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Taxonomy and phylogeny of a new species of *Pseudocercospora* on *Solanum nigrum* from India

Archana SINGH*, Sanjay YADAV, Raghvendra SINGH, Nawal Kıshore DUBEY
Center of Advanced Study in Botany, Banaras Hindu University, Varanasi , UP, India

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Abstract: A new species *Pseudocercospora solanicola* is discovered causing severe leaf spot disease on *Solanum nigrum* from Sonebhadra. Species is described and illustrated based on morphology, molecular sequence analysis and phylogeny of the large subunit (LSU) and internal transcribed spacer region of the nuclear ribosomal RNA (rRNA) gene. Morphologically, new species has similarity with allied species in *Pseudocercospora* but differs in having black circular, velvety leaf spot and size of stromata, conidia and conidiophores. A key to *Pseudocercospora* species on *Solanum* has been provided.

Key words: Taxonomy, *Pseudocercospora,* morphology, molecular phylogeny, Mycosphaerellaceae, Capnodiales

1. Introduction

Pseudocercospora is second largest genus in capnodiales with pathogenic species infecting various important crop and medicinal plants useful for mankind (Bakhshi et al., 2014). The genus was established by Segazzini (1910). Since then, many species have been added to the genus (Deighton, 1976; Braun, 1995; Pons and Sutton, 1988; Crous and Braun, 2003, 2009; Kamal, 2010). The introduction of molecular technique in taxonomic studies has widened the generic concept and distinguished two clades, one with species having similarity with *Pseudocercospora*, called *Pseudocercospora* sensu lato and other showing alignment with type species *Pseudocercospora viti* called *Pseudocercospora senu stricto* (Crous et al., 2009). The majority of species were interpreted as host specific, confined to a single host genus or even to a single species, although some may have wide host range (Deighton, 1976). The fact was supported by multigene analysis (Crous et al., 2013). Today consolidated species concept is practiced by mycologists to describe new species (Bakhshi et al., 2014; Nakashima et al., 2016; Shivas et al., 2015; Park et al., 2015; Yadav et al., 2021; Meswaet et al., 2021; Singh et al., 2021, 2022).

Solanum nigrum L. commonly known as black nightshade is an erect divaricated, branched, unarmed, suffrutescent annual herb distributed as a weed throughout dry parts of India. The plant is a rich source of riboflavin, nicotinic acid and vitamin C, β carotene and citric acid. Beside this, fruit also contains aglycone having steroidal glycol-alkaloids i.e. solamargine and α & β solanigrine. The plant is variously used in Ayurvedic and ethnomedicine to treat jaundice, dysentery, fever, various stomach and liver ailments (Jain, 1991; Prajapati et al., 2003).

Many species of *Pseudocercospora* have been reported on the host genus. *Ps. trichophila* (F. Stevens) Deighton, 1976 was reported on *Solanum ferox* and *S. verbasafolium* from Mysore (Karnataka). *Ps. venezuelae* (Chupp) Deighton, 1976 was reported to cause leaf spot on *Solanum indicum* from Hosamande, Nilgiris (Tamil Nadu). *Ps. egenula* (Syd.) Crous and Braun, 2003 was reported on *Solanum melongena* and it has a wide distribution, reported from Bangalore (Karnataka), Hyderabad (Telangana), Kolhapur (Maharastra) and Lakshadweep Island. Single species, *Ps. atromarginalis* (G. F. ATK.) Deighton, 1976 was reported infecting host species *Solanum nigrum* is widely distributed throughout India, reported from Annamalai Nagar (Tamil Nadu), Gorakhpur, Jaunpur (Uttar Pradesh), Hassan (Karnataka), Jabalpur (Madhya Pradesh), Mannanore forest (Andhra Pradesh), Pune (Maharastra) and West Bengal. Present species is collected from Sonebhadra forest. A survey was conducted to study leaf inhabiting cercosporoid fungi during October 2019–February 2020 at Sonebhadra district, Uttar Pradesh, India. Sonebhadra forest is spread in wide area of 6788 Km on Kaimur range and Vindhayan plateau. Many species have been reported earlier from the study area (Singh 2012; Singh and Dubey 2012a, 2012b; Singh et al., 2012; Singh et al., 2013; Singh et al., 2014).

* Correspondence: drarchanabhu@gmail.com

2. Material and methods

2.1. Isolation and morphology

Symptoms were minutely observed on the host plants and leaves showing the lesions were collected in a sterile paper bag from the area under investigation. Samples were brought to the laboratory and slides were prepared for morphological observations (color, size and shape of stromata, conidia and conidiophores). Leaf spots were scraped with a fine needle and mounted in lactophenol cotton blue. Thirty measurements of morphological characters of taxonomic importance were taken under Olympus CH20i compound microscope mounted with Magnus MIPS CMOS camera. Hand sections were cut with a sharp razor blade through infection spot of leaf to study histopathological analysis of the host and the pathogen. Preparation of pure culture and growth on potato dextrose agar (PDA), Sisco Research Laboratories Pvt. Ltd. (SRL) were done as described by Singh et al. (2019). Scanning electron microscopy was done for examining the plant surface at high resolution (Pathan et al., 2010). Leaves samples were dehydrated by pressing in blotting paper and hot air treatment. Samples were cut into pieces around symptoms and made conducting by gold coating with a sputter coater (Quorum Q150RES). Scanning electron microscopic images were taken by ZEISS-EVO-18-Research. The holotype specimens were deposited in Ajrekar Mycological Herbarium (AMH), MACS' Agharkar Research Institute, Pune, India. The extype culture was deposited in the National Fungal Culture Collection of India (NFCCI-WDCM932). The taxonomic novelties of the taxon were submitted in MycoBank (http://www.MycoBank.org) (Crous et al., 2004).

