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Short Article

# First report of a member of *Balladyna* (*Ascomycota*: *Balladynaceae*) on *Celastrus paniculatus* (*Celastraceae*) from India

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## ABSTRACT

Microscopic examination of an epiphyllous fungus on *Celastrus paniculatus* (*Celastraceae*) revealed characteristic features of the genus *Balladyna*, including unicellular appressoria, mycelial setae, globose ostiolate perithecia, and successively maturing asci containing uniseptate, brown ascospores. *Balladyna*, a genus within the family *Balladynaceae* (*Ascomycota*: *Dothideomycetes*), is commonly referred to as one of the 'black mildews' and typically exhibits host specificity, predominantly infecting members of a few dicotyledonous plant families. Currently, approximately 17 species of *Balladyna* are known, primarily associated with hosts in the families *Rubiaceae*, *Annonaceae*, *Hippocrateaceae*, *Strychnaceae* and *Verbenaceae*. This study reports, for the first time, the occurrence of a *Balladyna* species on *Celastrus paniculatus* (*Celastraceae*) from India. It is suggested to represent a new species within the genus; however, due to the lack of comprehensive data, particularly DNA sequence information, it is provisionally reported here as an unidentified species of *Balladyna.*

## KEYWORDS

Black mildew, Epiphyllous fungi, Foliicolous fungi, Dothideomycetes.



## INTRODUCTION

The genus *Balladyna* stands out as a relatively obscure but scientifically significant group of fungi. Belonging to the family *Balladynaceae*, *Balladyna* species are primarily known as foliar pathogens, often forming dark, superficial colonies on the leaves of various host plants (Boonmee et al. 2017, Bhise et al. 2021). About 39 species of *Balladyna* have been described on different plant families (Index Fungorum, https://www.indexfungorum.org, Bhise et al. 2021, Hosagoudar 2002, 2004, 2013, Hosagoudar et al. 2009). Despite their inconspicuous appearance and limited attention in mycological literature, *Balladyna* fungi are important for understanding plant-pathogen interactions, fungal biodiversity, and the ecological dynamics of tropical and subtropical environments. During a survey of the foliicolous fungi in the Western Ghats region of Kerala state, India, author came across an endemic plant, *Celastrus paniculatus* (*Celastraceae*) endemic to southern Western Ghats–South Sahyadri and between Palakkad hills to Coorg Region in Central Sahyadris (Henry et al. 1987, Gamble 1997, Sasidharan 2004), infected with a black mildew fungus from the Malabar Wildlife Sanctuary of Kozhikode district. Microscopic examination of the fungus revealed the presence of unicellular appressoria, mycelial setae, globose ostiolate perithecia, having successively maturing asci with uniseptate brown ascospores. These are the characteristics of the genus *Balladyna.*

## MATERIALS AND METHODS

In the field, infected plants were collected, and observations were documented about the nature of the colonies, the type of infection, and the collection site. A

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distinct field number was allocated for each collection. In the field, each infected plant was collected separately in polythene bags along with the host twig (preferably with the reproductive parts to facilitate the identification of the corresponding host). Infected plant tissues were systematically pressed and dried using blotting papers. Following the drying process, they were utilized for microscopic analysis. Scrapings were taken directly from the infected host and immersed in a 10% KOH solution. After 30 minutes, the KOH was replaced with Lactophenol (Hosagoudar and Kapoor, 1985). Both mountants functioned effectively as clearing agents, allowing for the visibility of septa for measurement. In order to examine the entire colony in its natural state, a drop of high-quality natural colored or clear nail polish was applied to the chosen colonies and carefully thinned with a delicate brush, guaranteeing that the colonies were not disturbed. Colonies exhibiting hyperparasites with a woolly appearance were excluded from this process. The treated colonies, along with their host plants, were placed in a dust-free chamber for a duration of thirty minutes. After the nail polish on the colonies had fully dried, a delicate, transparent or subtly apple rose-tinted layer was created, contingent upon the color of the nail polish, with the colonies firmly encased within it. For softer host materials, the film could be gently lifted off by applying slight pressure on the opposite side of the leaves, just beneath the colonies. In contrast, for harder host materials, the film was carefully detached using a razor or scalpel. A drop of DPX was applied to a clean slide, onto which the film was properly spread. Additional drops of DPX were added on top of the film, followed by the placement of a clean cover glass. By applying gentle pressure to the cover glass, any excess DPX was removed after drying, ensuring that air bubbles were avoided. The slides were then labeled and stored in a dust-free environment for one to two days to allow for drying (Hosagoudar and Kapoor, 1985). These permanent slides were subsequently utilized for further research. For intrinsic fungi, sections were prepared and stained with cotton blue. After analyzing each collection, a portion of the material was preserved in the regional herbarium, Mar Thoma College Herbarium, Thiruvalla (MTCHT).

