Some *Annulohypoxylon* spp. (*Xylariaceae*) from French Guiana, including three new species

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Abstract: This survey deals with *Annulohypoxylon* spp. collected in two French Guiana locations during two weeks in June 2012. Two species, *A. leptascum* var. *macrosporum* and *A. stygium*, previously reported, were collected. In addition, we collected *A. moriforme s.l.*, *A. purpureopigmentum* (first collected in Brazil), and three new species, *A. fulvum*, *A. nouraguense* and *A. subnitens*. All species are described and illustrated; a dichotomous key is provided. In conclusion, some problems in description of morphological features of *Annulohypoxylon*, especially the ostiolar discs, are discussed.
Keywords: Ascomycota, *Xylariales*, *Hypoxyloideae*, Nouragues, Paracou, pyrenomycetes, saproxylic, taxonomy, tropical mycology.

Résumé : cette étude concerne les espèces d'*Annulohypoxylon* récoltées en Guyane dans deux localités différentes pendant deux semaines en juin 2012. Deux espèces déjà connues de Guyane, *A. leptascum* var. *macrosporum* et *A. stygium*, furent récoltées à nouveau. De plus, nous signalons la présence d'*A. moriforme s.l.*, *A. purpureopigmentum* (connu initialement du Brésil) et trois espèces nouvelles, à savoir : *A. fulvum*, *A. nouraguense* et *A. subnitens*. Toutes ces espèces sont décrites et illustrées, et une clé dichotomique d'identification est proposée. En conclusion, certaines difficultés rencontrées dans la description des caractères morphologiques des *Annulohypoxylon*, en particulier celle des disques ostiolaires, sont discutées.
Mots-clés : Ascomycota, *Xylariales*, *Hypoxyloideae*, mycologie tropicale, Nouragues, Paracou, pyrénomycètes, saproxyliques, taxinomie.

Introduction

Annulohypoxylon Y.-M. Ju, J.D. Rogers & H.-M. Hsieh is morphologically characterized and separated from *Hypoxylon* Bull. by usually strongly carbonaceous stromata, conic-papillate ostioles surrounded by a discoid area resulting from a dehiscence of stromatal tissue and ascospores with a smooth perispore bearing a thickening at ca. ⅓ ascospore length when dehiscent in 10% KOH. The asexual morph of *Annulohypoxylon* is occasionally found on the natural substrate or more regularly obtained in culture on artificial media; it is like in *Hypoxylon*, hyphomycetous and referable to nodulisporium-like as defined by Ju & Rogers (1996). Another important feature common to both genera is the presence of stromatal granules yielding pigments in 10% KOH (Ju & Rogers, 1996).

Theissen (1908) was the first to recognize a relationship between ten tropical representatives of *Hypoxylon* with papillate ostioles encircled by a disc, forming an aggregate around *H. annulatum* (Schwein.) Mont. A section *Annulata* of *Hypoxylon* was later resumed by Miller (1961), including eight species and three varieties and by Ju & Rogers (1996), including twenty-two species and seven varieties. The great progress in understanding the section *Annulata* of *Hypoxylon* made by Ju & Rogers (1996) resulted primarily from their use of KOH-extractable pigments and asexual morphs obtained in culture as differential characters, coupled with thorough observations on the stromatal morphology and on the process of formation of the ostiolar discs. They unravelled the confusion resulting from the misinterpretation of the names *H. annulatum* (Schwein. : Fr.) Mont. and *H. truncatum* (Schwein.) J.H. Miller, that had been applied in the past to many unrelated species, by clearly delimiting these two species. They also included in their section *Annulata* those temperate species with highly reduced ostiolar discs like *H. cohaerens* (Pers.) Fr. and *H. multiforme* (Fr.) Fr. and their varieties, that were previously accommodated in Miller's section *Papillata* (Miller, 1961). Further additions were made by Ju & Rogers (1999), Whalley *et al.* (2000), Ju *et al.* (2004), raising the number of accepted epithets in *Hypoxylon* section *Annulata* to thirty-three.

While a former phylogenetic study based on ITS sequences had given inconclusive results as to the delimitation of both genera (Sánchez-Ballesteros *et al.*, 2000), the genus *Annulohypoxylon* was reliably segregated from *Hypoxylon* and from other related hypoxyloid genera based on phylogenetic analyses of α-actin and β-tubulin sequences (Hsieh *et al.*, 2005).

Following the erection of *Annulohypoxylon* by Hsieh *et al.* (2005), a new taxon related to *A. leptascum* var. *macrosporum* (Y.-M. Ju & J.D. Rogers) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh was described from South Africa, as *A. leucadendri* Marincowitz, M.J. Wingf. & Crous (Marincowitz *et al.*, 2008). Subsequently, Hladki & Romero (2009a) introduced the new variety *A. moriforme* var. *macrosporum* Hladki & A.I. Romero and after examination of the holotypes housed in LPS, recombined *H. apiahynum* Speg. and *H. subeffusum* Speg. with *Annulohypoxylon* as *A. apiahynum* (Speg.) Hladki & A.I. Romero and *A. subeffusum* (Speg.) Hladki & A.I. Romero respectively (Hladki & Romero, 2009b). One year later, Fournier *et al.* (2010) and Pereira *et al.* (2010) introduced respectively two new species of *Annulohypoxylon* from Thailand and four new species from Brazil. More recently, *A. orientale* Lar.N. Vassiljeva & S.L. Stephenson was described from southern Russian Far East (Vasilyeva & Stephenson, 2014).

