Phenotypic and Molecular Characterization of two *Phyllactinia* species (Ascomycota: Erysiphaceae) from Pakistan

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Abstract—This study investigated the prevalence and impact of powdery mildew fungi on *Platanus orientalis* and *Vaccinium arboreum* in Pakistan. Two significant powdery mildew pathogens, *Phyllactinia alnicola* and *Ph. kakicola*, were identified on the bases of morpho-anatomical and phylogenetic analyses. *Ph. kakicola* was found on *Platanus orientalis* in Battagram district, representing a new record for Pakistan as well as new host record for this fungal pathogen. *Ph. alnicola*, a previously unrecorded species in Pakistan, was found and identified on *Vaccinium arboreum*. These findings contribute to our understanding of fungal diversity in Pakistan and the potential threats to these economically important plant species.

Index Terms—Phylogeny, *Phyllactinia*, *Platanus*, *Vaccinium*, ITS, Pakistan.

1 INTRODUCTION

Powdery mildew is a common fungal disease impacting a broad range of plant species, including those from the *Platanaceae* and *Ericaceae* families, such as *Platanus orientalis* (Oriental plane tree) and *Vaccinium arboreum* (Blueberry). Both species are particularly vulnerable to this disease. *Phyllactinia* Lév., a widespread powdery mildew fungus, exhibits a unique lifestyle. It can live both internally within the leaf tissue (endophytically) and externally on the leaf surface (epiphytically). During its sexual reproduction, *Phyllactinia* produces distinct fruiting bodies called chasmothecia, which are significantly larger than those produced by other powdery mildews. Chasmothecial appendages resemble needle-like structures with bulbous bases that emerge from the equator (middle) of the chasmothecia. These appendages play a crucial role in orienting the chasmothecia during dispersal through the air, allowing them to travel more effectively on wind currents (Shin and Lee 2002). These unique characteristics of *Phyllactinia*'s sexual stage serve as a distinguishing hallmark, allowing for easy identification and differentiation from other powdery mildews that might infect Plane trees.

About seven species of this genus are previously reported in Pakistan viz. *Phyllactinia babayanii*

Simonyan on *Pyrus amygdalus* Batsch, *Ph. dalbergiae* Piroz. on *Dalbergia sissoo* Roxb. ex DC., *Ph. guttata* (Wallr.) Lév. on *Betula utilis* D. Don, *Jasmanium humile* L., *Lonicera quinquelocularis* Hardw., *Salix tetrasperma* Roxb., *Ph. mali* on *Cotoneaster bacillaris* Wall., *Ph. moricola* on *Morus alba* L., *Ph. cornicola* on *Cornus macrophylla* Wall. and *C. capitata* Wall. ex Roxb., and *Ph. himalayensis* on *Juglans regia* L., as detailed in previous studies (Afshan et al. 2021; Zafar et al. 2022, 2023).

This study delves into the occurrence and impact of this disease on two valuable plant species in Pakistan. By elucidating the characteristics of the causative fungi and identifying contributing factors, we may develop effective strategies for managing powdery mildews and safeguarding these plants.

2 MATERIALS AND METHODS

2.1 Sample collection

During a comprehensive study of phytopathogenic fungi across various regions of Pakistan, plant leaves from the Platanaceae and Ericaceae families, affected by powdery mildews, were collected**.** The infected leaves were carefully dried on blotting papers and preserved in paper envelopes with detailed collection information for future research work. Subsequently, these specimens were deposited in the herbarium of Hazara University in Mansehra (HUP), Khyber Pakhtunkhwa, Pakistan.

2.2 Morphological Characterization

For morphological assessments, photographs of the infected plant samples were taken in both field conditions and under a stereomicroscope, specifically the EMZ-5TR by Meiji Techno in Saitama, Japan. Examination of the infected plant parts, mounted in lactic acid, was conducted using a LABOMED light microscope sourced from Labo America Inc. in Fremont, CA. Microscopic features like chasmothecia, chasmothecial appendages, asci and ascospores were measured utilizing ScopeImage 9.0 (X5) Exe.

2.3 Molecular Characterization

Conidiophores, conidia, mycelium, chasmothecia, asci, and ascospores were carefully extracted from the dried plant material and placed in Eppendorf tubes. DNA was isolated using the Gene Jet Plant Genomic DNA Purification mini-Kit (Thermo Fisher Scientific, Waltham, USA), following the established protocol (Andersson et al. 2017). The internal transcribed spacer (ITS) region was amplified using the forward primer PMITS1 (5'TCGGACTGGCCAGGGAGA–3') and the reverse primer PMITS2 (5'TCACTCGCCGTTACTGAGGT–3'), as previously described (Cunnington et al. 2003).

