# *Hydropisphaera heliconiae,* a new species from Martinique (French West Indies)

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**Abstract:** A detailed description of *Hydropisphaera heliconiae* sp. nov. is presented based on two collections on dead leaves of *Heliconia caribea* (*Heliconiaceae*) in Martinique (FWI). The fungus was cultured and sequenced and its placement in the genus *Hydropisphaera* confirmed by analysis of LSU sequences. This species primarily differs from all known species in having ascomata nearly black when mature and deeply cupulate when dry. The new combination *ljuhya boothii* is proposed for a taxon examined during this study that was formerly placed in *Hydropisphaera*.

Keywords: Ascomycota, Bionectriaceae, Hypocreales, ribosomal DNA, taxonomy.

**Résumé :** Une description détaillée d'*Hydropisphaera heliconiae* sp. nov. est présentée à partir de deux récoltes sur feuilles mortes d'*Heliconia caribea (Heliconiaceae)* en Martinique (Petites Antilles françaises). Le champignon a été cultivé et séquencé et son placement dans le genre *Hydropisphaera* est confirmé par l'analyse des séquences LSU. Cette espèce diffère tout d'abord de toutes les espèces connues en ayant des ascomes presque noirs à maturité et profondément cupulés par le sec. La nouvelle combinaison *ljuhya boothii* est proposée pour un taxon examiné pendant cette étude qui était auparavant placé dans *Hydropisphaera*. **Mots-clés :** ADN ribosomal, Ascomycota, *Bionectriaceae*, Hypocréales, taxinomie.

## Introduction

In continuation of a research program on the fungal diversity of the Lesser Antilles (LECHAT & FOURNIER, 2017), we collected a surprising species of *Hydropisphaera* (Tode: Fr.) Dumort. with blackish and deeply cupulate ascomata occurring on dead leaves of *Heliconia caribea* Lamb. Usually, the ascomata of *Hydropisphaera* are pale yellow to dark orange or brownish-orange but none are dark brown to nearly black, which prompted a thorough study of both the sexual morph and the asexual morph obtained in culture. The genus *Hydropisphaera* is distinguished from other genera in the *Bionectriaceae* by morphological characters defined by ROSSMAN *et al.* (1999), LECHAT *et al.* (2010) and LECHAT & FOURNIER (2016, 2017). Based on these characters, phylogenetic analysis and comparison with known species of *Hydropisphaera*, the specimen described herein is determined to represent a previously undescribed species of *Hydropisphaera* for which the new name *H. heliconiae* is proposed.

## **Materials and methods**

The specimen was examined, cultured, sequenced and phylogenetically analysed using the methods described in LECHAT & FOURNIER (2015).

#### Taxonomy

Hydropisphaera heliconiae Lechat & J. Fourn., sp. nov. Fig. 2 Mycobank: MB 819916

**Diagnosis:** Differs from all known species of *Hydropisphaera* in having dark brown to blackish ascomata.

**Holotype:** FRENCH WEST INDIES, MARTINIQUE. Le Prêcheur, Anse Couleuvre, path to the Couleuvre waterfall, on dead leaf of *Heliconia caribea*, 3 Jun. 2014, *leg*. Christian Lechat CLLM14011 (LIP), ex-type culture: CBS 138704. GenBank LSU: KY656904.

Etymology: The epithet is derived from the host Heliconia L.

**Perithecia** solitary, superficial, subglobose, (200-)220-260(-280) µm high × (220-)280-300 µm diam. (X = 250 × 290 µm, n = 15), dark brownish orange, becoming dark brown to nearly black and collapsing deeply cupulate when dry, not changing colour in 3% KOH or lactic acid. **Basal hyphae** densely radiating from ascomatal base and extending over substratum, yellowish to pale brownish orange, 2.5-3.5 µm diam., flexuous, smooth. Perithecial apex with short, acute papilla, margin with fasciculate, thick-walled hairs 27-45  $\mu m$  long, 2.5-3  $\mu m$  wide, brownish-orange, cylindrical, slightly flexuous with wall 1–1.5 µm thick, rounded at tips, septate, arising from cells of ascomatal wall, agglutinated to form sparse, triangular teeth 10-14 µm wide at base, arranged in an irregular, stellate fringe around upper margin of perithecia. Perithecial wall 40-60 µm thick, composed of two regions: outer region 30-45 µm wide, of globose to ellipsoidal  $10-16 \times 8-12 \,\mu m$  cells with brownish orange walls 1–1.5  $\mu$ m thick, with outermost coating of a thin layer of amorphous brown material; inner region 10-15 µm wide, of elongate, flattened cells 8–14  $\times$  5–8  $\mu m$ , with hyaline walls 1–1.5  $\mu m$ thick. Asci evanescent, (55–)60–70(–74)  $\times$  (9–)10–11(–12)  $\mu$ m (X =  $64.5 \times 10.5 \ \mu\text{m}$ , n=20), fusiform-clavate, apices rounded, without ring, with 6-8 biseriate ascospores. Ascospores (13.5-)14.5- $17.8(-18.5) \times (-3.8)4.5-5(-5.3) \ \mu m (x = 16 \times 4.9 \ \mu m, n=30), narrowly$ ellipsoid, rounded at ends, equally 1-septate, hyaline, longitudinally striate, completely filling each ascus.

