetes, Ascomycota*; Abdollahzadeh et al. 2020), and covering about 120 genera are accepted within this family (Videira et al. 2017). The hyphomycetous ramularioid complex of this includes genera with colourless conidiophores and conidia. Morphologically, the most closely related genera of this complex are *Cercosporella* Sacc., *Pseudocercosporella* Deighton, and *Ramularia* Unger. These genera are very difficult to distinguish based on characteristics of conidiophores and conidia under light microscope, resulting in frequent transfers of species between these genera. The taxonomic problems related to this complex were extensively discussed by several workers (Hughes 1949; Sutton and Waller 1988; Braun 1990, 1991a,b, 1994, 1995, 1998; Verkley et al. 2004; Kirschner 2009; Videira et al. 2015, 2016, 2017).

In *Ramularia*, the conidial scars and hilum are slightly thickened while in those of *Cercosporella*, they are thickened. The ultrastructure of conidiogenous loci are smooth (flat conidiogenous loci shaped as a truncated cone) in *Cercosporella* and circular rim with a central dome in *Ramularia* (*Cladosporium*-type) under scanning electron microscope (Kirschner 2009; Bensch et al. 2012). *Cercosporella* produces cup-shaped appressoria while it is lacking in *Ramularia* (Kirschner 2009; Videira et al. 2016). *Pseudocercosporella* is characterised by unthickened and inconspicuous conidial loci as well as hila both (Deighton 1973; Braun 1995; Frank et al. 2010).

The hyaline genera with conspicuous conidial loci include *Cercosporella, Hawksworthiana, Neoovularia, Phacellium, Pseudodidymaria, Ramularia* and *Ramulariopsis*, while genera with inconspicuous conidial loci include *Monodidymaria, Neoramularia* and *Pseudocercosporella* (Videira et al. 2016). The Phylogenetic placement of *Ramularia* and allied genera within the order *Capnodiales* was established by Videira et al. (2016, 2017) using polyphasic approaches based on multilocus DNA sequence, morphological and cultural data.

To date, there have been several studies focused on diversity of phytopathogenic fungi in India, related to the genera of *Mycosphaerellaceae* (Singh et al. 2007, 2008, 2011, 2012, 2013, 2014a,b, 2020a, 2022; Kumar et al. 2013, 2014; Awasthi et al. 2015, 2016; Kharwar et al. 2015; Kumar and Singh 2015, 2016; Singh and Kumar 2017; Kushwaha et al. 2020). However, all previous studies have relied exclusively on morphological data, and very few records are supported by cultures and DNA sequence data (Singh et al. 2020b; Verma et al. 2021a, b; Yadav et al. 2021).

During survey of foliicolous fungi in the Afchand forest of Sagar, M.P., India, in December 2019, a colourless hyphomycete was reported on *Peristrophe bicalyculata*, represent a new lineage in *Mycosphaerellaceae* that was originally collected from same locality in 2013 and described as *Pseudocercosporella andrographidis* (Awasthi et al. 2016). Due to lack of phylogenetic analysis, the true generic affinity of *P. andrographidis* was quite unclear and unproven. In view of the limitation of using morphological traits for the elucidation of generic affiliations (Videira et al. 2017), phylogenetic examinations of the type materials were performed, showed that it could not be placed in any of the genera already described in *Mycosphaerellaceae*. Therefore, the new genus *Neocercosporella* is proposed. The addition of this novel strain segregated closely related species in *Mycosphaerellaceae*, resulted, establishment of new genera and combinations in this study.

Materials And Methods

Isolates and morphology

During the course of a survey of foliicolous fungi in the Afchand forest of Sagar, M.P., India, in December 2019, a colourless hyphomycete was found on *Peristrophe bicalyculata*. Infected leaves were collected in separate sterilized polyethylene bags and kept in dry paper envelopes and brought to the laboratory along with collection details. Close-up photographs of the infected host parts were taken with a Stereo Zoom Microscope (Magnus: MSZ-TR) with attached camera (CatCam300EF). For light microscopy spores were excised from the infected part of leaves and mounted on clear glass slides in both 50% glycerin and lactophenol cotton-blue mixture. Fungal propagules were photographed using Olympus compound microscope (CH20i-TR) equipped with Magnus camera (MIPS CMOS). The Scanning Electron Microscopy (SEM) was done with Fieldemission Scanning Electron Microscope (FEI Nova Nano SEM-450). Detailed observations of morphological characters were carried out at different magnifications through light microscopy (450× and 1000×) and scanning electron microscopy (up to ~ 18K×). For SEM micrographs specimens were coated with gold-paladium using a POLARON Sputter coater (180 sec in nitrogen atmosphere of 20 mA, 30 mm distant from the electrode) and examined with a LEO-430 scanning electron microscope. The holotype material is deposited in the Ajrekar Mycological Herbarium (AMH), Agharkar Research Institute (ARI), Pune, India and isotype material is retained in the Mycological Herbarium of the Department of Botany of Banaras Hindi University, Varanasi, U.P., India (MH-BHU).

For the cultivation of samples of *Neocercosporella* AMH 9671 (epitype) and AMH 10363 (topoepitype), conidia from collected samples were transferred to Petri dishes containing malt extract agar (2% w/v malt extract, 1.5% w/v agar agar). The dishes were placed at room temperature and diffuse daylight. Because culture from both the type grew about 1 mm in 4 wk and ceased to grow a living culture therefore, it could not be deposited.

Dna Extraction, Polymerase Chain Reaction (Pcr) And Sequencing

For isolation, amplification and sequencing of nuclear DNA of both the type materials of *Neocercosporella* (AMH 9671 and AMH10363), were used. DNA was isolated from freshly scrapped mycelia and spores from the heavily infected surface of living leaves using a sterile scalpel blade. Harvested mycelium approximately 200 mg of wet-weight was transferred to 2 ml microcentrifuge tubes kept in liquid nitrogen for two minutes and then grinded to a fine powder using pestle and mortar. From powdered form, DNA was extracted using Himedia DNA Isolation Kit (HiPurA™ Fungal DNA Purification Kit) following the manufacturers' protocols. Isolated DNA fragments were visualised by electrophoresis in 1% agarose gel (w/v) stained with ethidium bromide under Gel Documentation system (Bio-Rad Universal Hood II) and DNA concentration was quantified by using NanoDrop microvolume spectrophotometers (Thermo ScientificTM NanoDropTM One/OneC Microvolume UV-Vis Spectrophotometer with Wi-Fi).

Internal Transcribed Spacer (ITS) region, large subunit nuclear ribosomal DNA (LSU) gene and partial DNA-directed RNA polymerase II subunit (*RPB2*) gene were amplified by using ITS1/ITS4 (White et al. 1990), LROR/LR7 (Vilgalys and Hester 1990; Rehner and Samuels 1994) and RPB2-5F2/RPB2-7cR (Liu et al. 1999, Sung et al. 2007) primer pairs respectively. PCRs mixtures included the following ingredients for each 50 µL reaction: 5 µL of template DNA (~ 7 ng/µL), 5 µL PCR buffer containing $MgCl_2$, 1.5 µL of each forward and reverse primer (10 pmol), 1 µL dNTP (10 mM), 0.3 µL Taq DNA polymerase (5 Unit/µL) and 35.7 µl milli-Q water. The PCRs were carried out in Thermal Cycler (Bio-Rad T100™). Conditions for the PCR amplification consisted of an initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 94°C for 1 min; annealing at 54.7°C for ITS, 51.9°C for LSU and 53.9°C for *RPB2* for 1 min and extension at 72°C for 1 min. The final extension step was done at 72°C for 8 min. The amplified amplicon was run in 1.2% agarose gel and visualised in the Gel Documentation system (Bio-Rad Universal Hood II) for the product size and purity. The PCRs products were purified with FavorPrep™ PCR purification Kit. Sequencing was done at AgriGenome Labs Private Ltd., Kerala by the Sanger sequencing method using BigDye® Terminator v3.1 Cycle sequencing Kit and ABI 3100 DNA analyzer.

Sequence Alignment And Phylogenetic Analysis

The obtained ITS, LSU and *RBP2* sequences from both the type materials AMH 9671and AMH 10363 were assembled and edited with Chromas v.2.6.6. The manually edited sequences were submitted in NCBI GenBank (Table 1) and were subjected to a megablast search of the NCBI GenBank nucleotide database and sequences of related strains were retrieved. Reference sequences were also selected based on sequence availability from relevant published literature (Table 1). From the strains listed in Table 1, only those with the complete dataset of genes were used in the subsequent phylogenetic analyses, with the exception of *Cercospora rodmanii* (5H-GTOX), *Cercosporella pfaffiae* (Vic31849), and *Sonderhenia* sp. (CPC 17710) which were missing the *RPB2* sequence. Sequence alignments were generated with

MUSCLE in MEGA-X v.10.1.8 (Kumar et al. 2018). The alignments were manually checked, improved and concatenated where necessary with using BioEdit v.7.0.9 (Hall 2007) and MEGA-X v.10.1.8 (Kumar et al. 2018).

Table 1
Taxa included in molecular phylogenetic analyses and their GenBank accession numbers. The sequences in bold were generated in this study

Taxon	ID (isolate, strain, status, voucher)	GenBank accession no			Substrata	Location	References
		ITS	LSU	*RPB2*			
Acervuloseptoria fraxini	CPC 36558/ CBS 145992	MT223773	MT223870	MT223673	*Fraxinus* sp.	Russia	Crous et al. 2020
Acervuloseptoria ziziphicola	CBS 138009/ CPC 23707	KJ869164	KJ869221	MF951425	*Ziziphus mucronata*	South Africa	Crous et al. 2014, Videira et al. 2017
Cercospora apii	CBS 118712	KF251296	GQ852583	KT216554	NA	Fiji	Crous et al. 2009a, Verkley et al. 2013, Ismail et al. 2016
Cercospora beticola	CBS 116456/ CPC 11557	NR_121315	NG_068999	KT216555	*Beta vulgaris*	Italy	Groenewald et al. 2005 Schoch et al. 2006, Ismail et al. 2016
Cercospora fagopyri	CBS 132623/ CPC 14541	JX143594	MF951143	MF951463	*Fagopyrum esculentum*	South Korea	Groenewald et al. 2013, Videira et al. 2017
Cercospora janseana	CBS 145.37	MH855860	MH867363	MF951464	NA	USA	Videira et al. 2017, Vu et al. 2019
Cercospora rodmanii	5H-GTOX	GQ884184	GQ884186	NA	*Pontederia crassipes*	Maxico	Montenegro-Calderón et al. 2011
Cercospora sojina	CBS 132615/ CPC 11353	JX143659	KX286969	KX288419	*Glycine soja*	South Korea	Videira et al. 2016, Groenewald et al. 2013
Cercospora zeina	CBS 118820/ CPC 11995	DQ185081	MF951147	MF951469	*Zea mays*	South Africa	Crous et al. 2006, Videira et al. 2017
Cercosporella catenulate	CBS 355.73	KX287281	KX286973	KX288424	*Phaseolus vulgaris*	Rwanda	Videira et al. 2016
Cercosporella dolichandrae	CBS 138101/ CPC 22948	KJ869140	KJ869197	KX288423	*Dolichandra unguis-cati*	South Africa	Crous et al. 2014, Videira et al. 2016

