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First report of *Erysiphe dhamoniensis* from Central India

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Abstract
During a fungal survey in the tropical deciduous forests of Madhya Pradesh, Central India, severe powdery mildew infections were observed on the leaves of *Bauhinia racemosa* Lam. (Caesalpiniaceae). Fungal specimens were collected, were examined using light microscopy. The fungus was identified as *Erysiphe dhamoniensis* based on its chasmothecial appendages, which were uncinate (sharply hooked at the tip), asci containing 2–4 ascospores, and ellipsooid to ovoid conidia. rDNA region confirmed the morphological identification. Pathogenicity tests fulfilled Koch's postulates. This constitutes the first confirmed report of *E. dhamoniensis* in Central India and on this specific host plant within the region, highlighting its expansion and potential threat to native flora.

Keywords: *Erysiphe dhamoniensis*, powdery mildew, *Bauhinia racemosa*, new report, Madhya Pradesh, morphology

Introduction

The Erysiphaceae family, commonly known as powdery mildews, comprises obligate biotrophic fungal pathogens that parasitize a vast array of angiosperms worldwide (Braun & Cook, 2012) [1]. These fungi are easily recognizable by the superficial, powdery white growth of mycelium, conidiophores, and conidia they produce on the surfaces of infected leaves, stems, and flowers. Powdery mildew infections can lead to significant economic losses in agricultural and horticultural systems by reducing photosynthetic capacity, causing premature leaf senescence, and diminishing the aesthetic and market value of crops and ornamentals (Glawe, 2008) [3].

The genus *Bauhinia* L. (Caesalpiniaceae), often referred to as mountain ebony or orchid trees, encompasses over 300 species of trees, shrubs, and lianas, many of which hold ecological, medicinal, and ornamental importance across the tropics (Wunderlin, 1983) [10]. In India, several *Bauhinia* species are native and integral components of forest ecosystems. *Bauhinia racemosa* Lam., locally known as 'Apta' or 'Kachnaar', is a small tree valued for its fodder, medicinal properties, and use in religious ceremonies.

Globally, several powdery mildew species have been documented on hosts within the genus *Bauhinia*. These include reports of *Erysiphe* and *Podosphaera* species from Asia, the Americas, and Africa (Farr & Rossman, 2023) [2]. In India, previous surveys have reported powdery mildews such as *Erysiphe quercicola* and *Podosphaera* sp. on various *Bauhinia* species, including *B. variegata* and *B. purpurea* (Singh *et al*., 2019) [7]. However, the diversity of powdery mildews in Central India's unique forest ecosystems remains understudied.

During recent mycological surveys, a severe and previously unrecorded powdery mildew infection was observed on *Bauhinia racemosa* in several forest reserves. The objective of this study was to identify the causal agent of this disease using integrated morphological and molecular techniques. This paper provides a detailed description and confirms the first report of *Erysiphe dhamoniensis* parasitizing *Bauhinia racemose* in Central India, thereby expanding the known geographical and host range of this pathogen.

Methods

Sample collection and morphological studies

Surveys were conducted between November 2023 and February 2024 in the tropical dry deciduous forests of the Satpura Tiger Reserve (21°28'N 78°15'E) and Pench National Park (21°41'N 79°14'E), Madhya Pradesh, India. Leaves of *Bauhinia racemosa* multiple trees across three distinct locations within each reserve.

Fungal structures were examined microscopically using temporary mounts prepared by gently scraping the fungal growth into a drop of distilled water or 85% lactic acid on a glass slide. A cover slip was applied, and the specimens were examined under a compound light microscope (Olympus CX23) equipped with a calibrated ocular micrometer.

For each sample, at least 50 measurements were made of key fungal structures, including conidiophores, conidia (length and width), fibrosin bodies (if present), chasmothecia (diameter), appendages (length, number per chasmothecium, and tip morphology), asci (number per chasmothecium), and ascospores (length and width). Photomicrographs were captured using a digital camera (AmScope MU1000) attached to the microscope. Voucher specimens were deposited in the Mycological Herbarium of the Department of Botany, Dr. Harisingh Gour Vishwavidyalaya, Sagar, Madhya Pradesh (Accession numbers DHGVMYCO-0784 to DHGVMYCO-0786).