2.2. DNA extraction and amplification (PCR)

Genomic DNA was extracted from approximately 200 mg of 20 to 25 days old fresh cultures using Himedia DNA Isolation Kit (HiPurA Fungal DNA Purification Kit) following the manufacturer's protocols. DNA quality and quantity were determined using a NanoDrop Microvolume Spectrophotometers (Thermo Scientific NanoDrop One/One^{C} Microvolume UV-Vis Spectrophotometer with Wi-Fi).

The nuclear ribosomal DNA internal transcribed spacers (ITS) and large subunit rRNA (LSU) regions were amplified using the primer pairs ITS4/ITS5 (White et al., 1990) and LROR/LR7 (Vilgalys and Hester, 1990), respectively.

Polymerase chain reactions (PCR) were conducted in a total volume of 50 µL using PCR mixtures included the following ingredients for each 50µL reaction: 5µL PC buffer containing MgCl2, 1 µL each forward and reverse primer (10 pmol), 1 µL dNTP (10 mM), 0.3 µL Taq DNA polymerase (5 unit/µL) and 6 µL of DNA template (15.29 ng/µL). The PCRs were carried out in Thermal Cycler (BIO-RAD: T100 Thermal Cycler).

The thermal cycling programs were accomplished by an initial denaturation at 95 °C for 5 min; followed by 34 cycles of denaturation at 94 °C for 45 s; annealing at 48 °C for LSU and 51.9 °C for ITS 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 PCR products were run in 1.2% agarose gel and visualized in 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. DNA sequences were obtained manually and editing was done using Chromas Lite v. 2.01 (http:/www.technelysium.com.au). Obtained sequences were deposited in NCBI for accession number.

Sequences of the two gene markers including those of type strains were retrieved from GenBank (Table 1). Sequences were aligned with muscle. Both gene sequences were then concatenated using FABOX (1.6). Molecular phylogenetic analysis was done by the maximum likelihood method and Kimura 2-parameter as the best model with 1000 bootstrap replications (Tamura et al., 2011). The Bayesian analysis was performed using MrBayes v.3.2.6 (Ronquist et al., 2012). The analysis was performed for 10,000,000 generations until the standard deviation of split frequency was below 0.01. Burnin tree samples of 25% was discarded. The tree presented in the manuscript was obtained with a maximum likelihood approach. Tree reconstruction, visualization and editing were done with FIGTREE v1.4.4, TreeGraph_2.14.0 and MEGA 5.2

3. Results

3.1. Taxonomy

Pseudocercospora solanicola Archana Singh, Sanjay Yadav, N. K. Dubey sp. nov. Figures 1– 3.

MycoBank: 841403.

Etymology. Name refers to *Solanum*, the host genus from which the fungus was isolated

Culture characteristics– Conidia germinating on Potato Dextrose Agar (PDA). Colonies very slow growing, velvety, grayish black, reaching 10–11 mm diam. in 4 weeks at 27 °C, margin circular to irregular, reverse black, raising centrally with dense cottony mycelium and hard texture. Asexual and sexual spores were not formed.

Leaf spots Hypophyllous later amphiphyllous dark black, circular to subcircular, velvety on lower leaf surface and black with yellowing encircling area on the upper leaf surface (Figures 1b and 1c). **Caespituli** hypogenous later amphigenous, substomatal (Figure 1d). **Mycelium** internal branched. **Stromata** substomatal, pseudoparenchymatous, light yellowish brown to light brown, globular, 25–38 µm filling stomatal opening. **Conidiophores** macronematous, mononematous, olivaceous brown to light brown, paler

Table 1. GenBank number of strains of *Pseudocercospora* used in phylogenetic analyses.

