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journal homepage: www.elsevier.com/locate/myc**Short communication****A new endophytic fungus, *Chrysofolia barringtoniae* sp. nov., from Thailand**

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ABSTRACT

A new species of endophytic fungi, described herein as *Chrysofolia barringtoniae*, was isolated from leaves of *Barringtonia acutangula* in Lampang Province, Thailand. Morphological investigations revealed that its conidia are shorter than in *Chrysofolia colombiana*. Phylogenetic analyses also support the morphological results. A description and an illustration of *C. barringtoniae* are provided.

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The genus *Chrysofolia* was described by Crous et al. (2015) with *C. colombiana* Crous, Rodas & M.J. Wingf. as the type species. This genus belongs to the order Diaporthales, family Cryphonectriaceae (Crous et al. 2015). Currently, there is only one *Chrysofolia* species record in Index Fungorum (<http://www.indexfungorum.org/names/names.asp>), which it is from Colombia with plant pathogenic habits (Crous et al. 2015). During our investigation of endophytic fungi on *Barringtonia acutangula* (L.) Gaertn., we found an interesting species of *Chrysofolia* whose morphological observation and phylogenetic analysis revealed it as a new species.

Leaf samples of *B. acutangula* were collected from a natural forest in Mae Moh District, Lampang Province, northern Thailand (18°25'46.15"N, 99°46'22.82"E), in Jan 2016. The

samples were then taken to the laboratory and processed within 24 h. Samples were washed in running tap water for 15 min. Endophytic fungi were isolated using a triple sterilization method and cultured on potato dextrose agar (PDA) as described by Suwannarach et al. (2012). The fungal isolates were identified according to their macroscopic and microscopic structures. At least 50 measurements were made of each structure. A pure culture of the fungus was air-dried to prepare the holotype specimen. The holotype material and its original (ex-type) strains were deposited in the Culture Collection of Sustainable Development of Biological Resources Laboratory (SDBR), Faculty of Science, Chiang Mai University, Chiang Mai and Thailand Bioresource Research Center (TBRC), Thailand.

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Chrysofolia barringtoniae Suwannar., Kumla & Lumyong, sp. nov.

Fig. 1.

MycoBank no.: MB816193.

Diagnosis: This species can be distinguished from *C. colombiana* by its shorter conidia.

Etymology: *barringtoniae*, refers to the generic name of the host plant, *Barringtonia*.

Holotype: THAILAND, Mae Moh District, Lampang Province, 18°40'52.7"N, 98°52'10.2"E, isolated as an endophyte from leaves of *Barringtonia acutangula*, Jan 2016, Suwannarach N., dried culture: SDBR-ENBA048; ex-type living culture: TBRC 5647.

Gene sequences (from holotype): KU948046 (ITS) and KU948045 (LSU).

Fungal colonies on potato dextrose agar (PDA), oatmeal agar (OA) and malt extract agar (MEA) reaching 60–75, 85–90 and 85–90 mm diam at 25 °C after 1 wk (Fig. 1A–C). Colonies on PDA effuse, white to light brown, reverse dark brown. Colonies on OA profuse cottony, concentric rings, white to smoke-gray surface and reverse smoke-gray to olive brown. Colonies on MEA flat, scarce surface, white to smoke-gray mycelium and reverse smoke-gray to olive brown. Mycelium superficial and immersed, hyphae branched, septate, hyaline, 2–3 µm wide. Conidial masses and conidiomata were observed in both PDA and MEA cultures after 1 wk at 25 °C. Masses of conidia pale orange to orange color, exuding from conidiomata (Fig. 1D). Conidiomata pycnidial, globose, 50–110 µm diam, separated to aggregated, light-brown in

lactic acid, 2–3 wall layers of brown to dark brown color; neck short, with central ostiole surrounded by loose hyphal element, 1.5–2 µm diam (Fig. 1E). Conidiophores reduced to conidiogenous cells. Conidiogenous cell hyaline, smooth, cylindrical to ampulliform, straight to curved, wider at the base, 3–7.5 × 2–3 µm (Fig. 1F). Conidia hyaline, smooth, guttulate, ellipsoidal, straight, apex obtuse, base tapering to flat protruding scar, (3–)4–5(–7.5) × (2–)2.5(–3) µm (Fig. 1G).