The PCR product was sequenced at TsingKe (China) using the PMITS1 and PMITS2 primers. Consensus sequences were generated by combining the forward and reverse primer sequences using Bio-Edit (Hall 1999). These consensus sequences were then compared to

similar ITS region sequences from the GenBank database using NCBI BLAST.

Multiple sequence alignment was performed using the online MUSCLE alignment tool. Phylogenetic tree reconstruction was carried out using MEGA 7.0, employing the maximum likelihood model. One thousand bootstrap replicates were conducted to infer the evolutionary history of the species.

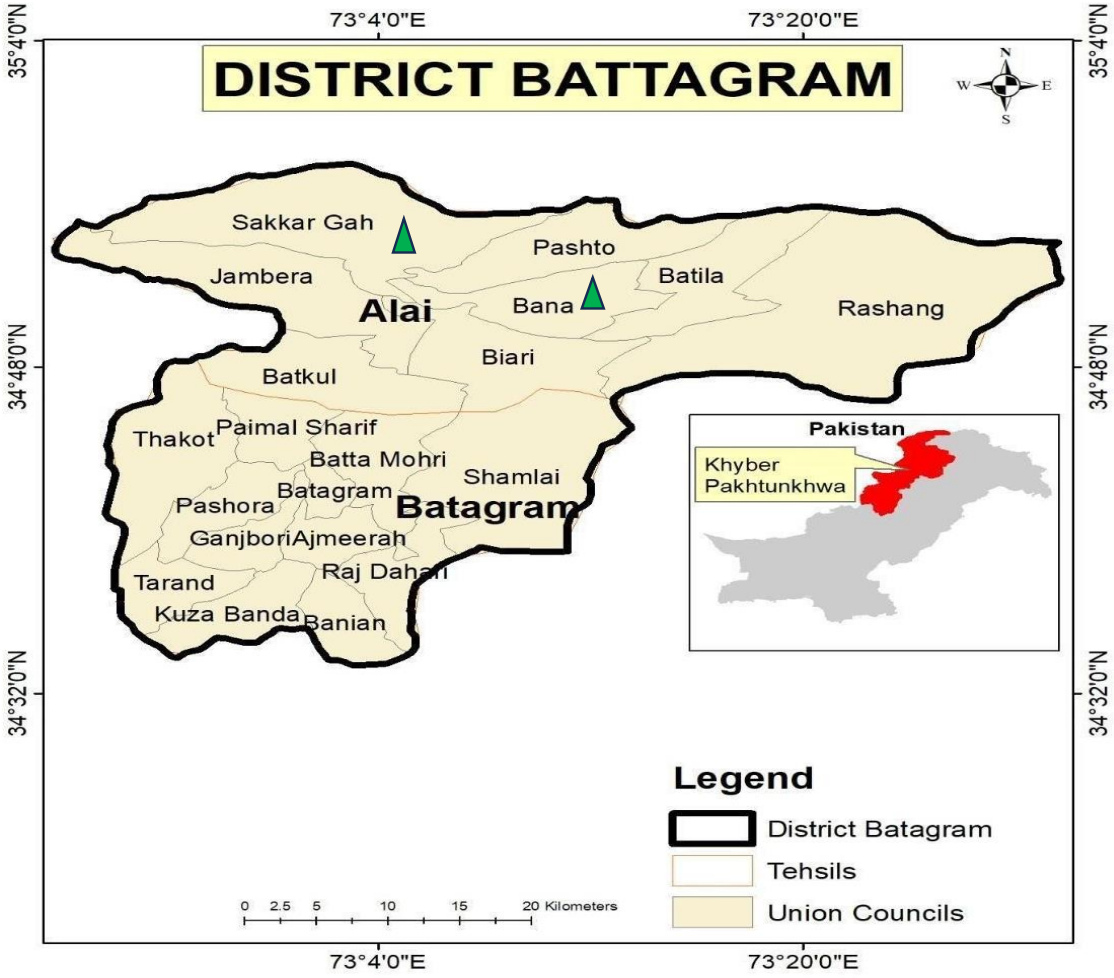

Fig. 1 Map of collection site Battagram district, KP, Pakistan

3 RESULTS

3.1 Phylogeny (Fig. 2)

The internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) was amplified and sequenced for all specimens. Sequence alignment software (e.g., BioEdit) was employed to assemble the forward and reverse sequences. A nucleotide similarity search was performed using a publicly available tool like the Basic Local Alignment Search Tool (BLAST) offered by the National Center for Biotechnology Information (NCBI). The most closely related sequences were

retrieved from GenBank for further phylogenetic analysis.

