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New record of *Rhopalostroma brevistipitatum* (Hypoxylaceae) and checklist of *Rhopalostroma* species from India

Nuevo registro de *Rhopalostroma brevistipitatum* (Hypoxylaceae) y lista de verificación de especies de *Rhopalostroma* de la India

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ABSTRACT

Rhopalostroma is one of genera bearing unique taxonomic characters in the family Hypoxylaceae, within the order Xylariales of the class Sordariomycetes. Species of *Rhopalostroma* predominantly grow on dead wood under saprophytic conditions and are restrictedly distributed in tropical and subtropical areas, including Africa and South Asia. In India, *Rhopalostroma* was previously reported only from the southern part of the country. This paper presents a new record of *R. brevistipitatum* from India, specifically from the northeastern state of Arunachal Pradesh (AP). The paper also includes a morphological description and documentations of the species accompanied by photographs, and provides a checklist of *Rhopalostroma* species found in India.

Keywords — Fungi; saprophytic; taxonomy.

RESUMEN

Rhopalostroma es uno de los géneros con caracteres taxonómicos únicos en la familia Hypoxylaceae, que se encuentra en el orden Xylariales de la clase Sordariomycetes. Las especies de *Rhopalostroma* crecen predominantemente en troncos de madera muerta en condiciones saprófitas y tienen una distribución restringida en áreas tropicales y subtropicales, incluyendo África y el sur de Asia. En India, *Rhopalostroma*

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se ha reportado previamente solo en la parte sur del país. En este artículo se presenta un nuevo registro de *Rhopalostroma brevistipitatum* en la India, específicamente en el estado nororiental de Arunachal Pradesh (AP). Se incluye una descripción morfológica y documentación de la especie, fotografías, y una lista de las especies de *Rhopalostroma* encontradas en la India.

Palabras clave — Hongos; saprófitos; taxonomía.

INTRODUCTION

In 1977, Hawksworth established the genus *Rhopalostroma* with the type species *Rhopalostroma indicum* Hawksw. & Muthap from Karnataka, India. The genus *Rhopalostroma* is characterized by pedicellate, erect stromata, which are either simple or occasionally branched, melanized, and mature stromata that become carbonaceous with expanded convex heads. The internal flesh is dark-brown to black without concentric zones. Multi-perithecia are regularly and superficially arranged in a single-layer form (monostichous). The asci are evanescent, meaning they are destroyed at maturity (except for *Rhopalostroma brevistipitatum*); the asci are cylindrical, amyloid, or inamyloid. Usually, fresh stromal tissue in a 10% KOH solution yields a greenish or olivaceous pigment or is absent.

A conidial state is produced in the culture of a few species, resembling nodulisporium-like (Hawksworth & Whalley, 1985; Stadler *et al.*, 2010; Daranagama *et al.*, 2014). Morphologically, this genus *Rhopalostroma* is closely related to *Phylacia* due to its globose to subglobose stromata; however, it differs from *Phylacia* in having a single layer of ascomata in the stroma, asci with apical apparatus, and ascospores with a germ slit. Based on asexual morph, phylogenetic analysis and secondary metabolite profiling, this genus is firmly placed within the family Hypoxylaceae (Wendt *et al.*, 2018).

Globally, this genus comprises a total of 11 validly published species, of which five species were previously reported from India.

MATERIALS AND METHODS

Morphological traits were examined using a stereozoom microscope (Stemi58). Slide were prepared for compound microscopy to observe various structures of the stromata, including perithecia, ostioles, asci, paraphyses, periphyses, and ascospores, by using a compound microscope (Zeiss A1). The 10% KOH test was conducted by adding a few drops of 10% KOH solution to a small amount of stromatic tissue (Ju & Rogers, 1996). Color designation was determined by Rayner's guidelines (1970). To determine the ascial apical apparatus, Melzer's reagent reaction was employed. Measurements of different characters such as stromata (n=10), ascomata (n=10), and ascospores (n=40) were taken from materials mounted in water, and the mean values were utilized in the descriptions. The validity of the species name was cross-checked using Index Fungorum and MycoBank. Photographs were produced using Adobe Photoshop.

