

Protocreopsis caricicola (Hypocreales, Bionectriaceae), the first species of *Protocreopsis* reported from a temperate area of the northern hemisphere

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Ascomycete.org, 8 (1) : 30-32.
Février 2016
Mise en ligne le 16/02/2016



Abstract: *Protocreopsis caricicola* sp. nov. is described and illustrated based on a collection on *Carex acutiformis* in Germany. The placement of *P. caricicola* in *Protocreopsis* is supported by morphological characters of both sexual and asexual morphs and analysis of LSU sequences. This species has smooth ascospores as does *Protocreopsis korffii* Lechat & J. Fourn. but differs from it in having significantly smaller ascospores and a different ecology.

Keywords: Ascomycota, Bionectriaceae, *Carex*, ribosomal DNA, taxonomy.

Résumé : *Protocreopsis caricicola* sp. nov. est décrit et illustré d'après une récolte sur *Carex acutiformis* en Allemagne. Son placement dans le genre *Protocreopsis* est corroboré par les caractères morphologiques du stade sexué et du stade asexué et l'analyse de séquences LSU. Cette espèce possède des ascospores lisses comme *Protocreopsis korffii* Lechat & J. Fourn. mais en diffère par des ascospores significativement plus petites et une écologie différente.

Mots-clés : Ascomycota, Bionectriaceae, *Carex*, ADN ribosomal, taxinomie.

Introduction

A comprehensive survey of *Protocreopsis* Yoshim. Doi including descriptions and a key to the known species was made by ROSSMAN *et al.* (1999). All species cited in this paper as well as the recently added *P. korffii* Lechat & J. Fourn. (2015) were reported from tropical and pantropical areas. A hypocrealean fungus collected in Germany on *Carex acutiformis* Ehrh. (*Cyperaceae*) featuring morphological characters typically encountered in *Protocreopsis* is presented as the new species *P. caricicola*, based on morphological, cultural and molecular data.

Materials and methods

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

Taxonomy

Protocreopsis caricicola Lechat & J. Fourn., *sp. nov.* – Pl. 1; Fig. 2 – Mycobank: MB 815276

Diagnosis: Differs from other species of *Protocreopsis* in having smooth and smaller ascospores as well as its occurrence in a north temperate area.

Holotype: GERMANY, Westmecklenburg, 5 km northeast Rehna, meadow pond 1 km east-southeast of Strohkirchen MTB 2132/434 (53.810055° N / 11.111687° E); tall sedge swamps on the banks of a eutrophic pond to wet past, last year's leaf residues of *Carex acutiformis*, 25 May 2015, coll. Torsten Richter, CLL15081 (LIP); ex-type culture CBS 140572; GenBank KU198184.

Etymology: The specific epithet refers to the host *Carex*.

Perithecia solitary or in groups of 2–6, superficial, completely immersed in cottony mycelium, subglobose, (140–)160–220(–250) high × (140–)150–200(–220) 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 2.5–3.2 µm wide, of indefinite length with wall 1–1.2 µm thick, rounded at free ends, at first white, becoming pale yellowish when mature. **Perithecial wall** 15–20(–25) µm thick, composed of two regions: outer region 10–15 µm wide, of globose to ellipsoidal 3.5–10.5 × 2–5 µm cells, with pale orange walls 1.5–2 µm thick; inner region 5–10 µm wide, of elongate, flattened cells 3–11 × 2.5–4 µm, hyaline, walls 1–1.5 µm thick. **Asci** evanescent (45–)50–60(–65) × (5.5–)6–7(–7.5) µm ($X = 55 \times 6.5 \mu\text{m}$, $n=20$), clavate, apically rounded with a faint apical ring-like thickening, with eight irregularly biseriolate ascospores completely filling each ascus. **Ascospores** (11.5–)12–13.5(–14.5) × 3–3.5 µm ($X = 13 \times 3.3 \mu\text{m}$, $n=30$), fusiform, slightly curved, hyaline, 1-septate, smooth.

Asexual morph: acremonium-like.

Cultural characteristics: Colony after two weeks on PDA, 28–40 mm diam, aerial mycelium white, with white radiating strands abundantly sporulating, reverse pale yellow to pale yellowish brown. Floccose aerial mycelium composed of smooth to finely rugulose, branched, thin-walled, septate, hyphae 2–3 µm wide, with wall 1–1.5 µm thick, rounded at free ends. Conidiophores borne on aerial hyphae, macronematous, mononematous, unbranched, flexuous, hyaline, surface smooth to roughened. Conidiogenous cells monophialidic, terminal, subulate towards apex 16–35 µm long, 1.5–1.8 µm wide at apex with a slightly flared collarette, 2.5–3 µm wide at base. Conidia solitary or grouped at tip of phialide to form a mucous head, aseptate, narrowly ellipsoidal to subcylindrical with rounded apex, attenuated at base with a flat abscission scar, smooth, hyaline, 4–6(–7) × 2.2–3 µm ($X = 5.8 \times 2.5 \mu\text{m}$, $n = 30$).

Known distribution: Germany.