Taxon	ID (isolate, strain, status, voucher)	GenBank accession no			Substrata	Location	References
		ITS	LSU	*RPB2*			
Cercosporella pfaffiae	Vic31849	JQ990331	JQ990330	NA			Machado et al. 2012
Cercosporella virgaureae	CBS 113304	GU214658	GQ852585	KX348051	*Erigeron annuus*	South Korea	Crous et al. 2009b, Videira et al. 2016
Cercosporella virgaureae	CPC 11461	KX287284	KX286977	KX288427	*Erigeron annuus*	South Korea	Videira et al. 2016
Cercosporella virgaureae	CPC 11456	MF951303	KX286974	KX348050	*Erigeron annuus*	South Korea	Videira et al. 2016, Videira et al. 2017
Cercosporella virgaureae	CPC 19492	KX287288	KX286981	KX288431	*Conyza canadensis*	Brazil	Videira et al. 2016
Cercosporella virgaureae	CPC 10287	KX287286	KX286979	KX288429	*Erigeron annuus*	South Korea	Videira et al. 2016
Cercosporella virgaureae	CPC 10286	KX287285	KX286978	KX288428	*Erigeron annuus*	South Korea	Videira et al. 2016
Cercosporella virgaureae	CPC 10288	KX287287	KX286980	KX288430	*Erigeron annuus*	South Korea	Videira et al. 2016
Cercosporella virgaureae	CPC 11460	KX287283	KX286976	KX288426	*Erigeron annuus*	South Korea	Videira et al. 2016
Cercosporella virgaureae	CPC 11457	KX287282	KX286975	KX288425	*Erigeron annuus*	South Korea	Videira et al. 2016
Clypeosphaerella calotropidis	CBS 129.30	MF951308	MF951153	MF951477	*Calotropis procera*	Egypt	Videira et al. 2017
Clypeosphaerella quasiparkii	CBS 123243/ CPC 15409	MH863287	MH874811	MF951478	*Eucalyptus* sp.	Thailand	Videira et al. 2017, Vu et al. 2019
Graminopassalora geissorhizae	CPC 38623/ CBS 146788	MW175336	MW175376	MW173111	*Geissorhiza splendidissima*	South Africa	Crous et al. 2020
Graminopassalora graminis	CBS 113303	GU214666	GQ852621	MF951502	*Alopecurus aequalis* var. *amurensis*	South Korea	Crous et al. 2009b, Crous et al. 2009c, Videira et al. 2017
Miuraea degenerans	MAFF 239265/ MUCC 1514	NR_156373	NG_070425	MF951523	*Miuraea degenerans*	Japan	Videira et al. 2017

Taxon	ID (isolate, strain, status, voucher)	GenBank accession no			Substrata	Location	References
		ITS	LSU	*RPB2*			
Miuraea persicae	CBS 131935/ CPC 10828	GU269844	JQ324939	MF951524	*Prunus armeniaca*	South Korea	Crous et al. 2013
Neocercosporella peristrophes	**AMH 9671**	**MZ311866**	**MZ311874**	**OL773683**	Peristrophe bicalyculata	**India**	**In this study**
Neocercosporella peristrophes	**AMH 10363**	**ON310831**	**ON310846**	**ON376994**	Peristrophe bicalyculata	**India**	**In this study**
Neodeightoniella phragmiticola	CPC 22057	KF777170	KF777223	MF951542	*Phragmites australis*	South Africa	Crous et al. 2013, Videira et al. 2017
Neodeightoniella phragmiticola	CBS 136418/ CPC 22059	NR_137606	NG_058043	MF951543	*Phragmites australis*	South Africa	Crous et al. 2013, Videira et al. 2017
Neopseudocercosporella brassicicola	CBS 228.32	MH855297	MH866752	KX348058	*Brassica oleraceae*	Denmark	Videira et al. 2016, Vu et al. 2019
Neopseudocercosporella capsellae	CPC 14774	KX287294	KX286993	KX288449	*Raphanus sativus*	South Korea	Videira et al. 2016
Parapallidocercospora colombiensis	CBS 110968/ CPC 1105	NR_156502	NG_069187	MF951581	*Eucalyptus urophylla*	Colombia	Crous et al. 2004b, Quaedvlieg et al. 2014, Videira et al. 2017
Parapallidocercospora thailandica	CBS 120723/ CPC 13478	MF951353	KF442667	MF951582	*Eucalyptus camaldulensis*	Thailand	Crous et al. 2013, Videira et al. 2017
Passalora bacilligera	CBS 131547/ CPC 19944	MF951356	MF951210	MF951585	*Alnus glutinosa*	Poland	Videira et al. 2017
Phloeospora ulmi	CBS 613.81	GU269825	GU253842	MF951601	*Ulmus* sp.	Austria	Crous et al. 2013, Videira et al.2017
Phloeospora ulmi	CBS 101564	KF251200	KF251703	MF951602	*Ulmus* sp.	Netherlands	Quaedvlieg et al. 2013, Videira et al. 2017

Taxon	ID (isolate, strain, status, voucher)	GenBank accession no			Substrata	Location	References
		ITS	LSU	*RPB2*			
Pseudocercospora punctata	CBS 132116/ CPC 14734	GU269765	GU253791	MF951622	*Syzygium* sp.	Madagascar	Crous et al. 2013, Videira et al. 2017
Pseudocercospora vitis	CBS 132012/ CPC 11595	DQ073923	GU214483	KX348076	*Vitis vinifera*	South Korea	Ayala-Escobar et al. 2006, Crous et al. 2009b, Videira et al. 2016
Pseudocercosporella bakeri	CBS 119488	KX287306	KX287005	KX288462	*Ipomoea indica*	New Zealand	Videira et al. 2016
Ramularia acroptili	CBS 120252	GU214689	GU214689	KX288472	*Rhaponticum repens*	Turkey	Crous et al. 2009b, Videira et al. 2016
Ramularia endophylla	CBS 113265	AY490763	AY490776	KP894673	*Quercus robur*	Netherlands	Verkley et al. 2004, Videira et al. 2015
Ramularia nyssicola	CBS 127665	KJ504765	KJ504724	KJ504636	*Nyssa ogeche* × *sylvatica hybrid*	USA	Videira et al.2015
Ramularia pusilla	CBS 124973	KP894248	KP894141	KP894687	*Poa annua*	Germany	Videira et al. 2015
Ramulariopsis gossypii	CBS 141099/ CPC 25909	KX287540	NG_059692	KX288702	*Gossypium* sp.	Brazil	Videira et al. 2016
Ramulariopsis pseudoglycines	CBS 141100/ CPC 18242	NR_154439	NG_059693	KX288705	*Gossypium* sp.	Brazil	Videira et al.2016
Ramulariopsis pseudoglycines	CPC 20036	KX287541	KX287244	KX288703	*Gossypium barbadense*	Togo	Videira et al. 2016
Ramulispora sorghi	CBS 110578/ CPC 905	MF951383	GQ852653	MF951653	*Sorghum bicolor*	South Africa	Crous et al. 2009a, Videira et al. 2017
Ramulispora sorghiphila	CBS 255.82	NR_156642	NG_058497	MF951656	NA	India	Videira et al. 2017
Septoria cucurbitacearum	CBS 178.77	KF251399	KF251903	MF951662	*Cucurbita maxima*	New Zealand	Verkley et al. 2013, Videira et al. 2017

Taxon	ID (isolate, strain, status, voucher)	GenBank accession no			Substrata	Location	References
		ITS	LSU	*RPB2*			
Septoria dysentericae	CPC 12328/ CBS 131892	GU214699	GU214699	KX348088	*Inula britannica* var. *chinensis*	South Korea	Crous et al. 2009b, Videira et al.2016
Septoria lycopersici	CBS 128654	KF251462	KF251966	KX348091	*Lycopersicum esculentum*	South Korea	Verkley et al. 2013, Videira et al. 2016
Septoria protearum	CBS 135477/ CPC 19675	KF251524	KF252029	MF951663	*Zantedeschia aethiopica*	South Africa	Verkley et al. 2013, Videira et al. 2017
Sphaerulina aceris	CBS 652.85	KF251594	GQ852673	MF951676	*Acer pseudoplatanus*	Netherlands	Crous et al. 2009a, Verkley et al. 2013, Videira et al. 2017
Sphaerulina koreana	CBS 131898/ CPC 11415	KF251639	KF252144	KX348096	*Vicia amurensis*	South Korea	Verkley et al. 2013, Videira et al. 2016
Sphaerulina tirolensis	CBS 109018	KF251638	KF252143	MF951680	*Rubus idaeus*	Austria	Verkley et al. 2013, Videira et al. 2017
Sonderhenia eucalypticola	CPC 112502/ CPC 3749	KF901677	KF902019	MF951672	*Eucalyptus* sp.	Portugal	Quaedvlieg et al. 2014, Videira et al. 2017
Sonderhenia eucalyptorum	CBS 120220	KF901505	KF901822	MF951673	*Eucalyptus coccifera*	Australia	Quaedvlieg et al. 2014, Videira et al. 2017
Sonderhenia sp.	CPC 17710	MN162025	MN162215	NA	*Sonderhenia* sp.	Australia	Crous et al. 2019
Outgroup							
Cladosporium cladosporioides	CBS 112388	HM148003	KX286982	KX288432	Indoor air	Germany	Videira et al. 2016, Bensch et al. 2012
Cylindroseptoria ceratoniae	CBS 477.69	KF251151	KF251655	MF951419	*Ceratonia siliqua*	Spain	Quaedvlieg et al. 2013, Videira et al. 2017

Taxon	ID (isolate, strain, status, voucher)	GenBank accession no			Substrata	Location	References
		ITS	LSU	*RPB2*			
Exopassalora zambiae	CBS 112971/ CMW 14782/ CPC 1227	AY725523	EU019273	MF951421	*Eucalyptus globulus*	Zambia	Crous et al. 2004a, Crous et al. 2007, Videira et al. 2017
Ramichloridium apiculatum	CBS 156.59	EU041791	EU041848	GU371770	Forest soil	USA	Arzanlou et al. 2007, Schoch et al. 2009
Readeriella nontingens	CPC 14444	GQ852786	GQ852663	MF951741	*Eucalyptus oblonga*	Australia	Crous et al. 2009a, Crous et al. 2009c, Videira et al.2017
Schizothyrium pomi	CBS 486.50	EF134948	EF134948	MF951735	*Fallopia sachalinensis*	Netherlands	Batzer et al.2008, Videira et al.2017
Stenella araguata	CBS 105.75	EU019250	EU019250	MF951742	*Stenella araguata*	Venezuela	Crous et al. 2007, Videira et al. 2017
Teratosphaeria stellenboschiana	CBS 125215/ CPC 13764	KF901733	KF937247	MF951743	*Eucalyptus punctata*	South Africa	Quaedvlieg et al. 2014, Videira et al. 2017
Uwebraunia australiensis	CBS 120729/ CPC 13282	KF442513	KF442553	KX348105	*Eucalyptus platyphylla*	Australia	Videira et al. 2016

The phylogenetic methods used in this study included a Bayesian analysis (BI) performed with MrBayes v.3.2.7 (Ronquist et al. 2012), maximum likelihood (ML) analysis performed with RAxML v.8.2.10 (Stamatakis 2014) and maximum parsimony (MP) analysis performed with PAUP v. 4.0b10 (Swofford 2003). The phylogenetic analyses were individually applied to two datasets: dataset 1 consisted of a concatenated alignment of LSU and *RPB2* sequences and datasets 2 consisted of concatenated alignments of LSU, *RPB2* and ITS sequences from 19 genera currently known to belong in the *Mycosphaerellaceae*, and from closely related families. All trees were rooted with *Cylindroseptoria ceratoniae* (CBS 477.69).