Multiple sequence alignment (MSA) was conducted using a suitable MSA software (e.g., MAFFT) with default settings. Maximum Likelihood analysis was then performed using phylogenetic analysis software (e.g., MEGA). A total of 31 ITS rDNA sequences were analyzed, with 28 obtained from the NCBI GenBank database. The aligned sequences comprised 648 unambiguous nucleotide positions, including160 conserved regions, 466 variable sites, 196 parsimony-informative sites, and 255 singletons.

The resulting phylogram (Fig. 2) showed that the sequences from specimens collected on *Platanus orientalis* (GenBank accession numbers PP528445 and PP535394) clustered with *Phyllactinia kakicola.* This cluster exhibited 100% similarity with previously reported sequences (AB080491) from Japan (2008). Similarly, the sequence excised from *Vaccinium arboreum* (Genbank accession number PQ201942) clustered with *Phyllactinia alnicola* (AB080554).

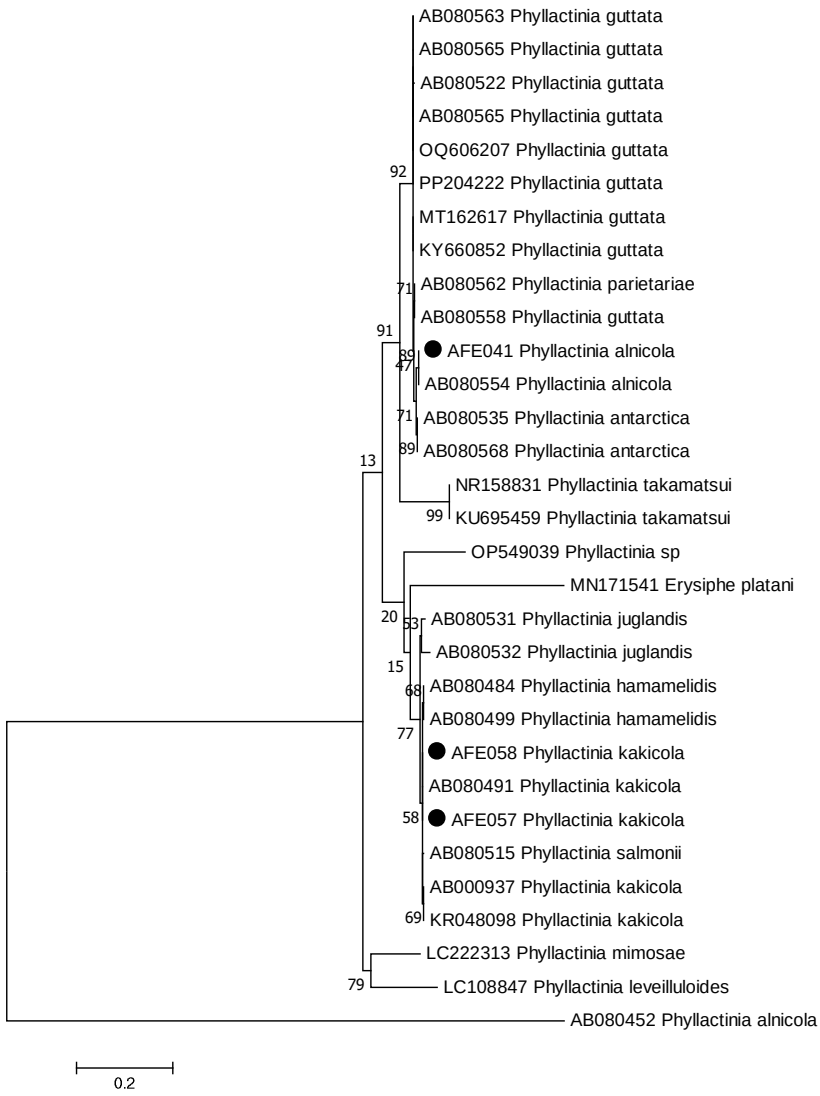

Fig. 2 Molecular phylogenetic analysis of *Phyllactinia kakicola* and *Phyllactinia alnicola* by the maximum likelihood method based on rdna sequences, including ITS1, 5.8S and ITS2. Numbers below branch node represent ml bootstrap (>50%) based on 1000 replicates. The sequences generated from Pakistan are marked with ●

3.2 Taxonomy

Phyllactinia kakicola Sawada, Rep. Dept. Agric. Gov. Res. Inst. Formosa 49: 80, 1930

(Figs. 3 & 4)

