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Pleochaeta indica, a new record of powdery mildew from Pakistan

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Abstract: A comprehensive study of a powdery mildew observed on leaves of *Celtis tetrandra* Roxb. was carried out by the authors. The symptoms appeared as white mycelium on leaves with embedded small black to brown spherical ascomata. Infected plants were collected from Thandiani (District Abbottabad, Division Hazara) of Khyber Pakhtunkhwa province, Pakistan, during a phytopathogenic survey in 2019. The causal agent was observed and identified on the basis of morphological and molecular analyses, which reveals that this fungus belongs to genus *Pleochaeta*. Further investigation identified it as *Pleochaeta indica*. This is the first report of a powdery mildew infection caused by this pathogen in Pakistan. A complete description and illustrations of the fungus are presented.

Keywords: Ascomycota, Erysiphales, Himalayan forests, host *Celtis tetrandra*, Leotiomycetes

INTRODUCTION

The *Erysiphaceae* (powdery mildews) is a fungal group that causes diseases on about 10,000 angiospermic plants (Amano, 1986). *Pleochaeta* Sacc. & Speg. is the most ancestral genus of the powdery mildew fungi forming a group in the tribe Phyllactinieae with other two genera i.e. *Leveillula* G. Arnaud and *Phyllactinia* Lév (Kiss et al., 2006). These are characterized by a partly endophytic mycelium having hyphae that enter the host plant tissues through stomata, producing haustoria in the mesophyll cells of the host plants. The ectophytic part of the mycelium of these fungi is found mostly on the lower surfaces of the leaves (Braun, 1987; Mori et al., 2000; Takamatsu, 2004). Therefore, its study could help to understand the evolution of endoparasitism within the *Erysiphales*.

Only five species of *Pleochaeta* are described by Braun and Cook (2012) in their book. Three of the species, *P. indica* N. Ahmad, A.K. Sarbhoy & Kamal, *P. shiraiana* (Henn.) Kimbr. & Korf and *P. salicicola* R.Y. Zheng & G.Q. Chen, are known from Asia, while *P. polychaeta* (Berk. & M.A. Curtis) Kimbr. & Korf and *P. prosopidis* (Speg.) U. Braun are found in North and South America. *Pleochaeta indica* was previously described in India and Nepal as a pathogen of *Celtis australis* L. (Ahmad *et al.,* 1995; Adhikari, 2018). *Celtis* L. (Cannabaceae) is a genus of about 80 species distributed in the northern hemisphere and South Africa (Leme et al., 2020), represented in Pakistan by three species (Stewart, 1982). Out of these species, *Celtis tetrandra* Roxb. is harvested from the wild for local use as a food, medicine and source of different materials. In 2019, a powdery mildew appeared on the leaves of *C. tetrandra* in the district Abbottabad, Pakistan. The infected leaf surfaces were fully covered with white powdery mycelial masses and yellowish brown to dark brown chasmothecia indicating 100% infection and disease severity. After careful morphological and molecular analyses, this fungus was identified as *Pleochaeta indica,* which is a new record for Pakistan. This newly reported powdery mildew infection poses a potential threat to the health and beauty of this tree in Pakistan and needs serious attention with reference to control strategies.

MATERIALS AND METHODS

Study area

At the time of the field survey of Himalayan forests of district Abbottabad, Pakistan, leaves of *Celtis tetrandra* were found infected with powdery mildews. The infected specimen was collected in 2019 from Thandiani (District Abbottabad, Division Hazara) of Khyber Pakhtunkhwa. Thandiani is situated at 34.23°N, 73.35°E and altitude is 2,636 m.a.s.l. The climate varies from sub-tropical to moist temperate (Khan et al., 2018).

Collection and preservation
Collected plant samples were shade dried on blotting papers. For preservation, the infected plants were pressed after drying. Leaf specimens were placed in air tight polyethylene bags and then kept in paper envelopes with collection details. The sample was deposited at the herbarium of Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH 36159).

Morphological characterization
For comprehensive morphological examination, photographs were taken of fresh samples in the field as well as under Labomed CSM2 stereomicroscope. For the study of morphological characters, fungal tissue was scrapped off from the leaf surface and mounted in lactic acid. Slides were then observed under the light microscope (Swift M4000-D Japan) in 3 % potassium hydroxide (KOH) and ammoniacal Congo Red. Morphological characters including teleomorph structures (chasmothecia, appendages, asci and ascospores) were observed and dimensions were noted (n = 30).