Bayesian inference was implemented with the GTR + I + G model. Bayesian inference was calculated using a Markov Chain Monte Carlo (MCMC) algorithm with Bayesian posterior probabilities (Rannala and Yang 1996). The analysis was performed till the standard deviation of split frequency was below 0.01. The first 25% of generated trees representing the burn-in phase were discarded, and the remaining trees were used to calculate posterior probabilities of the majority rule consensus tree. ML analysis was also performed using a GTR model of site substitution, including GAMMA + P-Invar model of rate heterogeneity and a proportion of invariant sites (Stamatakis 2014). The ML support values were evaluated with a bootstrapping method of 1000 replicates.. For the maximum parsimony analysis, a heuristic search option with 100 random sequence additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm was used. Alignment gaps were treated as fifth character states, and all characters were unordered and of equal weight. Maxtrees were set up to

5000, branches of zero length were collapsed, and all multiple, equally most parsimonious trees were saved. The robustness of the most parsimonious trees obtained was evaluated by 1000 bootstrap replications (Hillis and Bull 1993). Descriptive tree statistics for parsimony tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), homoplasy index (HI) and G-fit were calculated. These analyses involved 69 nucleotide sequences.

Presented trees were obtained with the ML approach. Tree reconstruction, visualization and editing were done using FigTree v.1.4.4 and TreeGraph_2.15.0. The multigene phylograms are shown in Figs. 4, 5.

Result

The data for the trees conducted in the different analyses are shown in table 1. Phylogenetic trees obtained from the combined gene analyses are supplied below (Figs 5, 6).

Dataset 1 (LSU & *RPB2* phylogeny):

This dataset consisted of a concatenated alignment of two loci (LSU, *RPB2*). The final alignment contained a total of 1439 characters divided in two partitions containing 748 (LSU) and 691 (*RPB2*) characters respectively, including alignment gaps. Phylogenetic trees generated from Bayesian analyses (BI), maximum likelihood (ML), and maximum parsimony (MP) produced trees with similar overall topology. A best scoring RAxML tree is presented in Fig. 6, with the Likelihood value of −21290.719845. The most parsimonious TL = 6393, CI = 0.297513, RI = 0.576999, RC = 0.171665, HI = 0.702487 and G-fit is −491.819875. From the analyzed characters, 530 were constant, 78 were variable and parsimony-uninformative, and 831 were parsimony-informative. In this analysis, some species of *Cercosporella* that were earlier considered as a member of *Cercosporella*, namely, *C. catenulata* (CBS 35573) and *C. dolichandrae* (CBS 138101), are now separated from this clade and make a separate sister branch of *Ramulariopsis* (Fig 6). Both the species of *Acervuloseptoria,* namely, *A. fraxini* (CPC 36558) and *A. ziziphicola* (CBS 138009) are distantly related from each other. *A. ziziphicola* is clustered closer and separated as a single-strain sister branch of *Neocercosporella peristrophes* with high bootstrap support (BI-PP/ML-BS/MP-BS: 1/100/100).

Dataset 2 (LSU, *RPB2* and ITS phylogeny):

The final alignment of this dataset contained a total of 1979 characters divided in three partitions containing 748 (LSU), 691 (*RPB2*), 540 (ITS) characters respectively, including alignment gaps. Phylogenetic trees generated from Bayesian analyses, ML, and MP produced trees with similar overall topology. A best scoring RAxML tree is presented in Fig. 7, with the Likelihood value of −27134.491457. The most parsimonious TL = 7894, CI = 0.321257, RI = 0.575503, RC = 0.184884, HI = 0.678743 and G-fit is −615.475663. From the analyzed characters, 744 were constant, 181 were variable and parsimony-uninformative, and 1054 were parsimony-informative. The results of analysis of dataset 2 (Fig 7), fully supports the dataset 1 (Fig 6).

The phylogenetic analysis based on both the datasets implied the same results. *Acervuloseptoria, Cercosporella, Neoacervuloseptoria, Neocercosporella, Neoramulariopsis* and*Ramulariopsis* form distinct lineage of a monophyletic group in *Mycosphaerellaceae* with high bootstrap support.

Taxonomy

Neoacervuloseptoria Raghv. Singh & Sanjay, *gen. nov.*

MycoBank MB840502

Etymology: Derived from genus name *Acervuloseptoria.*

Diagnosis: Differs from the genus *Acervuloseptoria* by its pycnidial type conidiomata opened via central ostioles and intermingled among spermatogonia.

Description: Plant pathogenic, foliicolous. Conidiomata pycnidial, intermingled among spermatogonia, black, opening via ostiole; wall brown, textura angularis. Conidiophores reduced to conidiogenous cells lining the inner cavity. Conidiogenous cells subcylindrical to ampulliform, hyaline, smooth, proliferating percurrently and sympodially at apex. Conidia solitary, subcylindrical, hyaline, smooth, granular, straight to curved, apex subobtuse, base truncate with basal marginal frill, septate. Adapted from Crous et al. (2020).

Type species: *Neoacervuloseptoria fraxini* (Crous & Bulgakov) Raghv. Singh & Sanjay (≡ *Acervuloseptoria fraxini* Crous & Bulgakov)

Neoacervuloseptoria fraxini (Crous & Bulgakov) Raghv. Singh & Sanjay, *comb. nov.*

MycoBank MB840503

Basionym: *Acervuloseptoria fraxini* Crous & Bulgakov, Fungal Syst. Evol. 6: 175 (2020)

Description and illustration: Crous et al. (2020)

Materials examined: Russia, Rostov region, Shakhty city district, trees near Atyukhta river, on living leaves of *Fraxinus pennsylvanica* Marshall (*Oleaceae*), 7 Oct. 2018, T.S. Bulgakov, HPC 2609 = Myc-45 (holotype CBS H-24228, culture ex-type CPC 36558 = CBS 145992).

Notes: *Acervuloseptoria* was established as a type species *A. ziziphicola* Crous & Jol. Roux (Crous et al. 2014). Only 3 species names are validly accepted to *Acervuloseptoria* (https://www.mycobank.org, queried 8 December 2021). In Videira et at. (2017), the *A. ziziphicola* Crous & Jol. Roux (CBS 138009) separated as a single-strain sister lineage of *Cercosporella* based on LSU-*RPB2* sequence data while clustered among the *Cercosporella* species based on LSU-*RPB2*-ITS sequence data. In 2020, another new species of *Acervuloseptoria, A. fraxini* Crous & Bulgakov(CPC 36558) was introduced thatclustered closer to *A. ziziphicola* based on LSU-*RPB2* sequence data (Crous et at.2020). According to Crous et al. (2020), *A. fraxini* does not show morphological similarity with *A. ziziphicola* but tentatively maintained as a new species of *Acervuloseptoria*.

In this study, based on both the datasets, *A. fraxini* clustered apart from *A. ziziphicola* and separated as an independent single-strain lineage with low bootstrap support (Figs 6, 7). *A. ziziphicola* has conidiomata that are black, erumpent, multilocular, with the upper layer disintegrating upon maturity, open irregularly and making conidiomata to have acervular appearance (Crous et al. 2014), while in those of *A. fraxini,* conidiomata are pycnidial type opened via central ostiole, intermingled among spermatogonia and never appear like acervular (Crous et at.2020). Therefore, it is worthwhile to establish this strain (CPC 36558) as a new genus *Neoacervuloseptoria* in *Mycosphaerellaceae*. *A. ziziphicola* separated as a single-strain sister lineage of *Neocercosporella* with high bootstrap support (BI-PP/ML-BS/MP-BS: 1/100/100) (Figs 6, 7). The differences in morphology are significant enough for retaining *Acervuloseptoria* (a coelomycete) as distinct from *Neocercosporella* (a hyphomycete). No molecular sequence data is available for *A. capensis* (G. Winter) Crous (Crous et al. 2015), therefore, it could not be incorporated in this study to know the exact placement with other clades.

Neocercosporella Sanjay & Raghv. Singh, *gen. nov.* Figs. 1–4

MycoBank MB840500

Etymology: Derived from the genus name *Cercosporella.*

Diagnosis: Differs from *Cercosporella s. str.,* by its conidiogenous loci which is conical in shape having very small rim-like depression on the top encircling a small flat protuberant like structure. In *Cercosporella,*conidiogenous cells are terminal and conidia formed singly, while in those of *Neocercosporella* conidiogenous cells are terminal and intercalary and weak catenation is found in conidia. It also differs from *Acervuloseptoria* due to its hyphomycetous nature, while later represents coelomycetous fungi.

Description: Plant pathogenic, foliicolous. Hyphae restricted to intercellular spaces. Colonies hypogenous. Stromata substomatal or subcuticular to erumpent. Conidiophores macronematous, fasciculate, arising from stromata, initially coming out of the leaf through stomata and later on by rupturing epidermis, erect to procumbent, hyaline to very light olivaceous, smooth, thin-walled to thick-walled, unbranched, rarely branched, straight to slightly curved, geniculate at the tip, septate. Conidiogenous cells integrated, terminal and intercalary, polyblastic, sympodial, conidiogenous loci slightly protuberant, thickened and darkened, loci conical having very small rim like depression on the top encircling a small flat protuberant like structure (Ultrastructure). Conidia formed singly, rarely catenate, mostly hyaline, rarely light olivaceous, dry, obclavate to obclavate-cylindrical, straight to curved, smooth, thin-walled, euseptate, base obconically truncate to rounded, tip obtuse, hila unthickened, sometimes slightly thickened and darkened.

Type species: *Neocercosporella peristrophes* (Syd.) Sanjay & Raghv. Singh (≡ *Cercosporella peristrophes* Syd.)

Notes: Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits using the **ITS** sequence had highest similarity to *Acervuloseptoria ziziphicola* (strain CBS 138009, GenBank NR_156287; Identities = 461/484 (95 %), 8 gaps (1%)), *Cercosporella dolichandrae* (strain CBS 138101, GenBank NR_156282; Identities = 459/495 (93%), 11 gaps (2 %)), and *Cercosporella virgaureae* (strain CBS 113304, GenBank GU214658; Identities = 461/484(95%), 8 gaps (1 %)). Closest hits using the **LSU** sequence are *Cercosporella virgaureae* (strain CBS 113304, GenBank GU214658; Identities = 1096/1133 (97%), 6 gap (0%)), *Septoria obesa* (strain CBS

354.58, GenBank GU214493; Identities = 1095/1133 (97%), 6 gap (0 %)), and *Septoria dysentericae* (strain CBS 12328, GenBank GU214699; Identities = 1092/1133(96%), 6 gap (0 %)). Closest hits using the ***RPB2*** sequence had highest similarity to *Acervuloseptoria ziziphicola* (strain CBS 138009, GenBank MF951425; Identities = 815/891 (91%), 0 gaps (0 %)), *Cercosporella virgaureae* (strain CBS 113304, GenBank KX348051; Identities = 746/893 (84%), 2 gaps (0 %)), and *Cercosporella catenulata* (strain CBS 355.73, GenBank KX288424; Identities = 655/795 (82%), 4 gaps (0 %))

Neocercosporellaperistrophes (N. Awasthi, Raghv. Singh & Sh. Kumar) Sanjay & Raghv. Singh, *comb. nov.* Figs. 1–4

MycoBank MB840501

Basionym: *Cercosporella peristrophes* Syd., Ann. Mycol. 31: 93 (1933)

Synonyms: *Cercosporella peristrophes* var. *microspora* N.D. Sharma & R.P. Mishra, J. Indian Bot. Soc. 56: 133 (1977)

Pseudocercosporella andrographidis N. Awasthi, Raghv. Singh & Sh. Kumar, Sydowia 68: 30 (2016)

Description: Infection spots amphiphyllous, white, circular to irregular, 1–10 mm in diam., later on covering the entire leaf surface and necrotic (Fig. 1a–f). Colonies hypogenous, white, velvety (Fig. 1e–f). Mycelium internal. Stromata present, globose to somewhat angular, substomatal or subcuticular to erumpent, hyaline, (9)15–25(35) × (10)15–20(25) µm diam (Fig. 2a–b). Conidiophores macronematous, densely fasciculate, arising from stromata, initially coming out of the leaf through stomata (Fig. 4a–c) and later on by rupturing epidermis, erect to procumbent, hyaline to very light olivaceous, smooth, thin-walled to thick-walled, unbranched, rarely branched, straight to slightly curved, geniculate at the tip, 0–3-euseptate, (10)15–40(53) × (2)3–4(6) µm (Fig. 2a–h). Conidiogenous cells integrated, terminal and intercalary, polyblastic (Fig. 4d), cylindrical, conidiogenous loci slightly protuberant, thickened and darkened (Fig. 2c–h), loci conical having very small rim-like depression on the top encircling a small flat protuberant like structure (Ultrastructure: Fig. 4e–i), 1.5–2.0 µm wide. Conidia formed singly, rarely catenate, mostly hyaline, rarely light olivaceous, dry, obclavate to obclavate-cylindrical, straight to curved, smooth (Fig. 4j–k), thin-walled, (0)1–6(12)-euseptate, base obconically truncated to rounded (Fig. 4l–m), tip obtuse, (18)30–80(117) × (2)3–5(6.5) µm, hila unthickened, sometimes slightly thickened and darkened, 1–2 µm wide (Fig. 3a–p).

