

***ERYSIPHE AUSTRALIANA*: THE CAUSE OF POWDERY MILDEW ON CRAPE MYRTLE TREE IN PAKISTAN**

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Abstract

A powdery mildew belonging to the genus *Erysiphe* was collected on the leaves of *Lagerstroemia indica* at the two sites in Azad Jammu & Kashmir, Pakis on the leaves of *Lagerstroemia indica* tan. Molecular phylogenetic analyses were carried out based on sequence data of rDNA ITS. After morphological and molecular examinations, the causal agent of this powdery mildew was identified as *Erysiphe australiana*, which is a new record for Pakistan. This contribution to the taxonomic knowledge of powdery mildews may serve as a help to create awareness amongst the growers of ornamentals to adopt control measures.

Key words: Azad Jammu & Kashmir, *Erysiphaceae*, Molecular phylogeny.

Introduction

Lagerstroemia indica L. (Crape myrtle), also commonly known as “Pride of India”, hails from the family Lythraceae and has been extensively used as ornamental tree in public parks, gardens and parking lots. It is commonly grown in temperate regions (Kim, 2021).

A common plant disease affecting leaves of Crape myrtle is the powdery mildew *Erysiphe australiana* (McAlpine) U. Braun & S. Takam. (Lee *et al.*, 2017). Powdery mildews are common widespread detrimental plant pathogenic fungi globally. About 900 species from 19 genera have been described worldwide (Takamatsu *et al.*, 2016; Braun *et al.*, 2000; Jin *et al.*, 2021). From Pakistan, 52 powdery mildew taxa belonging to 10 genera have been reported. Approximately 80 host plants infected by powdery mildews in Pakistan are so far known, including cereals, ornamentals, fruit trees, nuts, etc. (Afshan *et al.*, 2021, 2022a, 2022b, 2022c, 2023; Zafar *et al.*, 2022, 2023a, 2023b; Afzal *et al.*, 2023; Riaz *et al.*, 2024).

In the course of fungal surveys in October to December 2020 and 2021 in different regions of Azad Jammu & Kashmir, this landscape ornamental tree was found to be infected with *Erysiphe* species. Previously, four powdery mildew species have been reported on *L. indica* worldwide (Braun & Cook, 2012; Farr & Rossman, 2022). This species is described morpho-anatomically along with molecular analyses. After careful investigations, the above said fungus was identified as *Erysiphe australiana*, a new report for Pakistan.

Material and Method

Study area, collection & preservation: Plants infected with powdery mildews were collected from District Bagh and Muzaffarabad of Azad Jammu & Kashmir during October and December of the years 2020 and 2021. This area is located in the north-eastern part of Pakistan having an undulating terrain with very rich vegetation, which is predominated by coniferous forests (Dar *et al.*, 2012). For preservation the infected plants were dried, pressed, placed in an air tight polythene bag, kept in envelopes, and deposited at the Herbarium of Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Macro-morphological characterization: Morphologically, infected samples were observed using a stereomicroscope (Meiji Techno, EMZ-5TR, Japan). Slides were prepared in lactic acid. Hyphae on the host, shape and size of asci and ascospores were examined by a compound microscope (SWIFT M4000-D) having a 9MP camera system. At least twenty (20) measurements were taken for each diagnostic feature.

Extraction of DNA and PCR amplification: Fructifications were taken from fresh fungal specimens with the help of a razor blade. These were ground in liquid nitrogen and stored in Eppendorf tubes at -18°C . Extraction of DNA was done via Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit #K0791. Using PMITS1 as forward primer and PMITS2 as reverse primer and the ITS region was amplified (Cunnington *et al.*, 2003). Through a gel documentation system (Sambrook & Russel, 2001), visualization of products of PCR was done with agarose gel having 1% concentration. PCR products were sent for sequencing to Tsingke, China. Raw sequenced data were edited on BioEdit (Hall, 1999) and sequences were searched on BLAST against the database of GenBank (www.ncbi.nlm.nih.gov). Maximum query coverage and percent identity of sequences with related species were noted. All sequences were aligned along with the new sequences through MAFFT (multiple sequence alignment tool). Alignment and trimming of sequences were done at conserved sites from both 5' and 3' ends. The phylogenetics tree was executed within MEGA 6.0 (Tamura *et al.*, 2013), using ML (Maximum Likelihood Method) based on Kimura 2-parameter with 1000 rapid bootstrap replicates. The selection of the model of evolution was done by searching for the best model of DNA for ML analysis in MEGA 6.0 (Tamura *et al.*, 2013). *Golovinomyces ambrosiae* (Schwein.) U. Braun & R.T.A. Cook served as out-group in the analysis of *E. australiana* (Lee *et al.*, 2017).

