First report of a lichenicolous species of *Hypomyces* (*Hypocreaceae*), *H. peltigericola* sp. nov.

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Abstract: *Hypomyces peltigericola* sp. nov. is described and illustrated based on material collected in France on thalli of the lichen *Peltigera canina*. In culture the ascospores yielded a cladobotryum-like asexual morph that was sequenced. The placement of this new species in the aurofusarin-group of the genus *Hypomyces* is based on the combination of morphological characters of sexual and asexual morphs as well as the comparison of its ITS rDNA sequence with those of this group available in GenBank. **Keywords:** Ascomycota, aurofusarin, *Hypocreales*, ribosomal DNA, taxonomy.

Résumé : *Hypomyces peltigericola* sp. nov. est décrit et illustré d'après du matériel récolté en France sur thalles du lichen *Peltigera canina*. Les ascospores ont produit un stade asexué de type cladobotryum en culture, lequel a été séquencé. Le placement de cette nouvelle espèce dans le groupe aurofusarine du genre *Hypomyces* repose sur la combinaison des caractères morphologiques des stades sexué et asexué, ainsi que sur la comparaison phylogénétique de la séquence rDNA ITS avec celles de ce groupe disponibles dans Gen-Bank.

Mots-clés : ADN ribosomal, Ascomycota, aurofusarine, Hypocréales, taxinomie.

Introduction

During a survey of lichenicolous fungi in France, with emphasis on the east-central part, a surprising specimen of *Hypomyces* (Fr.) Tul. & C. Tul. occurring on *Peltigera canina* (L.) Willd. was repeatedly collected on the same trunk (Fig. 1), prompting a detailed morphological characterization and a successful single ascospore isolation for one collection. Aside from its unusual lichenicolous habitat, this fungus showed all the key features of the aurofusarin-group of the genus *Hypomyces*.

The genus *Hypomyces*, as defined by RossMAN *et al.* (1999), accommodates fungicolous hypocrealean ascomycetes whose sexual morphs feature ascomata partly embedded in a subiculum, either changing colour in 3% KOH and in lactic acid or not, cylindrical unitunicate asci with a thickened apex and fusiform, usually two-celled, biapiculate and warted ascospores; asexual morphs were assigned to various hyphomycetous genera including acremonium-like, *Cladobotryum*, *Mycogone*, papulaspora-like, *Stephanoma*, *Sepedonium* and verticillium-like. HELFER (1991) introduced the term "aurofusarin-group" to accommodate *Hypomyces* spp. having in common the production of aurofusarin, the molecule responsible for the red colour, either during the sexual or the asexual stage. The monophyly of this group was supported by subsequent phylogenetic analyses (POLDMAA *et al.*, 1999; POLDMAA, 2000; POLDMAA & SAMUELS, 2004). POLD-MAA (2011) and TAMM & POLDMAA (2013) revised *Hypomyces* spp. of the aurofusarin-group, introducing eight new tropical species and delimitating a group of thirteen monophyletic species within *Hypomyces*.



Fig. 1 – Hypomyces peltigericola in natural environment



0.05

Fig. 2 – Maximum likelihood phylogeny of *Hypomyces* species producing red pigments, based on ITS sequences, rooted on *H. aurantius*, which does not produce red pigments. Temperate species are highlighted in yellow and tropical species in blue.

Because of the typical crimson colour of ascomata and subiculum, the collection on *Peltigera canina* was assigned to the aurofusaringroup of the genus *Hypomyces*. This placement was confirmed by phylogenetic analyses of rDNA ITS sequences that revealed an isolated position of this collection among known species of this group (Fig. 2). BLAST search revealed only 93% similarity with the most similar sequences in GenBank. Besides the genetic distinctness, it is the first record of *Hypomyces* growing on a lichen. Based on this evidence a new species, *Hypomyces peltigericola*, is described below.

Materials and methods

The specimens were examined, sequenced and phylogenetically analysed using the methods described in LECHAT & FOURNIER (2015). To be consistent with previous studies, ascospore measurements include apiculi. The measurements given in parentheses correspond to the extreme values.