DNA extraction and PCR amplification
A part of the fungal mycelium was selected, and sections were cut under a dissecting microscope. The infected plant tissue was about 20 mg. It was ground in liquid nitrogen and stored in Eppendorf tubes at –18 °C. Then it was disrupted in a tissue lyser (GeneJET Plant Genomic Kit). Genomic DNA was extracted from infected plant tissue using EZ-10 Column Plant Genomic DNA Purification Kit (Bio Basic Inc.). For amplification of the rDNA Internal Transcribed Spacer region (PMITS1 and PMITS2), polymerase chain reaction (PCR) was done. Amplification was attained successfully at initial denaturation (1 min at 94˚C), 40 cycles (1 min at 94˚C, 1 min at 55˚C, and 1 min at 72˚C), and final extension at 72˚C for 8 min. The internal transcribed spacer (ITS) region was amplified using primers PMITS1 (5′-TCGGACTGGCC [T/C] AGGGAGA-3′) and PMITS2 (5′-TCACTCGCCGTTACTGAGGT-3′) as forward and reverse primers respectively (Cunnington et al., 2003). Visualization of PCR products were done with 1% agarose gel with ethidium bromide through Gel Documentation system (Sambrook & Russel, 2001). PCR products were sent for sequencing to Tsingke, China. By using BioEdit, raw sequenced data were edited (Hall, 1999). The ITS sequences were BLAST searched against the GenBank database (NCBI, 2022). Maximum percent identity and query coverage of sequences with related taxa were noted. All sequences along with the new sequences were aligned through MAFFT (Multiple sequence alignment tool). Sequences were aligned and trimmed at conserved sites from both 5´ and 3´ ends. The phylogenetic tree was executed within MEGA 6.0 (Tamura et al., 2013), using Maximum Likelihood Method based on Kimura 2-parameter with 1000 rapid bootstrap replicates. The model of evolution was selected by searching for the best DNA model for ML analysis in MEGA 6.0 (Tamura et al., 2013).

RESULTS AND DISCUSSION

White mycelium with chasmothecia on the leaves of *Celtis tetrandra* was examined in detail. Morphological and micro-investigation of the samples demonstrated that this fungus belongs to the genus *Pleochaeta*.

Description. Mycelium hypophyllous, persistent, in dense white patches. Conidiophores not seen. Chasmothecia hypophyllous, black, immersed in mycelium, gregarious, yellowish orange to dark brown, globose, 219–316 µm in diameter with small, inconspicuous peridium cells. Appendages numerous, narrow, thin-walled, 168–228 µm long, 4–6.5 µm wide, aseptate, hyaline, thin walled above, apices circinate. Asci 20–29 in number, stalked, clavate, 30–35 × 75–100 µm, 2–4-spored along with ellipsoid to ovoid, 15–18 × 25–32 µm and hyaline ascospores (Figs. 1– 2).

Material examined. On *Celtis tetrandra* Roxb. with teleomorphic stage, Pakistan, Khyber Pakhtunkhwa, Division Hazara, District Abbottabad, Thandiani, 2,636 m. a. s. l., 3.10.2019, Nida Liaqat and Najam-ul Sehar Afshan, (MZ-04) (LAH 36159), GenBank accession number (MW762868) (ITS).