Results

Taxonomy

***Erysiphe australiana*:** (McAlpine) U. Braun & S. Takam., *Schlechtendalia* 4: 17 (2000) (Fig. 1).

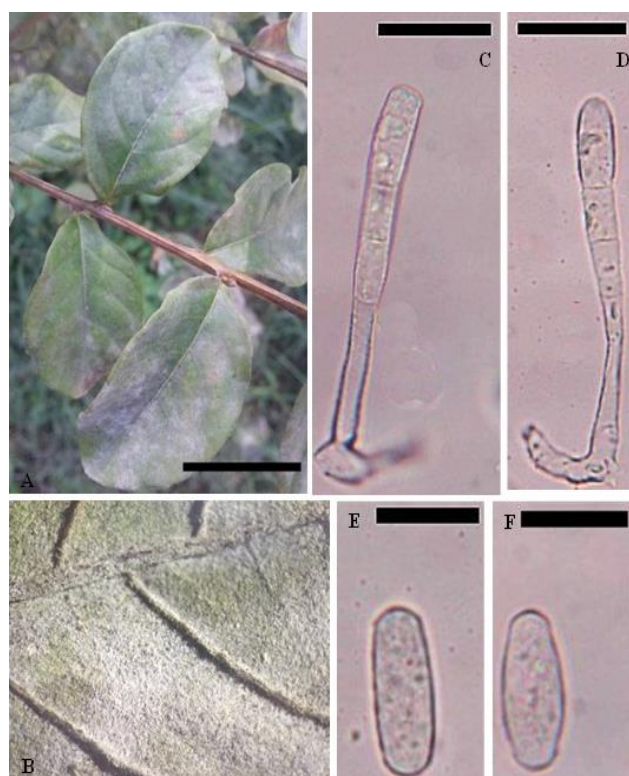


Fig. 1. *Erysiphe australiana*; A = Infected leaves of host plant; B = Infection under stereomicroscope; C–D = Conidiophores; E–F = Conidia; Scale Bars: A = 2 cm, C–D = 30 μ m, G–H = 20 μ m.

Mycelium on leaves and stalks, evanescent to persistent, dense, white or sometimes turning greyish in color, effused or in patches, amphigenous, sometimes conspicuous, covering the whole leaf. **Hyphae** thin, smooth, 2–6 μ m wide. **Conidiophores** erect, straight, arising centrally from the adaxial surface of the hyphal mother cell, up to 95 μ m long. **Foot cells** straight, 27–45 \times 5–8 μ m, followed by 1–2 shorter cells developing single conidia. **Conidia** ellipsoid to ovoid and cylindrical, 22–34 \times 8–11 μ m. **Hyphal Appressoria** multilobed, present on opposite position occasionally, 2–5 μ m diam.

Specimens examined-Pakistan. Azad Jammu & Kashmir, Muzaffarabad District: On *Lagerstroemia indica* L., with anamorphic stage, 737 m.a.s.l., Oct. 3rd, 2020, Irsa Zafar and Najam-ul-Sehar Afshan, (MZ-04) (LAH #37467), GenBank accession number (OP050107) (ITS); AZAD JAMMU & KASHMIR, Bagh District: On *Lagerstroemia indica* L., with anamorphic stage, 1676 m. a.s.l., Dec. 8th, 2021, Irsa Zafar and Najam-ul-Sehar Afshan, (MZ-05) (LAH #37468), GenBank accession number (OP055966) (ITS).

Phylogenetic analysis: Two powdery mildew samples MZ-04 (LAH37467) and MZ-05 (LAH37468) were genetically examined. The sequences of the nrDNA ITS region of these specimens were achieved using both forward and reverse primers. In this study, 34 sequences (Table 1) were used with 2 of them (ITS) being newly generated. BLASTn analysis of the NCBI revealed that these ITS sequences (OP050107) (OP055966) showed 100.00% similarity with *E. australiana* (KY611159, AB022408, MT892941) containing 87% query coverage. The final aligned dataset of the ITS region