RESULTS

Taxonomy

Rhopalostroma brevistipitatum Daranag. & K.D. Hyde, in Daranagama, Liu, Chamyuang, Stadler, Bahkali & Hyde. Fig. 1: A-K
MycoBank N° 550815

Teleomorph.— Stromata (3–)5–7(8) mm high x (3–)3.5–4.0(–4.3) (*av.* 5.8 x 3.7 mm), erect, scattered distribution, solitary, hard, not fused and not branched, light brown to purplish brown, carbonaceous stromata at mature. Stipe (1–)1.7–1.8(–2.6) × (1.8–)2.3–3.4(–3.8) mm (*av.* 1.6 × 2.8 mm), short and stout, tightly attached, infertile. Head (2–) 4.1–4.2(–5.2) × (2.8–)3.0–3.5(–4.0) mm (*av.* 4.15 × 3.2 mm), expanded, obtuse, clavate, fertile. Stromatal pigments is Dull Green (70) in 10% KOH test. Ascospores (0.213–)0.311–0.364(–0.383) × (0.151–)0.167–0.202(–0.212) mm (*av.* 0.341 × 0.187 mm), immersed directly beneath the ectostroma surface, regularly and superficially arranged in a single layer of perithecia, hemispherical to sub-cylindrical, perithecia with papillate ostioles. Paraphyses slightly longer than asci, filamentous, aseptate. Asci (78–)82–101(–106) μm (*av.* 96 μm) in total length, stipitate, cylindrical, unitunicatae, uniseriate with 8 ascospores, apical apparatus not cleared. Ascospores (9.12–)10.0–11.0(–11.73) × (3.5–)3.8–4.7(–5.0) μm (*av.* 10.5 × 4.1 μm), uniseriate, olivaceous (48), equilaterally-ellipsoidal, uniguttulated, with broadly rounded ends, epispore smooth, perispore indehiscent in 10% KOH, germ slit straight.

Asexual morph.— Mycelia simple to complex, hyaline, dichotomously branched, nodulisporium-like. Conidiogenous cells produced throughout the mycelia. Conidia (3–)4–5(–6.9) × (2–)2.7–3(–3.2) μm (*av.* 4.7 × 2.8 μm), hyaline, single, ellipsoidal, with one pointed end and one blunt end.

Distribution.— Thailand (Daranagama *et al.*, 2015).

Habitat.— Saprophytic on semi-decaying bamboo.

Material examined.— INDIA. Arunachal Pradesh, Papum Pare district, Rajiv Gandhi University (27°11'245"N 093°35'49"E; 577 masl.), on the bamboo host as a saprophytic condition, tropical forest, 03-II-2020, coll. by G. Dutta & R. K. Singh, specimen is deposited in Botanical Survey of India, Itanagar, Arunachal Pradesh, India, Voucher number: ARUN- 40951 and additional specimen is deposited in Department of Botany, Rajiv Gandhi University, Arunachal Pradesh, Voucher number: ARFR- 808.

Notes.— The newly reported species, *Rhopalostroma brevistipitatum*, is morphologically very similar to the holotype species in all aspects (Daranagama *et al.*, 2015). This species is characterized by small and solitary stromata with short and stout stipes, and cylindrical asci with a J+ discoid apical apparatus. However, in our examined species, we were unable to identify a clear apical apparatus. Furthermore, it's worth noting that the holotype species was originally described as saprobic on decaying bark, while the present species was discovered in saprophytic conditions on dead bamboo. Furthermore, this species bears a resemblance to *Xylaria badia* Pat. however, it exhibits notable differences. The most prominent distinction lies in the