Additional contributions to tropical records of *Annulohypoxylon* spp. include those of San Martín *et al.* (1999) for Mexico, Ju & Rogers (1999) for Taiwan, Suwannasai *et al.* (2005) for Thailand, Hladki & Romero (2009a) for Argentina and Rogers & Ju (2012) for Hawaiian Islands. A recent phylogenetic survey of *Annulohypoxylon* spp. from northeast Thailand suggests the presence of seven species morphologically and genetically different from known species, without introducing taxonomic novelties (Makornwattana *et al.*, 2013).

One of the two species so far reported from French Guiana is *A. leptascum* var. *macrosporum*, based on two collections including the holotype (Ju & Rogers, 1996). The other report of a species of *Annulohypoxylon* in French Guiana found in literature was that reported by Miller (1961), based on a collection made by Leprieur, as *Hypoxylon annulatum* var. *depressum* Fr., that Miller (1961) equated to *H. stygium* (Lév.) Sacc. As *A. stygium* (Lév.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh is a well-characterized species that is widespread in tropics and as its concept did not change since Miller's monograph we regard this record as reliable.

The present contribution aims at presenting the collections of *Annulohypoxylon* made during a short field trip in French Guiana in June 2012 (Fournier & Lechat, 2015). This genus was not the best represented in terms of frequency (28 samples) but most of collections turned out to be difficult to unequivocally equate to known species. Thorough morphological studies revealed the presence of three known species including the widespread pantropical *A. stygium* and two rarely recorded taxa viz.: *A. leptascum* var. *macrosporum* and *A. purpureopigmentum* Jad. Pereira, J.D. Rogers & J.L. Bezerra, respectively only known from French Guiana and from Bra-

zil (Ju & Rogers, 1996; Pereira *et al.*, 2010). Five collections matching the pantropical *A. moriforme* (Henn.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh as defined by Ju & Rogers (1996) were found to morphologically deviate from each other, although collected within the same restricted area. They are tentatively referred to *A. moriforme* until the taxonomic status of this species is more clearly delimited. Three new species are introduced to accommodate collections significantly different from known species. *Annulohypoxylon fulvum* sp. nov., known from two collections from Sinnamary in lowland rainforest, is diagnosed by yellow brown stromatal surface and KOH-extractable pigments, combined with relatively large dark brown ascospores. *Annulohypoxylon nouraguense* sp. nov., based on nine collections from Nouragues natural reserve in primary rainforest, appears to deviate from *A. purpureonitens* (Y.-M. Ju & J.D. Rogers) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh in KOH-extractable pigments and ascospore morphology after comparison with the isotype of *A. purpureonitens*. *Annulohypoxylon subnitens* sp. nov., based on a single collection from the Nouragues natural reserve, is primarily distinguished from its closest relative *A. nitens* (Ces.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh by different stromatal granules and different KOH-extractable pigments.

A dichotomous identification key to the species of *Annulohypoxylon* known from French Guiana is proposed. Comments on the issues encountered when identifying *Annulohypoxylon* spp. on morphological grounds are presented in conclusion and some suggestions are made to try to remedy them.

Material and methods

Material and methods, including colour charts, follow Fournier & Lechat (2015).

Taxonomy

Annulohypoxylon fulvum J. Fourn. & Lechat, *sp. nov.* – MycoBank MB815537 – Plates 2–3

Diagnosis: Differs from other *Annulohypoxylon* spp. by the combination of yellow-brown stromatal surface, isabelline to fawn KOH-extractable pigments and dark brown to blackish brown ascospores averaging 10.6 × 5 µm.

Holotype: French Guiana: Sinnamary, Saint-Elie, Arbocel plot, lowland rainforest, dead corticated branch on the ground, 26 Jun. 2012, *leg*. J. Fournier, GYJF 12214 (LIP).

Etymology: From Latin *fulvus* = fawn, for the unusual yellow-brown colour of the stromata and that of their KOH-extractable pigments.

Stromata effused to effused-pulvinate, with strongly exposed to almost perithecioid contours, 5–35 mm long × 5–20 mm wide × 0.7–0.85 mm thick, with fawn (87, oac743) sterile primordial zones at margins; outermost coating fawn (87, oac743), gradually fading at maturity, leaving a dull black and roughened squamulose surface; texture weakly carbonaceous, with subsurface orange brown, mainly composed of dull olivaceous yellow granules forming a thick layer above and around the perithecia, releasing isabelline (65, oac820) to fawn (87, oac743) KOH-extractable pigments within 1 min, slightly darkening to greyish sepia (106, oac640) upon prolonged incubation; subperithecial tissue inconspicuous to 0.35 mm thick, blackish brown. **Perithecia** spherical, 0.45–0.5 mm diam. **Ostioles** coarsely papillate, encircled with a flattened to slightly convex *truncatum*-type disc 0.25–0.35 mm diam with notched edges.