SN	*Pseudocercospora sp*	Isolate	Host	Countries	Nucleotide sequences	
					LSU	ITS
1	*Ps. acericola*	CBS 122279	*Acer albopurpurascens*	Taiwan	GU253699	GU269650
2	*Ps. ampelopsis*	CBS:131583	*Ampelopsis glandulosa var. brevipedunculat*	South Korea	GU253846	GU269830
3	*Ps. assamensis*	CBS:122467	*Musa*	India	GU253705	GU269656
4	*Ps. atromarginalis*	CPC: 11372	*Solanum nigrum*	South Korea	JX901883	JX901780
5	*Ps. breonadiae*	CBS: 143489	*Breonadia salicina*	South Africa	NG_069573	MH107913
6	*Ps. casuarinae*	CBS:128218	*Casuarina cunninghamiana*	Australia	HQ599604	HQ599603
7	*Ps. chengtuensis*	CBS: 131924	*Lycium chinense*	South Korea	MH877506	MH866053
8	*Ps. chiangmaiensis*	AGI: 094.3	*Eucalyptus camaldulensis*	Thailand	EU882144	EU882128
9	*Ps. cordiana*	CPC: 2552	*Cordia goeldiana*	Brazil	GU214472	GU269681
10	*Ps. cydoniae*	CBS:131923	*Chaenomeles lagenaria*	South Korea	GU253732	GU269691
11	*Ps. dovyalidis*	CBS: 126002	*Dovyalis zeyheri*	South Africa	NG_069071	MH863877
12	*Ps. eupatoriella*	CBS :113372	*Chromolaena odorata*	Jamaica	GU253743	GU269704
13	*Ps. flavomarginata*	CBS :124990	*Eucalyptus camaldulensis*	Thailand	GU253817	GU269799
14	*Ps. fraxinites*	MUCC: 891	*Fraxinus excelsior*	Japan	GU253748	GU269710
15	*Ps. fukuokaensis*	MUCC: 887	*Styrax japonica*	Japan	GU253751	GU269714
16	*Ps. fuligena*	MUCC: 533	*Solanum lycopersicum*	Japan	GU253749	GU269712
17	*Ps. haiweiensis*	CBS: 131584	*Eucalyptus sp.*	China	NG_069072	MH865906
18	*Ps. hakeae*	CBS: 112226	*Grevillea sp.*	Australia	GU253805	GU269784
19	*Ps. humuli*	MUCC: 742	*Humulus lupulus*	Japan	NG_069067	GU269725
20	*Ps. jagerae*	BRIP: 58549	*Jagera pseudorhus var. pseudorhus*	Australia	NG_069206	NR_147283
21	*Ps. kiggelariae*	CPC: 11853	*Kiggelaria africana*	South Africa	GU253762	GU269730
22	*Ps. macrospora*	CPC: 2553	*Bertholletia excelsa*	Brazil	GU214478	GU269745
23	*Ps. madagascariensis*	CBS: 124155	*Eucalyptus camaldulensis*	Madagascar	MH874880	MH863357
24	*Ps. myopori*	CBS: 114644	*Myoporum laetum*	New Zealand	KX287000	KX287302
25	*Ps. neriicola*	CBS: 138010	*Nerium oleander*	Italy: Potenza	KJ869222	KJ869165
26	*Ps. nymphaeacea*	TNM: R. Kirschner 3453	*Nymphaea sp.*	Taiwan	KY304494	GU269830
27	*Ps. paederiae*	CPC 10007	*Paederia scandens*	South Korea	GU253783	GU269757
28	*Ps. paraguayensis*	CBS:111317	*Eucalyptus nitens*	Brazil	GQ852634	JQ324978
29	*Ps. pini-densiflorae*	MUCC: 534	*Pinus thunbergii*	Japan	GU253785	GU269760
30	*Ps. prunicola*	CBS:132107	*Prunus yedoensis*	South Korea	GU253723	GU269676
31	*Ps. ravenalicola*	CBS: 122468	*Ravenala madagascariensis*	India	GU253828	GU269810
32	*Ps. rubi*	MUCC: 875	*Rubus allegheniensis*	Japan	GU253795	GU269773
33	*Ps. schizolobii*	CBS: 124990	*Eucalyptus camaldulensis*	Thailand	KF251827	KF251323
34	*Ps. securinegae*	CBS: 131930	*Securinega suffruticosa*	South Korea	MH877490	MH866043
35	*Ps. serpocaulonicola*	CPC:25077	*Serpocaulon triseriale*	Brazil	KT037566	KT037525
36	***Ps. solanicola* sp.nov.**	**AMH:10347**	***Solanum nigrum***	**India**	**MZ474953**	**MZ474952**
37	*Ps. solani-pseudocapsicicola*	CPC: 25229	*Solanum pseudocapsicum*	Brazil	KT290175	KT290148
38	*Ps. tabei*	YMM: 220	*Vigna unguiculata*	Benin	MW834434	MW834439
39	*Ps. tereticornis*	CBS: 124996	*Eucalyptus nitens*	Australia	KF251828	KF251324
40	*Ps. vitis*	CBS:132012	*Vitis vinifera*	South Korea	GU269829	GU214483
41	*Ps. xanthoxyli*	CPC: 10065	*Zanthoxylum ailanthoides*	South Korea	GU253848	GU269832
42	*Ps. zelkovae*	MUCC: 872	*Zelkova serrata*	Japan	GU253851	GU269835
43	*Coremiopassalora eucalypti*	CBS: 111318	*Eucalyptus saligna*	Brazil	GU253860	GU269845
44	*Pallidocercospora heimii*	CPC: 11716	*Eucalyptus* sp.	Brazil	KF442661	DQ302966

