Protocreopsis korfii (*Hypocreales, Bionectriaceae*), a new species from Martinique (French West Indies)

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Ascomycete.org, 7 (6) : 307-310.
Novembre 2015
Mise en ligne le 30/11/2015

Summary: *Protocreopsis korfii* sp. nov. is described and illustrated based on a collection on *Heliconia bihai* in Martinique. The placement of *P. korfii* in *Protocreopsis* is supported by morphological characters and analysis of LSU sequences. This species has smooth ascospores 35–46 × 7–8.5(–9) µm, shorter than the most closely related species, *P. fusigera*.
Keywords: Ascomycota, *Bionectriaceae*, *Heliconia*, ribosomal DNA, taxonomy.

Résumé : *Protocreopsis korfii* sp. nov. est décrit et illustré d'après une récolte sur *Heliconia bihai* en Martinique. Son placement dans le genre *Protocreopsis* est corroboré par les caractères morphologiques et l'analyse de séquences LSU. Cette espèce possède des ascospores lisses, 35–46 × 7–8.5(–9) µm, plus courtes que dans l'espèce la plus proche, *P. fusigera*.
Mots-clés : Ascomycota, *Bionectriaceae*, *Heliconia*, ADN ribosomal, taxinomie.

Introduction

During an ongoing research program on the fungal diversity of Lesser Antilles, conducted by Prof. R. Courtecuisse "Les champignons des Petites Antilles; diversité, écologie, protection" (Courtecuisse, 2006), hypocrealean ascomycetes appeared to feature a high diversity, especially in the families *Bionectriaceae* and *Nectriaceae*. New species in *Hydropisphaera* Dumort., *Ijuhya* Starbäck, *Lasionectria* (Sacc.) Cooke and *Verrucostoma* Hirooka, Tak. Kobay. & Chaverri collected during this inventorial survey have been described (Lechat *et al.*, 2010; Lechat & Courtecuisse, 2010; Lechat & Fournier, 2012; Lechat *et al.*, 2015). We introduce herein a new species of *Protocreopsis* Doi collected on *Heliconia bihai* (L.) L. (*Heliconiaceae*) in Martinique.

The most common *Heliconia* encountered in Martinique and Guadeloupe is *Heliconia caribaea* Lam. Its dead leaves and foliar sheaths still hanging above the soil level are frequently colonized by bionectriaceous fungi including the widespread *P. fusigera* (Berk. & Broome) Yoshim. Doi, the type species of the genus (Doi, 1977, 1978) and *P. pertusa* (Pat.) Samuels & Rossman. *Heliconia bihai* is much more rarely encountered than *H. caribaea* in natural environment and it was fairly unexpected to find on this host a *Protocreopsis* deviating from the largely prevailing *P. fusigera*.

The ascomata of *Protocreopsis* are pale yellow to pale orange or brownish-orange, not changing colour in 3% KOH or lactic acid and therefore the genus is accommodated in the *Bionectriaceae* (Rossman *et al.*, 1999). Protocreopsis is distinguished from other genera in the *Bionectriaceae* by the ascomata surrounded by a dense mat of white to tan, rarely greenish hyphae, the ascomatal wall more than 20 µm thick, typically striate ascospores, acremonium-like asexual morph and occurrence on monocotyledonous leaves, mostly palms and *Musaceae*. The most similar genus in the *Bionectriaceae* is *Stilbocrea* Pat., which also features ascomata embedded in a well-developed hyphal stroma. *Stilbocrea* can be readily distinguished from *Protocreopsis* by spinulose ascospores, synnematous or pycnidial asexual morphs and occurrence on woody substrates (Rossman *et al.*, 1999). A comprehensive survey of *Protocreopsis* including descriptions and a key to the known species was provided by Rossman *et al.* (1999) and since this time no other species was proposed. This survey served as a basis to evaluate the taxonomic status of our collection from Martinique.