Asci cylindrical, with eight uniseriate ascospores, 115–135 µm total length, the spore-bearing parts 74–94 × 6–7.5 µm, the stipes 34–45 (–68 µm) long, with a discoid, slightly wedge-shaped apical apparatus 0.8–1.2 × 2.3–3 µm, bluing in Melzer's reagent. **Ascospores** (9.5) 10–11.2 (–11.9) × (4.3–) 4.6–5.4 (–5.8) µm, Q = (1.9–) 2–2.3 (–2.6); N = 70 (Me = 10.6 × 5 µm; Qe = 2.1), ellipsoid-inequilateral with narrowly rounded ends, dark brown to blackish brown, with a conspicuous straight germ slit spore-length; perispore dehiscent in 10% KOH, smooth, with a thickening on the dorsal side at ca. ⅓ spore length; epispore smooth.

Asexual morph on the natural substrate: Present at margins of young and mature stromata, forming loose fawn (87, oac743) tufts; conidiophores upright with a stout brown to dark brown axis 0.35–0.6 mm high × 8–10 µm wide at base, coarsely roughened; conidiogenous structure nodulisporium-like, with conidiogenous cells 9–18 × 2.8–4.5 µm, finely roughened, pale brown, at times flattened and diverticulate, turning greenish in 3% KOH; conidia pale brown, smooth, ellipsoid, 4.5–5.5 × 2.2–2.7 µm.

Other specimen examined: French Guiana: Sinnamary, Saint-Elie, Arbocel plot, lowland rainforest, dead corticated branch on the ground, 26 Jun. 2012, *leg*. J. Fournier, GYJF 12215 (LIP, paratype).

Dichotomous key to *Annulohypoxylon* spp. known from French Guiana

1. KOH-extractable pigments inconspicuous, or olivaceous or green **2**
1. KOH-extractable pigments purple **6**
2. KOH-extractable pigments inconspicuous to faintly pale olivaceous grey ..... ***A. subnitens*** sp. nov.
2. KOH-extractable pigments conspicuous, dense olivaceous or green **3**
3. KOH-extractable pigments green to dark green **4**
3. KOH-extractable pigments olivaceous brown to greenish olivaceous **5**
4. Ostiolar discs 0.15–0.2 mm diam; ascospores 5–6 × 2.2–2.5 µm with long inconspicuous germ slit on the ventral side ..... ***A. stygium***
4. Ostiolar discs 0.35–0.4 mm diam; ascospores 21–25 × 4.8–5.8 µm with short germ slit originating from one end or inconspicuous ..... ***A. leptascum* var. *macrosporum***
5. Stromatal surface yellow brown; KOH-extractable pigments fawn; ascospores dark brown to blackish brown, 10–11 × 4.6–5.4 µm ..... ***A. fulvum*** sp. nov.
5. Stromatal surface olivaceous brown to blackish; KOH-extractable pigments greenish olivaceous; ascospores brown, 7–8.5 × 3–3.6 µm ..... ***A. moriforme*** *s. l.*
6. Stromata effused-pulvinate with fugacious dark vinaceous coating and shiny black subsurface; KOH-extractable pigments vinaceous grey to vinaceous purple, turning hazel ..... ***A. nouraguense*** sp. nov.
6. Stromata glomerate to pulvinate with umber to blackish brown, persistent, matt coating; KOH-extractable pigments dense livid purple, stable ..... ***A. purpureopigmentum***