Note: The conidia of *C. colombiana* [(4–)6–7.5(–10) × (2–)2.5(–3) µm] are longer than those of *C. barringtoniae* (Crous et al. 2015).

Genomic DNA was extracted from mycelial grew on PDA at 25 °C in darkness for 1 wk using the Genomic DNA Extraction Mini Kit (FAVORGEN, Taiwan). The large subunit (LSU) region of ribosomal RNA gene was also amplified with LROR and LRO5 primers under the following thermal conditions: 94 °C for 2 min, 30 cycles of 95 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min, and 72 °C for 10 min. In addition, the internal transcribed spacer (ITS) region of the ribosomal RNA gene was amplified by polymerase chain reaction (PCR) using ITS5 and ITS4 primers under the following thermal conditions: 95 °C for 2 min, 30 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min, and 72 °C for 10 min. PCR products were checked on 1% agarose gels stained with ethidium bromide under UV light and purified using NucleoSpin® Gel and PCR Clean-up Kit (Macherey–Nagel, Düren, Germany). The purified PCR products were directly sequenced. Sequencing reactions were performed and the sequences automatically determined with

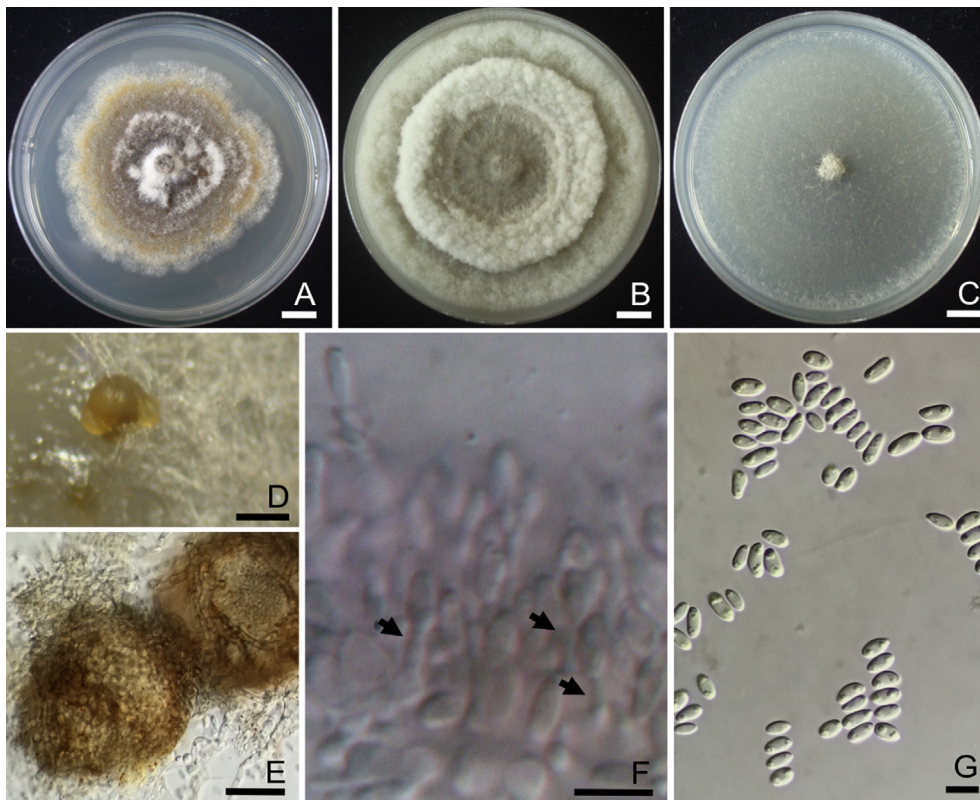


Fig. 1 – *Chrysofolia barringtoniae* (SDBR-ENBA048). A–C: Colonies on different media after 1 wk. A: Potato dextrose agar; B: Oatmeal agar; C: Malt extract agar. D: Mass of conidia. E: Conidiomata. F: Conidiogenous cells. G: Conidia. Bars: A–C 10 mm; D 1 mm; E 25 µm; F 10 µm; G 5 µm.

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