Asexual morph: acremonium-like.

**Cultural characteristics:** After one week at 25°C on Difco PDA containing 5 mg/L streptomycin, colony 4–5 cm diam, mycelium white, producing an abundant acremonium-like asexual morph at margin; conidiophores branched, 15–50  $\mu$ m long, 4–5  $\mu$ m diam, flexuous, smooth, arising from smooth, septate hyphae 5–7  $\mu$ m diam, each branch septate, with a simple conidiogenous cell 15–25  $\mu$ m long, 3–4  $\mu$ m diam at base producing conidia (4–)5–9(–11) × 2–3.5(–4)  $\mu$ m, ellipsoidal to subcylindrical, hyaline, smooth, non-septate, with rounded apex, attenuated at base with a basal abscission scar, grouped at tip of phialides to form a mucous head. After three weeks, colony proliferating irregularly, reaching 6–8 cm in diam, diffusing a pale reddish brown coloration into medium.

Additional specimen examined: FRENCH WEST INDIES, MARTINIQUE. Le Marigot, Forêt départementalo-domaniale de la Reculée, sentier du plateau d'Encens, on dead leaf of *Heliconia caribea*, 22. Aug. 2011, *leg*. Christian Lechat, CLLM11071 (LIP).

### Discussion

The fungus described above is characterized by superficial, nonstromatic, subglobose ascomata with a blackish wall composed of thin-walled cells not changing colour in 3% KOH or lactic acid, becoming strongly cupulate upon drying and bearing fasciculate hairs scattered around the apical region; its asci are unitunicate, fusiform-

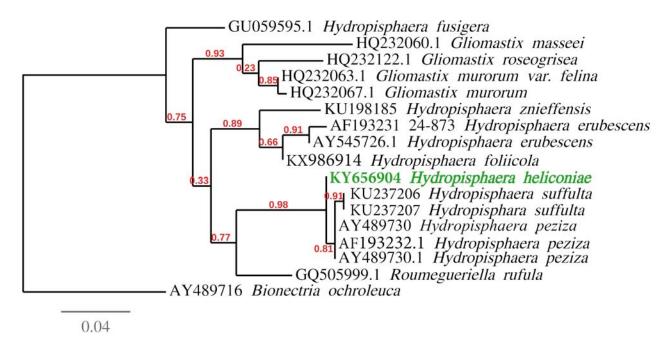


Fig. 1 – Maximum likelihood phylogeny of Hydropisphaera heliconiae based on LSU sequences, rooted with Bionectria ochroleuca

clavate, ascospores averaging  $16 \times 4.9 \,\mu\text{m}$  are ellipsoid, one-septate, hyaline and longitudinally striate, and the asexual morph obtained in culture is acremonium-like. This combination of morphological and cultural features clearly place it in the genus Hydropisphaera, which is confirmed by phylogenetic analysis of its LSU sequence showing it is nested within members of this genus (Fig. 1). When run through the updated key to Hydropisphaera spp. with fasciculate hairs proposed by LECHAT & FOURNIER (2016), based on colour of ascomatal wall and hairs, dimensions, septation and ornamentation of ascospores, the closest match appears to be with *H. suffulta* (Berk. & M.A. Curtis) Rossman & Samuels. Hydropisphaera suffulta is a common, widespread pantropical species, often present on dead Heliconia leaves, which primarily differs from our fungus by yellow to orange ascomatal wall composed of thin-walled (< 1 µm thick) cells, bearing fasciculate hairs scattered all over the ascomatal surface and smaller ascospores  $11.2-13.8 \times 4-5.5 \mu m$  (SAMUELS *et al.*, 1990).

Maximum likelihood phylogeny based on LSU sequences (Fig. 1) shows that the closest species to the fungus described above are *H. peziza* (Tode) Dumort. (type species of *Hydropisphaera*) and *H. suf-fulta*. *Hydropisphaera peziza* is different in having yellow to orange, glabrous ascomata (rarely with sparse hairs) containing numerous orange oily droplets in ascomatal wall and hymenium as well as shorter ascospores  $11-14 \times 5-7 \mu m$ , while *H. suffulta* is different for the reasons expressed above.

Based on morphological comparison and phylogenetic analysis, the new taxon *H. heliconiae* Lechat & J. Fourn. is thus proposed to accommodate this collection from Martinique.