Description: Chasmothecia are hypophyllous, appearing scattered and depressed, globose, measuring 170–290 µm in diameter. The **peridium cells** exhibit irregular polygonal shapes with a diameter of 10–20 µm. **Chasmothecial appendages**, situated equatorially, range from (5–)8 to 17(–20) and are acicular with a bulbous basal swelling measuring 25–40 µm in diameter. The apex is obtuse to subacute, with a length of 145–465 µm, equivalent to (0.8–)1–2(–2.5) times the chasmothecial diameter. **Penicillate cells** are hyaline, with a thickened wall in the upper half, numerous, measuring 50–90 µm in length,

including the filaments. The stem is subcylindrical, somewhat narrowed towards the apex or irregular, with dimensions of 35–75 × 10–20 µm. The apex is undivided or bears 2–4 short, subcylindrical–conical branchlets, and filaments are shorter than the stem, measuring 2–3 µm in width. The **asci** ranging from 4 to 19, are ellipsoid–obovoid, saccate, and measure 50–105 × 25–50 µm, being stalked and 2-spored. **Ascospores** are ellipsoid–ovoid, appear subhyaline, pale olivaceous, or olivaceous-brown. The anamorphic stage was not found.

Material examined

On *Platanus orientalis* (*Platanaceae*), with teleomorphic stage, Pakistan, Khyber Pakhtunkhwa, Division Hazara, Bana tehsil Allai, District Battagram, 1360 m. a. s. l., 10th October, 2021, A. Akbar, M. Fiaz & Jan Alam (AFE057) (Voucher number HUP 16048). GenBank Acession number (PP528445); *P. orientalis* (*Platanaceae*), with teleomorphic stage, Pakistan, Khyber Pakhtunkhwa, Division Hazara, Bana tehsil Allai, District Battagram, 1360 m. a. s. l., 20th November, 2022, A. Akbar, M. Fiaz & Jan Alam (AFE058) Voucher number. HUP 16096. GenBank Acession number (PP535394).

Comments: *Phyllactinia kakicola* is a new record for Pakistan.

