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ORIGINAL ARTICLE

Addition of three new lineages in *Mycosphaerellaceae*: *Neoacervuloseptoria* gen. nov., *Neocercosporella* gen. nov. and *Neoramulariopsis* gen. nov.

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Abstract

A colourless hyphomycetous fungus was found on living leaves of *Peristrophe bicalyculata* in India. Molecular phylogenetic analyses of several loci (LSU-*RPB2*-ITS) indicated a relationship with cercosporoid fungi (*Mycosphaerellaceae*). Since there is no other known lineage with similar morphology or DNA sequences, the new genus *Neocercosporella* is proposed with *N. peristrophes* comb. nov., based on *Cercosporella peristrophes* as type species. *Pseudocercosporella andrographidis* is recognized as new synonym. Similar ultrastructure of conidiogenous loci and hila confirm that *N. peristrophes* and *P. andrographidis* are conspecific. The conidiogenous loci are conical and have a small rim-like depression on the top encircling a small flat protuberant structure, which differs from other closely related members of the ramularioid complex. *Neocercosporella* and *Cercosporella s. str.* are paraphyletic as are several other genera in the *Mycosphaerellaceae*. Accordingly, we propose three new genera and four new combinations. The new genera are *Neoacervuloseptoria* gen. nov., *Neocercosporella* gen. nov. and *Neoramulariopsis* gen. nov. The new combinations are *Neoacervuloseptoria fraxini* comb. nov., *Neocercosporella peristrophes* comb. nov., *Neoramulariopsis catenulata* comb. nov. and *Neoramulariopsis unguis-cati* comb. nov.

Keywords Anamorph · *Mycosphaerellales* · *Dothideomycetes* · Multigene-phylogeny · Nomenclature · New taxa

Introduction

Most of the cercosporoid genera with and without connection with mycosphaerella-like sexual morphs belong to *Mycosphaerellaceae* (*Mycosphaerellales*, *Dothideomycetes*, *Ascomycota*; Abdollahzadeh et al. 2020). They cover about 120 genera within this family (Videira et al. 2017; Crous et al. 2020; Bakhshi et al. 2021; Rajeshkumar et al. 2021; Bakhshi and Braun 2022; Chen et al. 2022; Yadav et al. 2022). The hyphomycetous ramularioid complex 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 using the light microscope. Accordingly, numerous species have been transferred repeatedly from one of these genera to the other. The taxonomic problems related to this complex were extensively discussed in several studies (Hughes 1949; Sutton and Waller 1988; Braun 1990, 1991a, b, 1994a, 1995, 1998; Verkley et al. 2004; Kirschner 2009; Videira et al. 2015, 2016, 2017).

In *Ramularia*, conidial scars and hila are slightly thickened and darkened while those of *Cercosporella* are clearly thickened. The ultrastructure of the conidiogenous loci are smooth and shaped as a truncated cone in *Cercosporella*, while a cladosporium-type circular rim with a central dome is seen in *Ramularia* (Kirschner 2009; Bensch et al. 2012). *Cercosporella* produces cup-shaped appressoria which are lacking in *Ramularia* (Kirschner 2009; Videira et al. 2016). *Pseudocercosporella* is characterised by unthickened and inconspicuous conidial loci and hila (Deighton 1973; Braun 1995; Frank et al. 2010).

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Cercosporella, *Hawksworthiana*, *Neoovularia*, *Phacellium*, *Pseudodidymaria*, *Ramularia* and *Ramulariopsis* have hyaline conidiophores and conspicuous conidial loci. *Monodidymaria*, *Neoramularia* and *Pseudocercosporella* have inconspicuous conidial loci (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 multi-locus DNA sequences and morphological and cultural data.

Several studies focus on the diversity of phytopathogenic fungi in India that are related to the *Mycosphaerellaceae* (Singh et al. 2007, 2008, 2011, 2012, 2013, 2014a, b, 2020a, 2022; Kamal 2010; 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 a survey for foliicolous fungi in the Afchand forest of Sagar, M.P., India, in December 2019, a colourless hyphomycete was found on *Peristrophe bicalyculata* (Retz.) Nees. The same fungus was originally collected from the same locality in 2013 and described as *Pseudocercosporella andrographidis* Awasthi et al. (Awasthi et al. 2016). Due to lack of phylogenetic analysis, the true generic affinity of *P. andrographidis* is 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 materials showed that this fungus could not be placed in any of the genera already described in the *Mycosphaerellaceae*. Therefore, the new genus *Neocercosporella* is proposed. The recognition of this novel genus segregated closely related genera in the *Mycosphaerellaceae* and rendered several of these paraphyletic.

Materials and methods

Isolates and morphology

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 under a Stereo Zoom Microscope (Magnus: MSZ-TR) with attached camera (CatCam300EF). For light microscopy, fungal structures were transferred from the infected part of leaves and mounted on clear glass slides in a 1:1 mixture of glycerin and lactophenol cotton-blue. Fungal propagules were photographed using an Olympus compound microscope (CH20i-TR) equipped with a Magnus camera (MIPS CMOS). Scanning electron microscopy (SEM) was done with a field emission 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 ~ 18 K×). For SEM micrographs, specimens were coated with gold-paladium using a POLARON Sputter coater (180 s in nitrogen atmosphere of 20 mA, 30 mm distant from the electrode) and examined with a LEO-430 scanning electron microscope. Size ranges of morphological features derived from at least 25 measurements, and 95% confidence intervals were calculated, with the extreme values given in parentheses. 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 Hindu University, Varanasi, U.P., India (MH-BHU).

For the cultivation of samples of *Neocercosporella* AMH 9671 and AMH 10363, conidia 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 cultures from both specimens grew about 1 mm in 4 week and ceased to grow, a living culture was not deposited.

DNA extraction, PCR and sequencing

For isolation, amplification and sequencing of nuclear DNA, specimens AMH 9671 and AMH 10363 were used. DNA was isolated from mycelia and spores freshly scrapped from the heavily infected surface of collected leaves using a sterile scalpel blade. Approximately 200 mg of wet-weight was transferred to 2-mL microcentrifuge tubes kept in liquid nitrogen for 2 min and then grinded to a fine powder using pestle and mortar. DNA was extracted using Himedia DNA Isolation Kit (HiPurA™ Fungal DNA Purification Kit) following the manufacturers' protocols. Isolated DNA fragments were visualized by electrophoresis in a 1% agarose gel (w/v) stained with ethidium bromide under a Gel Documentation system (Bio-Rad Universal Hood II) and DNA concentration was quantified by using NanoDrop microvolume spectrophotometers (Thermo Scientific™ NanoDrop™ One/OneC Microvolume UV–Vis Spectrophotometer with Wi-Fi).

