Pseudocosmospora hypoxylicola (*Nectriaceae*), a new species from the French Alps

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Abstract: *Pseudocosmospora hypoxylicola* is described and illustrated based on material occurring on stromata of *Hypoxylon fuscum* on *Alnus alnobetula* in France. The placement of this fungus in the genus *Pseudocosmospora* and its status as a distinct species are based on the study of its sexual and asexual morphs, colour of colony in culture and phylogenetic comparison of ITS1-5.8S-ITS2 and LSU sequences with those of cosmospora-like fungi having an acremonium-like asexual morph.
Keywords: Ascomycota, fungicolous *Nectriaceae*, *Hypocreales*, *Hypoxylon*, rDNA, taxonomy.

Résumé : *Pseudocosmospora hypoxylicola* est décrite et illustrée d'après du matériel récolté sur des stromas d'*Hypoxylon fuscum* sur *Alnus alnobetula* en France. Le placement de ce champignon dans le genre *Pseudocosmospora* et son statut d'espèce nouvelle reposent sur l'étude des stades sexué et asexué, la couleur de la colonie en culture et sur la comparaison phylogénétique des séquences ITS1-5.8S-ITS2 et LSU avec celles d'espèces de type cosmospora ayant une forme asexuée de type acremonium.
Mots-clés : ADN ribosomal, Ascomycota, Hypocréales, *Hypoxylon*, *Nectriaceae* fongicoles, taxinomie.

Introduction

In the course of a field survey of Ascomycota in the French Alps in August 2019, initiated by Ascomycete.org, a cosmospora-like species was collected on dead or effete stromata of *Hypoxylon fuscum* (Pers.) Fr. (*Hypoxylaceae*, *Xylariales*) on *Alnus alnobetula* (Ehrh.) K. Koch. This fungus was assigned to the *Nectriaceae* based on ascomata changing colour in 3% KOH and lactic acid. The new species described herein is morphologically similar to the known cosmospora-like species occurring on dead or effete stromata of *Diatrypaceae* and *Hypoxylon* as recently revised and delimited by Gräfenhan *et al.* (2011), Herrera *et al.* (2013; 2015), Lombard *et al.* (2015), and Lechat *et al.* (2019). Here we explain our morphological, cultural and phylogenetic results leading to the placement of this species in the genus *Pseudocosmospora* C.S. Herrera & P. Chaverri and discuss the features supporting the segregation of *P. hypoxylicola* from its relatives.

Material and methods

Dry specimens were rehydrated and examined using the method described by Rossman *et al.* (1999). Microscopic observations and measurements were made in water. The holotype is deposited in LIP herbarium (University of Lille, France). Cultures of 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 cultures 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 65ºC. 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_2O. PCR amplification was performed with the primers ITS1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) for ITS, while LR0R and LR5 (Vilgalys & Hester, 1990) were used to amplify the 28S nLSU region. PCR reactions were performed under a program consisting of a hot start at 95ºC for 5 min, followed by 35 cycles at 94ºC, 54ºC and 72ºC (45, 30 and 45 s respectively) and a final 72ºC step 10 min. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with primer ITS4. Chromatograms were checked searching for putative reading errors, and these were corrected. Phylogenetic 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 (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands).

Taxonomy

Pseudocosmospora hypoxylicola Lechat & J. Fourn., *sp. nov.* – Fig. 3 – MB 833853.

Diagnosis: Ascomata bright red, occurring on dead or effete stromata of *Hypoxylon fuscum*, non-stromatic, with verrucose ascospores (7.5–)8–9.5(–10) × 4–4.5(–5) µm; greyish white colony and acremonium-like asexual morph *in vitro*.

Holotype: France, Isère, Le Bourg-d'Oisans, RB du Lac du Lauvitel, 1505 m asl., on *Hypoxylon fuscum* on *Alnus alnobetula*, 29 Aug. 2019, *leg*. A. Mombert, CLL19020 (LIP), ITS and LSU GenBank sequences: MN886606 and MN885608.

Etymology: The epithet *hypoxylicola* refers to the host *Hypoxylon* and the Latin suffix *-cola* = inhabitor.

