



MORPHOLOGICAL EVIDENCE REVEALS A NEW RECORD SPECIES IN THE GENUS *PHYLLACTINIA* FROM PAKISTAN

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Abstract

*The specimens were gathered in the Agror Valley of District Mansehra, Pakistan, during the period from July to September 2019. *Phyllactinia desmodii* is reported here for the first time from Pakistan. This study contributes significant input to fungal taxonomy through the integration of detailed morphological approaches. A comprehensive morphological description along with illustrations is provided. This species represents a new record and an addition to the mycobiota based on morphological evidence. The present study enhances our understanding of fungal diversity through a detailed examination of morphological characteristics.*

Keywords: Phyllactinia, New Record, Powdery Mildews, Morphology, Mansehra, Pakistan.



Introduction

The family Erysiphaceae comprises a group of fungi responsible for diseases in approximately 10,000 angiosperm plant species (Amano, 1986). Within the phylum Ascomycota, the order Erysiphales is regarded as one of the most significant groups causing plant diseases. The Erysiphales were defined, monophyletic order of about 873 species, which all were of obligatory biotrophic pathogens of plants (Braun and Cook, 2012). Erysiphales fungi on about ten thousand flowering plants including many economically important cultivated plants cause important plant diseases (Powdery mildew) (Braun, 2011; Amano, 1986). Hirata, (1986) estimated that nearly 40,000 angiosperm species were affected by these pathogens, with a large proportion belonging to dicotyledonous plants distributed across approximately 40 orders. *Phyllactinia desmodii* species possesses very large chasmothecium with relatively few appendages. A *Phyllactinia* sp. was recorded on various hosts of the genus Desmodium from the USA, but America material was not available for an examination (Braun and Cook, 2012). Previously, this taxon had been reported from other countries; however, the present study documents its first record from Pakistan. This research aims to contribute to the knowledge of powdery mildew fungi with particular emphasis on the forest ecosystems of the Agror Valley, District Mansehra, Pakistan.

Materials and Methods

Sampling Site: All powdery mildew infected samples were collected from the Agror Valley, District Mansehra. The Agror Valley is located in District Mansehra, Khyber Pakhtunkhwa, Pakistan. Sampling was carried out during the peak of the rainy season, when moisture levels were highest. The average temperature during this period was around 20 °C, which favors the rapid growth and reproduction of various fungal pathogens. The valley extends approximately ten miles in length and six miles in width, and was situated between latitude 34.5101° N and longitude 73.0273° E.

Collection of Samples: Infected plants specimens with pathogenic fungi were collected from various sampling sites of Agror valley district Mansehra. Powdery mildew were gathered and properly tagged. Their habitat and growth habit were recorded, photographs were taken and detailed field notes were made during July–September 2019. Plants were independently pressed among blotting papers, equally marked and all the specimens were then transported to the Plant Mycology Research Laboratory. The bolting papers were changed on daily bases to drying the collected specimens. The collected plant specimens were identified by comparing them with reference material preserved in the herbarium of the Department of Botany, Hazara University, Mansehra, Pakistan for further examination. Afterwards, all samples were deposited in a public herbarium.



Microscopic characterization: Images of the infected plants were captured using a stereomicroscope at magnifications ranging from $25\times$ to $50\times$. Sections of the infected plant material were carefully prepared by hand and mounted in lactic acid to create semi-permanent slides. The cover slips were sealed using nail lacquer to preserve the specimens (Dade and Gunnell, 1996). For anamorph stage, presence (either abaxial or adaxial side of leaf) abundance, pattern and colour of mycelium was noted. For microscopic examination, hyphae, conidiophores, conidia, and chasmothecia were carefully removed from the leaf surface using a clean needle and mounted in lactic acid. The prepared slides were then observed under a biological microscope.