The internal transcribed spacer (ITS) region was amplified by using ITS1/ITS4 (White et al. 1990), large subunit nuclear ribosomal DNA (LSU) gene with primers LROR/LR7 (Vilgalys and Hester 1990; Rehner and Samuels 1994) and partial DNA-directed RNA polymerase II subunit (*RPB2*) with primers RPB2-5F2/RPB2-7cR (Liu et al. 1999; Sung et al. 2007). PCR 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

Table 1 Taxa included in molecular phylogenetic analyses and their GenBank accession numbers. Newly generated sequences are in bold

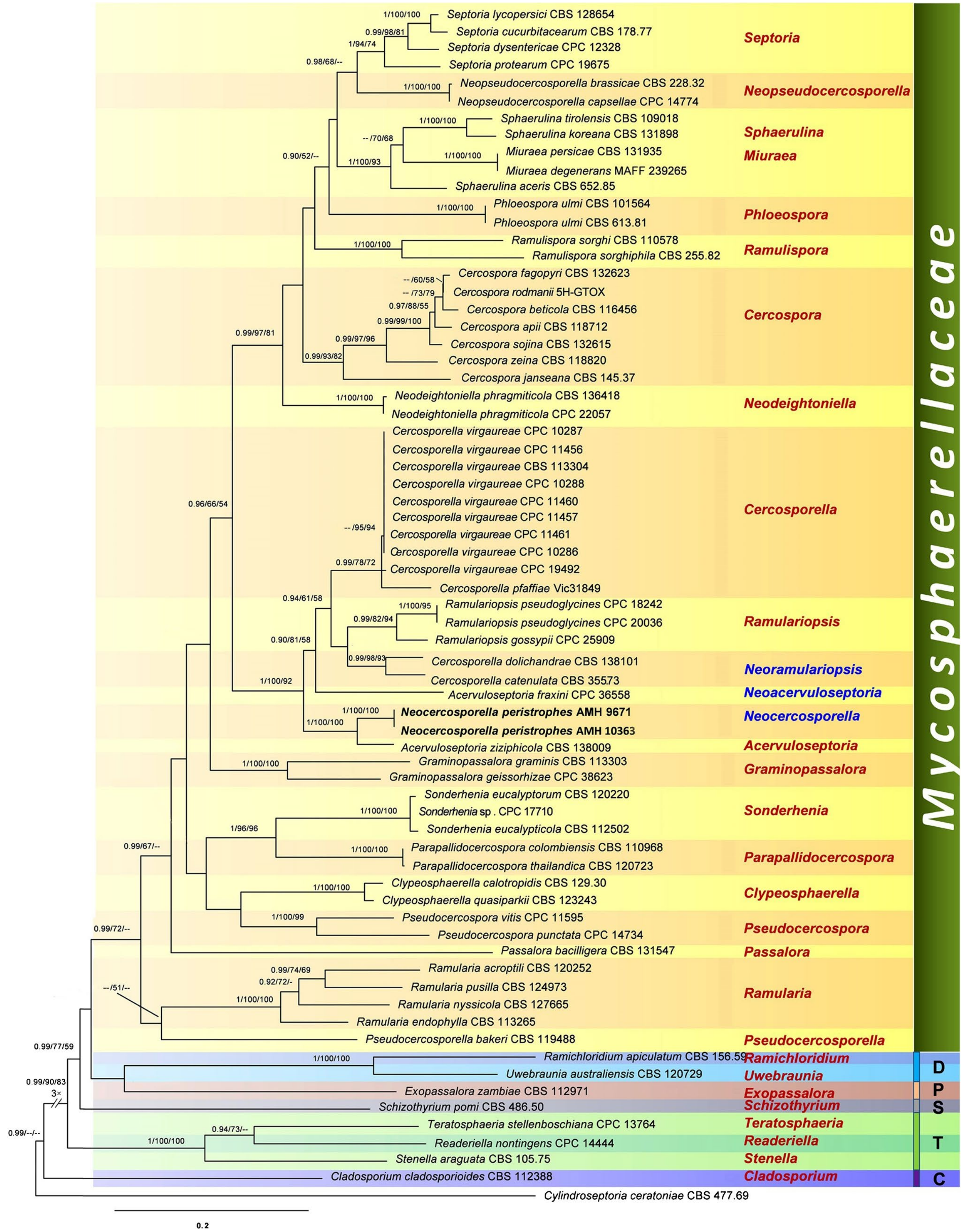
Taxon	ID (isolate, strain, status, voucher)	GenBank accession no			Substrata	Location	References
		ITS	LSU	*RPB2*			
Acervuloseptoria fraxini	CPC 36558/CBS 145992	MT223773	MT223870	MT223673	*Fraxinus* sp.	Russia	Crous et al. (2020)
Acervuloseptoria ziziphicola	CBS 138009/CPC 23707	KJ869164	KJ869221	MF951425	*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*	Mexico	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)
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)

Table 1 (continued)

Taxon	ID (isolate, strain, status, voucher)	GenBank accession no			Substrata	Location	References
		ITS	LSU	*RPB2*			
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)
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)
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)

Table 1 (continued)

Taxon	ID (isolate, strain, status, voucher)	GenBank accession no			Substrata	Location	References
		ITS	LSU	*RPB2*			
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)
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)
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)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0.99/98/81
Septoria cucurbitacearum CBS 178.77
1/94/74
Septoria dysentericae CPC 12328
0.98/68/--
Septoria protearum CPC 19675
Septoria
1/100/100
Neopseudocercosporella brassicae CBS 228.32
Neopseudocercosporella capsellae CPC 14774
Neopseudocercosporella
1/100/100
Sphaerulina tirolensis CBS 109018
-- /70/68
Sphaerulina koreana CBS 131898
Sphaerulina
0.90/52/--
1/100/100
Miuraea persicae CBS 131935
Miuraea
1/100/93
Miuraea degenerans MAFF 239265
Sphaerulina aceris CBS 652.85
1/100/100
Phloeospora ulmi CBS 101564
Phloeospora ulmi CBS 613.81
Phloeospora
1/100/100
Ramulispora sorghi CBS 110578
Ramulispora sorghiphila CBS 255.82
Ramulispora
-- /60/58
Cercospora fagopyri CBS 132623
-- /73/79
Cercospora rodmanii 5H-GTOX
0.97/88/55
Cercospora beticola CBS 116456
Cercospora
0.99/99/100
Cercospora apii CBS 118712
0.99/97/81
0.99/97/96
Cercospora sojina CBS 132615
0.99/93/82
Cercospora zeina CBS 118820
Cercospora janseana CBS 145.37
1/100/100
Neodeightoniella phragmiticola CBS 136418
Neodeightoniella
Neodeightoniella phragmiticola CPC 22057
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Cercosporella virgaureae CPC 11456
Cercosporella virgaureae CBS 113304
Cercosporella virgaureae CPC 10288
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Cercosporella virgaureae CPC 11460
Cercosporella
Cercosporella virgaureae CPC 11457
-- /95/94
Cercosporella virgaureae CPC 11461
Cercosporella virgaureae CPC 10286
0.99/78/72
Cercosporella virgaureae CPC 19492
Cercosporella pfaffiae Vic31849
0.94/61/58
1/100/95
Ramulariopsis pseudoglycines CPC 18242
0.99/82/94
Ramulariopsis pseudoglycines CPC 20036
Ramulariopsis
0.90/81/58
Ramulariopsis gossypii CPC 25909
0.99/98/93
Cercosporella dolichandrae CBS 138101
Neoramulariopsis
Cercosporella catenulata CBS 35573
1/100/92
Acervuloseptoria fraxini CPC 36558
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Sonderhenia eucalypticola CBS 112502
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1/100/100
Parapallidocercospora colombiensis CBS 110968
Parapallidocercospora
Parapallidocercospora thailandica CBS 120723
1/100/100
Clypeosphaerella calotropidis CBS 129.30
Clypeosphaerella
Clypeosphaerella quasiparkii CBS 123243
1/100/99
Pseudocercospora vitis CPC 11595
Pseudocercospora
0.99/72/--
Pseudocercospora punctata CPC 14734
Passalora bacilligera CBS 131547
Passalora
0.99/74/69
Ramularia acroptili CBS 120252
0.92/72/-
Ramularia pusilla CBS 124973
--/51/--
Ramularia
1/100/100
Ramularia nyssicola CBS 127665
Ramularia endophylla CBS 113265
0.99/77/59
Pseudocercosporella bakeri CBS 119488
Pseudocercosporella
Mycosphaerellaceae
1/100/100
Ramichloridium apiculatum CBS 156.59
Ramichloridium
0.99/90/83
Uwebraunia australiensis CBS 120729
Uwebraunia
D
3×
Exopassalora zambiae CBS 112971
Exopassalora
P
Schizothyrium pomi CBS 486.50
Schizothyrium
S
0.99/--/--
0.94/73/--
Teratosphaeria stellenboschiana CPC 13764
Teratosphaeria
1/100/100
Readeriella nontingens CPC 14444
Readeriella
T
Stenella araguata CBS 105.75
Stenella
Cladosporium cladosporioides CBS 112388
Cladosporium
C
Cylindroseptoria ceratoniae CBS 477.69
0.2