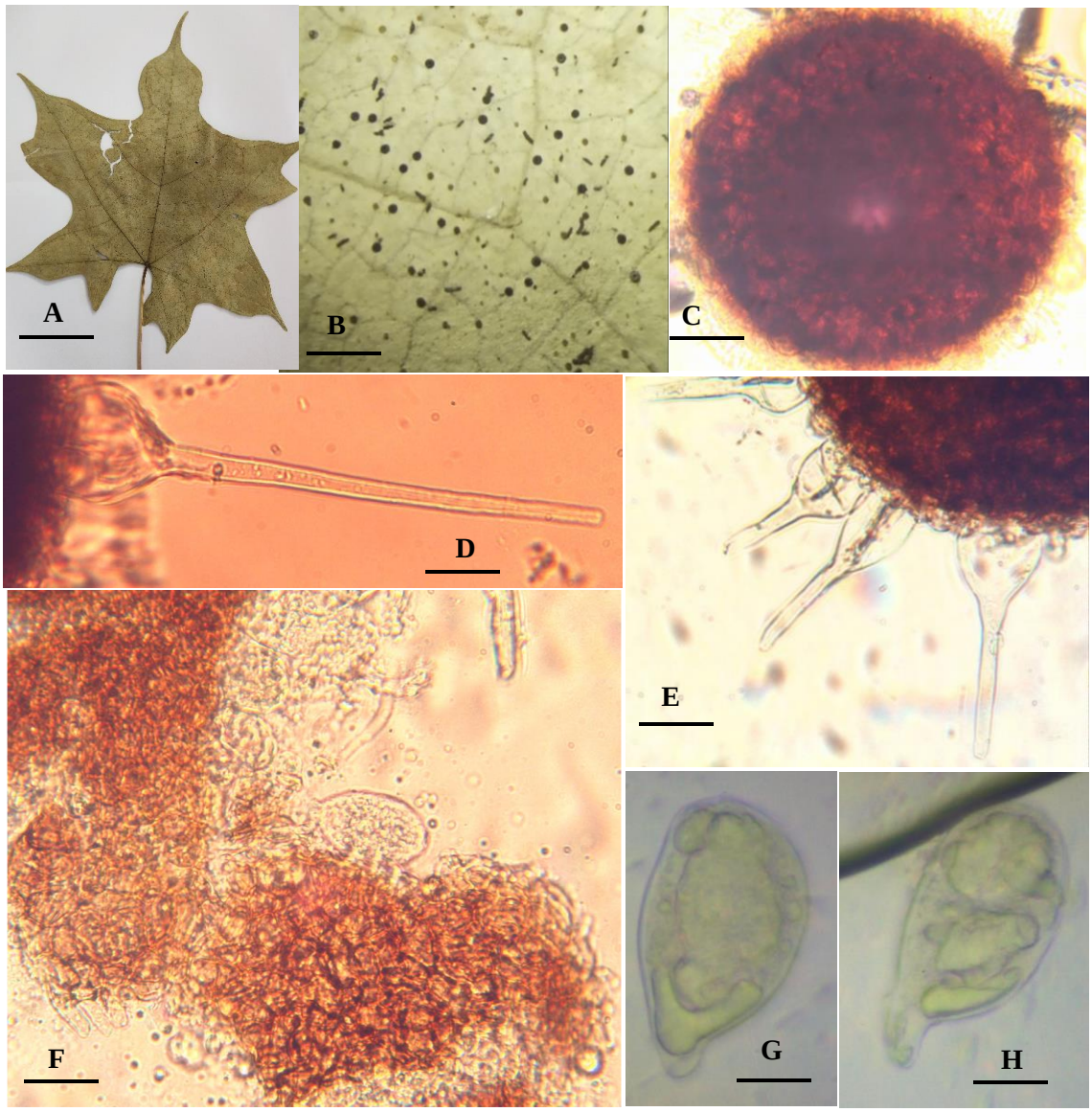

Fig. 3 *Phyllactinia kakicola* on *Platanus orientalis* A. Infected leaves of *P. orientalis* (AFE057). B. Infection under stereomicroscope. C. Chasmothecium. D & E. Chasmothecial appendages. F-H. Asci.