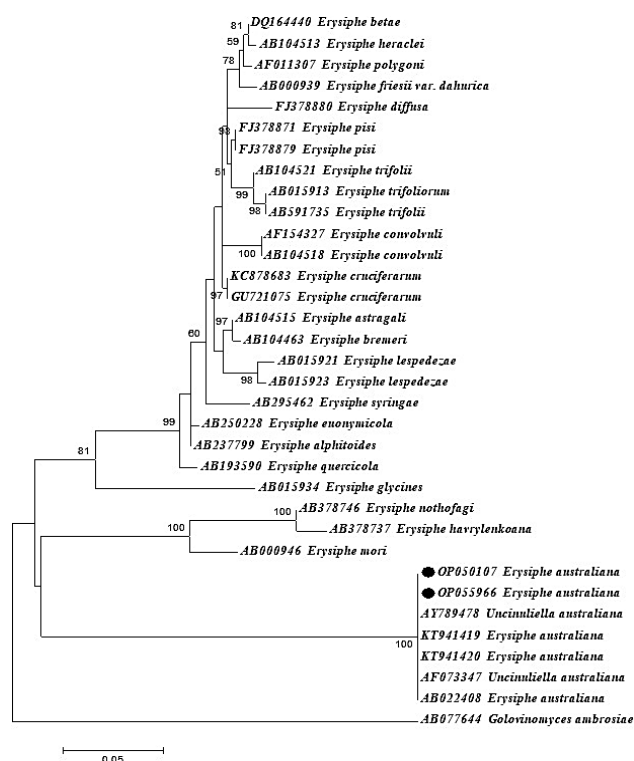


Fig. 2. Maximum Likelihood phylogenetic analysis of *Erysiphe* drawn from dataset of 34 ITS sequences, including one sequence of *Golovinomyces ambrosiae* as outgroup. The amplified sequences are highlighted by a black circle.

consisted of 629 characters with gaps; out of these 370 were conserved, 250 were variable, 188 were parsimony informative, and 62 were singleton sites. Entire aligned gaps which are ambiguous in the final aligned dataset were treated as ‘missing’. The Maximum Likelihood analysis assembled the sequences of the Pakistani *Erysiphe* collections (OP050107, OP055966) with numerous sequences of *E. australiana* (AY78947, KT941419, KT941420, AF073347, AB022408) showing 100% bootstrap support (Fig. 2). Morpho-anatomically, our species agrees well with *E. australiana* on *Lagerstroemia indica* (Braun & Cook, 2012). On the basis of these results, we identified this powdery mildew fungus as *E. australiana* on *L. indica* in Pakistan. This is a new report for Pakistan.

Discussion

Based on morphological and phylogenetic analyses, the powdery mildew fungus on *Lagerstroemia indica* was identified as *Erysiphe australiana*. This fungal species has been formerly recorded on *Lagerstroemia* spp. causing powdery mildew in Argentina (Delhey *et al.*, 2003), Australia, Brazil (Fonesca *et al.*, 2015), China, India (Baiswar *et al.*, 2009), Italy, Japan, Korea, New Zealand, Portugal, Russia, South Africa, Spain, Switzerland, Turkey (Göre, 2009), Taiwan, USA, United Kingdom, and Ukraine (Braun & Cook, 2012). The morphological features of our anamorphic collections agreed well with the published description of *Erysiphe australiana* (Braun & Cook, 2012), except for the shorter and narrower conidia (22–34 \times 8–11 μ m vs. 28–40 (–47) \times (12–) 13–18 μ m). Based on ITS barcoding data, this is a new report of *E. australiana* for Pakistan.