◂**Fig. 1** Phylogenetic tree resulting from a RAxML analysis of the combined LSU/*RPB2* sequence alignment (dataset 1). The Bayesian posterior probabilities (≥0.90; 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). The newly introduced lineage is represented in bold and novel genera denoted in blue. The family name *Mycosphaerellaceae* is unabbreviated while others are abbreviated as follows: D = *Dissoconiaceae*, P = *Phaeothecoidiellaceae*, S = *Schizothyriaceae*, T = *Teratosphaeriaceae*, C = *Cladosporiaceae*. The tree is rooted to *Cylindroseptoria ceratoniae* (CBS 477.69)

reverse primer (10 pmol), 1 µL dNTP (10 mM), 0.3 µL Taq DNA polymerase (HiMedia: 5 Unit/µL) and 35.7 µL milli-Q water. The PCRs were carried out in a 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 55, 52 and 54 °C for 1 min and extension at 72 °C for 1 min. The final extension step was done at 72 °C for 8 min. The amplicon was run in 1.2% agarose gel and visualized in a Gel Documentation system (Bio-Rad Universal Hood II) for the product size and purity. The PCR 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 with the same primers as for the PCR.

Sequence alignment and phylogenetic analysis

The obtained ITS, LSU and *RBP2* sequences from AMH 9671and AMH 10363 were assembled and edited with Chromas v.2.6.6. The manually edited sequences were submitted to NCBI GenBank (Table 1) and were subjected to a megablast search of the NCBI GenBank nucleotide database to retrieve sequences of related strains. 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) lacking 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 using BioEdit v.7.0.9 (Hall 1999) and MEGA-X v.10.1.8 (Kumar et al. 2018) and deposited as electronic supplementary materials in TreeBASE, study number 30079.

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 as different combinations of genes may result in alternative phylogenetic hypotheses as described by Videira et al. (2017). All trees were rooted with *Cylindroseptoria ceratoniae* (CBS 477.69). Dataset 1 consisted of a concatenated alignment of LSU and *RPB2* sequences, and dataset 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 other families.

Model GTR + I + G was selected for BI using a Markov chain Monte Carlo (MCMC) algorithm (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 as a majority rule consensus tree. ML analysis was also performed using a GTR model of site substitution, including GAMMA with 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 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.

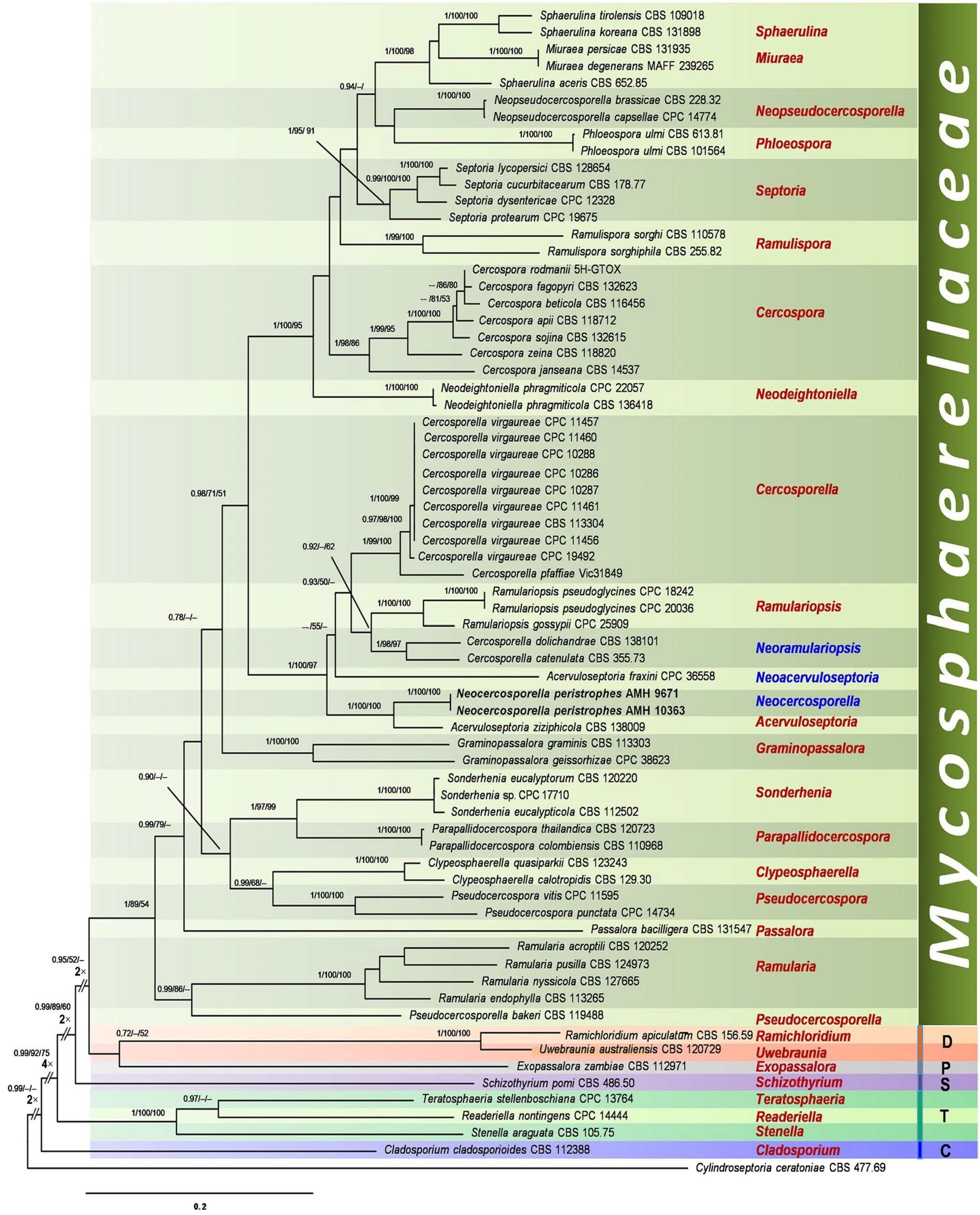
The trees presented here were obtained with the ML approach. Tree reconstruction, visualization and editing were done using FigTree v.1.4.4 and TreeGraph_2.15.0. The multigene phylograms are shown in Figs. 1 and 2.