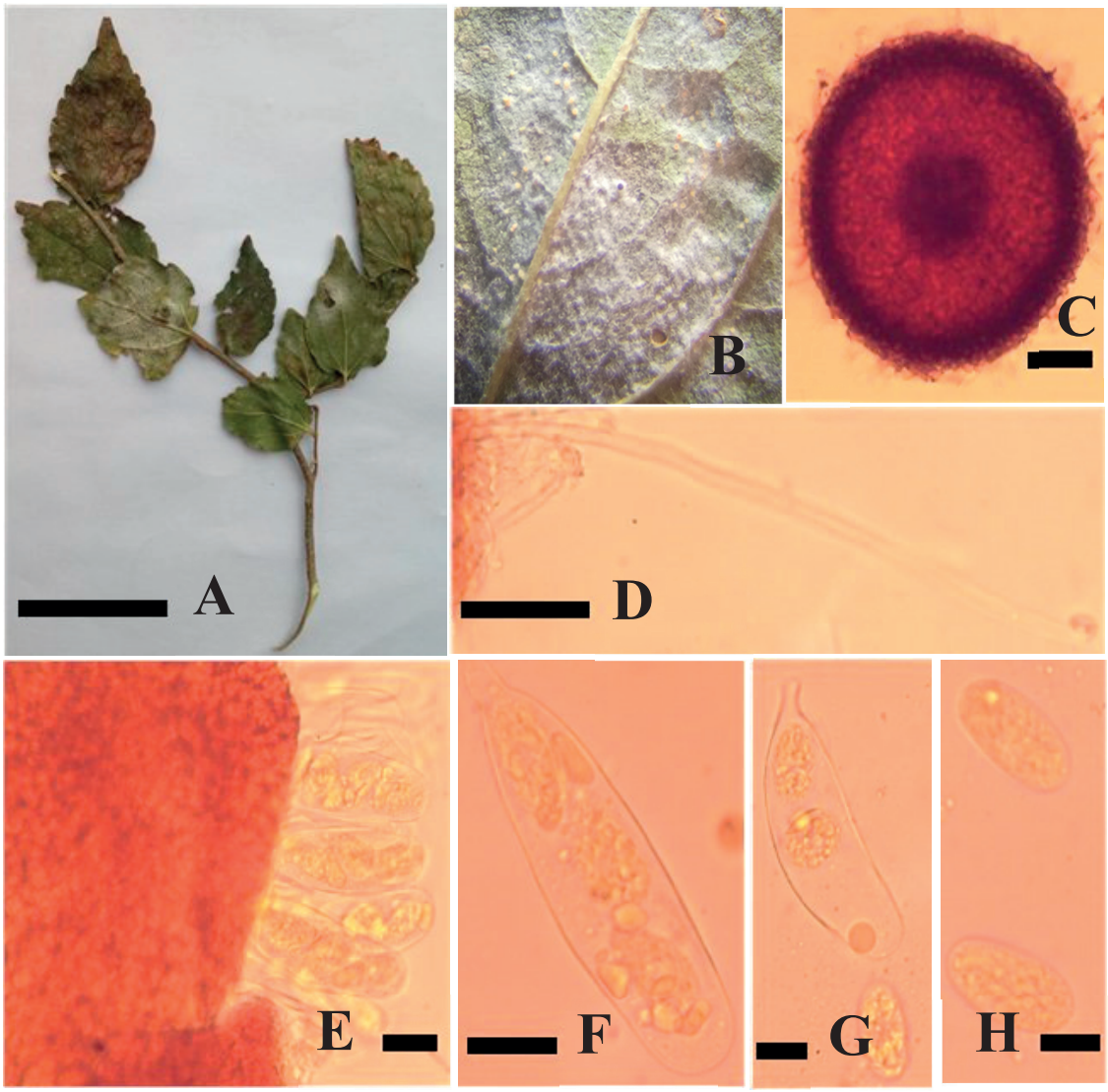

Fig. 1. *Pleochaeta indica* on *Celtis tetrandra*; **A –** infected host plant; **B –** infection under stereomicroscope; **C –** chasmothecium; **D –** chasmothecial appendage; **E –** chasmothecium releasing asci; **F–** ascus containing ascospores; **G –** ascus releasing ascospores; **H –** ascospores. Scale bars: A = 2 cm, C = 50 µm, D = 40 µm, E = 30 µm, F = 20 µm, G = 15 µm, H = 15 µm C-H in Congo red.

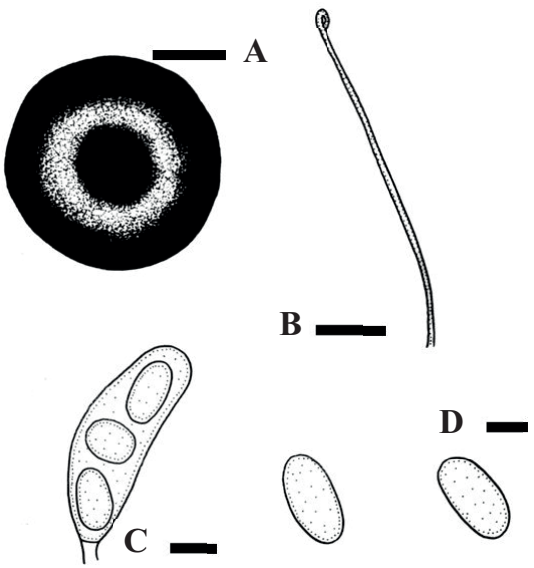

Fig. 2. Camera lucida drawings of *Pleochaeta indica*; **A –** chasmothecium; **B –** chasmothecial appendage; **C –** ascus containing ascospores; **D –** ascospores. Scale bars: A = 30 µm, B = 40 µm, C = 20 µm, D = 15 µm.