## TAXONOMY OF THE FUNGUS

### *Balladyna* sp.

Colonies epiphyllous, dense, velvety, up to 5 mm in diam., confluent and cover all surface of the leaves. Hyphae straight to undulate, branching alternate to irregular at acute to wide angles, closely reticulate to form a mycelial net, cells 12–28 × 2.4–5 µm. Appressoria numerous, alternate to unilateral, unicellular, antrorse to retrorse, straight to curved, entire to slightly angular, ovate, clavate, globose, subglobose, straight to variously curved, 12–18 × 7–10 µm. Mycelial setae numerous, scattered, simple, straight, flexuous, acute to obtuse at the tip, up to 70 µm long. Perithecia scattered, fairly numerous, initially stipitate, later become subsessile, ovate, globose, ostiolate, 100–120 × 60–70 µm; asci few, globose to ovate, interspersed with hyaline paraphyses, 4–6 spored, bitunicate, 40 µm in diam., wall thick; ascospores conglobate, oblong, brown, uniseptate, strictly constricted at the septum, 19–24 × 12–14 µm, wall smooth (Fig. 1).

**Specimen examined:** MTCHT 105, India, Kerala, Malabar Wildlife Sanctuary of Kozhikode, Janakikadu, on the living leaves of *Celastrus paniculatus* (*Celastraceae*), 18 January 2015, Collected by Lini K Mathew.

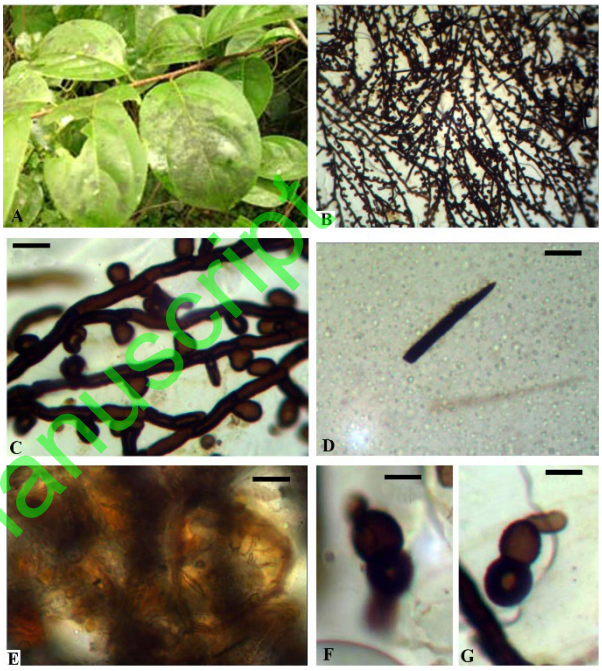


**Fig. 1.** *Balladyna* sp. on *Celastrus paniculatus*. (A) infected leaves, (B) mycelial colony with perithecia, (C) appressoriate mycelium, (D) mycelial seta, (E) asci in the perithecium, (F) and (G) germinating ascospores. Scale bars = 8 µm.

## DISCUSSION

The genus *Balladyna* (*Balladynaceae*) belongs to the obligate biotrophs of the group 'Black mildews', and their host range is restricted to the members of the corresponding host family. It is taxonomically placed under *Dothideomycetes* of *Ascomycota* (Cannon and Krick 2007). This genus represents 17 species infecting the members of the family *Rubiaceae*, *Annonaceae*, *Hippocrataceae*, *Strychnaceae,* and *Verbenaceae* (Hosagoudar 2004, Hosagoudar et al. 2009, Bhise et al. 2021).