Anatomical Characterization: Anatomical features were measured, and corresponding line drawings were prepared using Scope Image 9.0 (X5) software with a $40\times$ objective lens. For anamorph, shape and dimensions of appressoria, foot cell, conidiophore and conidia, type of conidiophore (Pseudoidium or Euoidium), number of conidial initials and presence or absence fibrosin bodies in conidia were noted. When the teleomorph stage was observed, the type of chasmothelial appendages, their number and dimensions, as well as the shape and size of chasmothecia, ascii, and ascospores, were carefully examined. Additionally, the number of ascii per chasmothecium and the number of ascospores per ascus were recorded. For accurate analysis, twenty-five measurements were taken for each of the following: conidia, conidiophore foot cells, chasmothecia, ascii, and ascospores.

Identification: To identify the Erysiphales, subsequent key & monograph/literature were consulted Ascomycete from Pakistan (Ahmad, 1978) as well as a monograph was used in Erysiphales (Powdery Mildews) (Braun, 1987). Many Fungal species have previously been reported from various regions of Pakistan (Ahmad et al., 1997) and a detailed taxonomic framework for the order Erysiphales was provided by (Braun and Cook, 2012). All powdery mildews (Erysiphales) from Europe Braun, (1995) were also considered. Specially, the host plant which was ranging in Geographical areas of the Powdery Mildew Fungi was also described by (Amano, 1986).

Results

Taxonomy: *Phyllactinia desmodii* J. F. Tao, J.Z. Qin & Y.Z. Shen, Acta Microbiol. Sin. 20(2): 123, 1980 (Figure 1& 2).

Description: Infection abaxial, *Chasmothecia hypophylloous*, scattered, about $190 - 320 \mu\text{m}$ diam. Chasmothecia were round in shape and brown in colour (Figure 1& Figure 2). Appendages equatorial, 6 – 14, mostly 6 – 9, acicular with bulbous basal swelling, apex pointed, length $300 - 520 \mu\text{m}$, Ascii numerous, about 14 – 26, ellipsoid-obovoid, saccate, $65 - 95 \times 20 - 35 \mu\text{m}$, stalked, 2-spored; ascospores ellipsoid-oblong, green in colour, $25 - 33 \times 12 - 18 \mu\text{m}$.



Habit and Habitat: On abaxial surface of *Desmodium sequax*; KP Province, Mansehra District, Manchora, September, 2019, Bano, M. Fiaz, No. B 10. (Herbarium No 9886.).

Comments: *Phyllactinia desmodiiis* first time reported from Pakistan.

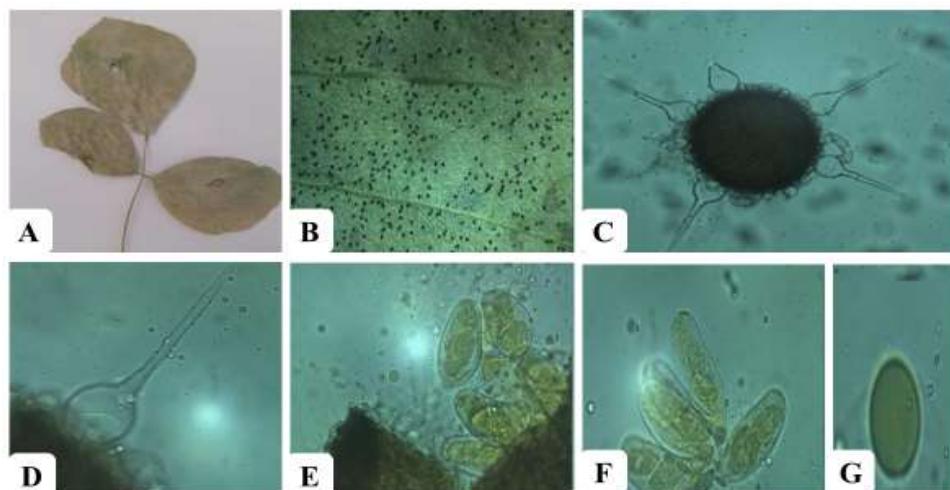


Figure 1. A – Infected leave of *Desmodium sequax* B – Stereomicroscope C – Chasmothecia D – Appendages E – Chasmothecium with ascus F – ascus G an Ascospore. Scale bar: A-1 cm B –1 cm C - 126 μ mD - 100 μ mF – 106 μ mG – 44 μ m.