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

Species	Accession number	Host plant	Country
<i>Erysiphe betae</i>	DQ164440	<i>Beta vulgaris</i> subsp. <i>vulgaris</i>	USA
<i>E. heraclei</i>	AB104513	<i>Pimpinella affinis</i>	Iran
<i>E. polygoni</i>	KJ703014	<i>Polygonum arenastrum</i>	USA
<i>E. friesii</i> var. <i>dahurica</i>	AB000939	<i>Rhamnus japonica</i>	Japan
<i>E. diffusa</i>	FJ378880	<i>Pisum sativum</i>	USA
<i>E. pisi</i>	FJ378871	<i>Pisum sativum</i>	USA
<i>E. pisi</i>	FJ378879	<i>Pisum sativum</i>	USA
<i>E. trifolii</i>	AB104521	<i>Trifolium pratense</i>	Iran
<i>E. trifoliorum</i>	AB015913	<i>Trifolium vulgare</i>	Japan
<i>E. trifolii</i>	AB591735	<i>Trifolium pratense</i>	Japan
<i>E. convolvuli</i>	AF154327	<i>Convolvulus</i> sp.	Australia
<i>E. convolvuli</i>	AB104518	<i>Convolvulus arvensis</i>	Iran
<i>E. cruciferarum</i>	KC878683	<i>Brassica rapa</i> subsp. <i>pekinensis</i>	China
<i>E. cruciferarum</i>	GU721075	<i>Brassica oleracea</i> var. <i>acephala</i>	South Korea
<i>E. astragali</i>	AB104515	<i>Astragalus</i> sp.	Iran
<i>E. bremeri</i>	AB104463	<i>Alhagi</i> sp.	Iran
<i>E. lespedezae</i>	AB015921	<i>Lespedeza cuneata</i>	Japan
<i>E. lespedezae</i>	AB015923	<i>Lespedeza thunbergii</i>	Japan
<i>E. syringae</i>	AB295462	<i>Syringa</i> sp.	Japan
<i>E. euonymicola</i>	AB250228	<i>Euonymus japonicus</i>	Japan
<i>E. alphitoides</i>	AB237799	<i>Mangifera indica</i>	Japan
<i>E. quercicola</i>	AB193590	<i>Quercus phillyraeoides</i>	Japan
<i>E. glycines</i>	AB015934	<i>Amphicarpaea edgeworthii</i> var. <i>japonica</i>	Japan
<i>E. nothofagi</i>	AB378746	<i>Nothofagus pumilio</i>	Japan
<i>E. havrylenkoana</i>	AB378737	<i>Nothofagus alpina</i>	Argentina
<i>E. mori</i>	AB000946	<i>Morus bombycis</i>	Japan
<i>E. australiana</i>	OP050107	<i>Lagerstroemia indica</i>	Pakistan
<i>E. australiana</i>	OP055966	<i>Lagerstroemia indica</i>	Pakistan
<i>Uncinuliella australiana</i>	AY789478	<i>Lagerstroemia indica</i>	USA
<i>E. australiana</i>	KT941419	<i>Lagerstroemia speciosa</i>	Brazil
<i>E. australiana</i>	KT941420	<i>Lagerstroemia indica</i>	Brazil
<i>Uncinuliella australiana</i>	AF073347	<i>Lagerstroemia indica</i>	Australia
<i>E. australiana</i>	AB022408	<i>Lagerstroemia indica</i>	Japan
<i>Golovinomyces ambrosiae</i>	AB077644	<i>Xanthium strumarium</i>	Japan

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References

- Afshan, N.S., A. Anwar, J. Majeed, M. Riaz, A.N. Khalid and Sirajuddin. 2022a. First report of *Erysiphe bunkiniana* on *Isodon rugosus* from Pakistan. *Mycotaxon*, 137(3): 569-575.
- Afshan, N.S., I. Zafar and A.N. Khalid. 2021. Checklist of powdery mildew fungi (*Erysiphaceae*) in Pakistan. *Mycotaxon*, 136(3): 689-689.
- Afshan, N.S., I. Zafar, M. Jabeen, N. Liaqat and A.N. Khalid. 2022b. New records of powdery mildews on Oak trees in Pakistan. *Nova Hedwigia*, 115(1-2): 79-88.
- Afshan, N.S., M. Riaz, N. Liaqat, J. Majeed, M. Jabeen, A.R. Niazi, A.N. Khalid, I. Zafar and S. Afzal. 2022c. *Erysiphe pakistanica* (*Erysiphaceae*), a new species on Indian willows (*Salix tetrasperma*) from Pakistan. *Phytotaxa*, 545(1): 57-68.
- Afshan, N.S., S. Afzal, M. Riaz, A. Saleem, A.R. Niazi and A.N. Khalid. 2023. Occurrence of *Erysiphe friesii* var. *dahurica* on purple buckthorn (*Rhamnus purpurea*) from Pakistan. *Can. J. Plant Pathol.*, 45(5-6): 427-432.
- Afzal, S., N.S. Afshan and A.N. Khalid. 2023. First report of *Erysiphe verniciferae* causing Powdery Mildews on Smoke Tree (*Cotinus coggygia* Scop.) from Pakistan. *Can. J. Plant Pathol.*, 45(1): 35-40.
- Baiswar, P., S. Chandra and S.V. Ngachan. 2009. First report of anamorphic *Erysiphe australiana* on *Lagerstroemia speciosa* in India. *Australas. Plant Dis. Notes*, 4(1): 49.
- Braun, U. and R.T.A. Cook. 2012. *Taxonomic manual of Erysiphales (powdery mildews)*, CBS Biodiv. Series, 11: 1-707.
- Braun, U., R.T.A. Cook, A.J. Inman and H.D. Shin. 2000. The taxonomy of the powdery mildew fungi. *The Powdery Mildews: A Comprehensive Treatise*, 2002: 13-55.
- Cunnington, J.H., S. Takamatsu, A.C. Lawrie and I.G. Pascoe. 2003. Molecular identification of anamorphic powdery mildews (*Erysiphales*). *Australas. Plant Pathol.*, 32(3): 421-428.
- Dar, M.E.U.I., R. Cochard, R.P. Shreshta and S. Ahmad. 2012. Spatiotemporal analysis of vegetation change in Himalayan foothills, a case from Machiara National Park, Azad Jammu and Kashmir, Pakistan. *J. Food. Agric. Environ.*, 10(3&4): 1139-1148.
- Delhey, R., U. Braun and M. Kiehr. 2003. Some new records of powdery mildew fungi from Argentina. *Schlechtendalia*, 10(1): 79-90.
- Farr, D.F. and A.Y. Rossman. 2022. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. 2022, Online document at: <https://nt.ars-grin.gov/fungaldbases/> (Retrieved July 16, 2022).