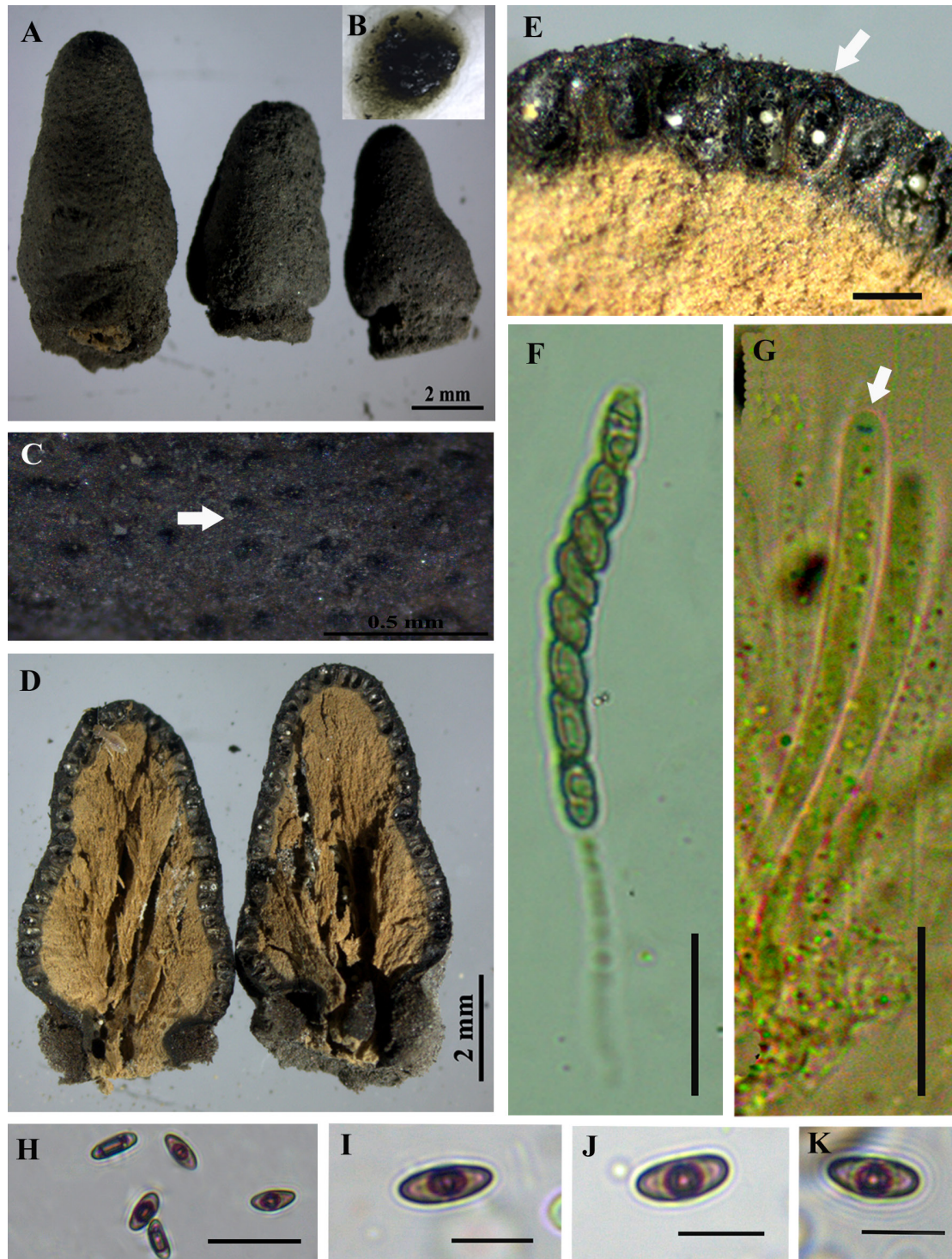


Fig. 1. Macroscopic and microscopic features of *Rhopalostroma brevistipitatum*: A) Stromata. B) Extractable pigments in 10% KOH. C) Ostioles (arrow) on stroma surface. D) Longitudinal section showing perithecial ascomata at the periphery of the stroma with superficial perithecia. E) Perithecia (arrow). F) Ascus. G) Immature asci with apical apparatus (arrow). H) Different shapes of ascospores. I-K) Uni-guttulate ascospores. Scale: E= 0.2 mm. F,G,H= 20 μ m. I-K= 10 μ m.