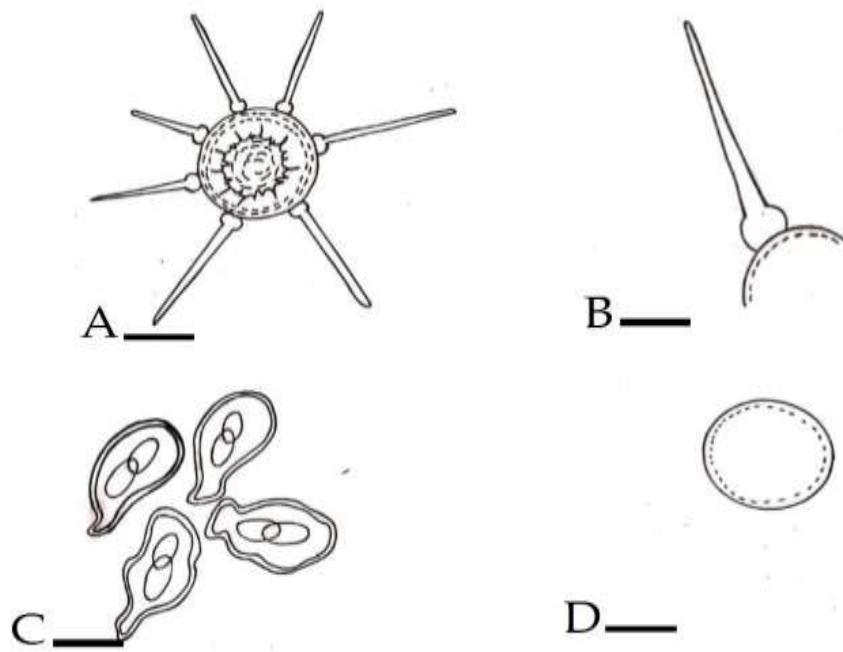


Figure 2. A – Chasmothecia B – Appendages C – Ascii D: –Spore. Scale bar: A – 300 μ m B – 111 μ m C – 106 μ m D – 48 μ m.



Discussion

Phylum ascomycota characterizes the largest group of pathogens responsible for most of phytopathogenic fungal diseases. Ascomycetes cause important plant diseases including Chestnut blight & other tree canker diseases & powdery mildews of many plants (Cooper and Gardener, 2007). Erysiphales is the most important order causing disease in plants, among the phylum Ascomycota. The Erysiphales were defined, monophyletic order of about 873 species, which all were obligatory biotrophic pathogens of plants (Braun and Cook, 2012). Erysiphales fungi on about ten thousand flowering plants including many economically important cultivated plants cause important plant diseases (Powdery mildew) (Braun, 2011; Amano, 1986). Hirata, (1986) estimated that approximately 40,000 species of angiosperms many of which were dicotyledonous and belong to about 40 orders were affected by these pathogens. Present investigation was done in Agror valley of District Mansehra of KP Province. *Phyllactinia desmodii* was a new record for Pakistan. This species possesses very large chasmothecium with relatively few appendages. A *Phyllactinia* sp. was recorded on various hosts of the genus Desmodium from the USA but America material was not available for an examination (Braun and Cook, 2012). This was first time reported from Pakistan.

Conclusion

Phyllactinia desmodii was reported as a new record from Agror Valley of District Mansehra Pakistan. This study contributes significant input to fungal taxonomy through the integration of detailed morphological approaches along with illustrations, images and the mycobiota based on morphological evidence.

Conflict of interest

The authors declare that there are no conflicts of interest associated with this study.

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