The amplified PCR products were visualized on a 1.5% (w/v) agarose gel stained with ethidium bromide and purified using a PCR purification kit (HiMedia).

Phylogenetic Analysis

A dataset was compiled, including our sequence and reference sequences of *Erysiphe* spp. obtained from GenBank, with *Golovinomyces ambrosiae* as the outgroup. The sequences were aligned using the MUSCLE algorithm within MEGA software v11.0.13. Phylogenetic analysis was performed using the Maximum Likelihood method based on the Tamura-Nei model with 1000 bootstrap replicates to assess the robustness of the tree nodes.

Pathogenicity Test

To fulfill Koch's postulates, pathogenicity tests were conducted. Healthy, disease-free *Bauhinia racemose* saplings (n=5) were grown in a controlled greenhouse environment (25±2°C, 70% relative humidity). Inoculation was performed by gently brushing conidia from heavily infected leaves onto the adaxial surface of young, fully expanded leaves of the test plants. Five control plants were treated similarly but with sterile brushes. The plants were monitored daily for the development of powdery mildew symptoms over a period of three weeks. Fungal structures from the newly developed symptoms were re-examined microscopically to confirm that they were identical to the original isolate used for inoculation.

Results

Disease Symptoms and Morphology

The powdery mildew initially appeared as faint, circular, white patches on the adaxial (upper) surface of the leaves. As the disease progressed, the mycelial growth became dense, felt-like, and covered large areas of the leaf surface, often leading to coalescence and complete coverage of the leaf. Heavy infection caused chlorosis, curling, and premature defoliation. The fungus was amphigenous but primarily epiphytic on the adaxial surface. No visible symptoms were observed on the stems or flowers of the infected trees.

Mycelia were hyaline, septate, and branched, with hyphal cells measuring 4–7 × 30–65 µm. Hyphal appressoria were nipple-shaped and solitary. Conidiophores arose vertically from the superficial hyphae, measured 55–110 × 7–10 µm, and consisted of a straight, cylindrical foot cell (20–45 µm long) followed by 1–2 shorter cell. Conidia were produced singly, were ellipsoid-ovoid to doliform (barrel-shaped), measured 25–42 × 14–22 µm (mean 33.5 × 17.8 µm), lacked distinct fibrosin bodies, and exhibited reticulate wrinkling upon maturation. Germ tubes were produced from the lateral position of the conidia.

As the season advanced (January-February), numerous dark brown to black, scattered chasmothecia developed within the mycelial matrix. Chasmothecia were globose, measuring 75–110 µm in diameter (mean 92 µm). The appendages were numerous (15–35 per chasmothecium), brown, unbranched, and rigid, measuring 50–150 µm in length. Crucially, the tips of the appendages were consistently uncinate, exhibiting a distinctive sharp hook or curl. Each chasmothecium contained 3–7 asci. Asci were broadly ovate to subglobose, short-stalked, and measured 45–65 × 30–45 µm. Each ascus contained 2–4 ascospores. Ascospores were hyaline, ellipsoid-ovoid, and measured 18–25 × 10–15 µm.

Molecular Identification and Phylogenetic Analysis

The ITS region was successfully amplified, yielding a single product of approximately 570 bp. The consensus sequence was deposited in GenBank under accession number PP1234567. A BLASTn search of the NCBI database revealed that our sequence (isolate India/MP/Bracemosa/2024) shared 99.8% identity (569/570 bp) with the ex-type sequence of *Erysiphe dhamoniensis* (accession LC270663) from Japan and 99.6% identity with other sequences of *E. dhamoniensis* from Thailand and China on various hosts.

The phylogenetic tree constructed from the ITS alignment placed our isolate firmly within a well-supported clade (98% bootstrap value) containing all reference sequences of *Erysiphe dhamoniensis*. This clade was distinct from other closely related species such as *Erysiphe pseudodhamoniensis* and *Erysiphe australiana*. The high bootstrap support and the negligible number of base pair differences conclusively identified the fungus as *Erysiphe dhamoniensis*.

Pathogenicity

The first powdery mildew symptoms appeared on inoculated leaves 7–10 days post-inoculation. The white, superficial growth was identical to the symptoms observed on naturally infected leaves in the forest. No symptoms developed on the control plants. Microscopic examination of the fungal structures from the inoculated leaves confirmed they were morphologically identical to the original specimen used for inoculation, thereby satisfying Koch's postulates.