*Balladyna rubiacearum* Hosag. *Balladyna sydowii* Petrak, *Balladyna terennae* Ebon & Cane and *Balladyna velutina* (Berk. Curt.) Hohn. can be compared based on the length of the mycelial setae and ascospores, but, *Balladyna rubiacearum* differs from the latter two species in having shorter mycelial setae and ascospores, while, differs from the former species in having longer mycelial setae and larger ascospores (Hansford 1946, Petrak 1955, Müller and Arx 1962, Eboh and Cain 1973). *Balladyna magnifica* (Sydow) Hansf. and *B.*

*tenuis* Hansf. are known on *Canthium globgensis* and *C. vulgare*, respectively, from Africa (Hosagoudar 2004), and *B. indica* Hosag. is known from an unidentified *Rubiaceae* from India (Hosagoudar et al. 2009). *Balladyna canthiigena* M.R. Bhise et al. differs from these species and closely related species in having larger velvety colonies, mycelial setae and perithecia distinctly formed only at the center of colony and absent on peripheral hyphae; larger sized hyphal cells, perithecia and ascospores; and distinctly cylindrical to capsulate and slightly constricted ascospores (Bhise et al. 2021)

This study reports, for the first time, the occurrence of a *Balladyna* species on *Celastrus paniculatus* (*Celastraceae*) from India. Since there is no report of the genus *Balladyna* on the members of the family *Celastraceae* and based on the morphological comparison, it is suggested to represent a new species within the genus; however, due to the lack of comprehensive data, particularly DNA sequence information, it is provisionally reported here as an unidentified species of *Balladyna.*

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**DATA AVAILABILITY**

Requests for data and materials should be addressed to the author**.**

**DECLARATION**

The authors declare no conflicts of interest.

**FUNDING**

The author declares that no financial support was received during this research.

**ETHICS APPROVAL**

Not applicable.

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# اولین گزارش از وجود *Balladyna* (*Ascomycota*: *Balladynaceae*) روی گیاه *Celstrus paniculatus* (*Celastraceae*) از هند

**لینی کاماتیو**

بخش تحقیقات و تحصیلات تکمیلی گیاه‌شناسی، کالج سنت توماس، کوژنچری، پاتانامتیتا، کرالا، هند

## چکیده

بررسی میکروسکوپی یک قارچ رورست روی گیاه *Celastrus paniculatus* (*Celastraceae*) ویژگی‌های مشخصه جنس *Balladyna* را نشان داد. در این قارچ آپرسوریوم‌های تک سلولی و خارهای میسلیومی دیده می شوند. پریتسیوم‌های کروی روزنه‌دار و آسک‌های بالغ حاوی آسکوسپورهای قهوه‌ای تک دیواره بودند. بر این اساس جنس *Balladyna* تشخیص داده شد. *Balladyna* از خانواده *Balladynaceae* (*Ascomycota*: *Dothideomycetes*) است و معمولاً به عنوان یکی از "کپک‌های سیاه" شناخته می‌شود و معمولاً میزبان اختصاصی دارد و عمدتاً اعضای چند خانواده گیاهی دولپه را آلوده می‌کند. در حال حاضر، تقریباً ۱۷ گونه از *Balladyna* شناخته شده است که عمدتاً با میزبان‌هایی در خانواده‌های *Rubiaceae*، *Annonaceae*، *Hippocrateaceae*، *Strychnaceae* و *Verbenaceae* مرتبط هستند. این مطالعه برای اولین بار، وقوع یک گونه *Balladyna* را روی *Celastrus paniculatus* (*Celastraceae*) از هند گزارش می‌دهد. به نظر می‌رسد که این گونه، گونه جدیدی را در این جنس باشد، با این حال، به دلیل فقدان داده‌های جامع، به ویژه اطلاعات توالی DNA، در اینجا موقتاً به عنوان یک گونه ناشناخته از *Balladyna* گزارش می‌شود.

کلمات کلیدی: کپک سیاه، قارچ رورُست، قارچ برگ‌رُست، Dothideomycetes.



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