Materials examined: India, UP, Allahabad, on leaves of *Peristrophe bicalyculata* (Retz.) Nees (*Acanthaceae*), November 1928, leg. Tandon, HCIO 12215 (holotype); India, MP, Sagar, Afchand forest, on living leaves of *Peristrophe bicalyculata* (Retz.) Nees (*Acanthaceae*), September 2013, leg. Neha Awasthi, AMH 9671 (epitype); India, MP, Sagar, Afchand forest, 23.834030°N 78.746567°E, on living leaves of *Peristrophe bicalyculata* (Retz.) Nees (*Acanthaceae*), 1 December 2019, leg. Raghvendra Singh, AMH 10363 (topoepitype).

Notes: In 2019, a colourless hyphomycete was collected on *Peristrophe bicalyculata*. Molecular phylogeny, showed hitherto undescribed genus*Neocercosporella* in *Mycosphaerellaceae* (Figs. 6, 7). This novel strain was originally described as *Pseudocercosporella andrographidis* Awasthi et al. (2016) from the same locality. The host of *P. andrographidis* was mistakenly identified as *Andrographis paniculata* in place of *Peristrophe bicalyculata.* The true generic affinity of *P. andrographidis* was quite unclear and unproven, due to lack of molecular sequence data and lack of discussion of ultrastructure of type material AMH 9671, hence it was established as a member of *Pseudocercosporella,*solely based on morphological features (Awasthi et al. 2016). However, the phylogenetic position of *P. andrographidis*, quite distant from the *Pseudocercosporella s. str.* clade, does now allow to maintain this species in the latter genus. Both the type materials AMH 9671 and AMH 10363 failed to develop live culture therefore, it could not be deposited. DNA sequences data from both the type materials (AMH 9671, AMH 10363) are 100% identical and cluster together with high bootstrap support (BI-PP/ML-BS/MP-BS: 1/100/100) to represent an same strainand could not be placed in any of the genera already described in *Mycosphaerellaceae* (Figs. 6, 7). Hence, it is justified to introduce a new genus for this lineage, viz., *Neocercosporella*. *Neocercosporella* is a monotypic genus that forms a well-supported clade in this study. The ultrastructure of conidiogenous loci and hila of conidia also confirms that both the type materials represent an identical strain(Figs. 5) and addition of this novel strain segregated closely related species in *Mycosphaerellaceae,* resulted, establishment of new genera and combinations.

Cercosporella peristrophes, the name of a common cercosporoid hyphomycete on *Peristrophe bicalyculata*, is available for the leaf spot disease examined and used as type species for *Neocercosporella*. *Cercosporella peristrophes* var. *microspora*, described from India on *Peristrophe bicalyculata*, is morphologically indistinguishable from *Cercosporella peristrophes* (Braun 1995).

On the basis of both the datasets, it is confirmed that *Cercosporella, Neocercosporella, Pseudocercosporella* and *Ramularia* represent separate genera (Fig 6, 7). Morphologically, based on ultrastructure of conidiogenous loci, *Cercosporella, Neocercosporella* and *Ramularia* can be easily distinguished. *Cercosporella* have flat conidial loci in the shape of a truncated cone (Fig. 5a–b) (Kirschner 2009) while *Neocercosporella* have conical loci having very small rim-like depression on the top encircling a small flat protuberant like structure (Fig.

5c–d). Conidiogenous loci of *Ramularia* have a raised rim with a central dome (Kirschner 2009) that is cladosporium-like (Fig. 5e–f). In *Cercosporella*,conidiogenous cells are terminal and conidia formed singly, while in those of *Neocercosporella* conidiogenous cells are terminal and intercalary and show weak catenation in conidia.

Cercospora acanthi Pass., *C. peristrophes* Thirum. & Govinduand *C. peristrophigena* Narayan et al. are additional asexual species of the *Mycosphaerellaceae* reported on *Peristrophe bicalyculata* (Thirum. and Govindu 1953, Narayan et al. 1999, Crous and Braun 2003, Kamal 2010), but irrelevant for the new strain since they belong to the genus *Cercospora* Fresen. which is characterized by having pigmented conidiophores and thickened, darkened conidiogenous loci and hila.

Semipseudocercospora peristrophes-acuminatae (J.M. Yen) J.M. Yen is also reported on *Peristrophe acuminate* (Yen 1983) and differs from novel strain due to its coloured nature of conidia and conidiophores both. The conidiogenous loci are distinctly denticle-like, and the solitary conidia are didymo- to phragmosporous, i.e. not scolecosporous (Videira et al. 2017).

Another genus, *Acervuloseptoria* with type species *A. ziziphicola* Crous & Jol. Roux (Crous et al. 2014) separated as a single-strain sister lineage of *Neocercosporella* with high bootstrap support (BI-PP/ML-BS/MP-BS: 1/100/100) (Figs. 6, 7). The differences in morphology are significant enough for retaining *Acervuloseptoria* (a coelomycete) as distinct from *Neocercosporella* (a hyphomycete).

Neoramulariopsis Raghv. Singh & Kushwaha, *gen. nov.*

MycoBank MB840504

Etymology: Derived from genus name *Ramulariopsis.*

Diagnosis: Differs from the *Cercosporella* due to its highly branched catenation in conidia and closer to *Ramulariopsis* but latter differs in having frequently branched conidiophores with integrated, terminal, intercalary and pleurogenous conidiogenous cells.

Plant pathogenic, foliicolous. Stromata immersed to erumpent, substromatal, brown, pseudoparenchymatal cells that develop into ascomata, with central ostiole; wall multilayers of brown textura angularis. Asci bitunicate, hyaline, smooth, obovoid, stipitate, with minute apical chamber. Ascospores guttulate, septate. Mycelium composed of hyaline, septate, branched hyphae. Conidiophores arising from hyphae or stromata, simple or branched, straight and subcylindrical to flexuous or geniculatesinuous, septate, hyaline, thin-walled, smooth. Conidiogenous cells integrated, terminal or lateral, hyaline, subcylindrical to geniculate-sinuous, with a single to multiple conidiogenous loci, conspicuous, loci truncate, thickened to unthickened, not darkened or very slightly darkened. Conidia hyaline, smooth, formed singly or in branched chains, form ramoconidia, intercalary and terminal conidia, aseptate to septate, with hila thickened but not darkened. Adapted from Crous et al. (2014) and Videira et at. (2016).

Type species: *Neoramulariopsis dolichandrae* (Crous & den Breeÿen) Raghv. Singh & Kushwaha (≡ *Cercosporella dolichandrae* Crous & den Breeÿen)

Neoramulariopsis catenulata (Videira & Crous) Raghv. Singh & Kushwaha, *comb. nov.*

MycoBank MB840505

Basionym: *Cercosporella catenulata* Videira & Crous, Stud. Mycol. 83: 91 (2016)

Description and illustration: Videira et al. (2016)

Materials examined: Rwanda, Rubona, on leaves of *Phaseolus vulgaris* L.(*Leguminosae*), 10 Jan. 1973, D. Froment (holotype CBS H-17715, culture ex-type CBS 355.73).

Neoramulariopsis dolichandrae (Crous & den Breeÿen) Raghv. Singh & Kushwaha, *comb. nov.*

MycoBank MB840506

Basionym: *Cercosporella dolichandrae* Crous & den Breeÿen, Persoonia 32: 233 (2014)

Description and illustration: Crous et al. (2014)

Materials examined: South Africa, KwaZulu-Natal, Pietermaritzburg, S29°37'50.95" E30°25'51.67", on leaves of *Dolichandra unguis-cati* (L.) L.G. Lohmann(*Bignoniaceae*), 15 Nov. 2011, A. King (holotype CBS H-21700, culture ex-type CPC 22948 = CBS 138101).

Notes: In Videira et at. (2016, 2017), based on LSU-*RBP2* sequence data, both *Cercosporella catenulata* and *C. dolichandrae* clustered together with *Cercosporella virgaureae* (Thüm.) Allesch. (the type species of *Cercosporella*)and form a well defined cladecloser to *Acervuloseptoria* Crous & Jol. Rouxand*Ramulariopsis* Speg. in *Mycosphaerellaceae* (Spegazzini 1911, Crous et al. 2014). Phylogenetically, *Acervuloseptoria* is represented by a single-strain lineage that is closely related to *Cercosporella* and *Ramulariopsis* (Videira et al. 2017). However, phylogenetic position of *Acervuloseptoria* is not yet clear, since it clustered near *Cercosporella* based on LSU-*RBP2* sequence data, but clustered among the *Cercosporella* species and separate both *C. catenulata* and *C. dolichandrae* from *Cercosporella* clade based on LSU-*RBP2*-ITSsequence data (Videira et al. 2017). In the single-gene (LSU/ITS) Bayesian trees of dataset, *Acervuloseptoria* clusters outside both the *Cercosporella* and the *Ramulariopsis* clade with high posterior probability value for LSU (PP = 0.94), with a low support in the case of ITS (PP = 0.54) (Videira et al. 2017). In the single gene Bayesian tree of *RPB2*, *Acervuloseptoria* sits in a highly supported polytomy (PP = 0.84) including the *Cercosporella* strains (Videira et al. 2017). Thus, *Acervuloseptoria* appears as a single-strain lineage sister to both *Cercosporella* and *Ramulariopsis* (Videira et al. 2017).

Similar results were also observed in this study (Figs 6, 7). On the basis of both the datasets, *Ramulariopsis* form a well defined clade in between the species of *Cercosporella* and separate both *C. catenulata* and *C. dolichandrae* from *Cercosporella* clade. Recently, similar results was also reported by Crous et al. (2020) where *Ramulariopsis* form well defined clade and separate both *C. catenulata* and *C. dolichandrae* from *Cercosporella,* based on LSU-*RBP2* sequence data. Such results indicate that *C. catenulata* and *C. dolichandrae* are not congeneric with *Cercosporella s. str.*, since they produce branched catenation in conidia (Crous et al. 2014, Videira et at. 2016). The highly branched catenation in conidia in both shows resemblance with *Ramulariopsis* and separated as its sister lineage. The *Ramulariopsis* species differs from *C. catenulata* and *C. dolichandrae* in having frequently branched conidiophores with integrated, terminal, intercalary and pleurogenous (as short nodulose protuberances or subcylindrical branchlets) nature of conidiogenous cells with prominently thickened and darkened conidiogenous loci (Videira et at. 2016, 2017). Therefore, it is worthwhile to establish a new genus *Neoramulariopsis* in *Mycosphaerellaceae* to accommodate these two *Cercosporella* species. Therefore, molecular sequence data of all the morphologically established species of *Cercosporella* are required to know the exact position in the phylogram. Most probably, *Neoramulariopsis* may accommodate all those *Cercosporella*-likespecies that have branched conidiophores and catenation in conidia.