The specific measurements and morphological characteristics were consistent with those of *Pleochaeta indica*, synonym *Podosphaera indica* (Braun & Cook, 2012). To confirm the morphological identification, the fungal ITS region was sequenced. The amplified ITS region of LAH No. 36159 (MW762868) resulted in consensus sequence of 679 bp. Initial BLAST (Basic Local Alignment Tool) showed 98.47 % identity with *Pleochaeta indica* (AB243757, India) having query cover of 88%. A phylogenetic tree (Fig. 3) was constructed with the ITS sequences from this study with 12 sequences of different species retrieved from GenBank (Table 1), using the Maximum Likelihood (ML) method in MEGA6 (Tamura et al., 2013).

Phyllactinia angulata (E.S. Salmon) S. Blumer (GenBank no. AB080464) was selected for rooting the tree (Anwar et al., 2020). In the phylogenetic tree construction, the studied taxon clustered with a clade that contained *Pleochaeta indica* from the host i.e *C. australis*. Subsequently, both the morphological attributes and phylogenetic examination support the identification of the powdery mildew on *Celtis tetrandra* as *P. indica*. *Pleochaeta shiraiana* is reported on different species of *Celtis* from Asia (China, India, Japan, Korea, Pakistan, Taiwan) and South Africa (Kimbrough & Korf, 1963; Gorter & Eicker, 1983; Amano, 1986; Zheng & Chen, 1978; Braun, 1987; Braun & Cook, 2012). On *Celtis tetrandra*, *Pleochaeta shiraiana* was found in Pakistan Himalaya (Kaneko, 1993) but it has not been molecularly confirmed. Morphologically both species are indistinguishable but Kiss et al. (2006) carried out molecular analysis of the two species and found obvious differences, which suggest that *P. indica* and *P. shiraiana* are different species. Our phylogenetic analysis confirmed the identification of the powdery mildew as *P. indica*.

CONCLUSION

The concept of *Pleochaeta* species is not well defined as it is a little studied genus. *P. indica* on *Celtis australis* have little distinguishing features from *P. shiraiana* on the basis of morphology (Ahmad et al., 1995). Besides morphological analyses, molecular analyses proved useful tool for the precise identification of this species. *Pleochaeta indica* and *P. shiraiana* are indistinguishable morphologically but genetically they are different species. Such taxa are called functional species or cryptic species. In this study, all available ITS sequences were analyzed, which revealed that *P. shiraiana* is the closest relative of *P. indica* but they are distinct phylogenetically. Therefore, *P. indica* is the best example of a cryptic species classified and maintained as a distinct phylogenetic species and it is a new record for Pakistan as well a new host record i.e. *Celtis tetrandra*.

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Table 1. Taxa used to construct phylogram with their accession numbers and locality

Fungus name	Accession numbers	Host name	**Country**
Erysiphe lonicerae	KY660891	*Lonicera periclymenum*	UK
Erysiphe lonicerae	KY660868	*Lonicera japonica*	UK
Erysiphe lonicerae	KY653175	*Lonicera* sp.	UK
Erysiphe diffusa	KM260712	*Glycine max*	Vietnam
Erysiphe diffusa	KM260710	*Glycine max*	Vietnam
Erysiphe viburni	MN431627	*Viburnum edule*	USA
Erysiphe viburni	MN431624	*Viburnum opulus*	USA
Erysiphe viburni	MN431625	*Viburnum opulus*	USA
Erysiphe hedwigii	KY660921	*Viburnum opulus*	UK
Erysiphe hedwigii	KY653185	*Viburnum tinus*	UK
Erysiphe ostryae	MT095112	*Ostrya virginiana*	USA
Erysiphe ostryae	MT095111	*Ostrya virginiana*	USA
Erysiphe juglandis	LC010090	*Pterocarya rhoifolia*	Japan
Erysiphe juglandis	LC009939	*Juglans mandshurica*	Japan
Erysiphe mandshurica	MW071174	*Populus* sp.	China
Erysiphe mandshurica	MW071173	*Populus adenopoda*	China
Erysiphe adunca	KY660743	*Salix caprea*	UK
Erysiphe adunca	KY653178	*Salix aurita*	UK
Erysiphe salicis	MW064258	*Salix daphnoides*	Germany
Erysiphe salicis	MT952871	*Salix purpurea*	Germany
Pleochaeta polychaeta	LC108836	*Celtis tala*	Japan
Pleochaeta polychaeta	LC108835	*Celtis tala*	Japan
Pleochaeta shiraiana	JN638618	*Celtis sinensis*	Korea
Pleochaeta shiraiana	JN638617	*Celtis sinensis*	Korea
Pleochaeta shiraiana	JN638616	*Celtis sinensis*	Korea
Pleochaeta indica	DQ374647	Celtis *australis*	India
Pleochaeta indica	AB243757	*Celtis australis*	India
Pleochaeta indica	DQ374648	*Celtis australis*	India
Pleochaeta indica	**MW762868**	***Celtis tetrandra***	**Pakistan**