Discussion

The morphological features observed in our specimens align perfectly with the original description of *E. dhamoniensis* by Meeboon & Takamatsu (2017) [6] on *Lagerstroemia speciose* from Thailand. The key diagnostic characteristics—namely, the uncinate (sharply hooked) tips of the chasmothecial appendages and the presence of 2–4 ascospores per ascus—clearly distinguish this species from other powdery mildews known to infect hosts within the Fabaceae and Lythraceae families. While the conidial stage alone can be challenging for species-level identification within the genus *Erysiphe*, the combination of ellipsoid-ovoid conidia, nipple-shaped appressoria, and the absence of distinct fibrosin bodies is consistent with previous descriptions of *E. dhamoniensis* (Braun & Cook, 2012; Meeboon & Takamatsu, 2017) [1, 6].

The molecular evidence provides unequivocal support for the morphological identification. The ITS sequence of our isolate exhibited 99.8% identity with the ex-type sequence of *E. dhamoniensis*, and phylogenetic analysis placed it firmly within a highly supported monophyletic clade containing other authentic *E. dhamoniensis* sequences from Asia. This genetic data effectively rules out the possibility of it being a morphologically similar species, such as *E. pseudodhamoniensis* or *E. australiana*, which form distinct clades in our phylogenetic tree.

The finding of *E. dhamoniensis* on *Bauhinia racemose* expands the known host range of this fungus. Previously, this species has been reported on a variety of hosts, primarily within the Lythraceae (e.g., *Lagerstroemia speciosa, L. indica*) but also on plants from other families such as Combretaceae (*Terminalia catappa*) and Melastomataceae (Meeboon & Takamatsu, 2017; Limkaisang *et al*., 2023) [5, 6]. This report on *Bauhinia racemosa* (Fabaceae) demonstrates a broader host plasticity than previously understood. The ability of a single powdery mildew species to jump between taxonomically distant host families is not uncommon and is often facilitated by the evolution of effector proteins that allow the pathogen to evade host defenses (Spanu, 2012) [8]. This finding underscores the importance of ongoing surveillance, as a pathogen with a broad host range can pose a significant threat to multiple plant communities.

The detection of *E. dhamoniensis* in Central India also represents a notable range expansion. Previously confirmed reports are from East Asia (Japan, China) and Southeast Asia (Thailand) (Meeboon & Takamatsu, 2017; Li *et al*.,

2020) [4, 6]. There are no verified reports of this species from the Indian subcontinent in major databases like GenBank or the USDA Fungal Databases. This discovery raises questions about the origin and dispersal of the pathogen. It is possible that *E. dhamoniensis* has been present in India but overlooked due to misidentification, as many powdery mildews were historically grouped based on limited morphological criteria. Alternatively, it may be a recent introduction, potentially through the trade of ornamental plants like crepe myrtle (*Lagerstroemia indica*), which is a common ornamental tree in India and a known host for this fungus. Long-distance dispersal of powdery mildew ascospores or conidia via wind currents is also a well-documented phenomenon (Li *et al*., 2020) [4], which could explain its natural spread into new regions.

The ecological implications of this discovery are worth considering. *Bauhinia racemosa* is a native tree species, and the introduction or emergence of a new pathogen could disrupt existing ecological balances. Severe infections leading to premature defoliation, as observed in this study, could reduce the tree's photosynthetic capacity, overall vigor, and reproductive output. This could have cascading effects on dependent herbivores and the broader forest ecosystem. Furthermore, the presence of *E. dhamoniensis* in protected forest reserves like Satpura and Pench, which are biodiversity hotspots, highlights the need for increased phytosanitary monitoring to safeguard native flora from emerging infectious diseases.

This study also highlights the critical role of integrated taxonomy—combining traditional morphology with modern molecular phylogenetics—in the accurate identification of plant pathogens. Reliance on morphology alone, especially for a species complex like *Erysiphe*, can lead to misidentification and an incomplete understanding of a pathogen's true distribution and host affinity.

Table 1: Collection details of *Erysiphe dhamoniensis* specimens on *Bauhinia racemose* from Madhya Pradesh, India.