Ascomata gregarious, solitary to most often clustered in small to large groups, basally slightly immersed in host tissues, non-stromatic, subglobose to widely obpyriform 180–240 µm high, 170–220 µm wide (Me = 220 × 200 µm, n = 20), laterally pinched when dry, bright red, turning purple in 3% KOH, yellow in lactic acid, with a discoidal apex 80–110 µm diam, composed of clavate, thick-walled cells with orange wall, with a minute acute papilla 10 µm high, 20 µm diam., composed of cylindrical to slightly clavate cells with pale yellow wall. **Ascomatal surface** composed of cells of undefined shape forming *textura epidermoidea*. **Lateral ascomatal wall** in vertical section 20–30(–35) µm thick, of two regions: outer region 15–20(–25) µm thick, composed of subglobose to ellipsoidal, thick-walled cells, 6–12 × 5–8 µm, with orange wall 2–3 µm thick; inner region 10–15 µm thick, composed of ellipsoidal, elongate, hyaline cells 5–14 × 4–5 µm. **Asci** unitunicate, cylindrical, short-stipitate, 70–75 × 6–7 µm, apex flat to rounded with a ring-like apical thickening, containing 8 uniseriate ascospores, becoming multiseriate when mature; evanescent, narrowly moniliform paraphyses 2–2.5 µm diam, interspersed between asci. **Ascospores** ellipsoidal, (7.5–)8–10(–10.5) × 4–4.7(–5) µm (Me = 9.5 × 4.5 µm, n = 40), equally 1-septate, slightly constricted at septum, verrucose, hyaline, becoming pale yellowish brown when mature.

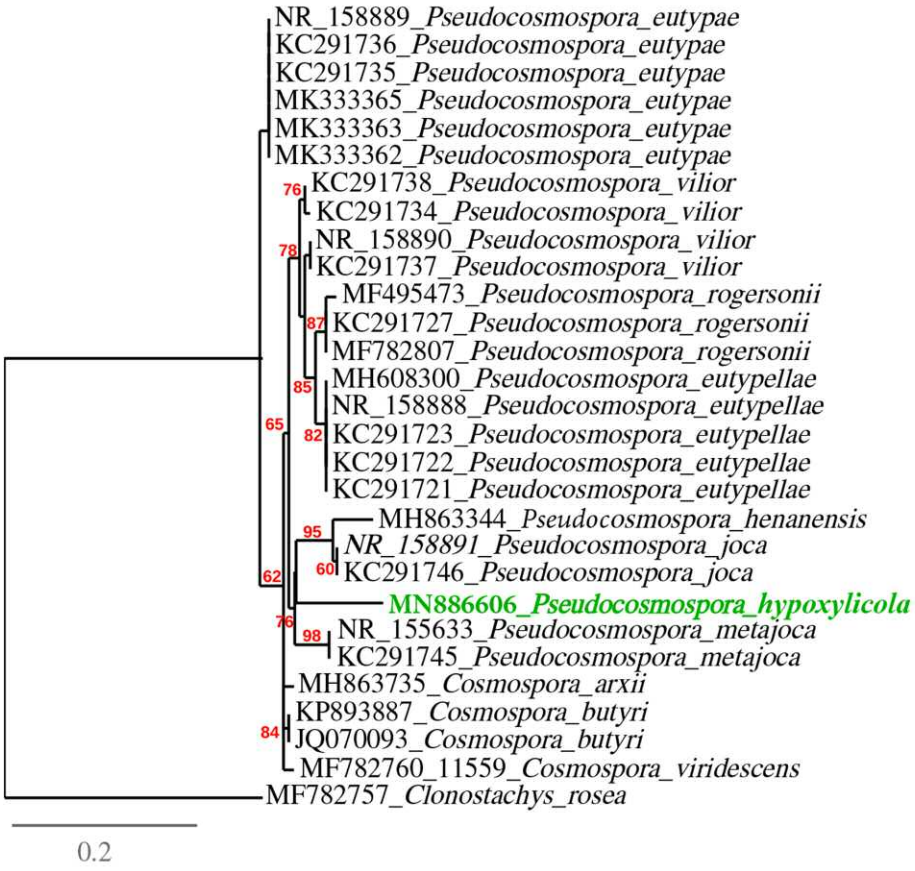

Fig. 1 – Maximum likelihood phylogeny (-lnL = 1550.92269) of *Pseudocosmospora* spp. inferred by PhyML 3.0, model HKy85 from a 610 bp matrix of ITS sequences, rooted with *Clonostachys rosea*.