Scale bars: A= 5 cm, B = 5 mm, C–G = 50 µm, G & H = 20 µm

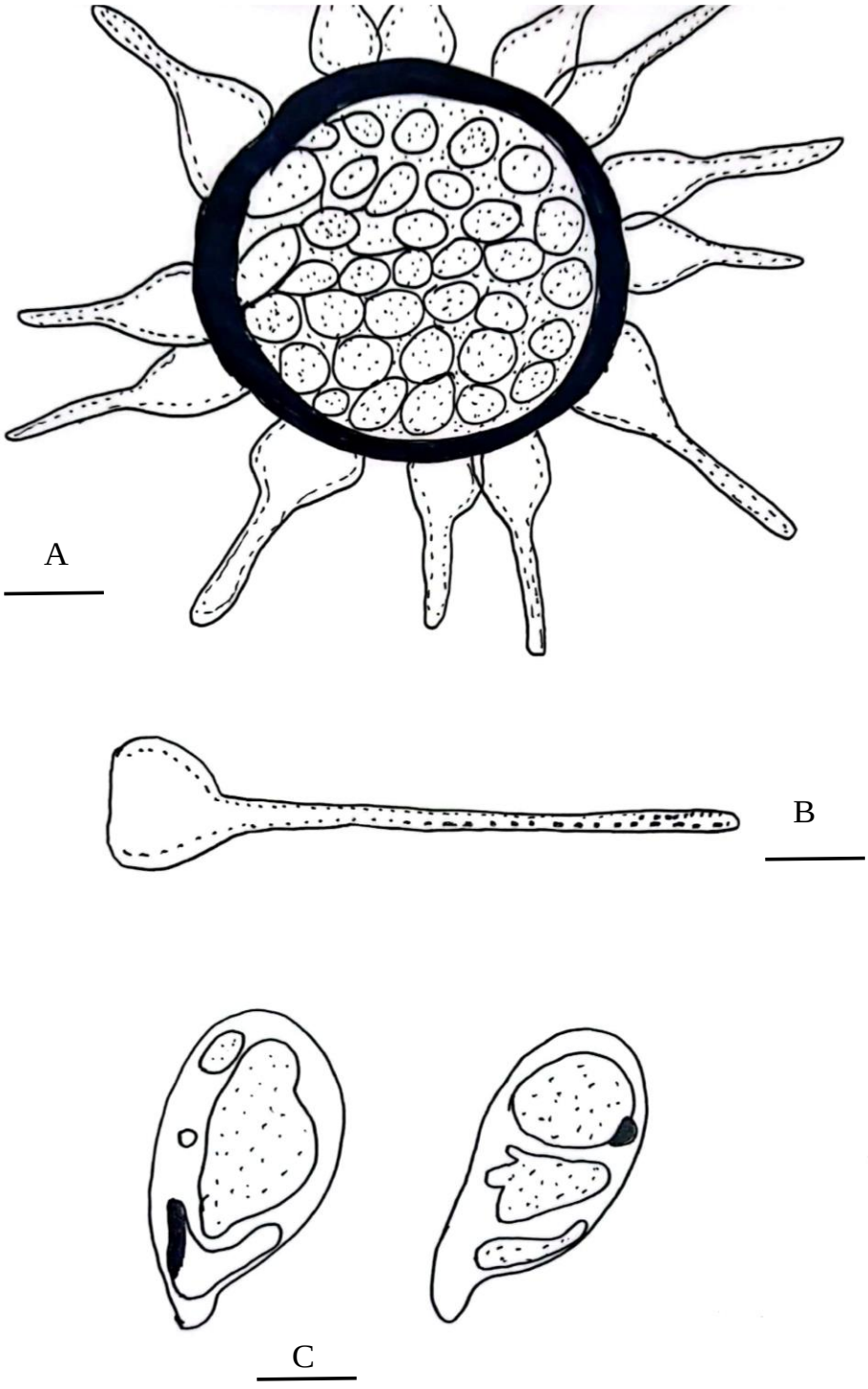

Fig. 4 *Phyllactinia kakicola* A. Chasmothecium B. An appendage C. Asci.

Scale bars. A = 5 cm, B = 5 mm, C = 20 µm

Phyllactinia alnicola U. Braun, Taxonomic Manual of the Erysiphales (Powdery Mildews): 226 (2012) **(Figs. 5 & 6)**

Description:

Infection abaxial**,** **Chasmothecia** hypophyllous scattered to gregarious, about 130 – 210 µm diam. Chasmothecium dark brown at center globose in shape; **Appendages** equatorial, acicular with 4 – 6 × 20 – 35 µm diameter bulbous basal swelling, straight, apex obtuse to pointed, length about 100-300 µm, 1-2.5 times the chasmothecial diam., hyaline, wall thickened, up to 4 µm below. **Asci** numerous , 40–90 × 23–30 µm ellipsoid - obovoid, short stalked, 2 to 5 spores, **Ascospores** ellipsoid–ovoid, 20–41 ×14–22 µm, initially yellowish green then greenish in colour.

Material examined: On lower surface of *Vaccinium arboreum* (Ericaceae), with telomorphic stage, Pakistan, Khyber Pakhtunkhwa, Battagram district, Sakkar gah, 2100 m a. s. l., 15th October 2022, A. Akbar, M. Fiaz, AFE041 (HUP Voucher No. 16756) Gen Bank Accession number (PQ201942).

Comments: *Phyllactinia alnicola* is new record for Pakistan.

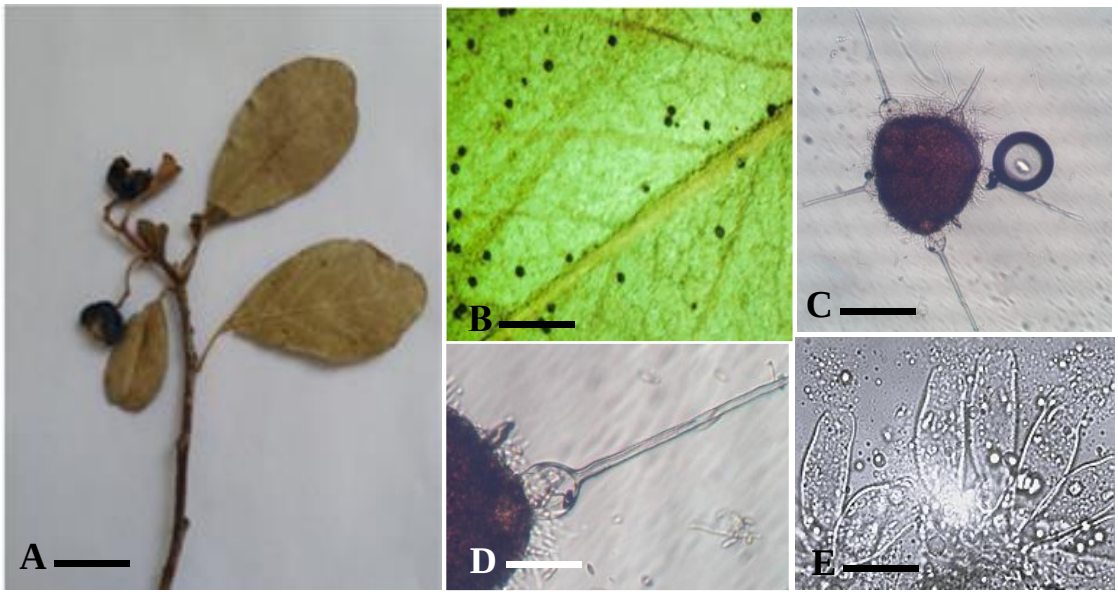

Fig. 5 A. *Vaccinium arboreum* B. Infection under stereoscope C. Chamothecium, D. Chasmothecial appendage E. Asci