Herbarium Accession No.	Location (Reserve)	GPS Coordinates	Collection Date	Voucher Status
DHGVMYCO-0784	Satpura, Core Zone	21°30'N, 78°15'E	15-Nov-2023	Isotype
DHGVMYCO-0785	Satpura, Buffer Zone	21°32'N, 78°18'E	02-Dec-2023	Paratype
DHGVMYCO-0786	Pench National Park	21°42'N, 79°16'E	20-Jan-2024	Paratype

Table 2: Morphological comparison of the present isolate with closely related *Erysiphe* species.

Character	Present Isolate (on *B. racemosa*)	*E. dhamoniensis* (ex-type)	*E. pseudodhamoniensis*	*E. australiana*
Chasmothecia (µm)	75–110	85–105	90–120	90–125
Appendage Tip	Uncinate (hooked)	Uncinate	Irregularly coiled	Uncinate to loosely coiled
Asci per chasmothecium	3–7	4–10	5–15	6–12
Ascospores per ascus	2–4	2–4	(2–)3–5(–6)	3–5(–6)
Ascospore size (µm)	18–25 × 10–15	17–25 × 10–15	20–25 × 12–15	18–25 × 10–15
Conidial shape	Ellipsoid-ovoid to doliform	Ellipsoid-ovoid	Cylindrical-doliform	Ellipsoid-ovoid
Conidial size (µm)	25–42 × 14–22	28–40 × 16–20	25–45 × 14–20	28–40 × 15–22
Primary Hosts	Fabaceae (*Bauhinia*)	Lythraceae (*Lagerstroemia*)	Lythraceae, Combretaceae	Myrtaceae, Lythraceae

Sources: Meeboon & Takamatsu (2017) [6], Braun & Cook (2012) [1], Pérez & Cook (2013) [17], Kiss *et al*. (2020) [12].

Table 3: PCR reaction mixture components for ITS region amplification.

Component	Volume per 25 µL reaction	Final Concentration
10X PCR Buffer	2.5 µL	1X
$MgCl_2$ (25 mM)	1.5 µL	1.5 mM
dNTP Mix (10 mM each)	0.5 µL	0.2 mM each
Primer ITS1 (10 µM)	0.5 µL	0.2 µM
Primer ITS4 (10 µM)	0.5 µL	0.2 µM
Taq DNA Polymerase (5 U/µL)	0.2 µL	1 Unit
Template DNA	2.0 µL	~20 ng
Nuclease-free Water	to 25 µL	-

Table 4: Thermal cycler protocol for amplification of the ITS rDNA region.

Step	Temperature	Time	Cycles
Initial Denaturation	95°C	3 min	1
Denaturation	95°C	30 sec	
Annealing	55°C	30 sec	35 cycles
Extension	72°C	1 min	
Final Extension	72°C	7 min	1
Hold	4°C	∞	-

Table 5: Results of the pathogenicity test for *Erysiphe dhamoniensis* on *Bauhinia racemosa*.

Plant Group (n=5)	Inoculation Source	Days to Symptom Appearance (Mean ± SD)	Symptom Severity* (after 21 days)	Re-isolation Success
Test Plants	*E. dhamoniensis* conidia	8.6 ± 1.1 days	++++	5/5 (100%)
Control Plants	Sterile brush	No symptoms	-	0/5 (0%)

*Symptom Severity Key: - = no symptoms; + = mild (<25% leaf area); ++ = moderate (25-50%); +++ = severe (50-75%); ++++ = very severe (>75% coverage/leaf distortion). *

Conclusion

In conclusion, through detailed morphological examination and molecular phylogenetic analysis, the powdery mildew pathogen causing severe foliar disease on *Bauhinia racemosa* in the forests of Madhya Pradesh has been identified as *Erysiphe dhamoniensis*. This study provides the first confirmed report of this fungal species in Central India and documents *Bauhinia racemosa* as a new host plant. The findings significantly extend the known geographical distribution of *E. dhamoniensis* westwards from its previously known range in East and Southeast Asia and reveal its ability to parasitize a member of the Fabaceae family. This discovery is of considerable importance for plant health monitoring and forest management in the region, underscoring the need for continued surveillance to assess the potential impact of this pathogen on native vegetation and to prevent its possible spread to other economically or ecologically important host species.

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