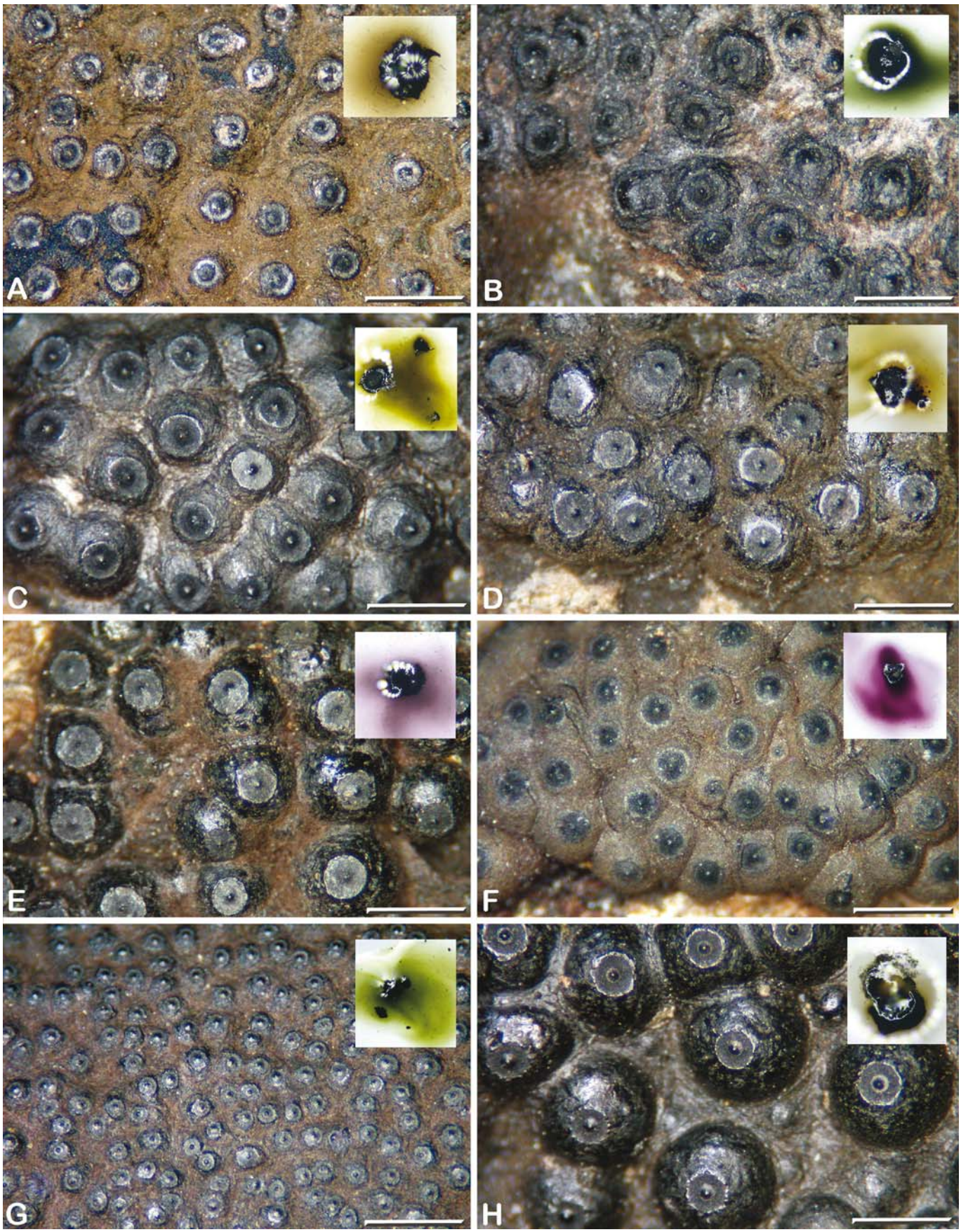

Plate 1 – Comparison of stromatal surfaces at the same scale and KOH-extractable pigments of *Annulohypoxylon* spp. known from French Guiana

A: *A. fulvum* GYJF 12214; B: *A. leptascum* var. *macrosporum* GYJF 12175; C: *A.* cf. *moriforme* GYJF 12181; D: *A.* cf. *moriforme* GYJF 12204; E: *A. nouraguense* GYJF 12057; F: *A. purpureopigmentum* GYJF 12178; G: *A. stygium* GYJF 12136; H: *A. subnitens* GYJF 12106. Scale bars = 1 mm.

Known distribution: French Guiana.

Discussion: This distinctive *Annulohypoxylon* is characterized by yellow-brown stromata with often strongly exposed to almost free perithecial contours, weakly carbonaceous texture, fairly small ostiolar discs that are sometimes slightly convex and have strongly notched edges, pale olivaceous brown KOH-extractable pigments and relatively large dark brown ascospores with a conspicuous germ slit. This combination of characters does not fit any known species in that genus and thus it is described as new.

It recalls *A. elevatidiscum* (Y.-M. Ju, J.D. Rogers & H.-M. Hsieh) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh, known from Taiwan, in having rather small ostiolar discs that are sometimes convex, a most unusual feature in *Annulohypoxylon* (Ju *et al*., 2004). However, *A. elevatidiscum* differs in having dull black stromata, greenish olivaceous KOH-extractable pigments, asci with a smaller apical apparatus faintly bluing or not bluing in Melzer's reagent and paler brown ascospores 8–10 × 3.5–4.5 µm.

While most of *Annulohypoxylon* spp. yield green or olivaceous green pigments in 10% KOH, two species feature pale olivaceous brown KOH-extractable pigments similar to those of *A. fulvum*, viz.: *A. bahnphadengense* J. Fourn. & M. Stadler and *A. moriforme* var. *microdiscum* (Y.-M. Ju & J.D. Rogers) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh. The former, so far known from Thailand only (Fournier *et al*., 2010), primarily differs from *A. fulvum* by a dark vinaceous outermost coating and pale brown smaller ascospores 6.5– 8.4 × 3–3.6 µm. The latter, known from Taiwan (Ju & Rogers, 1996) and from Martinique (JF, unpublished data) differs by a dark brown outermost coating, much smaller ostiolar discs 0.1–0.2 mm diam and narrowly ellipsoid medium brown ascospores 9.5–11.5 × 3.6–4.8 µm. A further species described from Malaysia, *A. gombakense* (M.A. Whalley, Y.-M. Ju, J.D. Rogers & A.J.S. Whalley) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh, resembles *A. fulvum* by isabelline KOH-extractable pigments and relatively large brown to dark brown ascospores (Whalley *et al*., 2000). It clearly differs from *A. fulvum* by host affiliation to bamboo, a reddish brown surface, larger perithecia 0.7–0.9 mm diam, smaller ostiolar discs 0.2 mm diam and larger ascospores 13–15 × 5–6 µm.