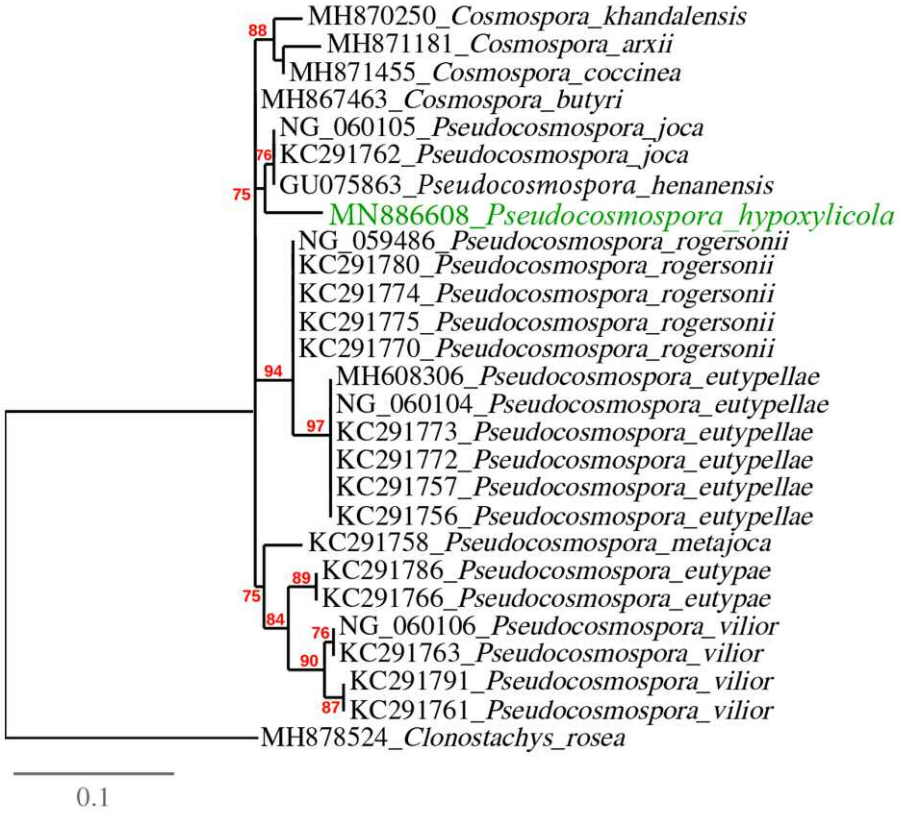

Fig. 2 – Maximum likelihood phylogeny (-lnL = 1765.86433) of *Pseudocosmospora* spp. inferred by PhyML 3.0, model HKY85 from a 825 bp matrix, based on LSU sequences, rooted with *Clonostachys rosea*.