In the field, a confusion with *H. fusigera* (Berk. & Broome) Rossman, L. Lombard & Crous (LOMBARD *et al.*, 2015) is possible when its reddish brown cupulate ascomata appear blackish due to the accumulation of dark brown to black conidia of its associated gliomastix-like asexual morph trapped in its ascomatal hairs. *Hydropisphaera fusigera* can be distinguished from *H. heliconiae* by its occurrence on bamboo, its larger one-celled ascospores averaging 23.6 × 6.7 µm and its gliomastix-like asexual morph (LECHAT *et al.*, 2010, as *H. bambusicola*).

Hydropisphaera boothii (D. Hawksw.) Rossman & Samuels, a further hairy species of Hydropisphaera excluded from the aforementioned key and unknown to us at that time, has been revised recently. HAWKSWORTH & MINTER (1980) described Nectria boothii as a species occurring on *Oenanthe crocata (Apiaceae)*, which was combined in *Hydropisphaera* as *H. boothii* by ROSSMAN & SAMUELS (1999). Examination of the holotype (K[M]) revealed that it belongs to *ljuhya* Starbäck, and all morphological characters of this specimen match well with those of *I. oenanthicola* Lechat & Hairaud (LECHAT & HAIRAUD, 2012). The synonymy is established here, and we introduce the new combination *ljuhya boothii* (D. Hawksw.) Lechat & J. Fourn. *comb. nov.*, MycoBank MB 819968 [basionym: *Nectria boothii* D. Hawksw., *in* Hawksworth & Minter, *Trans. Brit. mycol. Soc.*, 74 (3): 572 (1980)].

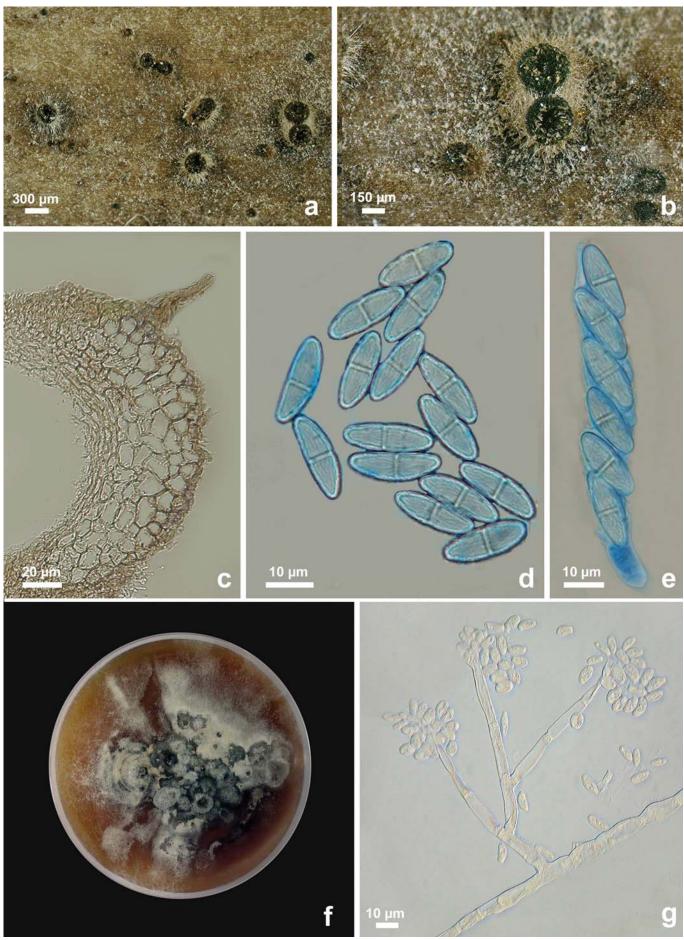
The known asexual morphs of *Hydropisphaera* spp. are considered acremonium-like (ROSSMAN *et al.*, 1999), but to date two species recently published have a gliomastix-like asexual morph as discussed by LECHAT *et al.* (2010) and LECHAT & FOURNIER (2016). Phylogenetic analysis (Fig. 1) shows that *H. heliconiae* is nested in the clade comprising the species having an acremonium-like asexual morph, which is in agreement with the asexual morph obtained in culture during this study.

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**Fig. 2** – **a-g:** *Hydropisphaera heliconiae* (holotype). a-b: Ascomata on the substratum, showing the blackish cupulate ascomata with the base surrounded by dense radiating hyphae; c: Lateral ascomatal wall in vertical section showing a fascicle of hairs toward the apex; d: Ascospores with striate wall, in lactic cotton blue; e: Ascus and ascospores, in lactic cotton blue; f: Culture at three weeks; g: Conidiophores and conidia, in lactic acid.

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