The pantropical and widespread *A. moriforme* (Henn.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh should be likewise considered because its young stromata frequently have olivaceous tones on surface but it does not feature the typical long persistent yellow-brown coating of *A. fulvum*, its KOH-extractable pigments are greenish olivaceous to dull green and its ascospores are paler brown and significantly smaller 6–9 × 2.5–4 µm. The dark brown ascospores of *A. fulvum* make a good differential character since the ascospores of most *Annulohypoxylon* spp. with effused stromata are pale brown to brown and relatively narrower.

Annulohypoxylon leptascum Speg. **var. *macrosporum*** (Y.-M. Ju & J.D. Rogers) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh, *Mycologia*, 97(4): 859 (2005). Plate 4

Stromata effused-pulvinate, with inconspicuous perithecial contours, 10–25 mm long × 8–22 mm wide ×1.2–1.9 mm thick; surface tan to silvery grey, matt, slightly roughened by the ostioles and the ostiolar discs; subsurface carbonaceous, brittle, extending downwards as a crust completely encasing the perithecia, composed of abundant yellowish brown waxy granules in a carbonaceous matrix, releasing dark green (21, oac82) KOH-extractable pigments, becoming slightly more blackish after 20 min incubation; subperithecial tissue 0.25–0.85 mm thick, dark greyish brown with black, vertically oriented carbonaceous streaks. **Perithecia** obovoid to tubular, 0.7–1 × 0.3–0.5 mm. **Ostioles** coarsely papillate, encircled with a slightly convex disc 0.35–0.4 mm diam *truncatum*-type with strongly notched edges.

Asci cylindrical, with eight uniseriate overlapping ascospores, frequently somewhat biseriate in the upper third, 125–160 µm total length, the spore-bearing parts 100–135 × 6.5–7.5 (–11) µm, the stipes 22–35 µm long, with a cuboid to cylindrical apical apparatus 3–3.5 × 2.4–2.7 µm, bluing in Melzer's reagent. **Paraphyses** filiform, 2.5–4.5 µm wide, simple to rarely ramified, abundant, embedded in mucilage. **Ascospores** (20.6–) 21.6–24.6 (–31.2) × (4.6–) 4.8–5.8 (–5.9) µm, Q = (3.7–) 4–4.8 (–5.8); N = 60 (Me = 23.4 × 5.3 µm; Qe = 4.4), fusoid-inequilateral, pale yellowish brown, heteropolar, with one end narrowly rounded and the other end more broadly rounded, the upper four to five ascospores oriented with the attenuated end downwards, the lower three to four ascospores oriented upside down, lacking a germ slit visible by LM but with a small blackish spot located towards the most narrowly rounded end on the flattened side, visible in PVA-lactophenol; perispore indehiscent in 10% KOH; epispore smooth.

Asexual morph on the natural substrate not observed.

Specimens examined: *Annulohypoxylon leptascum*: Guadeloupe: Saint-Claude, Matouba, Victor Hugues trail, rainforest, on bark, 4 Sept. 2005, *leg*. C. Lechat, CLL 5340 (LIP). *Annulohypoxylon leptascum* var. *macrosporum*: French Guiana: Sinnamary, Paracou, CIRAD field station, Guyaflux plot, lowland rainforest, on bark of a dead trunk lying on the ground, 24 Jun. 2012, *leg*. J. Fournier, GYJF 12175 (LIP).

Known distribution: French Guiana.

Discussion: *Annulohypoxylon leptascum* var. *macrosporum* was recognized as a variety of *A. leptascum* primarily based on its much larger ascospores 19.5–23.5 × 4.5–6.5 µm vs. 7.5–13 × 3–4 µm (Ju & Rogers, 1996, in *Hypoxylon* section *Annulata*). The typical variety is well-characterized by pulvinate carbonaceous stromata with inconspicuous perithecial contours, ostiolars discs 0.2–0.3 mm diam, green KOH-extractable pigments and pale brown, fusoid ascospores with a short germ slit originating from one end and a perispore indehiscent in 10% KOH. Besides its larger ascospores, the variety *macrosporum* also features larger ostiolar discs and larger asci with larger apical apparati. It is known from only two collections from French Guiana and our collection conforms well to the protologue, just deviating in two respects, viz.: The ascal apical apparati are not discoid but cuboid to cylindrical and a germ slit could not be detected, even after mounting the ascospores in PVA-lactophenol. Instead, ascospores observed in this medium exhibit a small blackish spot located next to the attenuated end, on the ventral side. Nothing suggests it might be a germination site and it rather looks like a local thickening of the ascospore wall, recalling the dark thickening typically observed on dehiscent perispores of *Annulohypoxylon*. In the specimen of *A. leptascum* from Guadeloupe examined during this survey for comparison, the observation of ascospores germ slits in water was inconclusive but after mounting in PVA-lactophenol, it became possible to make out short, light-coloured germ slits originating from the more attenuated end of ascospores, at the centre of a more pigmented area reminiscent of the blackish spots present in the variety *macrosporum*.