Results

The sequences from specimens AMH 9671 and AMH 10363 were 100% identical in each region. 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. 1 and 2).

Dataset 1 (LSU and *RPB2* phylogeny)

This dataset consisted of a concatenated alignment of two loci (LSU, *RPB2*). The final alignment contained a total


1/100/100
Sphaerulina tirolensis CBS 109018
Sphaerulina koreana CBS 131898
Sphaerulina
1/100/98
1/100/100
Miuraea persicae CBS 131935
Miuraea degenerans MAFF 239265
Miuraea
0.94/–/
Sphaerulina aceris CBS 652.85
1/100/100
Neopseudocercosporella brassicae CBS 228.32
Neopseudocercosporella capsellae CPC 14774
Neopseudocercosporella
1/95/ 91
1/100/100
Phloeospora ulmi CBS 613.81
Phloeospora ulmi CBS 101564
Phloeospora
1/100/100
Septoria lycopersici CBS 128654
0.99/100/100
Septoria cucurbitacearum CBS 178.77
Septoria dysentericae CPC 12328
Septoria protearum CPC 19675
Septoria
1/99/100
Ramulispora sorghi CBS 110578
Ramulispora sorghiphila CBS 255.82
Ramulispora
Cercospora rodmanii 5H-GTOX
– /86/80
Cercospora fagopyri CBS 132623
– /81/53
Cercospora beticola CBS 116456
1/100/100
Cercospora apii CBS 118712
1/100/95
1/99/95
Cercospora sojina CBS 132615
1/98/86
Cercospora zeina CBS 118820
Cercospora janseana CBS 14537
Cercospora
1/100/100
Neodeightoniella phragmiticola CPC 22057
Neodeightoniella phragmiticola CBS 136418
Neodeightoniella
Cercosporella virgaureae CPC 11457
Cercosporella virgaureae CPC 11460
Cercosporella virgaureae CPC 10288
Cercosporella virgaureae CPC 10286
Cercosporella virgaureae CPC 10287
0.98/71/51
1/100/99
Cercosporella virgaureae CPC 11461
0.97/98/100
Cercosporella virgaureae CBS 113304
1/99/100
Cercosporella virgaureae CPC 11456
0.92/–/62
Cercosporella virgaureae CPC 19492
Cercosporella pfaffiae Vic31849
Cercosporella
0.93/50/–
1/100/100
Ramulariopsis pseudoglycines CPC 18242
1/100/100
Ramulariopsis pseudoglycines CPC 20036
Ramulariopsis gossypii CPC 25909
Ramulariopsis
0.78/–/–
–/55/–
1/98/97
Cercosporella dolichandrae CBS 138101
Cercosporella catenulata CBS 355.73
Neoramulariopsis
1/100/97
Acervuloseptoria fraxini CPC 36558
Neoacervuloseptoria
1/100/100
Neocercosporella peristrophes AMH 9671
1/100/100
Neocercosporella peristrophes AMH 10363
Neocercosporella
Acervuloseptoria ziziphicola CBS 138009
Acervuloseptoria
1/100/100
Graminopassalora graminis CBS 113303
Graminopassalora geissorhizae CPC 38623
Graminopassalora
0.90/–/–
Sonderhenia eucalyptorum CBS 120220
1/100/100
Sonderhenia sp. CPC 17710
1/97/99
Sonderhenia eucalypticola CBS 112502
Sonderhenia
0.99/79/–
1/100/100
Parapallidocercospora thailandica CBS 120723
Parapallidocercospora colombiensis CBS 110968
Parapallidocercospora
1/100/100
Clypeosphaerella quasiparkii CBS 123243
0.99/68/–
Clypeosphaerella calotropidis CBS 129.30
Clypeosphaerella
1/100/100
Pseudocercospora vitis CPC 11595
1/89/54
Pseudocercospora punctata CPC 14734
Pseudocercospora
Passalora bacilligera CBS 131547
Passalora
Ramularia acroptili CBS 120252
Ramularia pusilla CBS 124973
1/100/100
Ramularia nyssicola CBS 127665
0.95/52/–
2×
0.99/86/–
Ramularia endophylla CBS 113265
Ramularia
0.99/89/60
2×
Pseudocercosporella bakeri CBS 119488
Pseudocercosporella
0.72/–/52
1/100/100
Ramichloridium apiculatum CBS 156.59
Ramichloridium
0.99/92/75
4×
Uwebraunia australiensis CBS 120729
Uwebraunia
D
Exopassalora zambiae CBS 112971
Exopassalora
P
0.99/–/–
2×
Schizothyrium pomi CBS 486.50
Schizothyrium
S
0.97/–/–
Teratosphaeria stellenboschiana CPC 13764
Teratosphaeria
1/100/100
Readeriella nontingens CPC 14444
Readeriella
T
Stenella araguata CBS 105.75
Stenella
Cladosporium cladosporioides CBS 112388
Cladosporium
C
Cylindroseptoria ceratoniae CBS 477.69
0.2
Mycosphaerellaceae

◂**Fig. 2** Phylogenetic tree resulting from a RAxML analysis of the combined LSU/*RPB2*/ITS sequence alignment (dataset 2). The Bayesian posterior probabilities (≥ 0.90; 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). The newly introduced lineage is represented in bold and novel genera denoted in blue. The family name *Mycosphaerellaceae* is unabbreviated while others are abbreviated as follows: D = *Dissoconiaceae*, P = *Phaeothecoidiellaceae*, S = *Schizothyriaceae*, T = *Teratosphaeriaceae*, C = *Cladosporiaceae*. The tree is rooted to *Cylindroseptoria ceratoniae* (CBS 477.69)

of 1439 characters divided in two partitions containing 748 (LSU) and 691 (*RPB2*) characters, 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. 1, with the Likelihood value of – 21,290.719845. The most parsimonious tree was characterized by TL = 6393, CI = 0.297513, RI = 0.576999, RC = 0.171665 and HI = 0.702487, and G-fit is – 491.819875. From the analysed characters, 530 were constant, 78 were variable and parsimony-uninformative, and 831 were parsimony-informative. In this analysis, *Cercosporella catenulata* (CBS 355.73) and *Cercosporella dolichandrae* (CBS 138101) are now separated from the *Cercosporella* clade and are placed in a separate sister branch of *Ramulariopsis* (Fig. 1). *Acervuloseptoria fraxini* (CPC36558) and *A. ziziphicola* (CBS138009) form a paraphyletic group. *Acervuloseptoria ziziphicola* and *N. peristrophes* form a statistically supported monophyletic group (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 into three partitions containing 748 (LSU), 691 (*RPB2*) and 540 (ITS) characters, including alignment gaps. Phylogenetic trees generated from BI, ML and MP analysis had similar overall topology. A best scoring RAxML tree is presented in Fig. 2, with the Likelihood value of – 27,134.491457. The most parsimonious tree was characterized by TL = 7894, CI = 0.321257, RI = 0.575503, RC = 0.184884 and HI = 0.678743, and G-fit is – 615.475663. From the analysed characters, 744 were constant, 181 were variable and parsimony-uninformative, and 1054 were parsimony-informative. The results of analysis of dataset 2 (Fig. 2) fully support the dataset 1 analysis (Fig. 1).