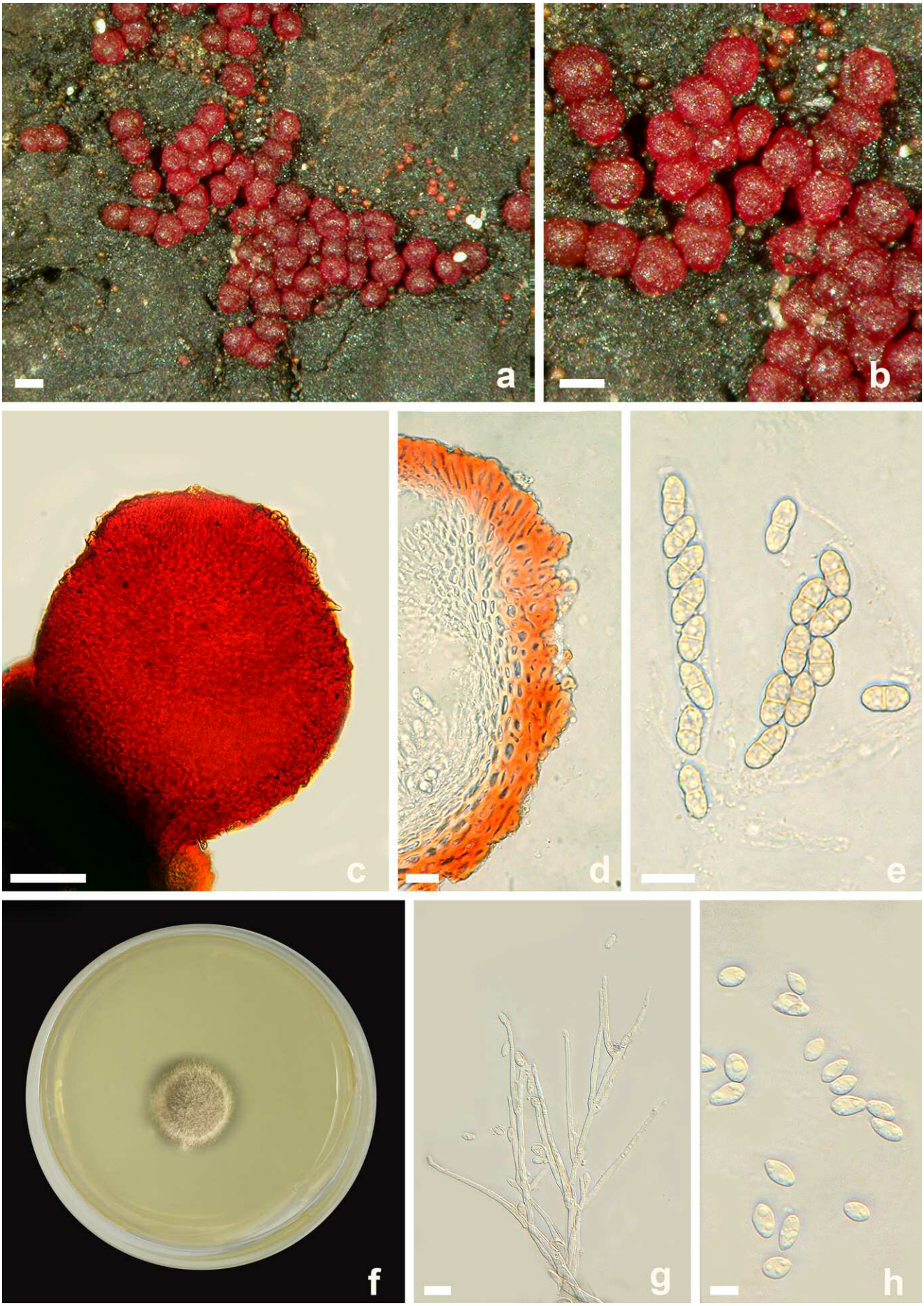

Fig. 3 – a-h: *Pseudocosmospora hypoxylicola* (CLL19020 Holotype); a-b: Ascomata in natural environment; c: Close-up of an ascoma in side view; d: Vertical section through lateral ascomatal wall; e: Asci and ascospores; f: Culture at two weeks (Petri dish 5 cm diam); g: Conidiophore, phialides and conidia from culture; h: Conidia from culture. All microscopic illustrations in water. Scale bars: a-b = 200 µm; c = 50 µm; d-e = 10 µm; g-h = 5 µm.

Asexual morph in nature not observed.

Cultural characteristics: colony 0.5–1.5 cm diam after two weeks, aerial mycelium floccose, pale greyish in centre, white and sporulating at margin, producing an acremonium-like asexual morph and diffusing a pale grey coloration in medium immediately around colony. Conidiophores simple, branched to verticillate, 60–150 µm long, 3–4 µm diam, flexuous, smooth, arising from smooth, septate hyphae 3.5–4 µm diam; Conidiogenous cells monophialidic, terminal, apically subulate, with a minutely flared collarette, 25–52 µm long, 2–3 µm diam at base, 1.5–2 µm diam at apex. Conidia ellipsoidal, 5–8 × 3–5 µm, rounded at ends or rounded at tip and attenuated at base, smooth, with or without a visible abscission scar.

Results and discussion

Fungicolous *Nectriaceae* formerly accommodated in *Cosmospora sensu* Rossman *et al.* (1999) were shown to be para/polyphyletic by Gräfenhan *et al*. (2011), who segregated *Cosmospora sensu stricto* typified by *C. coccinea* Rabenh. for species with acremonium-like asexual morphs. Based on a good correlation between phylogenetic results and the colours of the colonies in culture on PDA, *Pseudocosmospora* typified by *P. eutypellae* C.S. Herrera & P. Chaverri was introduced for ten cosmospora-like species occurring mostly on stromata of *Diatrypaceae*, having acremonium-like asexual morphs and producing rosy-buff, pale-luteous to orange or salmon-pink colonies (Herrera *et al*., 2013). In contrast, *Cosmospora* was restricted to species growing on polypores or *Xylariaceae*, forming green colonies in culture with *Cosmospora arxii* (Gams) Gräfenhan & Schroers being an exception in having a salmon to saffron colony (Herrera *et al*., 2015).