Fig. 1. Características macroscópicas y microscópicas de *Rhopalostroma brevistipitatum*: A) Estromas. B) Pigmentos extraíbles con KOH 10%. C) Ostiolo visible (flecha) en la superficie del estroma. D) Corte longitudinal del estroma mostrando ascomas periteciales en la periferia, con peritecios superficiales. E) Peritecio (flecha). F) Asco. G) Ascó inmaduro con aparato apical (flecha). H) Diferentes formas de ascosporas. I-K) Ascosporas uni-guttuladas. Escala: E= 0.2 mm. F,G,H= 20 μ m. I-K= 10 μ m.

smaller stromata height, av. (3–)5–7(8) mm vs. (2.5–)5–15(–28) mm. Additionally, the fertile portion of the stroma is shorter with obtuse apex, and the stipe is considerably shorter than that of *X. badia*. The surface color also sets them apart, with the present species displaying a light brown to purplish-brown, in contrast to *X. badia*'s grayish-brown to silvery-gray appearance. Moreover, while the inner stromata of *X. badia* are soft-textured, solid, spongy, and white to tan, the present species is predominantly hard, creamy white to brown, and characterized by cracks. The size of the perithecia and asci is nearly identical, with dimensions of 0.341×0.187 mm vs. $0.3\text{--}0.35 \times 0.2\text{--}0.3$ mm. However, there is a slight variation in the size of the ascospores, with the present species having slightly larger ascospores av. 10.5×4.1 μm vs. 9.4×3.9 μm (Fournier *et al.*, 2020).

DISCUSSION

The generic distribution is limited to tropical and subtropical regions in Africa and Asia (Hawksworth, 1977). The distribution of the species *Rhopalostroma brevistipitatum* is confined to the northern region of Thailand. Morphologically, this species resembles immature *Xylaria* spp. due to its very small clavate-shaped stromata. In comparison, *R. brevistipitatum* is much smaller than other *Rhopalostroma* species. Additionally, most *Rhopalostroma* species have deliquescent asci, making it challenging to properly identify the apical apparatus of the asci. However, *R. brevistipitatum* has a minute apical apparatus that appears blue in Melzer's reagent (Daranagama *et al.*, 2015). The anamorph produced is nodulisporium-like, similar to *R. angolense* and *R. lekae* (Hawksworth, 1977; Stadler *et al.*, 2010; Daranagama *et al.*, 2014). Before the addition of the presently reported species, *R. brevistipitatum*, India had only five different species of *Rhopalostroma*. With the addition of the present species *R. brevistipitatum*, the total number of species for the genus in India increases to six (Table 1).

Table 1. Checklist of *Rhopalostroma* species of India.

Tabla 1. Lista de verificación de las especies de *Rhopalostroma* de la India.

Name of the species	Substratum	Site	Reference
<i>Rhopalostroma africanum</i> (Wakef.) D. Hawksw	On bark of <i>Ficus benghalensis</i> L. (Moraceae)	Pune, Maharashtra	Patil <i>et al.</i> , 2012
<i>Rhopalostroma brevistipitatum</i> Daranag. & K.D. Hyde			This study
<i>Rhopalostroma dennisii</i> D. Hawksw., Zachariah & Sankaran	<i>Artocarpus integrifolia</i> S. Zachariah (Moraceae)	Calicut University, Kerala	Hawksworth <i>et al.</i> , 1979
<i>Rhopalostroma indicum</i> D. Hawksw. & Muthappa	<i>Ficus retusa</i> L., Mant. (Moraceae)	Karnataka	Hawksworth, 1977
<i>Rhopalostroma lekae</i> Whalley, Thienh., M.A. Whalley & Sihan.	On bark of <i>Memecylon umbellatum</i> Burm. (Melastomataceae)	Panhala, Kolhapur District, Maharashtra	Patil <i>et al.</i> , 2012
<i>Rhopalostroma sphaerocephalum</i> (Petch) D. Hawksw. var <i>indica</i> , var. nov.	On bark of <i>Bombax malabaricum</i> DC. (Bombacaceae)	Radhanagari, Kolhapur District, Maharashtra	Patil <i>et al.</i> , 2012

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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