Materials and methods

The specimen was examined using the method described by Rossman *et al.* (1999). Microscopic observations and measurements were made in water and the ascospore ornamentation was observed in lactic cotton blue not heated. The holotype specimen is deposited in LIP herbarium (Lille) and cultures at CBS (The Netherlands). Cultures of the living specimen were made on PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 9 cm diam. A mass of ascospores and asci was removed from a perithecium with a fine needle and placed in a drop of sterile water that was stirred with a needle to distribute the elements on the slide. A part of the drop containing ascospores was placed on PDA using a sterile micropipette, thereafter the Petri dish was incubated at 25 °C. DNA extraction, amplification, and sequencing were performed by ALVALAB (Santander, Spain): Total DNA was extracted from dry specimens blending a portion of them using a micropestle in 600 µl 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 centrifugated 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, centrifugated again for 2 min and dried. It was finally resuspended in 200 µl ddH2O. PCR amplification was performed with the primers LR0R 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 www.phylogeny.lirmm.fr (Dereeper *et al.*, 2008). Maximum likelihood phylogenetic analyses were performed with PhyML 3.0 aLRT (Zwickl, 2006), using the GTR + I + Γ model of evolution. Branch support was assessed using the non-parametric version of the approximate likelihood-ratio test, implemented in PhyML SH-aLRT (Anisimova & Gascuel, 2006). Nomenclature follows Mycobank (CBS-KNAW Fungal Biodiversity Center, Utrecht, The Netherlands).

Taxonomy

Protocreopsis korfii Lechat & J. Fourn., *sp. nov.* – Fig. 1, Plate 1
Mycobank: MB814620

Diagnosis: Differs from other species of *Protocreopsis* in having smooth ascospores 35–46 × 7–8.5 µm.

Holotype: French West Indies, Martinique, Fort-de-France, Maison forestière de la Donis, hygrophilic rainforest, ca. 440 m elevation, on *Heliconia bihai*, 14 Jun. 2014, CLLM14077 (LIP); ex-type culture CBS 138733; GenBank KT852955.