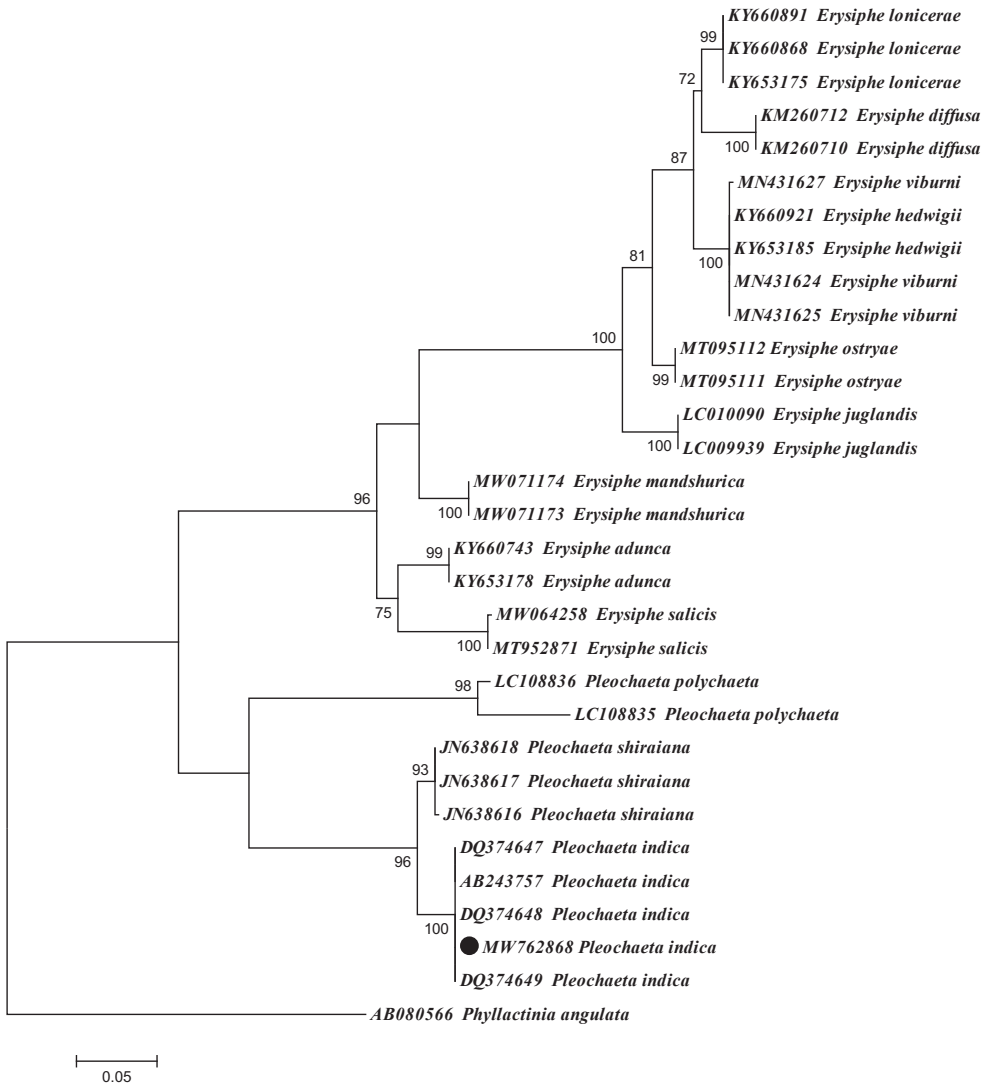

Fig. 3. Phylogenetic analyses of *Pleochaeta indica* based on ITS sequences of 12 different species including 1 species of *Phyllactinia* as outgroup. Maximum Likelihood bootstrap support values above 50% are cited on nodes (MLB). Amplified sequence is marked with bullet.

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