Acervuloseptoria, *Cercosporella*, *Neoacervuloseptoria*, *Neocercosporella*, *Neoramulariopsis* and *Ramulariopsis* formed a statistically supported monophyletic group.

Taxonomy

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

MycoBank MB840502

Etymology: derived from the genus name *Acervuloseptoria.*

Diagnosis: differs from the genus *Acervuloseptoria* by its pycnidial conidiomata opening via central ostioles and intermingled among spermatogonia.

Description: (adapted from Crous et al. 2020): 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.

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)

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

In this study, based on both datasets, *A. fraxini* clustered apart from *A. ziziphicola* (Figs. 1 and 2). *Acervuloseptoria ziziphicola* has acervular conidiomata that are black, erumpent and multilocular; their upper layer disintegrates upon maturity (Crous et al. 2014). Conidiomata in *A. fraxini* are pycnidial (thus, not acervular), have a central ostiole and are intermingled among spermatogonia (Crous et al. 2020). Therefore, a new genus *Neoacervuloseptoria* is to be introduced for the strain CPC 36558 in the *Mycosphaerellaceae*. *Acervuloseptoria ziziphicola* separated as a sister lineage of *Neocercosporella* with high bootstrap support (BI-PP/

ML-BS/MP-BS: 1/100/100) (Figs. 1 and 2). 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).

Neocercosporella Sanjay & Raghv. Singh, **gen. nov.** Figs. 3, 4, 5, and 6

MycoBank MB840500

Etymology: derived from the genus name *Cercosporella.*

Diagnosis: differs from *Cercosporella s. str.* by its conidiogenous locus, which is conical in shape and having a 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 conidiogenous cells in *Neocercosporella* are terminal and intercalary, and the conidia are produced at least initially in chains. It also differs from *Acervuloseptoria* due to its hyphomycetous nature, while the latter is coelomycetous.

Description: Plant pathogenic, foliicolous. Hyphae restricted to intercellular spaces. Colonies hypogenous. Stromata substomatal or subcuticular to erumpent. Conidiophores macronematous, fasciculate, arising from stromata, initially erumping through stomata, later by rupturing epidermis, erect to procumbent, hyaline to light olivaceous, smooth, thin- 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 a 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%)].

Neocercosporella peristrophes (Syd.) Sanjay & Raghv. Singh, **comb. nov.** Figs. 3, 4, 5, and 6

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), **syn. nov.**

Description: Infection spots amphiphyllous, white, circular to irregular, 1–10 mm in diam., later covering the entire, necrotic leaf surface. Colonies hypogenous, white, velvety. Mycelium internal. Stromata present, globose to somewhat angular, substomatal or subcuticular to erumpent, hyaline, (9)15–25(35) × (10)15–20(25) µm. Conidiophores macronematous, densely fasciculate, arising from stromata, initially erumping through stomata, later by rupturing epidermis, erect to procumbent, hyaline to 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. Conidiogenous cells integrated, terminal and intercalary, polyblastic, cylindrical, conidiogenous loci slightly protuberant, thickened and darkened, loci conical having small rim-like depression on the top encircling a small, flat, protuberant-like structure (ultrastructure), 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, thin-walled, (0)1–6(12)-euseptate, base obconically truncated to rounded, tip obtuse, (18)30–80(117) × (2)3–5(6.5) µm, hila unthickened, sometimes slightly thickened and darkened, 1–2 µm wide.

Materials examined: India, Uttar Pradesh, Allahabad, on leaves of *Peristrophe bicalyculata* (Retz.) Nees, Nov. 1928, *Tandon* (holotype HCIO 12215); India, Madhya Pradesh, Sagar, Afchand forest, on living leaves of *P. bicalyculata*, Sept. 2013, *N. Awasthi* (epitype designated here AMH 9671, MycoBank MBT10009148, gene sequence GenBank: MZ311866 (ITS), MZ311874 (LSU), OL773683 (*RPB2*); India, Madhya Pradesh, Sagar, Afchand forest, 23.834030°N 78.746567°E, on living leaves of *P. bicalyculata*, 01 Dec. 2019, *R. Singh* (AMH 10363, gene sequence GenBank: ON310831 (ITS), ON310846 (LSU), ON376994 (*RPB2*).