The pale greyish brown fusoid ascospores with perispore indehiscent in 10% KOH and with narrowly rounded ends and a short germ slit originating from one end are diagnostic within the genus *Annulohypoxylon* since they only occur in *A. leptascum*, its variety *macrosporum* and *A. urceolatum* (Rehm) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh. The latter, known from Asia, primarily differs by vinaceous purple KOH-extractable pigments. Large pale brown fusoid inequilateral ascospores with perispore indehiscent in 10% KOH are likewise encountered in *A. thouarsianum* (Lév.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh and its variety *A. thouarsianum* var. *macrosporum* (San Martín, Y.-M. Ju & J.D. Rogers) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh. Both feature ascospores with spore-length germ slit on the ventral side and are easily recognized by the hemispherical to spherical shape of their stromata up to 3 cm thick (Ju & Rogers, 1996). Interestingly, *A. urceolatum* appeared nested on the same branch as *A. thouarsianum* and

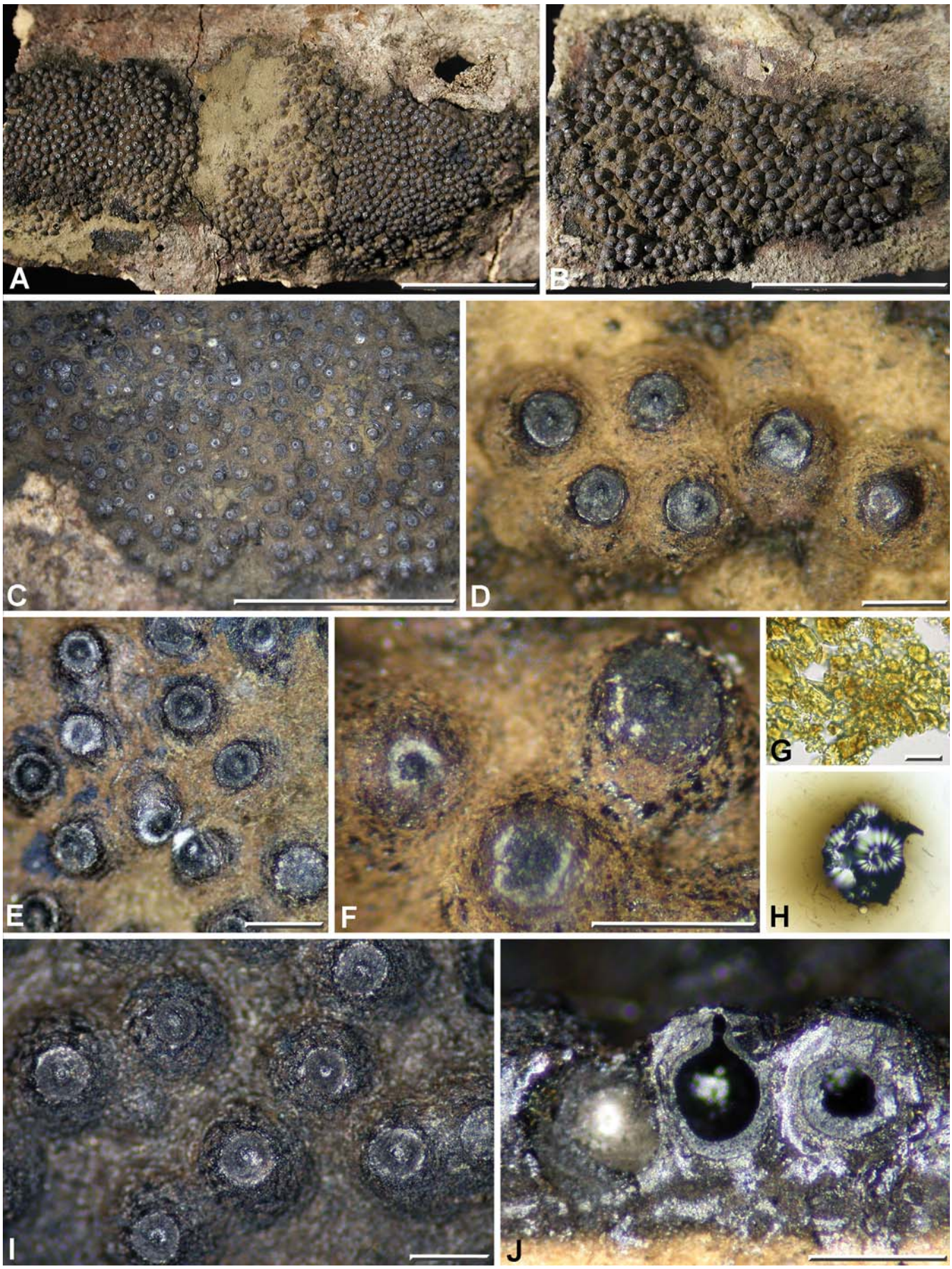

Plate 2 – *Annulohypoxylon fulvum*

A-H, J: Holotype GYJF 12214; I: Paratype GYJF 12215. A-C: Effused stromata on bark, with variously exposed perithecial contours; D, E: Stromatal surface in close-up showing the ostiolar discs and the yellow-brown coating; F: Ostiolar discs in close-up at three different stages of development showing the discs gradually flaking off (truncatum-type); G: Stromatal waxy granules in water; H: KOH-extractable pigments after 1 min incubation; I: Surface of a weathered stroma lacking the outermost yellow-brown coating; J: Stroma in vertical section showing the perithecia and the subperithecial tissue. Scale bars: A, B = 10 mm; C = 5 mm; D-F, I, J = 0.5 mm; G = 10 µm.