- Fonseca, N.R., L.M.S. Guimarães, R.P. Pires and A.C. Alfenas. 2015. *Erysiphe australiana*: the cause of powdery mildew on *Lagerstroemia speciosa* in Brazil. *For. Pathol.*, 46(4): 366-368.
- Göre, M.E. 2009. Powdery mildew caused by *Erysiphe* sp. on *Lagerstroemia indica* newly reported in Turkey. *Plant Pathol.*, 58(1): 397.
- Hall, T.A. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program, for windows 95/98/NT. *Nucl. Acid Symp. Series*, 41(1): 95-98.
- Jin, D.N., L. Kiss, S. Takamatsu, Z.Y. Zhang, M.G. Cabrera, Z. Li, Y. Li and S.Y. Liu. 2021. Hidden diversity of powdery mildews belonging to the recently re-discovered genus *Salmonomyces*. *Mycol. Prog.*, 20(9): 1009-1018.
- Kim, K.W. 2021. Morphology and surface characteristics of the anamorphic stage of powdery mildew *Erysiphe australiana* on crape myrtle leaves. *Micron*, 143(1): 103013.
- Lee, J.K., B.J. Cha, H.D. Shin, Y.J. La and K.W. Kim. 2017. *Forest and Shade Tree Pathology*, Hyangmunsa, Seoul, Korea, 342.
- Riaz, M., N.S. Afshan, S. Afzal, I. Zafar, A. Saleem and A.N. Khalid. 2024. DNA barcoding and Scanning electron microscopy reveals *Erysiphe ahmadii* sp. nov. and a new record *Erysiphe populicola* (Erysiphaceae, Helotiales) on salicaceous hosts from Pakistan. *Microsc. Res. Tech.*, 87(1): 21-30.
- Sambrook, J. and D.W. Russel. 2001. Rapid isolation of yeast DNA. In: Sambrook, J. & Russel, D.W. (Eds) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, New York, 2001: 631-632.
- Takamatsu, S., S.A. Siahaan, O. Moreno-Rico, M.G. Cabrera de Alvarez and U. Braun. 2016. Early evolution of endoparasitic group in powdery mildews: molecular phylogeny suggests missing link between *Phyllactinia* and *Leveillula*. *Mycologia*, 108(5): 837-850.
- Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.*, 30(12): 2725-2729.
- Zafar I., N.S. Afshan and A.N. Khalid. 2023a. *Phyllactinia cornicola*, a new species of powdery mildews from Pakistan. *Nord. J. Bot.*, 2023 (5): e03864.
- Zafar I., N.S. Afshan, A.N. Khalid and M. Götz. 2023b. *Erysiphe machilicola*: a new powdery mildew species on Duthie's Bay Tree. *Phytotaxa*, 594(4): 283-291.
- Zafar, I., A.N. Khalid, S.Y. Liu and Tang S.R. 2022. *Phyllactinia himalayensis*, a new species on *Juglans regia* from Pakistan. *Phytotaxa*, 548(2): 267-276.

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