Declarations

Acknowledgments The authors are indebted to anonymous reviewers for helpful comments and the curator of AMH for accepting material and providing an accession number there off. We are also thankful to the Head, CAS in Botany, Banaras Hindu University, Varanasi for Instrumental facilities.

Author's contribution All authors contributed to the conception and design of the study. Sanjay Yadav collected samples, tried to cultivate culture, isolated DNA and prepared samples for sequencing. Sanjeet Kumar Verma and Gargee Singh developed morphological features and surveyed concerned literature. Raghvendra Singh developed photo plates, performed phylogenetic analyses and developed the discussion part of the manuscript. Prakash Kushwaha wrote the first draft of the manuscript. All authors contributed to previous drafts of the manuscript and read and approved the final draft of the manuscript.

Funding RS thanks Science & Engineering Research Board (SERB), Department of Science & Technology (DST), Govt. of India (Scheme No. CRG/2020/006053) and Institution of Eminence (IoE) Scheme, Ministry of Human Resource and Development (MHRD), Govt. of India (Scheme No.6031) for providing financial support.

Data availability The sequences generated in this study have been submitted in GenBank with the accession numbers listed in table 1. The specimen studied in this work was deposited in the Ajrekar Mycological Herbarium (AMH), Agharkar Research Institute (ARI), Pune, Maharashtra, India.

Code availability Not applicable

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare that they have no conflict of interest in the develop of this research work.

References

1. Abdollahzadeh J, Groenewald JZ, Coetzee MPA, Wingfield MJ, Crous PW (2020) Evolution of lifestyles in *Capnodiales*. Stud Mycol 95:381–414. https://doi.org/10.1016/j.simyco.2020.02.004
2. Arzanlou M, Groenewald JZ, Gams W, Braun U, Shin HD, Crous PW (2007) Phylogenetic and morphotaxonomic revision of *Ramichloridium* and allied genera. Stud Mycol 58:57–93. https://doi.org/10.3114/sim.2007.58.03
3. Awasthi N, Singh R, Kumar S (2015) First report of a foliar disease caused by *Cercospora apii s. lat.* on *Spigelia anthelmia* from Madhya Pradesh, India. Schlechtendalia 28:53–57
4. Awasthi N, Singh R, Kumar S (2016) A new species of *Pseudocercosporella* on *Andrographis paniculata* from Central India. Sydowia 68:27–33
5. Ayala-Escobar V, Yánez-Morales de Jesùs M, Braun U, Groenewald JZ, Crous PW (2006) *Pseudocercospora opuntiae* sp. nov., the causal organism of cactus leaf spot in Mexico. Fungal Divers 21:1–9
6. Batzer JC, Arias MMD, Harrington TC, Gleason ML, Groenewald JZ, Crous P (2008) Four species of *Zygophiala* (*Schizothyriaceae, Capnodiales*) are associated with the sooty blotch and flyspeck complex on apple. Mycologia 100(2):246–258. https://doi.org/10.1080/15572536.2008.11832480
7. Bensch K, Braun U, Groenewald JZ, Crous PW (2012) The genus *Cladosporium*. Stud Mycol 72:1–401. https://doi.org/10.3114/sim0003
8. Braun U (1990) Studies on *Ramularia* and allied genera (III). Nova Hedwigia 50:499–521
9. Braun U (1991a) Taxonomic problems of the *Ramularia*/ *Cercosporella* complex. Stud Mycol 32:65–75
10. Braun U (1991b) Studies on *Ramularia* and allied genera (IV). Nova Hedwigia 53:291–305
11. Braun U (1994) Studies on *Ramularia* and allied genera (VII). Nova Hedwigia 58(1–2):191–222
12. Braun U (1995) A monograph of *Cercosporella, Ramularia* and allied genera (phytopathogenic hyphomycetes), vol 1. IHW-Verlag, Eching, Germany: IHW-Verlag, p 333
13. Braun U (1998) A monograph of *Cercosporella*, *Ramularia* and allied genera (phytopathogenic hyphomycetes), vol 2. IHW-Verlag, Eching, p 493
14. Crous PW, Braun U (2003) *Mycosphaerella* and its anamorphs: 1. Names published in *Cercospora* and *Passalora*. CBS Biodiversity Series, vol 1. Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands, p 571
15. Crous PW, Braun U, Groenewald JZ (2007) *Mycosphaerella* is polyphyletic. Stud Mycol 58:1–32. https://doi.org/10.3114/sim.2007.58.01
16. Crous PW, Braun U, Hunter GC, Wingfield MJ, Verkley GJM, Shin HD, Groenewald JZ (2013) Phylogenetic lineages in *Pseudocercospora*. Stud Mycol 75:37–114. https://doi.org/10.3114/sim0005
17. Crous PW, Groenewald JZ, Groenewald M, Caldwell P, Braun U, Harrington TC (2006) Species of *Cercospora* associated with grey leaf spot of maize. Stud Mycol 55:189–197. https://doi.org/10.3114/sim.55.1.189
18. Crous PW, Groenewald JZ, Mansilla JP, Hunter GC, Wingfield MJ (2004a) Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. Stud Mycol 50(1):195–214
19. Crous PW, Groenewald JZ, Pongpanich K, Himaman W, Arzanlou M, Wingfield MJ (2004b) Cryptic speciation and host specificity among *Mycosphaerella* spp. occurring on Australian *Acacia* species grown as exotics in the tropics. Stud Mycol 50:457–469
20. Crous PW, Schoch CL, Hyde KD, Wood AR, Gueidan C, De Hoog GS, Groenewald JZ (2009b) Phylogenetic lineages in the *Capnodiales*. Stud Mycol 64:17–47. https://doi.org/10.3114/sim.2009.64.02
21. Crous PW, Schumacher RK, Wingfield MJ, Lombard L, Giraldo A, Christensen M, Gardiennet A, Nakashima C, Pereira O, Smith AJ, Groenewald JZ (2015) Fungal Systematics and Evolution: FUSE 1. Sydowia 67:81–118
22. Crous PW, Shivas RG, Quaedvlieg W, van der Bank M, Zhang Y, Summerell BA, Guarro J, Wingfield MJ, Wood AR, Alfenas AC, Braun U, Cano-Lira JF, García D, Marin-Felix Y, Alvarado P, Andrade JP, Armengol J, Assefa A, den Breeÿen A, Camele I, Cheewangkoon R, De Souza JT, Duong TA, Esteve-Raventós F, Fournier J, Frisullo S, García-Jiménez J, Gardiennet A, Gené J, Hernández-Restrepo M, Hirooka Y, Hospenthal DR, King A, Lechat C, Lombard L, Mang SM, Marbach PAS, Marincowitz S, Marin-Felix Y, Montaño-Mata NJ, Moreno G, Perez CA, Pérez Sierra AM, Robertson JL, Roux J, Rubio E, Schumacher RK, Stchigel AM, Sutton DA, Tan YP, Thompson EH, van der Linde E, Walker AK, Walker DM, Wickes BL, Wong PTW, Groenewald JZ (2014) Fungal Planet Description Sheets: 214–280. Persoonia 32:184–306. https://doi.org/10.3767/003158514X682395
23. Crous PW, Summerell BA, Carnegie AJ, Wingfield MJ, Groenewald JZ (2009c) Novel species of *Mycosphaerellaceae* and *Teratosphaeriaceae*. Persoonia 23:119–146. https://doi.org/10.3767/003158509X479531

24. Crous PW, Summerell BA, Carnegie AJ, Wingfield MJ, Hunter GC, Burgess TI, Groenewald JZ (2009a) Unravelling *Mycosphaerella*: do you believe in genera? Persoonia 23:99–118. https://doi.org/10.3767/003158509X479487
25. Crous PW, Wingfield MJ, Cheewangkoon R, Carnegie AJ, Burgess TI, Summerell BA, Groenewald JZ (2019) Foliar pathogens of eucalypts. Stud Mycol 94:125–298. https://doi.org/10.1016/j.simyco.2019.08.001
26. Crous PW, Wingfield MJ, Schumacher RK, Akulov A, Bulgakov TS, Carnegie AJ, Jurjević Ž, Decock C, Denman S, Lombard L, Lawrence DP, Stack AJ, Gordon TR, Bostock RM, Burgess T, Summerell BA, Taylor PWJ, Edwards J, Hou LW, Cai L, Rossman AY, Wöhner T, Allen WC, Castlebury LA, Visagie CM, Groenewald JZ (2020) New and Interesting Fungi. Fungal Syst Evol 6 3:157–231. https://doi.org/10.3114/fuse.2020.06.09
27. Deighton FC (1973) Studies on *Cercospora* and allied genera. IV. *Cercosporella* Sacc., *Pseudocercosporella* gen. nov. and *Pseudocercosporidium* gen. nov. Mycol Pap 133:1–62
28. Frank J, Crous PW, Groenewald JZ, Oertel B, Hyde KD, Phengsintham P, Schroers H-J (2010) *Microcyclosporella* and *Microcyclospora*: novel genera accommodating epiphytic fungi causing sooty blotch on apple. Persoonia 24:93–105. https://doi.org/10.3767/003158510X510560
29. Groenewald JZ, Nakashima C, Nishikawa J, Shin HD, Park JH, Jama AN, Crous PW (2013) Species concepts in *Cercospora*: spotting the weeds among the roses. Stud Mycol 75:115–170. https://doi.org/10.3114/sim0012
30. Groenewald M, Groenewald JZ, Crous PW (2005) Distinct species exist within the *Cercospora apii* morphotype. Phytopathology 95(8):951–959. https://doi.org/10.1094/PHYTO-95-0951
31. Hall T (2007) Bioedit, Version 7.0.9. [Online]. Ibis Biosciences, Carlsbad, 1997–2007. http://www.mbio.ncsu.edu/ BioEdit/bioedit.html
32. Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol 42:182–192. https://doi.org/10.1093/sysbio/42.2.182
33. Hughes SJ (1949) Studies on some diseases of sainfoin (*Onobrychis sativa*) II. The life history of *Ramularia onobrychidis* Allescher. Trans Br Mycol Soc 32:34–59
34. Ismail SI, Batzer JC, Harrington TC, Crous PW, Lavrov DV, Li H, Gleason ML (2016) Ancestral state reconstruction infers phytopathogenic origins of sooty blotch and flyspeck fungi on apple. Mycologia 108(2):292–302. https://doi.org/10.3852/15-036
35. Kamal (2010) Cercosporoid fungi of India. Bishen Singh Mahendra Pal Singh, Dehra Dun, p 351
36. Kharwar RN, Singh A, Singh R, Kumar S (2015) Two new species of *Zasmidium* from Nepal. Mycotaxon 130:241–246. https://doi.org/10.5248/130.241
37. Kirschner R (2009) *Cercosporella* and *Ramularia*. Mycologia 101(1):110–119. https://doi.org/10.3852/07-038
38. Kumar S, Singh R (2015) *Passalora musicola*, sp. nov. – a new Indian hyphomycete. Sydowia 67:21–23
39. Kumar S, Singh R (2016) *Passalora caesalpiniicola* sp. nov. from India on. Caesalpinia bonduc Mycotaxon 131:25–30. https://doi.org/10.5248/131.25
40. Kumar S, Singh R, Upadhyaya PP, Saini DC (2013) First report of *Cercospora apii s. lat.* on *Ceiba pentendra* from northeastern Uttar Pradesh, India. J New Biol Rep 2(3):238–240. https://doi.org/10.5943/ppq/3/1/2
41. Kumar S, Singh R, Upadhyaya PP, Saini DC (2014) First report of foliar disease caused by *Cercospora apii s. lat.* on *Nymphaea nouchali* from Uttar Pradesh, India. J New Biol Rep 3(3):252–254
42. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096
43. Kushwaha P, Singh R, Chaurasia B (2020) *Ramularia titarpaniensis*—a new species of ramularioid complex from central India. Phytotaxa 429(4):274–280. https://doi.org/10.11646/phytotaxa.429.4.3
44. Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol Biol Evol 16:1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092
45. Machado AR, Pinh DB, Silva M, Pereira OL (2012) First report of leaf spot disease caused by *Cercosporella pfaffiae* on Brazilian ginseng (*Pfaffia glomerata*) in Brazil. Plant Dis 96(11):1702–1702. https://doi.org/10.1094/PDIS-06-12-0614-PDN
46. Montenegro-Calderón JG, Martínez-Álvarez JA, Vieyra-Hernández MT, Rangel-Macías LI, Razzo-Soria T, Chávez-Herrera R, Leal-Morales CA (2011) Molecular identification of two strains of *Cercospora rodmanii* isolated from water hyacinth present in Yuriria lagoon, Guanajuato, Mexico and identification of new hosts for several other strains. Fungal Biol 115(11):1151–1162. https://doi.org/10.1016/j.funbio.2011.08.001
47. Narayan S, Singh PN, Rao GP (1999) Sugarcane Pathology 1: Fungal Diseases. 308 pp

