# *Hydropisphaera palmicola* (*Bionectriaceae*), a new species from Saül (French Guiana)

**Christian LECHAT (†)** Abstract: A detailed description of Hydropisphaera palmicola sp. nov. is presented, based on a collection **Jacques FOURNIER** on dead leaves of Astrocaryum sp. (Arecaceae) in French Guiana. The acremonium-like asexual morph has been obtained in culture and sequenced. Its placement in the genus Hydropisphaera was confirmed by the **Delphine CHADULI** analysis of LSU sequences. Based on morphological and phylogenetic comparison with the known Hydro-**Anne FAVEL** pisphaera species, we propose H. palmicola as a new species. The similarity of our species with H. erubescens and H. foliicola is discussed. Ascomycete.org, 14 (2): 81-84 Keywords: Astrocaryum, Hypocreales, new species, ribosomal DNA, taxonomy, tropical mycology. Mise en ligne le 25/04/2022 doi 10.25664/ART-0349 Résumé : une description détaillée d'Hydropisphaera palmicola sp. nov. est présentée à partir d'une récolte sur feuilles mortes d'Astrocaryum sp. (Arecaceae) en Guyane française. La forme asexuée de type acremo-CC BY-NC-ND nium a été obtenue en culture et séquencée. Son placement dans le genre Hydropisphaera a été confirmé par l'analyse des séquences LSU. Sur la base d'une comparaison morphologique et phylogénétique avec les espèces d'Hydropisphaera connues, nous proposons H. palmicola comme une nouvelle espèce. La ressemblance de notre espèce avec H. erubescens et H. foliicola est commentée.

Mots-clés : ADN ribosomal, Astrocaryum, espèce nouvelle, Hypocréales, mycologie tropicale, taxinomie.

#### Introduction

In the continuity of an inventorial survey of fungi in French Guiana (GARDIENNET *et al.*, 2019; LECHAT *et al.*, 2019; LECHAT & FOURNIER, 2019a; 2019b; 2019c; 2020a; 2020b; 2020c), an hypocrealean fungus was collected on dead palm leaves of *Astrocaryum* sp., which proved to be different from known species. This fungus was morphologically characterised, cultured and phylogenetically analysed. Based on its characteristics, phylogenetic analysis and comparison with other genera in the *Bionectriaceae* and with known species of *Hydropisphaera*. The specimen described herein is determined to represent a previously undescribed species of *Hydropisphaera*.

#### **Materials and methods**

Dry specimens were rehydrated and examined using the method described by ROSSMAN et al. (1999). Microscopical observations and measurements were made in water and the ascospore ornamentation was observed in lactic cotton blue not heated. The holotype specimen was deposited in LIP herbarium (University of Lille). Cultures of the living specimens were made on PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 5 cm diam. incubated at 25°C. DNA extraction, amplification, and sequencing were performed by ALVALAB (Oviedo, Spain): Total DNA was extracted from pure culture, blending a portion using a micropestle in 600  $\mu l$ CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min. at 65°C. A similar volume of chloroform: isoamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifuged for 10 min at 13.000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in 70% cold ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µl ddH<sub>2</sub>O. PCR amplification was performed with the primers LROR and LR5 (VILGALYS & HESTER, 1990) to amplify the 28S nLSU region. PCR reactions were performed under a program consisting of a hot start at 95°C for 5 min, followed by 35 cycles at 94°C, 54°C and 72°C (45, 30 and 45 s respectively) and a final 72°C step 10 min. Chromatograms were checked searching for putative reading errors, and these were corrected.

Analyses were performed online at phylogeny.lirmm.fr (DEREEPER et al., 2008). Maximum likelihood phylogenetic analyses were performed with PhyML 3.0 aLRT (ZWICKL, 2006), using the GTR + I +  $\Gamma$ 

model of evolution. Branch support was assessed using the nonparametric version of the approximate likelihood-ratio test, implemented in PhyML SH-aLRT (ANISIMOVA & GASCUEL, 2006). Nomenclature follows MycoBank (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands).

## Taxonomy

*Hydropisphaera palmicola* Lechat & J. Fourn., *sp. nov.* Figs. 1–2 – MycoBank: MB843711

**Diagnosis:** Differs from the most similar species *H. erubescens* in having smaller, punctate-striate ascospores,  $(17-)18-20(-22) \times 2.8-3.2 \mu m vs.$   $(17-)18-26(-29) \times 3.5-4.5 \mu m.$ 

**Holotype:** FRENCH GUIANA, Saül, trail head to Roche-Bateau, 3.62056 N, -53.199899 E, ca. 240 m, on dead palm leaves of *Astrocaryum* sp. (*Arecaceae*), 30 Mar. 2021, leg. C. Lechat CLLG21119 (LIP), ex-type culture no longer viable. GenBank LSU: ON181664.