Our fungus exhibits a greyish white colony in culture and an acremonium-like asexual morph and appears to fit better in *Pseudocosmospora*, which is clearly supported by our phylogenetic analyses of ITS and LSU sequences (Figs. 1 and 2). When compared to the known species of *Pseudocosmospora*, it is set apart by an almost colourless colony in culture and occurrence on *Hypoxylon* Bull. Moreover, phylogenetic analyses show that it has respectively 94 and 98% similarity of its ITS and LSU sequences with *P. joca* (Samuels) C.S. Herrera & P. Chaverri, and 95 and 98.5% with *P. metajoca* C.S. Herrera & P. Chaverri, the most closely related species (Figs. 1 and 2). *Pseudocosmospora joca*, known from South America on *Biscogniauxia capnodes* Y.-M. Ju & J.D. Rogers, shares with the new species occurrence on a xylariaceous host, but differs by a salmon-pink to orange colony and significantly larger ascospores 12.8 × 7 µm on average; *P. metajoca*, known from New Zealand, has ascospores in the same size range (8.9 × 4.3 µm on average) but differs by its occurrence on *Eutypa* sp. (*Diatrypaceae*) and a salmon-pink colony in culture. *Pseudocosmospora henanensis* (Y. Nong & W.-Y. Zhuang) W.-Y. Zhuang & Z.Q. Zeng appears phylogenetically closely related to *P. joca*, possibly conspecific based on a similar ascospore size range (10.7–)11.2–13.4 × 6.4–7.5 µm (Nong & Zhuang, 2005). It thus clearly differs from *P. hypoxylicola* by its larger ascospores and association with a "beaked ascomycete".

The combination of morphological and cultural characters of our fungus and phylogenetic analyses shows that it is an undescribed species and leads us to propose *Pseudocosmospora hypoxylicola* Lechat & J. Fourn. as a new species.

Whenever a reliable identification of the host can be made, it more or less strongly suggests host-specificity of cosmospora-like fungi for a fungal species or genus (Herrera *et al*., 2013; 2015). Cosmospora-like fungi are rarely reported from *Hypoxylon*. Most *Pseudocosmospora* species occur on *Diatrypaceae* and *P. hypoxylicola* is the first species known to occur on *Hypoxylon*; *C. arxii* is the only *Cosmospora* species known to be associated with a *Hypoxylon*, occurring on both *H. fragiforme* (Pers.): J. Kickx fil. and *H. howeanum* Peck (Herrera *et al*., 2015).

The host of *P. hypoxylicola* is the common and widespread *H. fuscum*, on which, before this recent collection on *Alnus alnobetula*, a cosmospora-like fungus was never encountered on stromata collected in lowlands, mainly on *Alnus glutinosa* (L.) Gaertn., *Carpinus betulus* L. and *Corylus avellana* L. One could have expected that such a fairly conspicuous mycoparasite would have not remained overlooked for long, which implies two different interpretations until more collections of *P. hypoxylicola* become available.

Hypoxylon fuscum is suspected to be a complex of closely related species, occurring on several members of *Betulaceae* Gray and exhibiting a wide range of ascospore dimensions. An attempt to demonstrate a statistical correlation between host and ascospore size was inconclusive because of a large overlap of ascospore dimension between collections from various hosts. However, it was shown that ascospores of collections of *H. fuscum* on *Alnus alnobetula* [as *A. viridis* (Chaix. DC.)] were larger than those on other hosts and significantly larger than those on *Carpinus betulus* (Petrini *et al*., 1987). The peculiar ecology of *Alnus alnobetula*, ranging from subalpine to alpine zone, suggests a possible speciation process within the *H. fuscum* complex, which might involve a specific mycoparasite. If so, an active search on stromata of *H. fuscum* on *A. alnobetula* should lead to the discovery of additional collections of *P. hypoxylicola*.

On the other hand, this single collection on which we base *P. hypoxylicola* may just represent the fortuitous occurrence of a still unknown or misidentified *Pseudocosmospora* whose host range remains to be investigated. In both cases, it can be hoped that the present work will contribute to prompt further observations of cosmospora-like parasites on stromata of pyrenomycetes in subalpine and alpine zone.

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