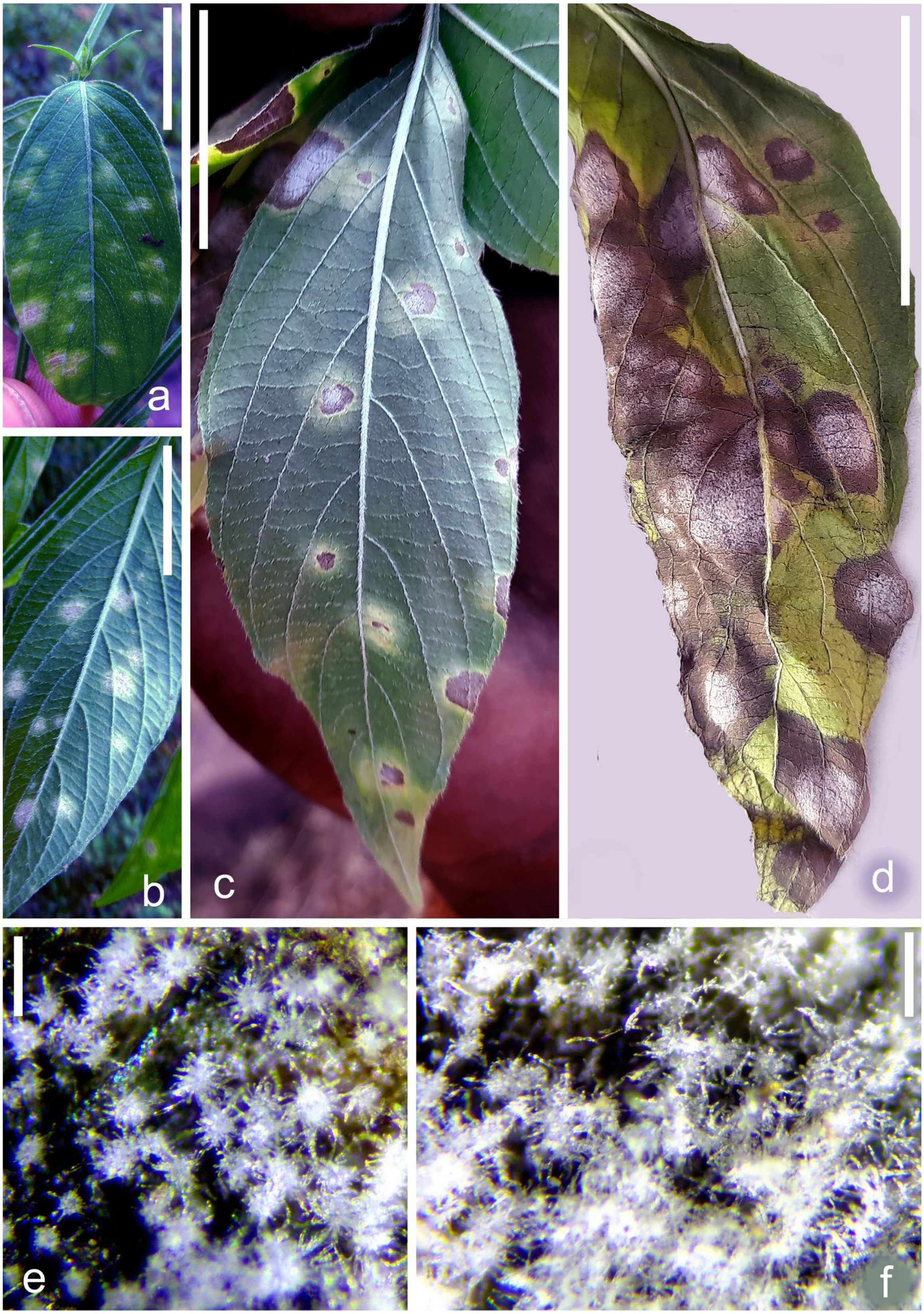


Fig. 3 Symptoms of infection of *Neocercosporella peristrophes* on *Peristrophe bicalyculata*. (**a**) Initial stage of symptom on upper surface of leaf, (**b**) initial stage of infection on lower surface of leaf, (**c**, **d**) late stage of infection on lower surface of leaves, (**e**, **f**) fascicles of conidiophores developed on the surface of leaves. Bars: (**a**–**d**) 20 mm, (**e**) 200 µm, (**f**) 100 µm

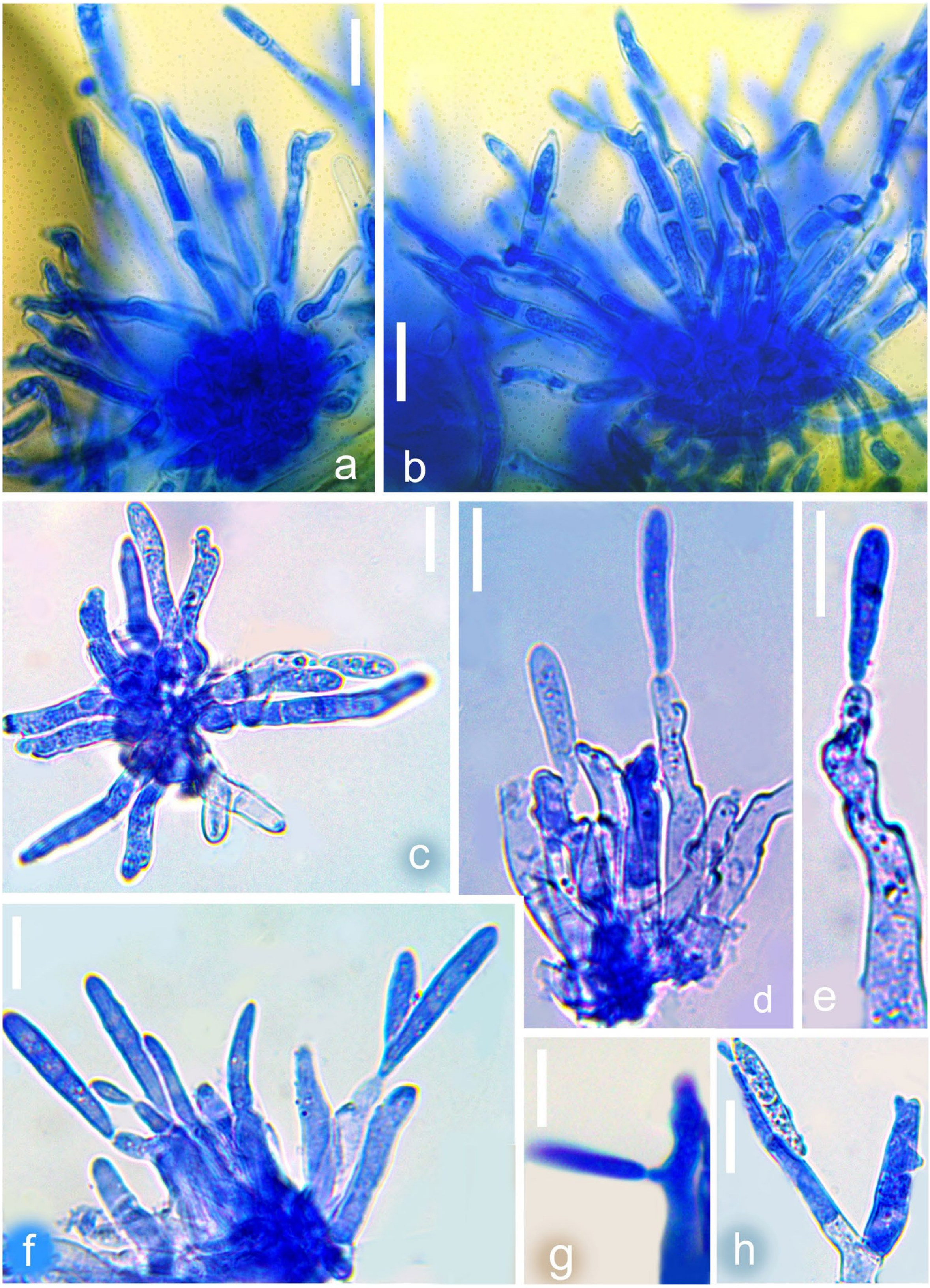


Fig. 4 Microphotographs of *Neocercosporella peristrophes* (AMH 10363). (**a**–**c**) Fascicles of conidiophores, (**d**–**g**) conidiophores with conidia, (**h**) branched conidiophores. Bars: 10 µm