**Etymology:** The specific epithet "*palmicola*" refers to its habitat on dead palm leaves.

Perithecia solitary, scattered on substratum, superficial, non-stromatic, globose, 160–180(–200) µm diam. (Me = 170 µm, n = 10), pale yellow to pale orange, not collapsing when dry, glabrous, smooth to slightly roughened, not changing colour in 3% KOH or lactic acid. Perithecial apex with a minute, acute papilla. Perithecial wall 35-50  $\mu$ m thick, composed of two regions: outer region 30–35  $\mu$ m wide, of subglobose cells,  $8-12(-14) \times 5-9 \mu m$ , with pale orange walls 1-1.5 µm thick; inner region 10–12 µm wide, of elongate, flattened cells, 7–15  $\times$  2–3.5  $\mu m$  , with hyaline wall. Asci 38–50  $\times$  8–10  $\mu m$ (Me =  $44 \times 9 \mu m$ , n=15), short-stipitate, clavate, attenuated at tip; apex simple, flattened, 8-spored, ascospores multiseriate, filling each ascus. Paraphyses hyphal, 2–3  $\mu$ m diam., inserted between asci. Ascospores (17–)18–20(–22)  $\times$  2.8–3.2  $\mu m$  (Me = 18  $\times$  3  $\mu m,$ n=40), fusiform, rounded at ends, (1-)3-septate, hyaline, punctatestriate, with an inconspicuous ornamentation, best seen in lactic cotton blue.

**Cultural characteristics:** After two weeks on PDA at 25°C, colony 15–20 mm diam., producing an acremonium-like asexual morph, cottony, pale buff in centre, pale greyish brown in middle area, white at margin, not diffusing colouration in medium, composed of white to pale brown, septate, smooth hyphae, 2.5–3  $\mu$ m wide. Conidiophores flexuous, rarely branched, 35–48  $\mu$ m long, 2.5–3.5  $\mu$ m diam., bearing a single phialide, 16–28 long, with a not flared collarette,



**Fig. 1** – a-d, g, h: *Hydropisphaera palmicola* (CLLG21119 Holotype); a: Ascomata on the substratum; b: Lateral ascomatal wall in vertical section; c: Asci and ascospores (b, c in water); d: Ascospores; e: Ascospores of *H. erubescens*; f: Ascospores of H. foliicola (d-f in lactic cotton blue); g: Culture at two weeks; h: Conidiophores and conidia from culture, in lactic acid. Scale bars:  $a = 200 \mu m$ ;  $b = 20 \mu m$ ; c,  $h = 10 \mu m$ ;  $d-f = 5 \mu m$ .

producing conidia in a slimy head. Conidia ellipsoidal,  $5-7 \times 2-2.5 \,\mu$ m, smooth, hyaline, attenuated at base, with or without abscission scar, forming small globose heads at apex of conidiogenous cells.

## Discussion

HYDROPISPHAERA PALMICOLA is placed in the genus Hydropisphaera based on the morphological characteristics of its sexual and asexual morphs, as well as the phylogenetic analysis of its LSU sequence. This fungus is morphologically characterised by its glabrous ascomata not changing colour in 3% KOH or lactic acid and fusiform, 3sepate ascospores. Several glabrous species of Hydropisphaera exhibit these characteristics, including *H. erubescens* (Roberge ex Desm.) Rossman & Samuels and H. foliicola Lechat & J. Fourn., which are morphologically separable only by size, ornamentation and ascospore septation, like all glabrous species of Hydropisphaera (LECHAT & FOURNIER, 2017). Our fungus was cultured, and the culture resulted in an acremonium-like asexual morph, which fits well with Hydropisphaera, as species in this genus are known to have an acremonium or gliomastix-like asexual morph (ROSSMAN et al., 1999; SUMMERBELL et al., 2011; LECHAT et al., 2010; LECHAT & FOURNIER, 2016). Our phylogenetic analysis based on LSU sequences (Fig. 2) shows that H. palmicola is nested within the Hydropisphaera clade. Phylogenetically, the closest species to H. palmicola is H. saulensis Lechat & J. Fourn., which differs mainly in its dark brownish orange to blackish brown ascomata and ellipsoidal, verrucose ascospores (LECHAT & FOURNIER, 2020b). The new species is morphologically similar to H. erubescens (Roberge ex Desm.) Rossman & Samuels, but differs from it in having significantly smaller ascospores  $(17-)18-20(-22) \times 2.8-3.2 \ \mu m \ vs.$  (17–)18–26(–29) × 3.5–4.5 µm (Fig. 1). Moreover, the ascospores of *H. erubescens* are spinulose as defined by LECHAT & FOURNIER (2017), while those of *H. palmicola* have a punctate-striate ornamentation. *Hydropisphaera foliicola* also resembles our fungus, but differs from it by having smaller, ellipsoidal, verrucose ascospores (14–)15–17 (–18) × 4–4.7(–5) µm vs. (17–)18–20(–22) × 2.8–3.2 µm (LECHAT & FOURNIER, 2017). Based on differences in microscopical characters and phylogenetic analysis, *H. palmicola* Lechat & J. Fourn. is therefore proposed as a new species.

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# **Authors' contribution**

Christian Lechat was responsible for the conception of the study, morphological studies, phylogenetic analyses, design of figures and plates and writing a first draft. Jacques Fournier critically reviewed the first draft and proposed an improved version, and took care of the registration at MycoBank. Delphine Chaduli and Anne Favel managed the culture collection in which the culture was to be deposited and took care of the registration at GenBank. All authors except CL read and approved the final manuscript.



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**Fig. 2** – Maximum likelihood phylogeny (-lnL = 3116.64105) of *Hydropisphaera* spp. inferred by Phyml 3.0, model hky85 from a 800 bp matrix of 28s rDNA sequence, rooted with *Dialonectria diatrypicola*.

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