48. Quaedvlieg W, Binder M, Groenewald JZ, Summerell BA, Carnegie AJ, Burgess TI, Crous PW (2014) Introducing the Consolidated Species Concept to resolve species in the *Teratosphaeriaceae*. Persoonia: Mol Phylogeny Evol Fungi 33:1–40. https://doi.org/10.3767/003158514X681981
49. Quaedvlieg W, Verkley GJM, Shin HD, Barreto RW, Alfenas AC, Swart WJ, Crous PW (2013) Sizing up *Septoria*. Stud Mycol 75:307–390. https://doi.org/10.3114/sim0017
50. Rannala B, Yang Z (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J Mol Evol 43:304–311. https://doi.org/10.1007/PL00006090
51. Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycol Res 98:625–634. https://doi.org/10.1016/S0953-7562(09)80409-7
52. Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Ohna SH, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Effcient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029
53. Schoch CL, Crous PW, Groenewald JZ, Boehm EWA, Burgess TI, De Gruyter J, De Hoog GS, Dixon LJ, Grube M, Gueidan C, Harada Y, Hatakeyama S, Hirayama K, Hosoya T, Huhndorf SM, Hyde KD, Jones EBG, Kohlmeyer J, Kruys Ã, Li YM, Lücking R, Lumbsch HT, Marvanová L, Mbatchou JS, McVay AH, Miller AN, Mugambi GK, Muggia L, Nelsen MP, Nelson P, Owensby CA, Phillips AJL, Phongpaichit S, Pointing SB, Pujade-Renaud V, Raja HA, Plata ER, Robbertse B, Ruibal C, Sakayaroj J, Sano T, Selbmann L, Shearer CA, Shirouzu T, Slippers B, Suetrong S, Tanaka K, Volkmann-Kohlmeyer B, Wingfield MJ, Wood AR, Woudenberg JHC, Yonezawa H, Zhang Y, Spatafora JW (2009) A class-wide phylogenetic assessment of Dothideomycetes. Stud Mycol 64:1–15. https://doi.org/10.3114/sim.2009.64.01
54. Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW (2006) A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98(6):1041–1052. https://doi.org/10.1080/15572536.2006.11832632
55. Singh A, Kharwar RN, Singh R, Kumar S (2014a) A new species of *Zasmidium* (*Mycosphaerellaceae*) from India. Sydowia 66(2):309–312
56. Singh A, Kumar S, Singh R, Agrawal DK (2008) Two new species of *Ramularia* from Indian Sub-continents. Ind Phytopath 61(3):348–352
57. Singh G, Yadav S, Singh R, Kumar S (2022) *Passalora golaghati* comb. nov. from India Mycotaxon 137(1):89–94. https://doi.org/10.5248/137.89
58. Singh R, Chaurasia B, Shukla K, Upadhyaya PP (2012) *Passalora aseptata*, a new cercosporoid fungus from northeastern Uttar Pradesh, India. Mycotaxon 120:461–463. https://doi.org/10.5248/120.461
59. Singh R, Kumar S (2017) *Passalora rhamnaecearum* comb. nov. (*Capnodiales, Mycosphaerellaceae*) from India. Kavaka 48(1):50–51
60. Singh R, Kumar S, Kamal (2011) Two new species of *Passalora* and *Pseudocercospora* from northeastern Uttar Pradesh, India. Mycotaxon 117:137–143. https://doi.org/10.5248/117.137
61. Singh R, Kumar S, Pal VK, Upadhyaya PP, Agrawal DK (2007) New taxa of foliicolous hyphomycetes-*Cercospora*, *Corynespora* and *Phaeotrichochonis* from North-Eastern U.P. Ind Phytopath 60(4):506–512
62. Singh R, Kumar S, Saini DC, Upadhyaya PP, Kamal, Braun U (2013) Diversity of *Passalora* on *Ficus*. Mycol Prog 12:637–643. https://doi.org/10.1007/s11557-012-0870-6
63. Singh R, Singh A, Kumar S, Upadhyaya PP, Castañeda-Ruíz RF (2014b) Two new species of *Zasmidium* from northeastern Uttar Pradesh, India. Nova Hedwigia 98(1–2):257–263. https://doi.org/10.1127/0029-5035/2013/0137
64. Singh R, Verma SK, Yadav S, Bhojak P, Kumar S (2020b) Morphology and phylogeny of *Pseudocercospora hamiltoniani*—A new species comparable to *Sirosporium from* Uttarakhand, India. Phytotaxa 458(4):281–293. https://doi.org/10.11646/phytotaxa.458.4.4
65. Singh R, Verma SK, Yadav S, Kumar S (2020a) *Cercosporella bundelkhandae* comb. nov. from India Mycotaxon 135:315–320. https://doi.org/10.5248/135.315
66. Spegazzini C (1911) Mycetes Argentinenses (Series V). Anales del Museo Nacional de Historia Natural Buenos Aires. ser. 3, 13:329–467
67. Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033
68. Sung G-H, Sung J-M, Hywel-Jones NL, Spatafora JW (2007) A multigene phylogeny of *Clavicipitaceae* (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. Mol Phylogenet Evol 44:1204–1223. https://doi.org/10.1016/j.ympev.2007.03.011

69. Sutton BC, Waller JM (1988) Taxonomy of *Ophiocladium hordei*, causing leaf lesions on *Triticale* and other gramineae. Trans Br Mycol Soc 90:55–61. https://doi.org/10.1016/S0007-1536(88)80180-3
70. Swofford DL (2003) PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b 10. Sinauer Associates, Sunderland, Massachusetts
71. Thirumalachar MJ, Govindu HC (1953) Notes on some *Cercosporae* of India - II. Sydowia 7(1–4):45–49
72. Verkley GJ, Crous PW, Groenewald JE, Braun U, Aptroot A (2004) *Mycosphaerella punctiformis* revisited: morphology, phylogeny, and epitypification of the type species of the genus *Mycosphaerella* (*Dothideales, Ascomycota*). Mycol Res 108(11):1271–1282. https://doi.org/10.1017/S0953756204001054
73. Verkley GJM, Quaedvlieg W, Shin HD, Crous PW (2013) A new approach to species delimitation in *Septoria*. Stud Mycol 75:213–305. https://doi.org/10.3114/sim0018
74. Verma SK, Kushwaha P, Yadav S, Singh R (2021a) Morphology and phylogeny of *Teratoramularia rumicis*—a new foliar pathogen of *Rumex crispus* from India and diversity of Ramularioid complex on *Rumex* spp. Phytotaxa 523(3):208–228. https://doi.org/10.11646/phytotaxa.523.3.2
75. Verma SK, Yadav S, Singh G, Singh R (2021b) *Pseudocercospora cappadocici*, a new *Stigmina*-like *Pseudocercospora* species on *Acer cappadocicum* from India. Sydowia 74:79–91
76. Videira SIR, Groenewald JZ, Braun U, Shin HD, Crous PW (2016) All that glitters is not *Ramularia*. Stud Mycol 83:49–163. https://doi.org/10.1016/j.simyco.2016.06.001
77. Videira SIR, Groenewald JZ, Verkley GJ, Braun U, Crous PW (2015) The rise of *Ramularia* from the *Mycosphaerella labyrinth*. Fungal Biol 119(9):823–843. https://doi.org/10.1016/j.funbio.2015.06.003
78. Videira SIR, Groenewald JZ, NakashimaC, Braun U, Barreto RW, de Wit PJGM, Crous PW (2017) *Mycosphaerellaceae*—chaos or clarity? Stud Mycol 87:257–421. https://doi.org/10.1016/j.simyco.2017.09.003
79. Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246. https://doi.org/10.1128/JB.172.8.4238-4246.1990
80. Vu D, Groenewald M, De Vries M, Gehrmann T, Stielow B, Eberhardt U, Verkley GJM (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud Mycol 92:135–154. https://doi.org/10.1016/j.simyco.2018.05.001
81. White TJ, Bruns T, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White JW (eds) A guide to molecular methods and applications. Academic Press, New York, pp 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
82. Yadav S, Verma SK, Singh VK, Singh R, Singh A, Kumar S (2021) Morphology and phylogeny of a new species, *Pseudocercospora haldinae* (*Mycosphaerellaceae*) on *Haldina cordifolia* from India. Phytotaxa 501(2):281–292. https://doi.org/10.11646/phytotaxa.501.2.3
83. Yen JM (1983) Studies on parasitic fungi from South East Asia, 49. Parasitic fungi from Malaysia, 25: *Semipseudocercospora* gen. nov. Mycotaxon 17:361–363

Figures

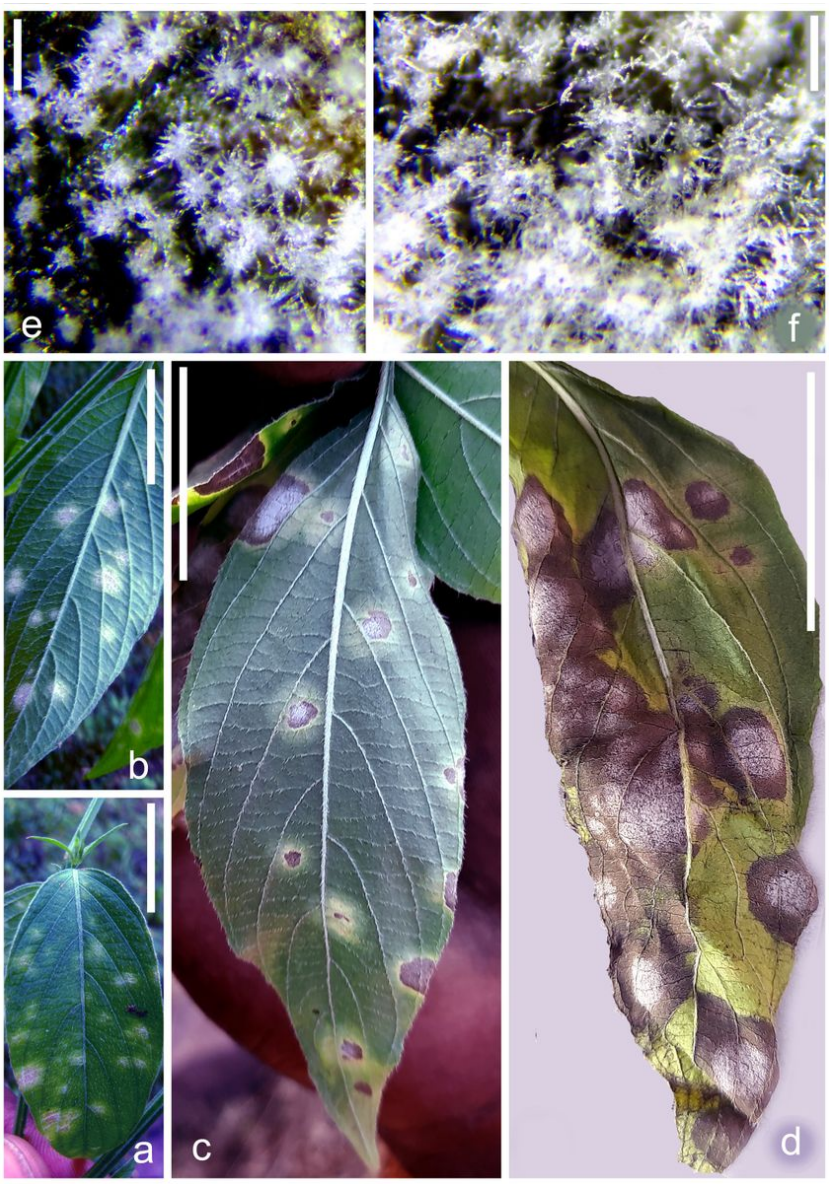

Figure 1

Symptoms of infection of *Neocercosporella peristrophes* on *Peristrophe bicalyculata*. **a** Initial stage of symptom on upper surface of leaf, **b** Initial stage of infection on lower surface of leaf, **c, d** Late stage of infection on lower surface of leaves, **e, f** Fascicles of conidiophores developed on the surface of leaves. Bars: **a**–**d** = 20 mm, **e** = 200 µm, **f** = 100 µm