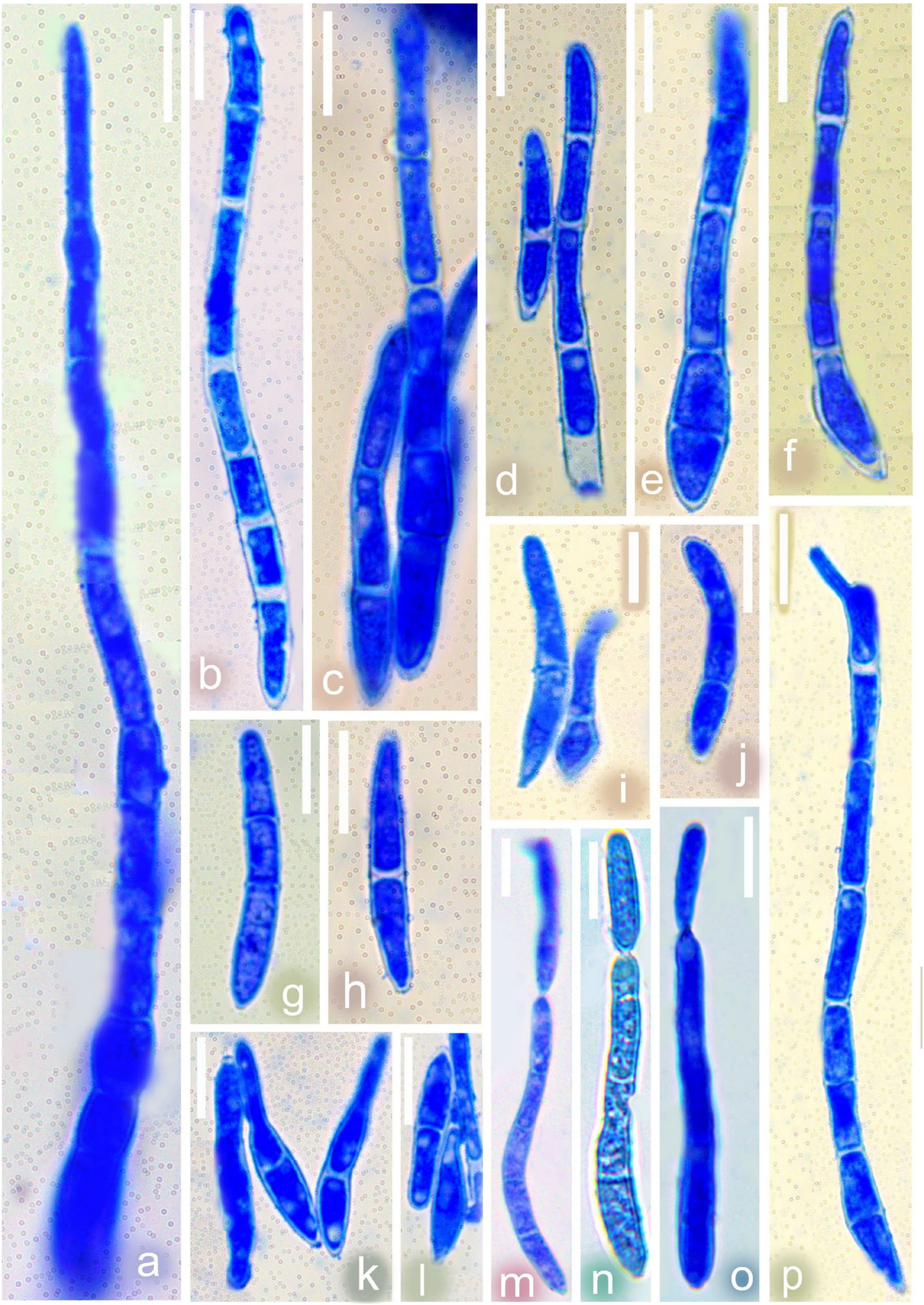


Fig. 5 Microphotographs of *Neocercosporella peristrophes* (AMH 10363). (**a**–**l**) Conidia, (**m**–**o**) catenate conidia, (**p**) germinating conidium. Bars: 10 µm

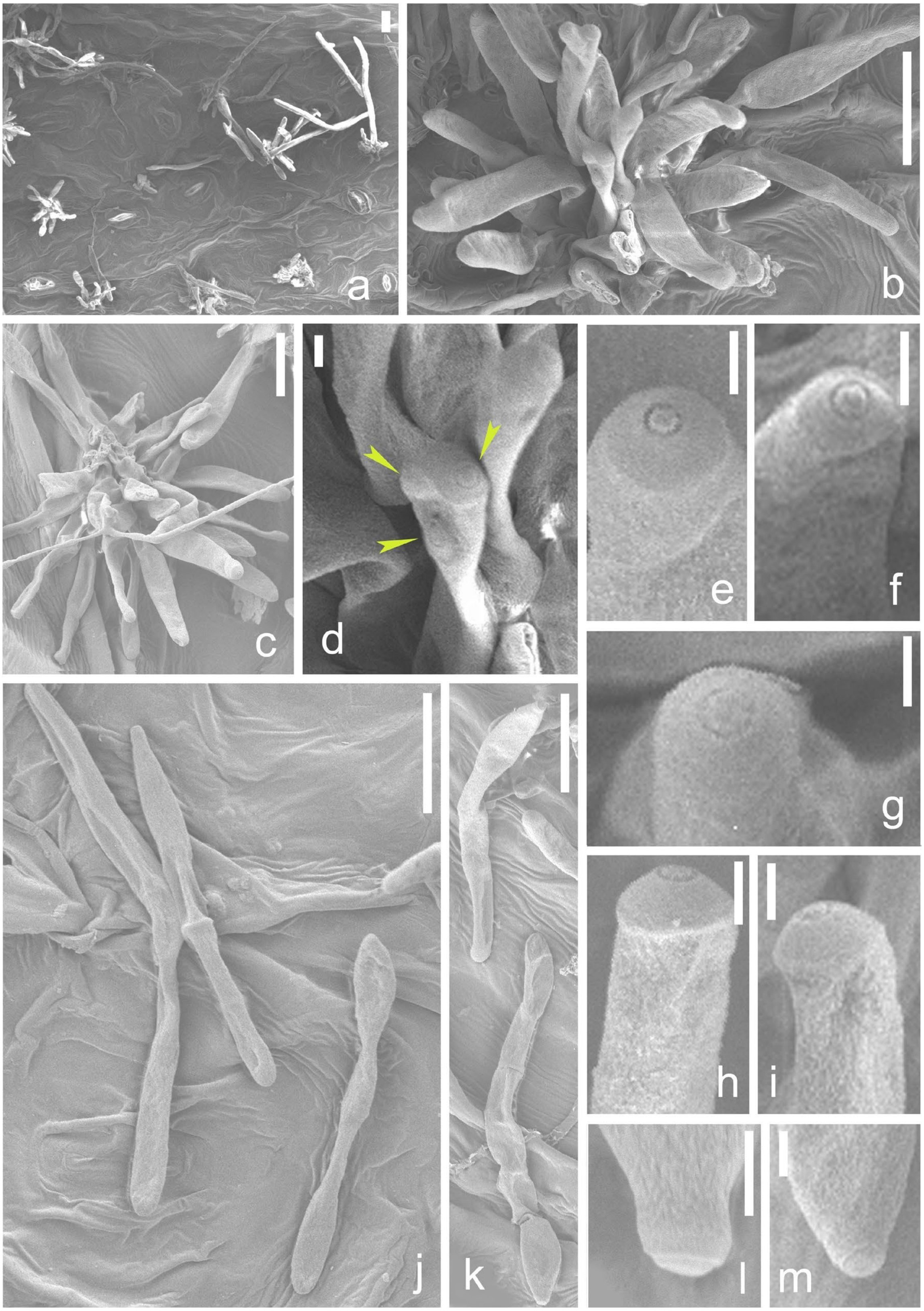


Fig. 6 Scanning electron microphotographs of *Neocercosporella peristrophes* (AMH 10363). (**a**) Initial stage of development of conidiophores through stomata, (**b**, **c**) fascicles of conidiophores, (**d**) polyblastic conidiogenous cell (yellow arrows), (**e**–**g**) top view of conidiogenous loci, (**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**) = 1 μm

Notes: Neocercosporella belongs to the *Mycosphaerellaceae* (Figs. 1 and 2). The type species of this genus was originally described as *Pseudocercosporella andrographidis* (Awasthi et al. 2016) from the same locality as the epitype. The host of *P. andrographidis* was mistakenly identified as *Andrographis paniculata* instead 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; 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 maintaining this species in *Pseudocercosporella*. We obtained cultures from specimens AMH 9671 and AMH 10363 but, unfortunately, they stopped growing after few subculturing events. DNA sequence data from specimens AMH 9671 and AMH 10363 are identical, cluster together with statistical support (BI-PP/ML-BS/MP-BS: 1/100/100), and could not be placed in any of the genera already described in the *Mycosphaerellaceae* (Figs. 1 and 2). Hence, it is justified to introduce a new genus for this monotypic lineage, viz., *Neocercosporella*.