Scale bars: A= 5 cm, B = 5 mm, C-D= 50 µm, E = 20 µm

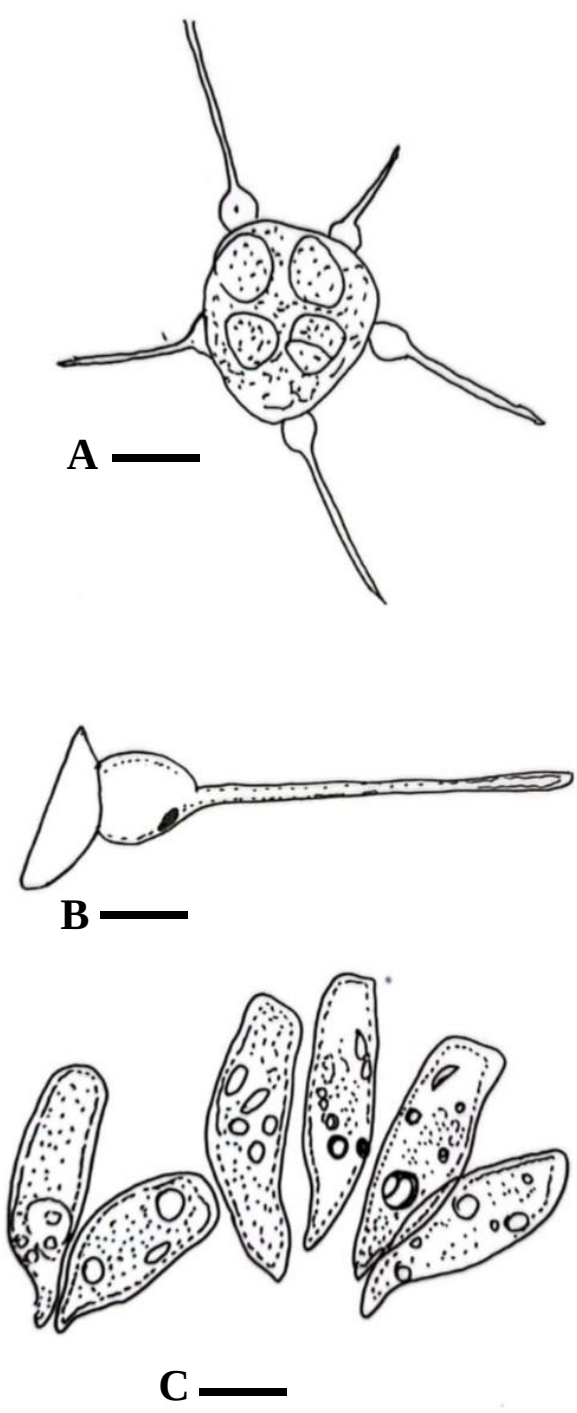

Fig. 6 *Phyllactinia alnicola* A. Chasmothecium B. Chasmothecial appendage C. Asci
Scale bars. A=5 cm, B = 5 mm, C = 20 µm

4 DISCUSSION

This study provides new insights into powdery mildew infections on *Platanus orientalis* and *Vaccinium arboreum* in Pakistan, with significant findings for both fungal pathogens.

For *Platanus orientalis*, the study identified *Phyllactinia kakicola* as the causative agent of powdery mildew. This species of powdery mildew was observed on *Platanus* leaves collected in Battagram district, Khyber Pakhtunkhwa, during the peak outbreak seasons of October 2021 and November 2022. Morphological characteristics of the collected specimens, along with internal transcribed spacer (ITS) rDNA sequencing, confirmed the presence of *Ph. kakicola*. This fungus was previously reported on persimmon (*Diospyros kaki*) in Taiwan and Japan (Takamatsu et al. 2008; Meebon et al. 2015) and is known to be a significant fungal pathogen in South Korea (Kwon et al. 2013). Although *Erysiphe platani* has been reported on various *Platanus* species in countries like South Korea (Lee et al. 2013), Ukraine (Kliuchevych et al. 2021), and China (Liang et al. 2008; Zhu and Pie 2017), this study represents the first documentation of *Phyllactinia kakicola* on *P. orientalis* in Pakistan.

The identification of *Phyllactinia kakicola* on *Platanus orientalis* not only adds to the fungal diversity known in Pakistan but also highlights the need for effective management strategies. Powdery mildew caused by *Ph. kakicola* can lead to reduced photosynthetic efficiency, stunted growth, and premature leaf drop, which adversely affect the health and aesthetic value of *Platanus* trees (Afshan et al. 2021; Zafar et al. 2022).