Taxonomy

Hypomyces peltigericola Lechat & Gardiennet, sp. nov. Fig. 3 MycoBank: MB819219

Diagnosis: Differs from known species of *Hypomyces* producing red pigments by long fusiform ascospores (29–)31–42(–46) × 5–6 μ m, asexual morph not changing colour in 3% KOH, conidia 0–1-septate and occurrence on a lichen.

Holotypus: FRANCE, Côte-d'Or, Salmaise, Ermitage Saint-Jean de Bonnevaux, on *Peltigera canina* occurring on *Salix caprea*, 21 Jun. 2016, *leg*. A. Gardiennet, AG16093 (LIP), ex-type culture CBS141848, rDNA ITS sequence GenBank KY088202.

Etymology: The specific epithet refers to the host, *Peltigera*.

Perithecia gregarious, semi-immersed in cottony, whitish, pale yellow to crimson subiculum which is composed of hyaline to pale purplish, thin-walled, septate hyphae, $4.5-6(-9) \mu m$ wide with some cells enlarged up to 12 μ m, scattered in small patches of 0.5–1.5 imes0.2-0.6 cm on the host surface. Perithecia subglobose to flask-shaped, 350-430(-460) µm high, 240-380 µm diam., bright red to crimson, turning purple in 3% KOH and yellow in lactic acid, with broadly conical, rounded, darker papilla 100–120 µm high, 120–150 µm wide at the base, composed of purplish red, globose, subglobose to clavate, thick-walled cells. Perithecia not collapsing when dry, with papilla appearing nearly black. Ascomatal wall 30-45 µm wide, composed of a single region of globose to ellipsoidal, thickwalled cells $15-25 \times 6-15 \mu m$ with wall 0.7-1.8 m thick, purplish red, becoming more flattened, thin-walled and hyaline toward the interior. Ascomatal wall in surface view composed of subangular, globose, subglobose to ellipsoidal thick-walled cells, up to 25 μ m in greatest dimension. Asci long cylindrical, 140–160(–170) × (6–)6.5– 8(-9) µm total length, shortly stipitate, apex thickened and with a pore, containing 8 uniseriate, partly overlapping ascospores. Ascospores long fusiform, equally 1-septate, finely verrucose, $(29-)31-42(-46) \times 5-6 \ \mu m$ including apiculi; apiculi strongly stained in cotton blue, conical, acute, straight to curved, 3-5.5 µm long, 2-3 um wide at base.

Cultural characteristics: Colony on PDA at 25°C, slow growing, reaching 40–50 mm diam. after two weeks, white to pale cream, not producing pigmentation in the medium, reverse pale buff in the centre, white to pale yellowish at the margin. No odour was detected. Aerial mycelium composed of sterile hyphal elements, 2.5–3.5 μ m in diam., smooth-walled, compacted towards the centre. Conidiophores arising at colony margin, indefinite in length, verticillately branched, hyaline, not changing colour in lactic acid or 3% KOH, 5.0–6.5 μ m diam., each branch ending in 1–3 conidiogenous cells. Conidiogenous cells simple, subulate, 30–60 μ m long, 4–5 μ m wide at base tapering to 2 μ m at tip, without collarette, producing 1–4 conidia joined by bases in short imbricate chains and forming "stellate heads". Conidia clavate, attenuated at the base to a narrow,



Fig. 3 – a-n: *Hypomyces peltigericola* (Holotype AG16093); a–b: Ascomata on the substratum; c: Close-up of a perithecium; d: Perithecium in water: e: Perithecium in 3% KOH; f: Perithecium in lactic acid; g: Vertical section of the apex of a perithecium; h: Longitudinal section of a perithecium; i: Lateral ascomatal wall in vertical section; j: Culture at two weeks; k–l: Conidia and conidiophores; m–n: Asci and ascospores.

slightly curved hilum, 0–1-septate, hyaline, smooth, (12–)15–25(–28) \times 5.5–8.5(–10) $\mu m.$ Chlamydospores not seen.