Cercosporella peristrophes, the name of a common cercosporoid hyphomycete on *Peristrophe bicalyculata*, is the agent of a leaf spot disease 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 the two datasets, it is confirmed that *Cercosporella*, *Neocercosporella*, *Pseudocercosporella* and *Ramularia* represent separate clades (Figs. 1 and 2). *Cercosporella*, *Neocercosporella* and *Ramularia* can be easily distinguished, based on the ultrastructure of their conidiogenous loci. *Cercosporella* has flat conidial loci in the shape of a truncated cone (Kirschner 2009), while *Neocercosporella* has conical loci with very small rim-like depressions on the top encircling a small, flat and protuberant-like structure. Conidiogenous loci of *Ramularia* have a raised rim with a central dome (Kirschner 2009) that is cladosporium-like. *Cercosporella* produces terminal conidiogenous cells forming conidia solitarily, while *Neocercosporella* produces terminal and intercalary conidiogenous cells and weak catenation of conidia.

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

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

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

MycoBank MB840504

Etymology: derived from the genus name *Ramulariopsis.*

Diagnosis: differs from *Cercosporella* due to its highly branched chains of conidia and its phylogenetic position that is closer to *Ramulariopsis*. The latter differs from *Neoramulariopsis* in having frequently branched conidiophores with integrated, terminal, intercalary and pleurogenous conidiogenous cells.

Description: (adopted from Crous et al. 2014 and Videira et al. 2016): plant pathogenic, foliicolous. Stromata immersed to erumpent, substomatal, brown, consisting of pseudoparenchymatal cells. Ascomata developing from stromata, 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 geniculate, sinuous, septate, hyaline, thin-walled, smooth. Conidiogenous cells integrated, terminal or lateral, hyaline, subcylindrical to geniculate-sinuous, with single to multiple conidiogenous loci, loci truncate, thickened to unthickened, not darkened or very slightly darkened. Conidia hyaline, smooth, formed singly or in branched chains; ramoconidia, intercalary and terminal conidia aseptate or septate, with thickened but not darkened hila.

Type species: Neoramulariopsis unguis-cati (Speg.) Raghv. Singh & Kushwaha (≡ *Cercosporella unguis-cati* Speg.)

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)

Neoramulariopsis unguis-cati (Speg.) Raghv. Singh & Kushwaha, **comb. nov.**

MycoBank MB840506

Basionym: Cercosporella unguis-cati Speg. 13: 422–423 (1911).

Synonyms: Pseudocercospora unguis-cati (Speg.) U. Braun, Mycotaxon 51: 49 (1994b).

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

Description and illustration: Crous et al. (2014)

Notes: Cercosporella catenulata and *C. dolichandrae* clustered together with *Cercosporella virgaureae* (Thüm.) Allesch. (the type species of *Cercosporella*) and formed a well-defined clade close to *Acervuloseptoria* and *Ramulariopsis* in *Mycosphaerellaceae* (Crous et al. 2014; Videira et al. 2016, 2017). Phylogenetically, *Acervuloseptoria* is represented by a single-strain lineage that is closely related to *Cercosporella* and *Ramulariopsis* (Videira et al. 2017). However, the phylogenetic position of *Acervuloseptoria* is not yet clear. It clustered near *Cercosporella* when LSU-*RBP2* sequence data were analysed, but among *Cercosporella* species based on LSU-*RBP2*-ITS sequence data (Videira et al. 2017). The latter dataset separated *C. catenulata* and *C. dolichandrae* from *Cercosporella*. In the single-gene analysis of either LSU or ITS, *Acervuloseptoria* clustered outside both the *Cercosporella* and the *Ramulariopsis* clades with high support in the LSU (BI, PP = 0.94) but without support in the ITS tree (Videira et al. 2017). Thus, *Acervuloseptoria* appears as a single-strain lineage sister to both *Cercosporella* and *Ramulariopsis* (Videira et al. 2017).

We obtained similar results (Figs. 1 and 2), as reported by Crous et al. (2020). Morphological characters support separating *C. catenulata* and *C. dolichandrae* from *Cercosporella s. str.* as the two species produce branched conidial chains (Crous et al. 2014; Videira et al. 2016), as does *Ramulariopsis. Ramulariopsis* species have frequently branched conidiophores, terminal and intercalary conidiogenous cells, forming small lateral projections or branchlets usually just below the septa, and cicatrized, thickened and darkened loci (Braun 1998). As *Cercosporella catenulata* and *C. dolichandrae* form terminal conidiogenous cells with truncate loci (Crous et al. 2014), it is clear that a new genus, *Neoramulariopsis*, is required to accommodate these two *Cercosporella* species and perhaps other cercosporella-like species producing terminal conidiogenous cells with truncate loci.

Silva et al. (2012) reported leaf spots on *D. unguis-cati* in Brazil caused by *Pseudocercospora unguis-cati* (Speg.) U. Braun (Braun 1994b). Similar leaf spots on the same host are also caused by *Cercosporella dolichandrae* (Crous et al. 2014), and questions emerged as to whether *Ps. unguis-cati* and *C. dolichandrae* are conspecific. A BLASTn search of the ITS sequence of *Ps. unguis-cati* (accession no. MW036753) showed a 99.83% identity (604/605 nt) to the type sequence of *C. dolichandrae* (NR_156282/ KJ869140; Crous et al. 2014). *Cercosporella unguis-cati* was originally described on *D. unguis-cati* by Spegazzini (1911) and is morphologically identical to *Ps. unguis-cati* Silva et al. (2012). The earlier name of *Cercosporella unguis-cati* Speg. takes priority over *Ps. unguis-cati* (Speg.) U. Braun and *C. dolichandrae* Crous & den Breeÿen, which are now considered synonyms (Colmán et al. 2020).

Similarly, *Neocercosporella* also differs from *Ramulariopsis* in having hyaline to very light olivaceous, mostly unbranched or rarely branched conidiophores. *Neocercosporella* does not form conidiogenous cells as small lateral projections or branchlets just below the septa, and its conidia are mostly formed solitary, although they can be rarely catenate. Its conidia are also mostly hyaline, rarely light olivaceous and hila are mostly unthickened or rarely slightly thickened and darkened. The phylogenetic analysis in this study also supports the separation of *Neocercosporella* from *Ramulariopsis*.

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Author contribution All authors contributed to the conception and design of the study. Sanjay Yadav collected samples, tried to cultivate strains, isolated DNA and prepared samples for sequencing. Sanjeet Kumar Verma and Gargee Singh developed morphological features and surveyed 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 the authors contributed to previous drafts of the manuscript and read and approved the final draft of the manuscript.

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Data availability Sequences generated in this study have been deposited 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.

Declarations

Ethics approval Not applicable.

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