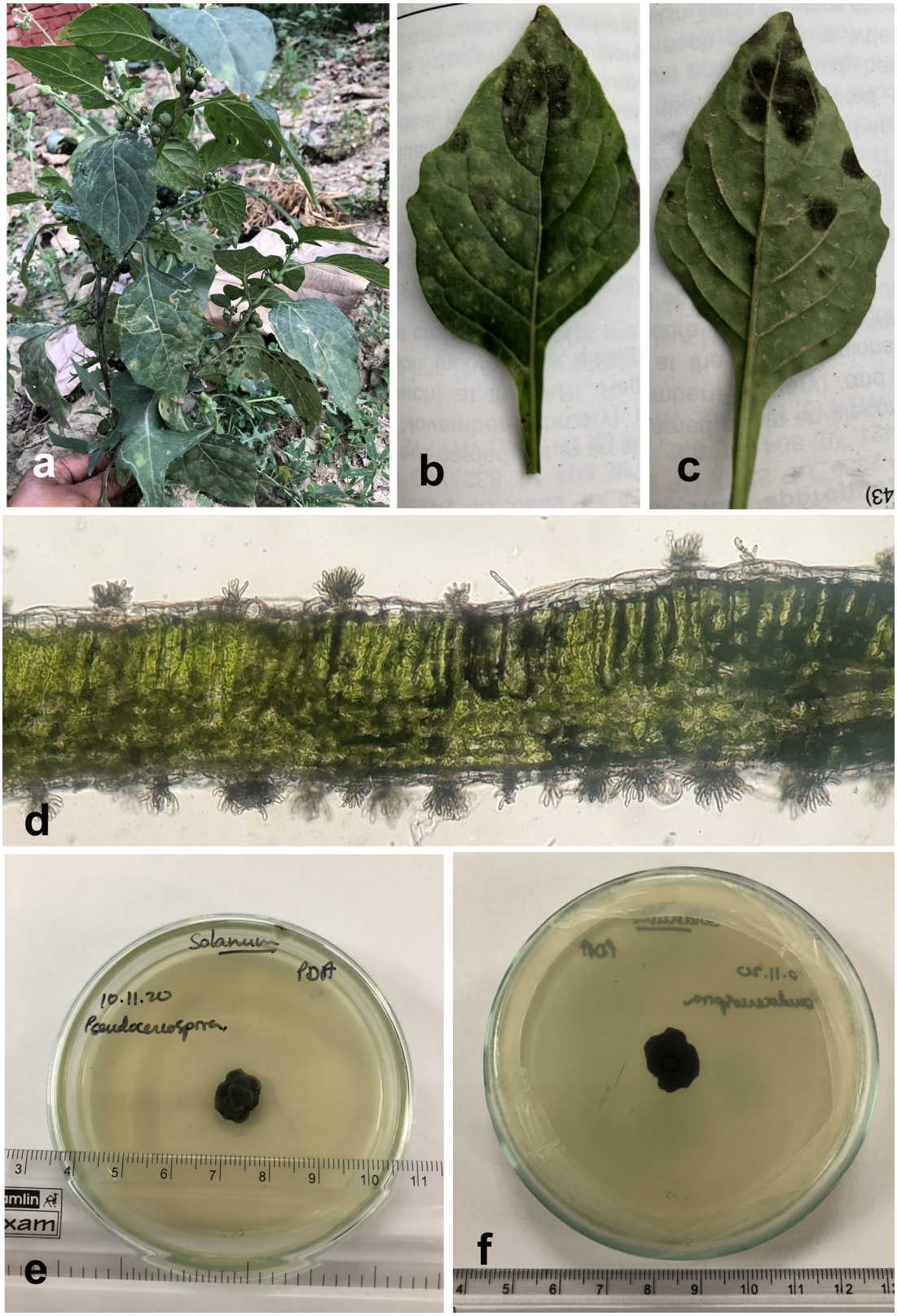

Figure 1. *Pseudocercospora solanicola* (Holotype AMH: 10347) **a.** Habit of the host plant *Solanum nigrum*. **b–c.** Symptoms on upper and lower surfaces of leaf. **d.** Transverse section through infection spots of leaf showing fungal colonies on lower and upper surfaces. **e–f.** Fungal culture on PDA front and reverse view.

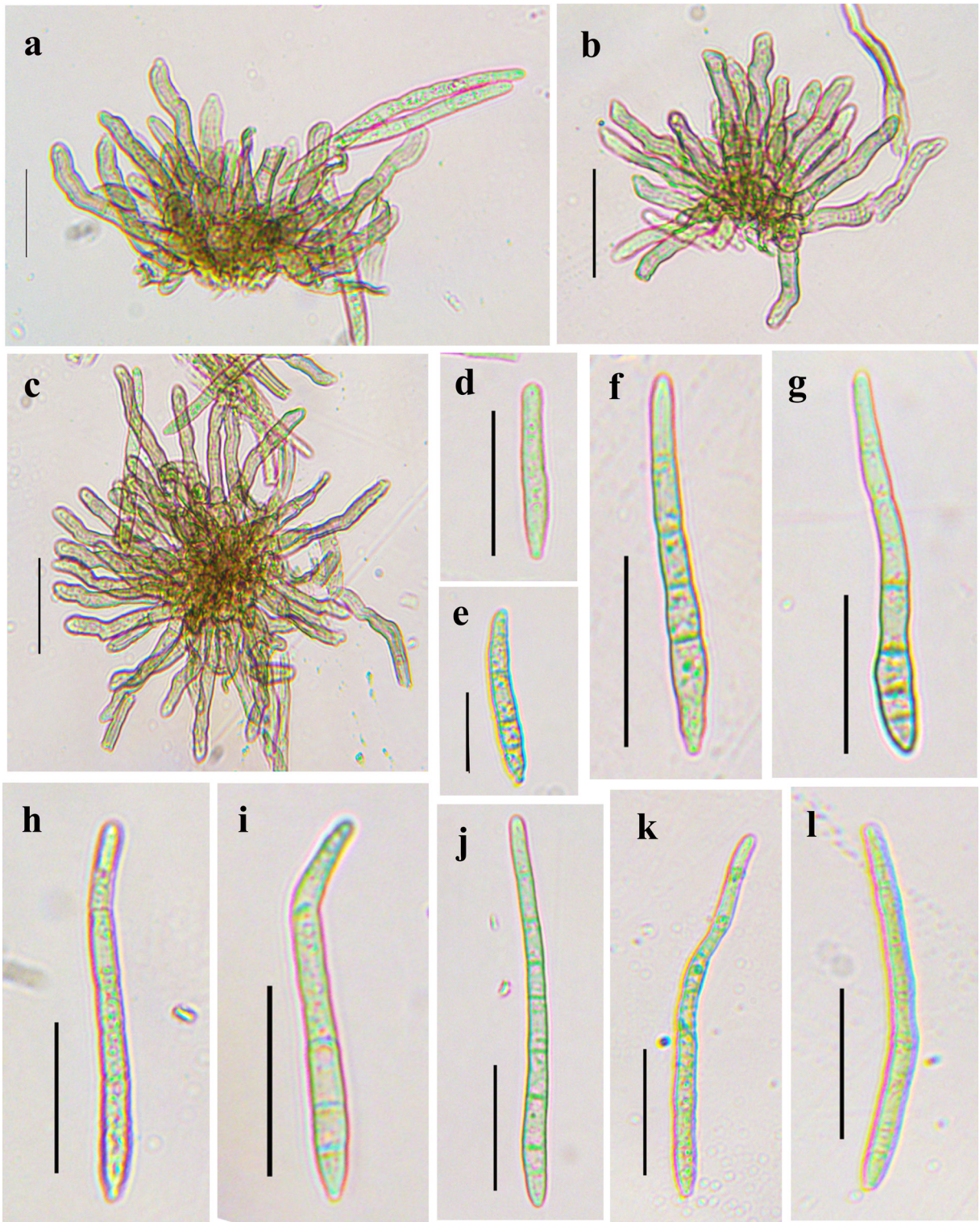

Figure 2. *Pseudocercospora solanicola* (Holotype AMH: 10347) **a–c.** Fascicle of conidiophores. **d–i** Conidia. Scale bar = 20 µm.