Discussion: Morphological characters of *Protocreopsis caricicola* such as superficial gregarious ascomata embedded in a cottony mycelium arising from the ascomatal wall, ascomatal wall pale orange, KOH- and lactic acid-, over 20 µm thick and clavate asci with fusiform, 1-septate and often ornamented ascospores fit well the genus *Protocreopsis* as defined by ROSSMAN *et al.* (1999). This placement is supported by the acremonium-like asexual morph obtained in culture and the phylogenetic analysis of LSU sequences (Fig.1). In the phylogenetic tree *P. caricicola* appears close to *P. pertusa* (Pat.) Samuels & Rossman, which differs from the new species in having larger and striate ascospores, besides its tropical distribution. The phylogenetically closest genus having an acremonium-like asexual

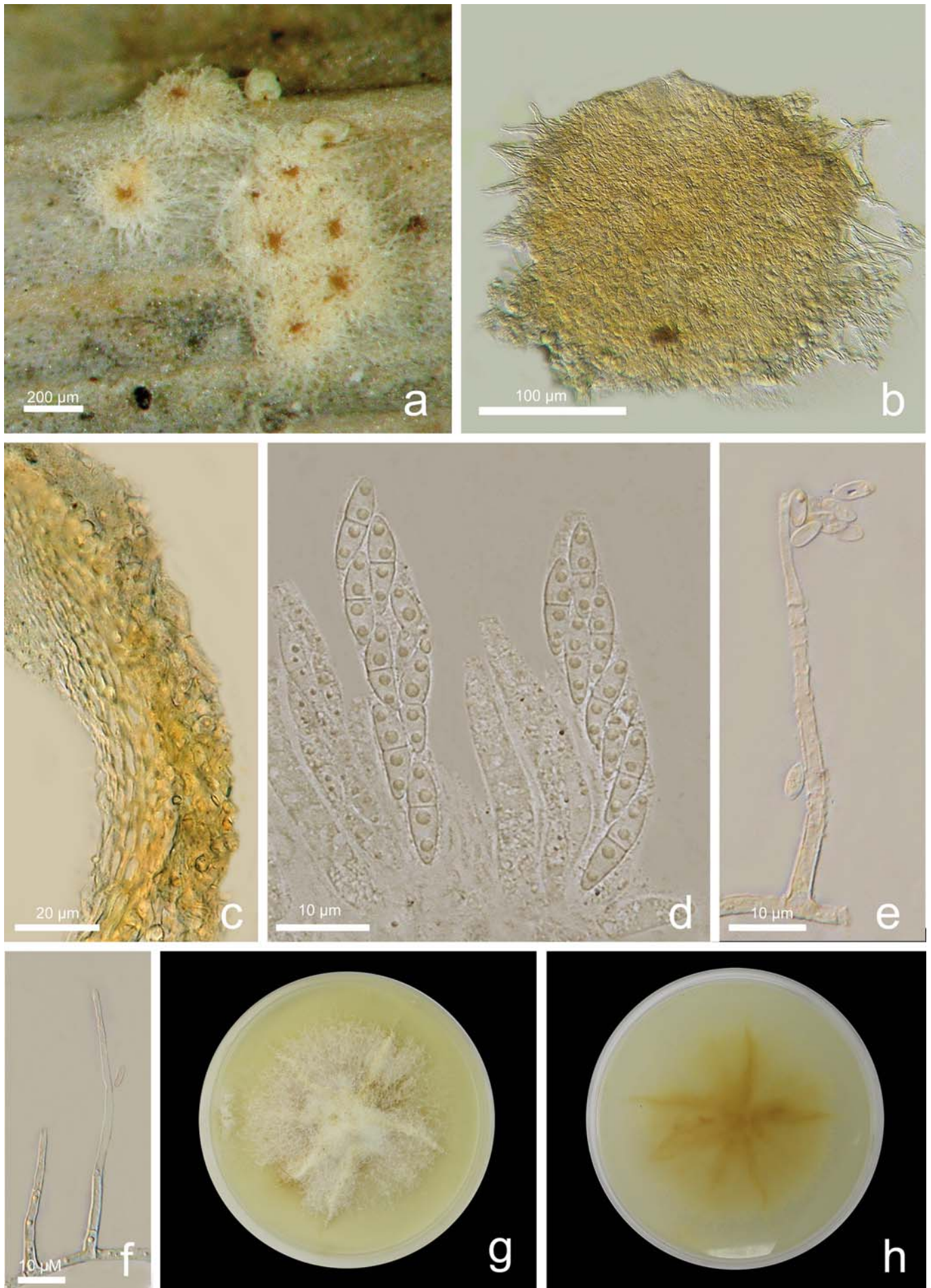


Plate 1: a-h: *Protocreopsis caricicola* (Holotype); a: Ascomata on the substratum; b: Close-up of perithecium observed in water; c: Lateral ascomatal wall in vertical section; d: Asci and ascospores; e-f: Conidiophores and conidia; g: Culture at two weeks; h: Culture reverse.