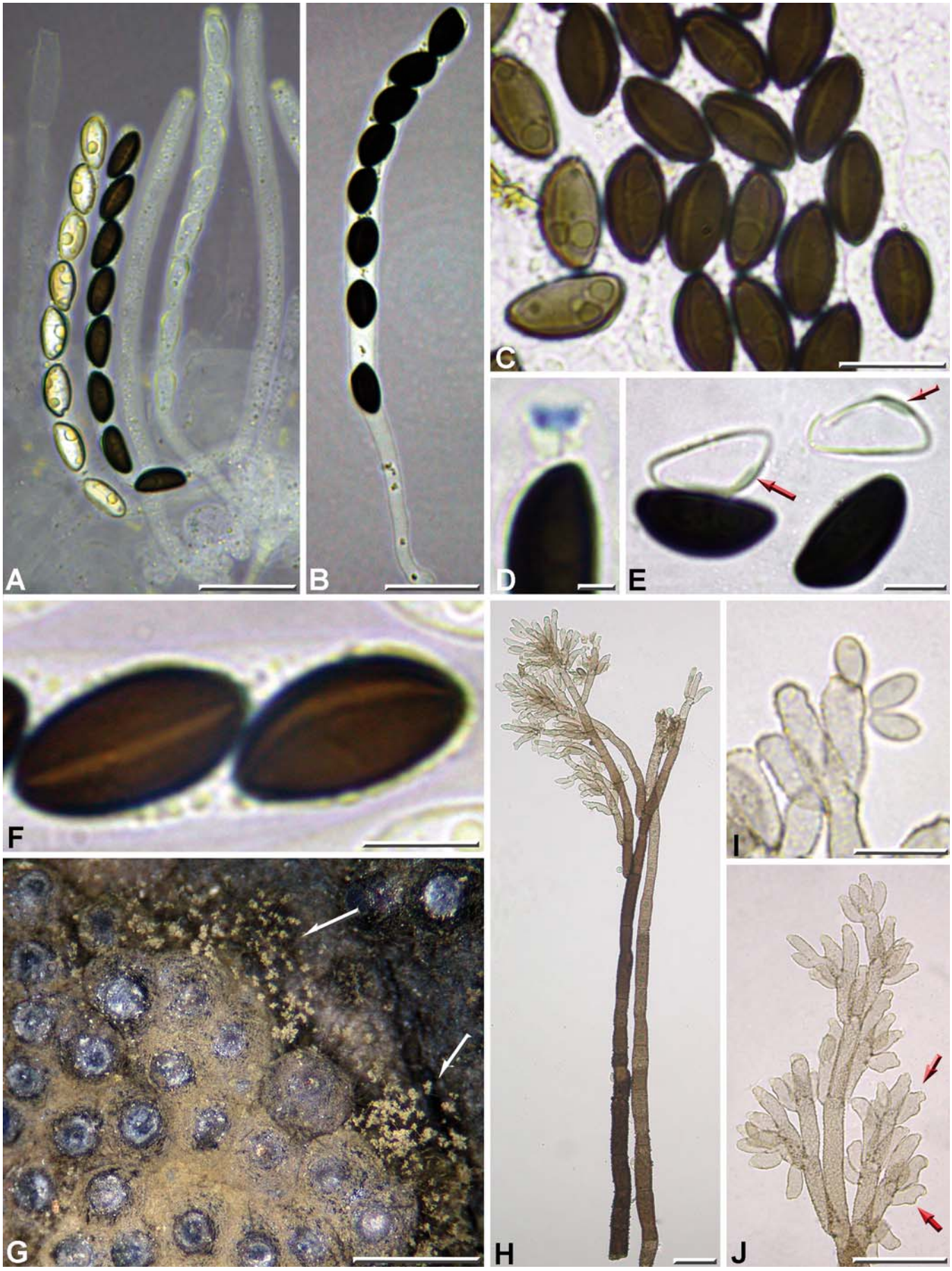

Plate 3 – *Annulohypoxylon fulvum*

Holotype GYJF 12214. A, B: Immature and mature asci in black Pelikan ink; C: Ascospores in water; D: Ascal apical apparatus in Melzer's reagent; E: Ascospores in 10% KOH showing the dehiscent perispore with a thickening on the dorsal side (arrows); F: Two ascospores in black Pelikan ink showing the germ slit; G: Tufts of the asexual morph (arrows) at