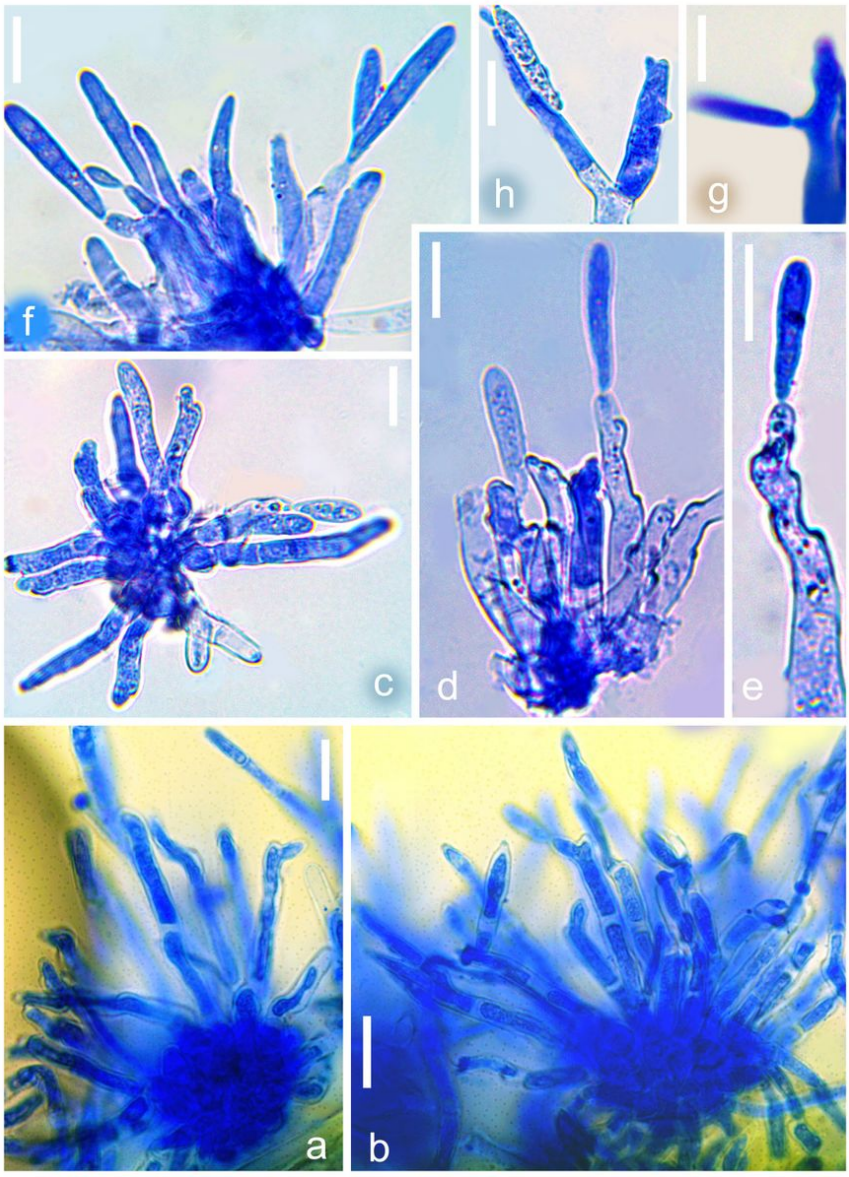

Figure 2

Microphotographs of *Neocercosporella peristrophes* (topoepitype, AMH 10363). **a–c** Fascicles of conidiophores, **d–g** Conidiophores with conidia, **h** Branched conidiophores. Bars: 10 µm

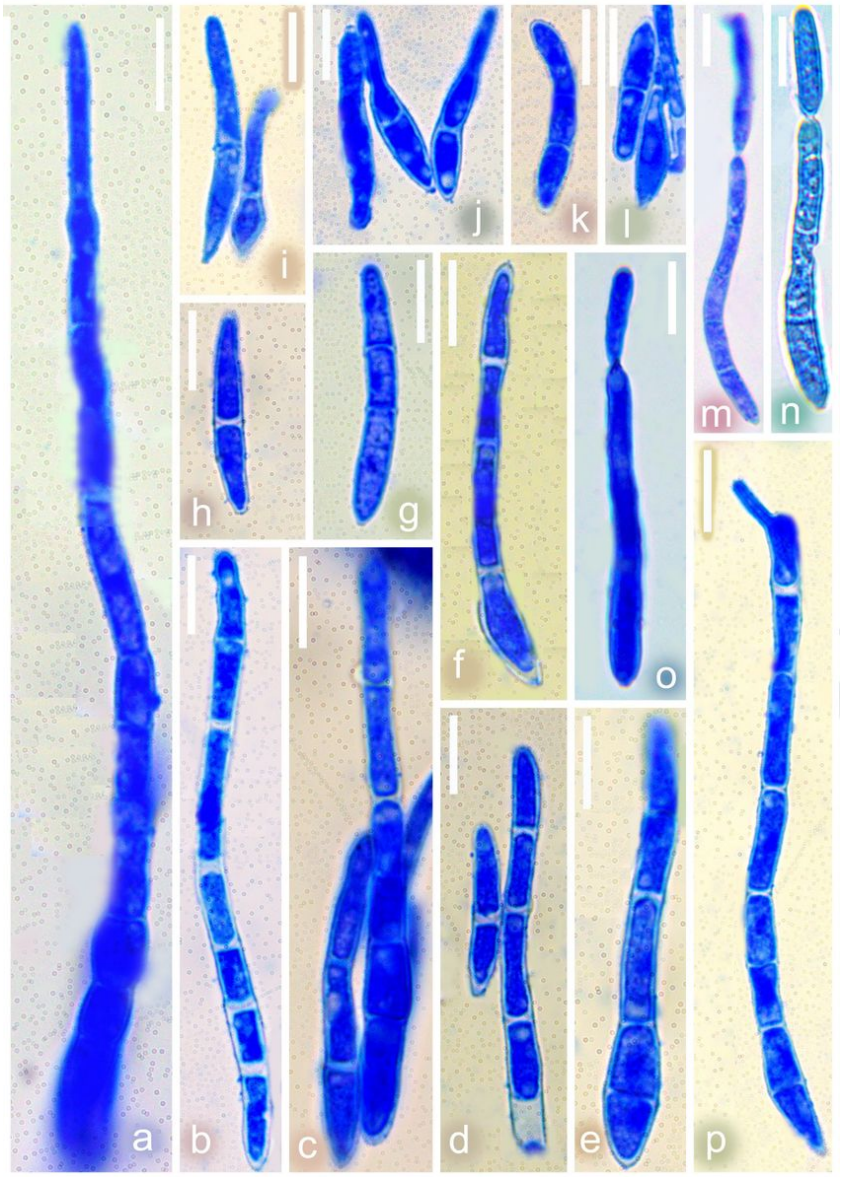

Figure 3

Microphotographs of *Neocercosporella peristrophes* (topoepitype, AMH 10363). **a–l** Conidia, **m–o** Catenate conidia, **p** Germinating conidium. Bars: 10 µm

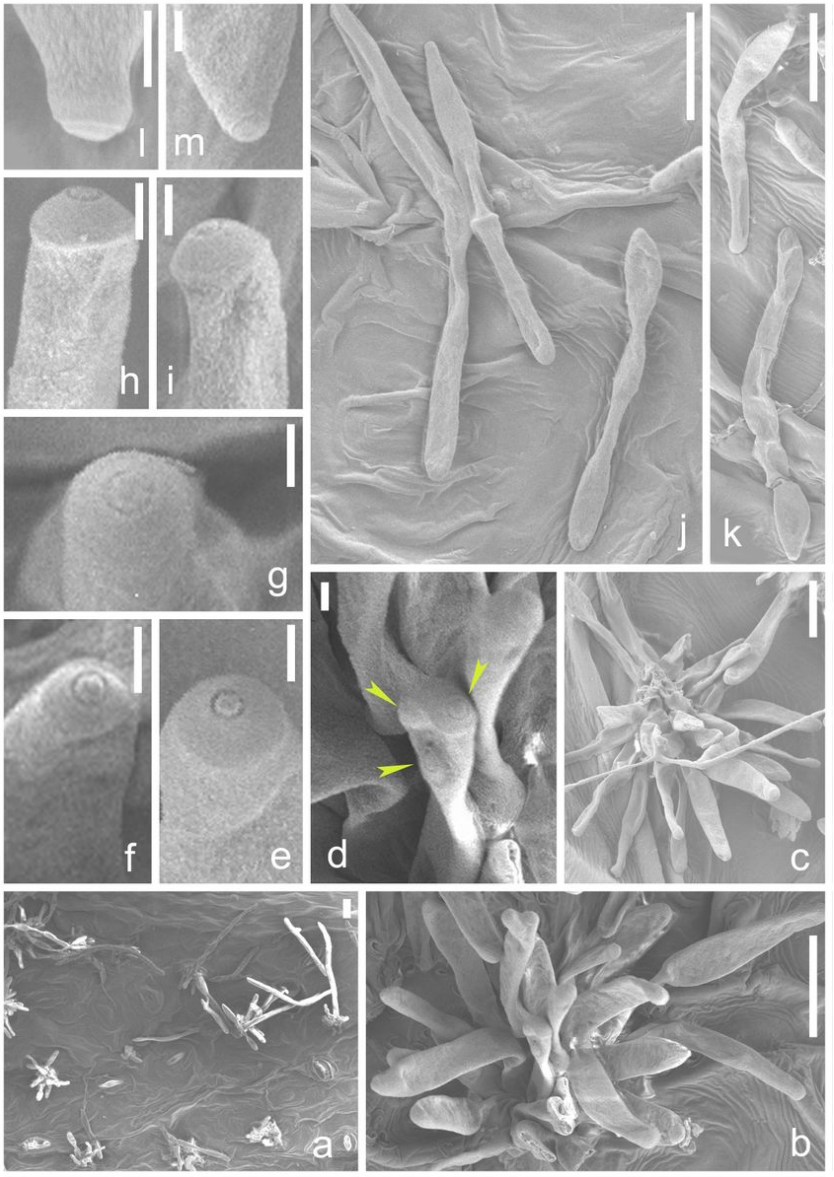

Figure 4

Scanning electron microphotographs of *Neocercosporella peristrophes* (topoepitype, AMH 10363). **a** Initial stage of development of conidiophores through stomata, **b, c** Fascicles of conidiophores, **d** Polyblastic conidiogenous cell (Yellow arrows), **e–g** Top view of conidiogenous loci, **h, i** Lateral view of conidiogenous loci, **j, k** Conidia, **l, m** Hila of conidia. Bars: **a–c** = 10 µm, **d–i** = 1 µm, **j, k** = 10 µm, **l, m** = 1µm