towards the tip, fasciculate 21–30 in divergent fasciles, smooth, 0–3 septate, rarely branched, straight to curved, geniculate, subcylindrical 20.5–45 × 3–4 µm (Figures 2a–2c). **Conidiogenous cells** integrated, terminal, polyblastic, pale olivaceous, smooth, geniculate, scar unthickened. **Conidia** solitary, acropleurogenous, holoblastic, pale

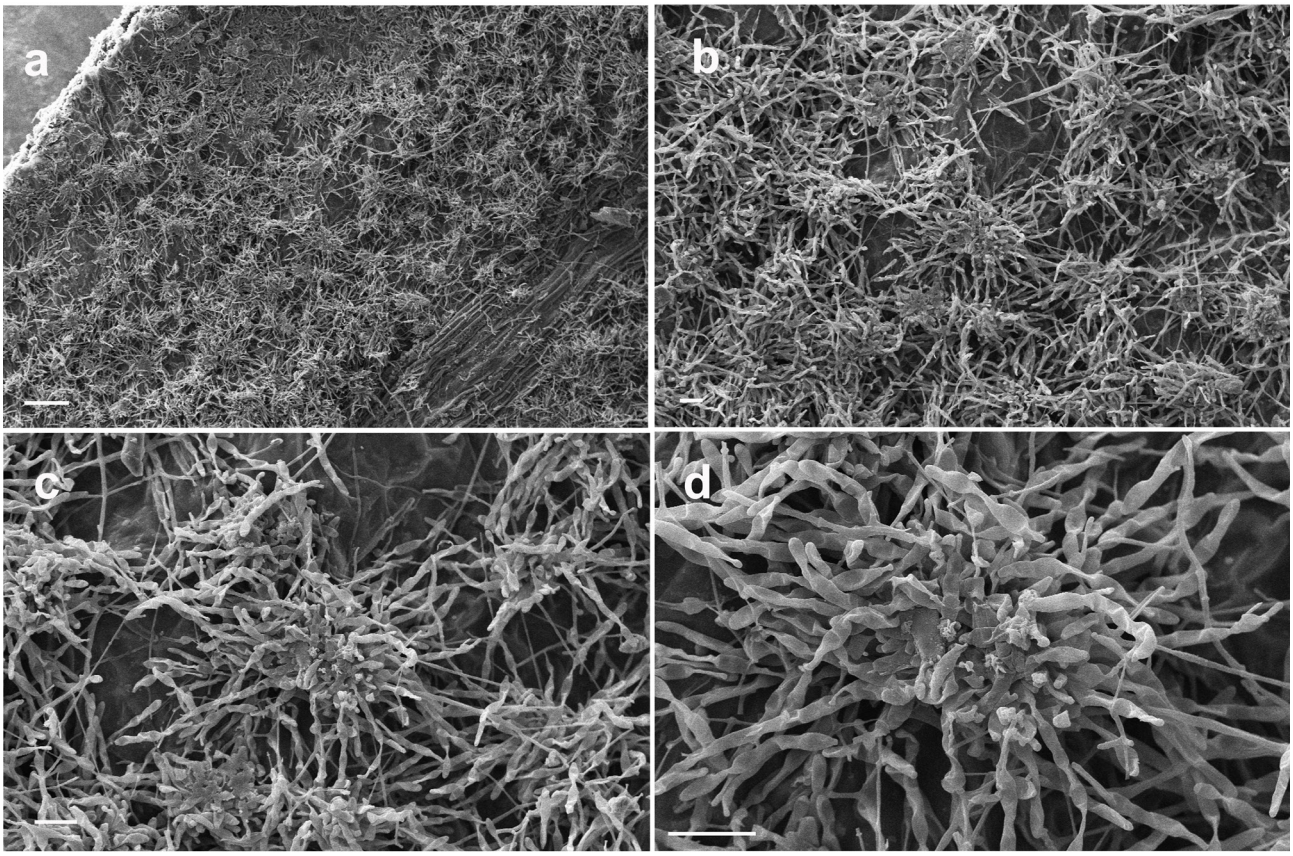

Figure 3 *Pseudocercospora solanicola* sp. nov. (Holotype AMH: 10347) **a–b**. SEM micrograph of surface view of host plant showing distribution of fascicles of conidiophores (in low magnification). **c.** Numerous fascicles of conidiophores. **d.** Single fascicle of conidiophores. Scale bars: a. 100 µm, b–d. 20 µm.

brown, subcylindrical, apex obtuse to subacute, base obconically truncate, straight to curved, 4–6 septate, 32.0–81.5 × 3.5–4.5 µm, hilum unthickened (Figures 2d–2i).

On living leaves of *Solanum nigrum* L. (Solanaceae) Sonebhadra, U.P. India, Oct 2020, collected by Archana Singh, AMH:10347 (Holotype), BHU Herb no. AS/20/96 Isotype, culture ex type NFCCI: 5015, GenBank deposition no. LSU: MZ474953, ITS: MZ474952

3.2. Phylogeny

Ps. solanicola sp. nov. has ribosomal DNA LSU sequence of 1112 bp with G+C content 49.6% and ITS sequence of 539 bp with G+C content 51.4%. NBLAST result with LSU sequence shows similarity with *Ps. macrospora* strain CPC 2553 (97.94%), with *Ps. cordiana* strain CPC 2552 (97.94%), with *Ps. atromarginalis strain* CPC 11372 (97.85%). NBLAST result with ITS sequence shows similarity with *Ps. fuligena* strain CPC 12296 (99.06%), with *Ps. chengtuensis* strain CPV 10785 (99.06%), with *Ps. atromarginalis* strain CPC 11372 (99.06%). The phylogenetic tree created with concatenated LSU and ITS sequences data set showed that *Ps. solanicola* sp. nov. was separated from other *Pseudocercospora* species, with *Ps. myopori* CBS: 114644 as closely related species (Figure 4).