Asexual morph: cladobotryum-like.

Discussion

Morphology of the fungus and the ITS sequence leave no doubt that the collection on *Peltigera* represents a new species that belongs to the aurofusarin-group of Hypomyces/Cladobotryum. Evidence of the former statement results from a blast search resulting best matches of <94% sequence similarity. The latter is based on phylogenetic analysis (ML, Fig. 2), revealing its position inside this group. Analyses with different methods (Bayes, ML) and alternation of included taxa each yield a tree with different position of the proposed species. However, any of its alternative positions on these trees is supported (by BS or pp) (PÕLDMAA, pers. comm.). Because to date no species of Hypomyces occurring on lichens has been reported in the literature (LAWREY & DIEDERICH, 2015; ROUX, 2012), the new species H. peltigericola is proposed, in reference to its unusual host as well as morphological and genetic distinctness.

As defined by PÕLDMAA (2011), Hypomyces species belonging to the "aurofusarin-group" feature "red-pigmented perithecia and/or colonies in culture" and occur on basidiomycetes. Externally, H. peltigericola is morphologically roughly identical to most known species of the "aurofusarin-group", but it is distinguishable from them by the characteristics of the ascospores and conidia, its occurrence on a lichen and its asexual morph not changing colour in 3% KOH, which is unusual in the aurofusarin-group. Maximum likelihood phylogeny of H. peltigericola based on ITS sequences shows that the new species is nested in the clade "aurofusarin-group" on a sister branch of the subclade including the temperate species H. odoratus G.R.W. Arnold and H. rosellus (Alb. & Schwein.) Tul. & C. Tul. Hypomyces peltigericola primarily differs from H. odoratus and H. rosellus in having longer and more finely verrucose ascospores and 0–1-septate conidia. The asexual morph of H. odoratus is very common on various basidiomycetous hosts (almost exclusively agarics) in northern Europe (Põldmaa, 1999; TAMM & Põldmaa, 2013) and produces 1(-3)-septate conidia; its sexual morph is only known from ascomata obtained in culture featuring ascospores $25.8-34.4 \times 6.0-7.7 \ \mu m$, and a strong camphor-like odour is associated with the culture (AR-NOLD, 1964). The larger ascospores reported by ROGERSON & SAMUELS (1994) from neotropical collections referred to H. odoratus were said to belong to different species (PÕLDMAA, 1999, 2011). The widespread H. rosellus differs from H. peltigericola in having shorter ascospores $(20-)25-30(-38) \times (3-)4-5(-7.5) \ \mu m \ and \ (2-)3-septate \ conidia \ (Ro-$ GERSON & SAMUELS, 1993; PÕLDMAA, pers. comm.). In our phylogenetic analysis, the three temperate species of the aurofusarin-group cluster on a separate branch distinct from that on which tropical species cluster, supporting the distribution pattern already noted by POLD-MAA (2011). However, more gene regions need to be analyzed to get a robust phylogeny indicating relationships of the new species in more detail, which should hopefully be accomplished in the future (PÕLDMAA, pers. comm.).

Based on several observations on the same trunk over five years, H. peltigericola appears to parasitize exclusively the lichen and gradually spreads over thalli of Peltigera on the bark, higher and higher on the trunk. We did not notice the presence of usual hosts of Hypomyces like polypores or corticioid fungi on the trunk where H. peltigericola was found, neither did we notice the presence of the asexual morph associated with the sexual morph on the host. It was collected for the first time on Jan. 31, 2011 but, unfortunately, this collection could not be cultured. Two additional specimens were collected, one on Jan. 24, 2012 and another on Jan. 10, 2013, but neither of them was in culturable condition. Finally, the last collection from Jun. 21, 2016 (holotype), collected at 3 meters high on the trunk, was in good culturable condition and produced a cladobotryum-like asexual morph. Our successive observations most likely account for the evolution of the same population of *H. peltigericola* over time.

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