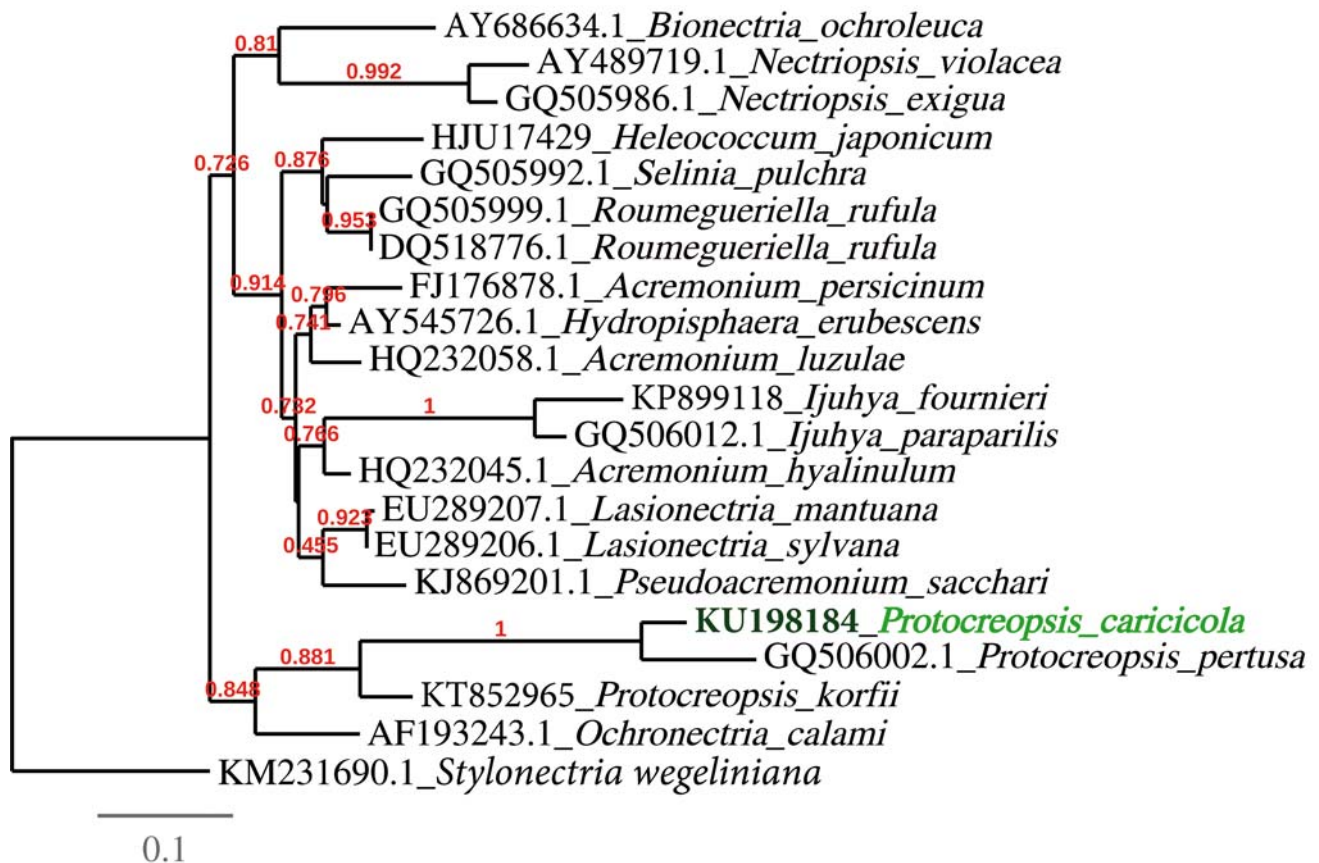


Fig. 2 – Maximum likelihood phylogeny of *Protocreopsis caricicola* based on LSU sequences, rooted with *Stylonectria wegeliniana*.

morph is *Ochronectria* Rossman & Samuels, but *Ochronectria* differs from *Protocreopsis* in having the ascomata seated on a thin subiculum and ascomatal wall more than 45 µm thick, made of three regions, with orange oily droplets between the cells of the middle layer (ROSSMAN *et al.*, 1999). The only known species of *Protocreopsis* featuring smooth ascospores is *P. korfii*, which differs from *P. caricicola* in having much larger ascospores (32–)35–46(–48) × (6.5–)7–8.5(–9) µm vs. (11.5–)12–13.5(–14.5) × 3–3.5 µm and a Caribbean origin.

It should be noted that *P. caricicola* slightly deviates from the generic concept delimited by ROSSMAN *et al.* (1999) by a two-layered ascomatal wall. The same feature was likewise observed in *P. korfii* (LECHAT & FOURNIER, 2015). The presence of the thin inner layer composed of flattened cells was observed in fresh collections of both latter species. It is assumed that this thin inner layer may be easily overlooked in old dry material, making it a differential character difficult to appraise.

Protocreopsis is a well-represented genus in the tropics but was so far unknown from temperate areas. *Protocreopsis caricicola* is the

first species of this genus reported from a temperate area of the northern hemisphere and from Europe.

Acknowledgements

The authors gratefully acknowledge Dr Amy Rossman (Oregon State University, USA) for her advices and scientific support.

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