4. Discussion

A search in MycoBank and Index Fungorum reveals that there are only two species of *Pseudocercospora* reported on the host species *Ps. atromarginalis* (G.F. Atk.) Deighton and *Ps. solanacea* Braun. Sequences of *Ps. atromarginalis* were available and included in phylogenetic studies whereas sequences of *Ps. solanacea* was not available therefore not included in the study. Morphological comparison of both species, based on published description has been provided in Table 2. It is evident from the Table 2 that new species is different from both described species having dark black velvety leaf spots spreading on both side of leaf surfaces, stromata filling stomatal opening, conidiophores arising in dense fascicles, 32–81.5 × 3.5–4.5 µm conidia.

Morphological and molecular analysis both attest to the fact that described species is new to the science.

5. Key to *Pseudocercospora* species described on the host genus *Solanum*

1a.Mycelium internal and external, superficial mycelium giving rise to solitary conidiophore 2

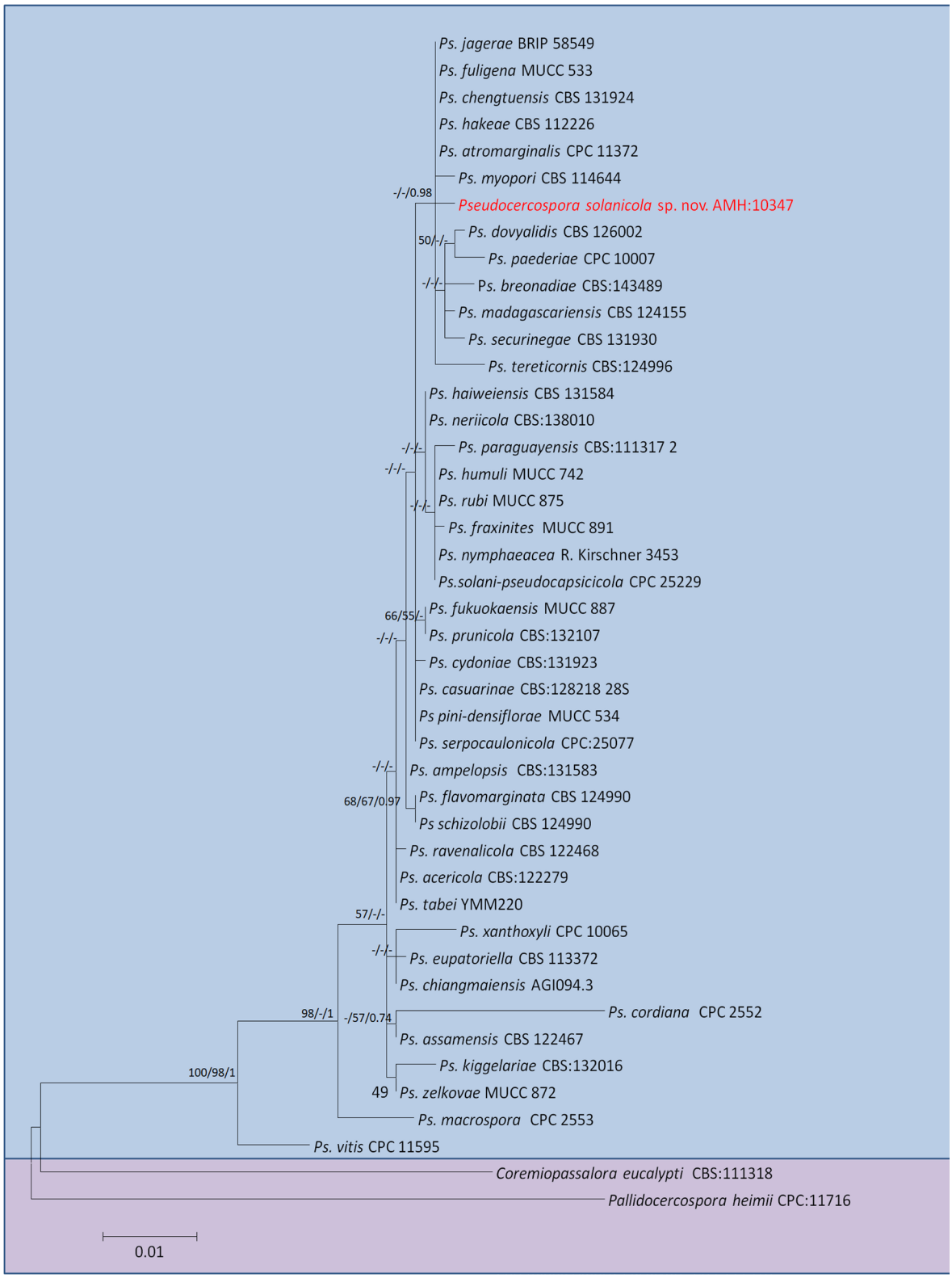

Figure 4. Maximum likelihood tree illustrating the phylogeny of *Pseudocercospora solanicola* (Holotype AMH: 10347) with related species in *Pseudocercospora* based on LSU and ITS concatenated sequences. Branches are labeled with maximum likelihood and parsimony bootstrap value higher than 50% and Bayesian posterior probabilities more than 0.95, respectively. Sequence of *Pallidocercospora heimii* and *Coremiopassalora eucalypti* are used as outgroup and new species is colored in blue.