For *Vaccinium arboreum* (Blueberry), this study marks the first occurrence of *Phyllactinia alnicola* in Pakistan. This report expands the known host range of this pathogen. *Ph. alnicola* poses a significant threat to blueberries by causing symptoms such as reduced photosynthetic efficiency, stunted growth, and premature leaf drop, which negatively impact plant health and yield (Shin and Lee 2002). The introduction of *Ph. alnicola* as a pathogen in the region underscores the importance of implementing effective management practices. Recommendations include applying fungicides and adopting cultural practices to reduce humidity and improve air circulation, thereby minimizing the conditions conducive to powdery mildew development (Hall 1999; Zafar et al. 2022).

The findings from this study emphasize the need for ongoing research to monitor the distribution and spread of these powdery mildew pathogens. Developing and refining integrated pest management strategies will be crucial for managing powdery mildew effectively and protecting both *Platanus orientalis* and *Vaccinium arboreum* cultivation in Pakistan. This research contributes to a deeper understanding of plant-pathogen interactions and highlights areas for further investigation and intervention.

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REFERENCES

[1] N.S. Afshan, I. Zafar, and A.N. Khalid, "Checklist of powdery mildew fungi (Erysiphaceae) in Pakistan," *Mycotaxon*, vol. 136, no. 3, pp. 689, 2021.

[2] J.H. Cunnington, S. Takamatsu, A.C. Lawrie, and I.G. Pascoe, "Molecular identification of anamorphic powdery mildews (Erysiphales)," *Aust P Pathol*, vol. 32, no. 3, pp. 421–428, 2003.

[3] T.A. Hall, "Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT," *Nucleic Acid Symposium Series*, vol. 41, pp. 95–98, 1999.

[4] M. Kliuchevych, S. Stoliar, P. Chumak, and O. Strygun, "New data on the expansion of *Erysiphe platani* (Howe) U Braun and S Takam (Erysiphales, Ascomycota) in Ukraine," *Ukr J Ecol*, vol. 11, no. 5, pp. 9–14, 2021.

[5] J.H. Kwon, H.S. Lee, S.T. Choi, C.K. Shim, and H.J. Jee, "Control of Powdery Mildew on Sweet Persimmon Using Cooking Oil and Yolk Mixture in the Orchard," *Korean J Organic Agri*, vol. 21, no. 2, pp. 265–270, 2013.

[6] H.B. Lee, H.W. Lee, and H.Y. Mun, "First Report of Powdery Mildew Caused by *Erysiphe platani* on Sycamore (*Platanus occidentalis*) in South Korea," *Plant Dis*, vol. 97, no. 6, pp. 41, 2013.

[7] C. Liang, G.Z. Lu, and H.D. Shin, "First report of powdery mildew of *Platanus orientalis* caused by *Erysiphe platani* in China," *Plant Pathol*, vol. 57, no. 2, pp. 375, 2008.

[8] H.D. Shin and H.J. Lee, "Morphology of penicillate cells in the genus Phyllactinia and its taxonomic application," *Mycotaxon*, vol. 83, no. 1, pp. 301–325, 2002.

[9] K. Tamura, G. Stecher, D. Peterson, A. Filipski and S. Kumar, "MEGA6: molecular evolutionary genetics analysis version 60," *Mol Bio Evol*, vol. 30, no. 12, pp. 2725–2729, 2013.

[10] S. Takamatsu, M. Inagaki, S. Niinomi, S.A. Khodaparast and H.D. Shin, "Comprehensive molecular phylogenetic analysis and evolution of the genus Phyllactinia (Ascomycota: Erysiphales) and its allied genera," *Mycol Res*, vol. 112, no. 3, pp. 299–315, 2008.

[11] I. Zafar, A.N. Khalid, S.Y. Liu, and S.R. Tang, "*Phyllactinia himalayensis*, a new species on Juglans regia from Pakistan," *Phytotaxa*, vol. 548, no. 2, pp. 267–276, 2022.

[12] M. Zafar, Q. Ali and M. Khan, "Characterization and management of powdery mildew in fruit crops," *Asian J Plant Sci*, vol. 21, no. 2, pp. 100–110, 2022.

[13] I. Zafar, N.S. Afshan and A.N. Khalid, "*Phyllactinia cornicola*, a new species of powdery mildews from Pakistan," *Nord J Bot*, pe03864, 2023.

[14] X.Q Zhu and D.L Pei,"First Report of Powdery Mildew of *Platanus* × *acerifolia* Caused by *Erysiphe platani* in China," *Plant Dis*, vol.101 , no .7 ,2027 .

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