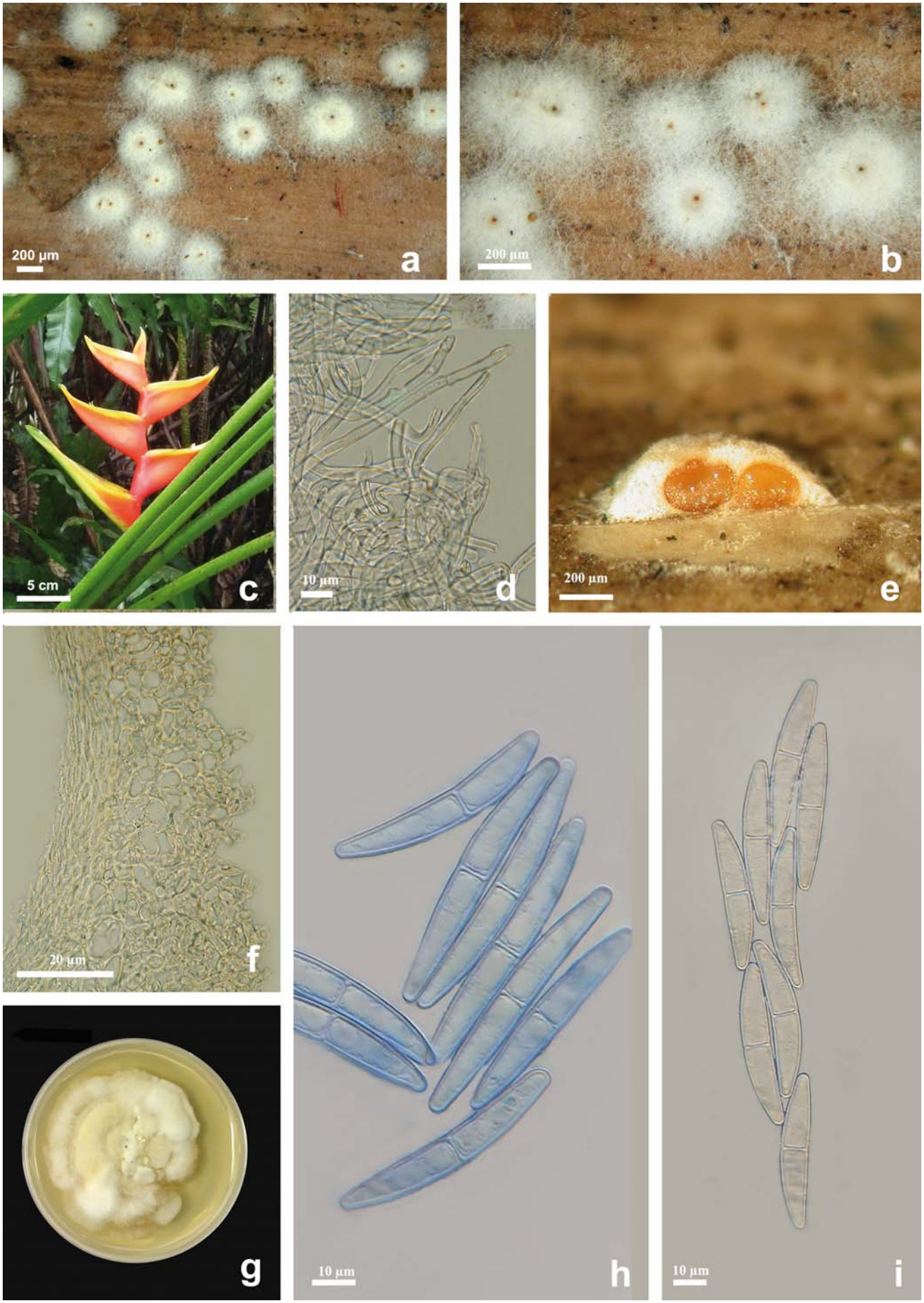

Plate 1 – a-h: *Protocreopsis korfii* (holotype) and host; a-b: Ascomata on the substratum; c: Inflorescence of *Heliconia bihai*; d: Hyphal elements of mycelium surrounding the perithecia; e: Ascomata in vertical section; f: Lateral ascomatal wall in vertical section; g: Culture after three weeks; h-i: Ascospores and ascus.

Etymology: The specific epithet refers to Professor Richard P. Korf to whom the authors dedicate this species to acknowledge his outstanding contributions to the taxonomy of Ascomycota.

Perithecia solitary or in groups of 2–3, superficial, completely immersed in cottony mycelium, subglobose, (250–)270–330(–350) high × (280–)300–360(–380) µm diam., pale yellowish to pale orange, not changing colour in 3% KOH or lactic acid, collapsing cupulate when dry with only papilla visible between hyphal elements of mycelium. **Mycelium** composed of smooth, branched, septate, thick-walled hyphae 3.5–5 µm wide, of indefinite length with wall 1–1.5 µm thick, at first white, becoming pale brownish orange over maturation. **Perithecial wall** 25–35(–40) µm thick, composed of two regions: outer region 18–22(–25) µm wide, of globose to ellipsoidal 4–10 × 4–6 µm cells, with pale orange walls 1–1.5(–2) µm thick; inner region 15–20 µm wide, of elongate, flattened cells 6–12 × 1.5–3 µm with a small lumen and hyaline walls 0.5–1 µm thick. **Asci** evanescent (85–)90–115(–120) × (14–)16–22(–25) µm (X = 110 × 19 µm, n=20), clavate, without ring, with eight irregularly biseriate ascospores completely filling each ascus. **Ascospores** (32–)35–46(–48) × (6.5–)7–8.5(–9) µm (X = 42.5 × 7.5 µm, n=30), fusiform, slightly curved, hyaline to pale orange en masse, 1-septate, smooth.

Asexual morph unknown.

Cultural characteristics: Colony after two weeks on PDA, 48–62 mm diam, pale yellow in center, white at margin, reverse pale yellow to pale yellowish brown, not sporulating. Floccose aerial mycelium composed of smooth, branched, septate, hyphae 2.5–4.5 µm wide, with wall 1–1.5 µm thick, rounded at free ends. No conidia produced in culture after four weeks.