Table 2. Morphotaxonomic comparative study of *Ps. solanicola* sp nov. with allied species of *Pseudocercospora* spp. reported on the host species.

SN	Fungus	Leaf spots	Stromata	Conidiophores	Conidia
1	*Ps. atromarginalis* (Atk.) Deighton (1976)	Indistinct, circular to irregular, pale brown to grey, with dark brown border	Lacking or few brown cells	4–15 in a fascicle, 1–4 septate, rarely geniculate, 10–50 × 3–5 µm	Cylindric to obclavato-cylindric, 3–9 septate, 15–90 × 2.5–5 µm
2	*Ps. solanacea* Braun (2017)	Pale subcircular to irregular	Well-developed, substomatal to immersed, 20–80 µm diam	Small to large dense fascicle, 0 to 2 septate, 5–40 × 2–5.5 µm	Obclavate to subcylindric, 3-9 septate, (30–) 40–60(–75) × 2.5–5.5 µm
3	*Pseudocercospora solanicola* sp. nov.	Dark black circular to subcircular, velvetty	Substomatal 25µm to 38 µm	21–30 in divergent fasciles, 0–3 septate, geniculate, 20.5–45 × 3–4 µm	Subcylindric, 4–6 septate, 32.0–81.5 × 3.5–4.5 µm

2a. leaf spot indistinct, fasciculate conidiophores upto 15 µm, conidia 20–100 × 2–4 µm ..... *Ps. rugosi*

2b.leaf spot distinct, conidiophores 10–80 µm 3

3a. Conidia cylindrical-linear, base rounded, truncate to short obconically truncate, but not abruptly attenuated hila ≥ 2 µm wide ..... *P. solani-torvicola*

3b. Conidia cylindrical to obclavate-cylindrical, attenuated towards the base, abruptly attenuated, hila 1–2 µm wide 4

4a. Caepituli mainly hypophyllous, effuse floccose-velutinous, deep olivaceous; stromata lacking .... *P. trichophila* var. *trichophila*

4b. Caespituli amphigenous, punctiform on the upper leaf surface, 10–60 µm diam, dark brown to blackish, with fasciculate conidiophores arising from stromata .... *P. trichophila* var. *punctata*

1b. Mycelium internal, superficial hyphae producing solitary conidiophores absent 5

5a. Conidiogenous loci conspicuous, slightly thickened and darkened at the outermost rim, visible in front view as minute circle Conidia obclavate-cylindrical, 40–90 × 2.5–5 µm, base distinctly obconically truncate, hila 1–2 µm diam ..... *P. carolinensis*

5b. Conidiogenous loci quite inconspicuous or subdenticulate, but always unthickened, not darkened, in front view not visible as minute circles 6

6a. Stromata lacking or small, only formed as small substomatal aggregations of swollen hyphal cells, to about 30 µm diam; conidiophores loosely fasciculate 7

7a. Conidia (2.5–) 3–5 µm wide; on a wide range of *Solanum* species almost worldwide ..... *P. atromarginalis* (incl. *P. fuligena* and *P. solani-melongenicola*)

7b Conidia narrower, 2 –3.5 µm; on *Solanum pseudocapsicum* ..... *P. solani-pseudocapsicicola*

6b. Stromata well-developed, 10–80 µm diam; often forming sporodochial conidiomata 8

8a. Conidiophores long and narrow, up to 110 × 2–3 µm, pale olivaceous; conidia narrowly obclavate-cylindrical, 60–110 × 2–3 µm ..... *P. fasciculata*

8b. Conidiophores much shorter, 5–60 µm long, or conidiophores and conidia much wider, 2.5–6 µm 9

9a Conidiophores and conidia narrow, 1.5–4 µm, on average < 3 µm 10

10a. Conidiophores uniformly short, 5–25 µm; conidiogenous loci inconspicuous or visible as truncate tip, but not denticle-like ..... *P. marcelliana*

10b. Conidiophores 8–60 µm long; conidiogenous loci denticle-like ..... *P. venezuelae*

9b. Conidiophores and conidia broader, 2.5–6 µm, on average > 3 µm 11

11a. Caespituli epiphyllous; conidiophores 15–75 µm long, not geniculate, septa indistinct, olivaceous brown; conidia pale fuliginous ..... *P. modesta*

11b. Caespituli amphigenous; conidiophores distinctly septate, pale, subhyaline to pale olivaceous 12

12a. Leaf spot not very conspicuous, pale, aseptate, occasionally with 1 – 2 septa at the base, Stromata 20–80 µm ..... *Ps. solanacea*

12b. Leaf spot very conspicuous, dark black, velvety spreading on entire leaf surface, stromata 25–38 µm .... ***Ps. solanicola* sp.nov**.

Note: Partial key source from Braun 2017.

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Conflict of interest

The Authors declare that they have no conflicts of interest.

References

Bakhshi M, Arzanlou M, Babai-Ahari A, Groenewald JZ, Crous, PW (2014). Multi-gene analysis of *Pseudocercospora* spp. from Iran. Phytotaxa 184: 245-264. doi: 10.11646/phytotaxa.184.5.1

Braun U (1995). A monograph of *Cercosporella*, *Ramularia* and allied genera (phytopathogenic hyphomycetes). Vol. 1. Eching: IHWVerlag

Braun U (2017). *Pseudocercospora solanacea* revisited and a survey of *Pseudocercospora* spp. on *Solanum* with a key to the species. Schlechtendalia 32: 51-65.

Crous PW, Braun U (2003). *Mycosphaerella* and its Anamorphs. 1. Names published in *Cercospora* and *Passalora*. CBS Biodiversity Series no. 1, Utrecht, CBS-KNAW Fungal Biodiversity Centre.