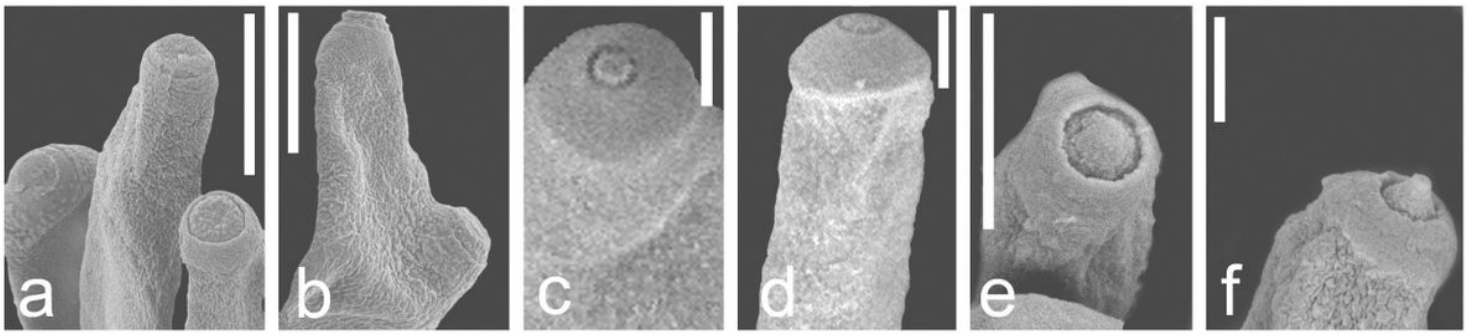

Figure 5

Scanning electron micrographs of oblique lateral and lateral view of scars formed by conidiogenous loci after conidium dehiscence in the type species. **a, b** *Cercosporella virgaureae* (Kirschner, 2009), **c, d** *Neocercosporella peristrophes,* **e, f** *Ramularia pusilla* (Kirschner, 2009). Bars: **a, b** = 4 µm, **c** = 2 µm, **d–f** = 1 µm

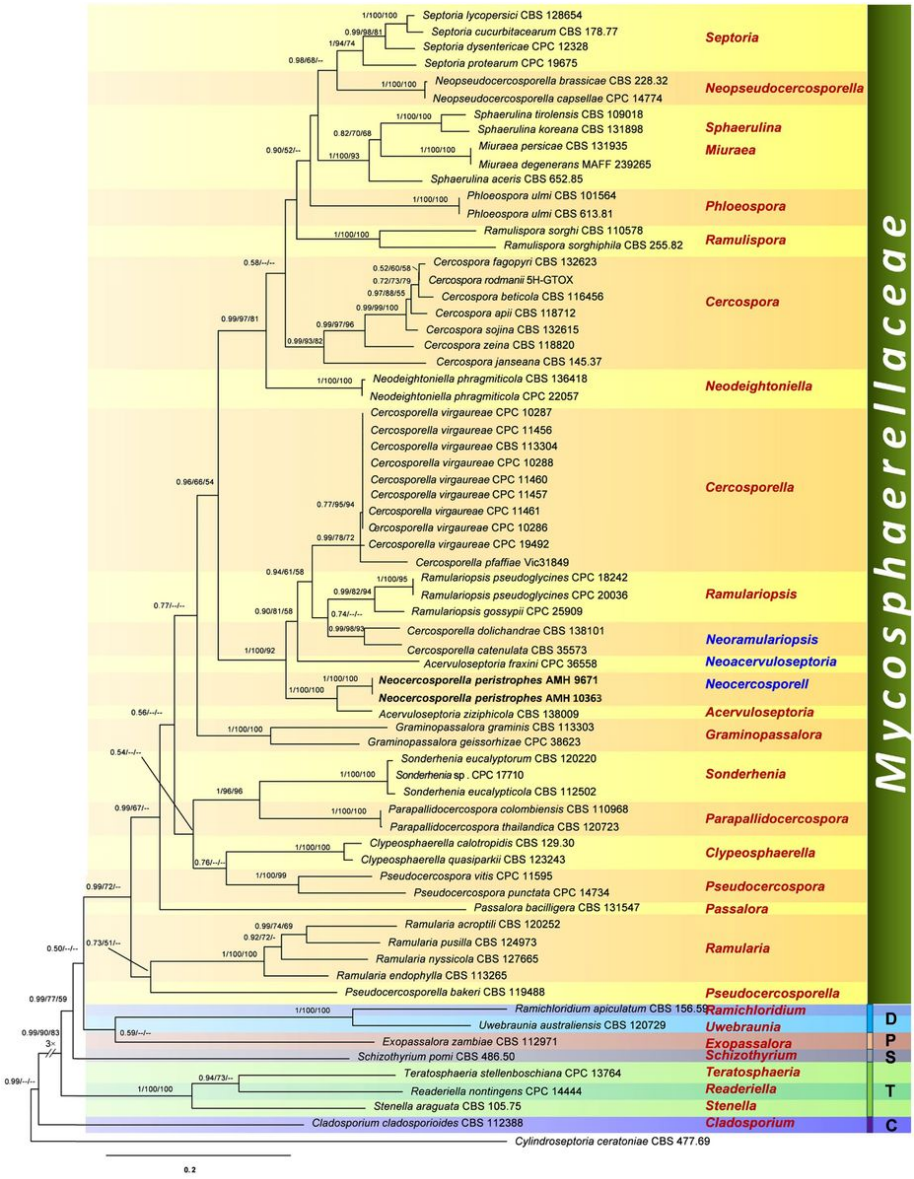

Figure 6

Consensus phylogram (50% majority rule) resulting from a maximum likelihood of the combined two-gene (dataset 1: LSU-*RPB2*) sequence alignment. The Bayesian posterior probabilities (≥ 0.50; BI-PP), maximum likelihood bootstrap support values (≥ 50%; ML-BS) and maximum parsimony bootstrap support values (≥ 50%; MP-BS) are given at the nodes (BI-PP/ML-BS/MP-BS). All taxa names are written in black, newly introduces strain is represented in bold and novel genera denoted in blue. A vertical bar is used to the right of the coloured boxes and encompasses all genera within their respective families. The family name *Mycosphaerellaceae* is unabbreviated while the rest are abbreviated as follows: D = *Dissoconiaceae*, P = *Phaeothecoidiellaceae*, S = *Schizothyriaceae*, T = *Teratosphaeriaceae*, C = *Cladosporiaceae*. The tree was rooted to *Cylindroseptoria ceratoniae* (CBS 477.69)

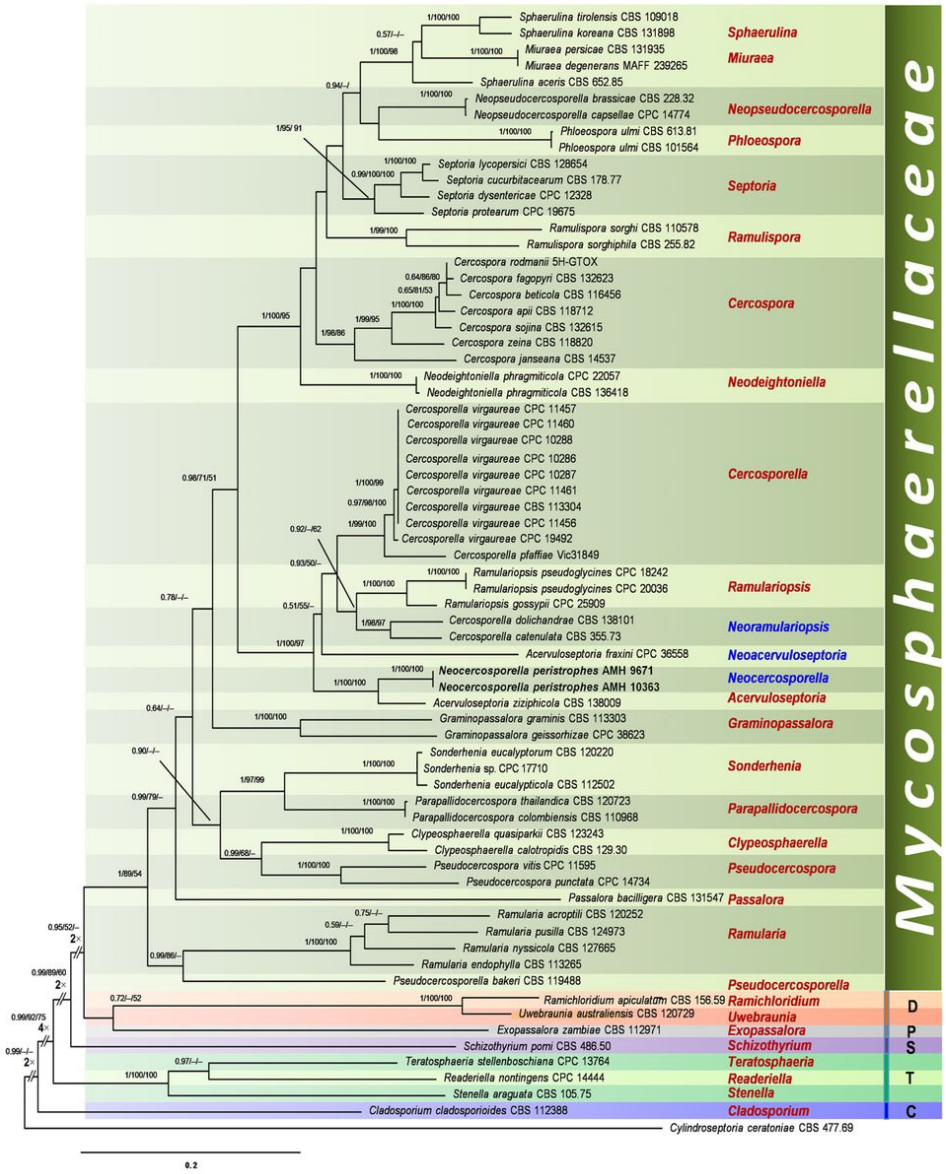

Figure 7

Consensus phylogram (50% majority rule) resulting from a maximum likelihood of the combined three-genes (dataset 2: LSU-*RPB2*-ITS) sequence alignment. The Bayesian posterior probabilities (≥ 0.50; BI-PP), maximum likelihood bootstrap support values (≥ 50%; ML-BS) and maximum parsimony bootstrap support values (≥ 50%; MP-BS) are given at the nodes (BI-PP/ML-BS/MP-BS). All taxa names are written in black, newly introduces strain is represented in bold and novel genera denoted in blue. A vertical bar is used to the right of the coloured boxes and encompasses all genera within their respective families. The family name *Mycosphaerellaceae* is unabbreviated while the rest are abbreviated as follows: D = *Dissoconiaceae*, P = *Phaeothecoidiellaceae*, S = *Schizothyriaceae*, T = *Teratosphaeriaceae*, C = *Cladosporiaceae*. The tree was rooted to *Cylindroseptoria ceratoniae* (CBS 477.69)