Known distribution: Martinique.

Discussion: *Protocreopsis korfii* is characterised by the combination of pale orange ascomata with wall 25–35 µm thick, surrounded by a conspicuous white to tan hyphal stroma and smooth ascospores, 35–46 × 7–8.5 µm. This set of characters fits well the genus *Protocreopsis* as defined by Rossman *et al.* (1999) and this is supported by the phylogenetic analysis of LSU sequences (Fig.1). In our phylogenetic tree, *Protocreopsis* appears related to *Lasionectria* and *Ochronectria* Rossman & Samuels, both known to have acremonium-like asexual morph. *Lasionectria* differs from *Protocreopsis* in having often hairy ascomata lacking a hyphal stroma, while in *Ochronectria* ascomata are seated on a thin subiculum and feature a 3-layered wall more than 45 µm thick, with orange oily droplets between the cells of the middle layer and ascospores are multiseptate (Rossman *et al.*, 1999).

The only species of *Protocreopsis* featuring ascospores over 30 µm long is *P. fusigera*. *Protocreopsis korfii* is proposed as a new species because it has smaller ascomata than *P. fusigera* (300–360 vs. 430–720 µm diam) and smaller ascospores 35–46 × 7–8.5 vs. 50–76 × 6.5–9 µm that are smooth-walled vs. striate in *P. fusigera*. Unfortunately LSU sequences of *P. fusigera* are not available in GenBank for comparison.

Based on this single collection, it is unknown whether its occurrence on *Heliconia bihai* reflects a host specificity, a host preference or is merely fortuitous.

Acknowledgements

Prof. Régis Courtecuisse (Laboratoire des sciences végétales et fongiques, Faculté des sciences pharmaceutiques et biologiques, Université de Lille 2, France) for having initiated, organized and carried out the ambitious project of exploration of the fungal diversity of French West Indies, with technical facilities by the French Mycological Society (Paris, France), with the financial support of the National Forest Office (ONF central office in Paris : Michel Hermeline, Patrice Hirbec; in Martinique: Philippe Richard and Jean-Baptiste Schneider) and the DREAL Martinique. The Regional Natural Park of Martinique (PNRM) is thanked for collecting facilities. Dr Amy Rossman (U.S.A.) is warmly thanked for her invaluable scientific help and her presubmission review. Dr. Pierre-Arthur Moreau (Laboratoire des sciences végétales et fongiques, Faculté des sciences pharmaceutiques et biologiques, Université de Lille 2, France) is warmly thanked for his precious help with phylogenetic analyses.

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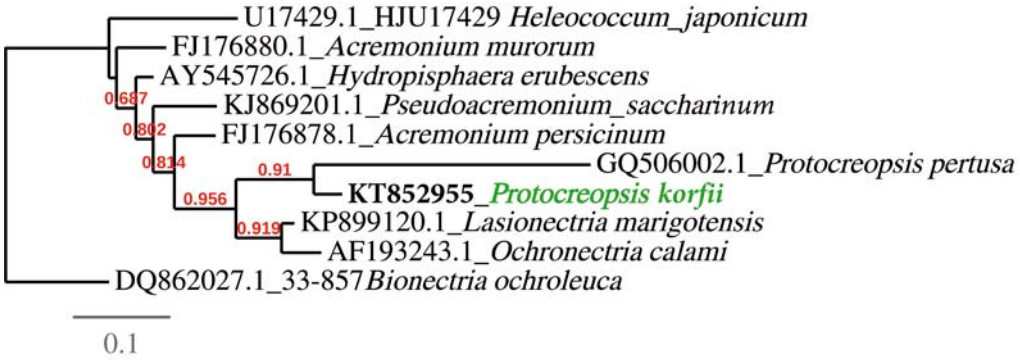

Fig. 1 – Maximum likelihood phylogeny of *Protocreopsis korfii* based on LSU sequences, rooted with *Bionectria ocholeuca*.

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