Crous PW, Braun U, Hunter, GC, Verkley GJM, Shin HD et al. (2013). Phylogenetic lineages in *Pseudocercospora*. Studies in Mycology 75: 37-114.doi:10.3114/sim0005

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004). MycoBank: an online initiative to launch mycology into the 21st century. Studies in Mycology 50: 19-22.

Crous PW, Summerell BA, Carnegie AJ, Wingfield MJ, Hunter et al. (2009). Unravelling *Mycosphaerella*: do you believe in genera? Persoonia 23: 99-118. doi: 10.3767/003158509X479487

Deighton FC (1976). Studies on *Cercospora* and allied genera. VI. *Pseudocercospora* Speg., *Pantospora* Cif. and *Cercoseptoria* Petr. Mycological Papers 140: 1-168.

Kamal (2010). Cercosporoid fungi of India. Bishan Bishen Singh Mahendra Pal Singh Publication, Dehradun (UK), India. 160 pp.

Jain SK (1991). Dictionary of Indian folk medicine and ethnobotany. Deep publication, 168.

Meswaet Y, Mangelsdorff R, Yorou NS, Piepenbring M (2021). Unravelling unexplored diversity of cercosporoid fungi (Mycosphaerellaceae, Mycospaerellales, Ascomycota) in tropical Africa. Mycokeys 81: 69-138. doi: 10.3897/mycokeys.81.67850

Nakashima C, Motohashi K, Chenm CY, Johannes Z, Groenewald JZ et al. (2016). Species diversity of *Pseudocercospora* from Far East Asia. Mycological Progress 15:1093-1117. doi :10.1007/s11557-016-1231-7

Park JH, Hong SB, Kim BS, Kim JY, Shin HD (2015). *Pseudocercospora* leaf spot caused by *Pseudocercospora nymphaeacea* on *Nymphaea tetragona*. Tropical Plant Pathology 40: 401-404. doi: 10.1007/s40858-015-0054-4

Pathan AK, Bond J, Gaskin RE (2010) sample preparation for SEM of Plant surface, Materials Today. 12: 32-43

Pons N, Sutton BC (1988). *Cercospora* and similar fungi on yams (*Dioscorea* species). Mycological Papers 160: 1-78.

Prajapati ND, Purohit SS, Sharma AK, Kumar T (2003). A handbook of medicinal plants: A complete source book; Agrobios (India). 480-481.

Ronquist F, Teslenko M, Mark PVD, Ayres DL, Darling A et al. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539-542. doi :10.1093/sysbio/sys029

Shivas RG, Marney TS, Tan YP, McTaggart AR (2015). Novel species of *Cercospora* and *Pseudocercospora* (Capnodiales, Mycosphaerellaceae) from Australia. Fungal Biology 119: 362-369. doi: 10.1016/j.funbio.2014.09.004

Singh A (2012). New report of *Pseudocercospora* Speg. on some medicinal plants from Sonebhadra forest UP. Biological forum-An International Journal 4 (1): 9-13.

Singh A, Dubey NK (2012a). An ethnobotanical study of medicinal plants in Sonebhadra District of Uttar Pradesh, India with reference to their infection by foliar fungi. Journal of Medicinal Plants Research 6(14): 2727-2746. doi:10.5897/JMPR10.895

Singh A, Dubey NK. (2012b). New host record of *Cercospora apii* s.lat. on medicinal plant: *Diplocyclos palmatus* (Linn.)Jeffery from India; Journal of New Biological Reports **1** (1): 09-11

Singh A, Kumar S, Singh R, Dubey NK (2012). A new species of *Corynespora* causing foliar disease on *Ficus religiosa* from forest flora of Sonebhadra, Uttar Pardesh, India. Mycosphere 3 (5): 890-892. doi:10.5943 /mycosphere/3/6/2

Singh A, Kumar S, Dubey NK (2013). *Corynespora clerodendrigena sp.nov*. causing foliar disease on *Clerodendrum viscosum* from Sonebhadra forest of Uttar Pradesh, India. Plant Pathology and Quarantine 3 (1): 15-17. doi:10.5943/ppq/3/1/3

Singh A, Kumar S, Singh R, Dubey NK (2014). A new species of *Corynespora* from Sonebhadra forest of Uttar Pradesh, India. Current Research in Environment and Applied Mycology 4(1): 149-151. doi: 10.5943/cream/4/1/14

Singh A, Singh NK, Singh PN, Singh R, Dubey NK (2019). Additions to *Ochroconis* from India. Phytotaxa 427 (3): 186-199. doi:10.11646/phytotaxa.427.3.2

Singh A, Singh PN, Dubey NK (2022). Morphology and Phylogeny of a new species *Pseudocercospora rauvolfiicola* on medicinal plant *Rauvolfia serpentina* from Sonebhadra Forest, Uttar Pradesh, India. Phytotaxa 545 (2): 128–138. doi: 10.11646/phytotaxa.545.2.2

Singh A, Singh PN, Nath G, Dubey NK (2021). Phylogeny and Taxonomy of a novel species of *Pseudocercospora* from India. Turkish Journal of Botany 45: 172-180. doi:10.3906/bot-2012-27

Spegazzini C (1910). Mycetes Argentinenses (Series V). Anales del Museo Nacional de Historia Natural, Buenos Aires 20: 329-467.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M et al. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731-2739. doi: 10.1093/molbev/msr121

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238-4246.

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylognetics. In: Innis MA, Gelfand DH, Sninsky JJ, White JW. (Eds.) A guide to molecular methods and applications. Academic Press, New York: 315-322.doi:10.1016/b978-0-12-372180-8.50042-1

Yadav S, Verma SK, Singh VK, Singh R, Singh A et al. (2021). A new species of *Pseudocercospora haldiniae* on *Haldina cordifolia* from Srinagar, Uttarakhand, India. Phytotaxa 501 (2): 281-292. doi:10.11646/phytotaxa.501.2.3