the margin of a mature stroma; H: Conidiophores in 1 % SDS; I: Conidiogenous cells with conidia, in 1 % SDS; J: Apex of a conidiophore with nodulisporium-like conidiogenous structure, with some diverticulate conidiogenous cells (arrows). Scale bars: A, B, H, J = 20 µm; C, I = 10 µm; D = 2 µm; E, F = 5 µm; G = 1 mm.

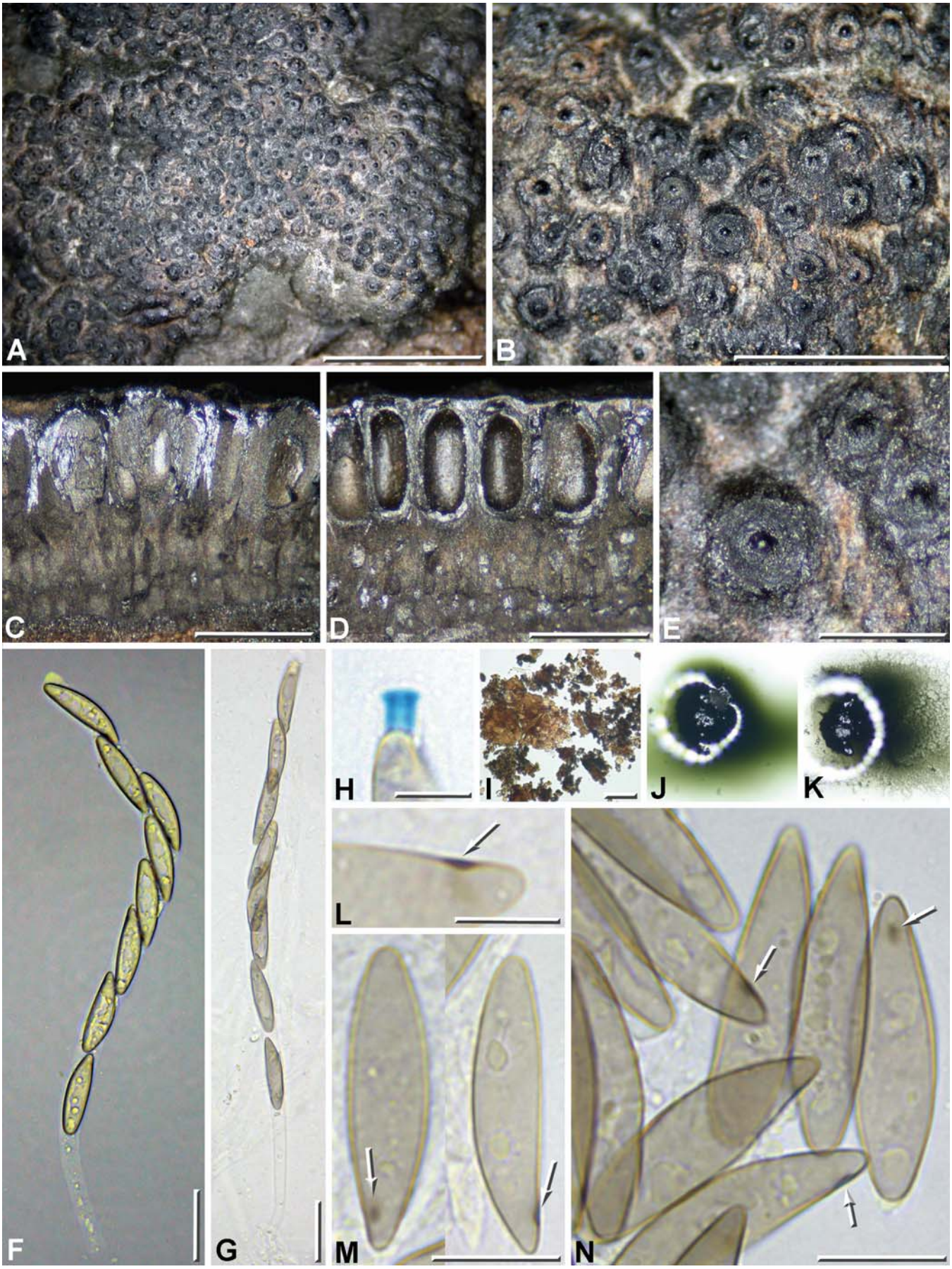

Plate 4 – *Annulohypoxylon leptascum* var. *macrosporum*

GYJF 12175. A: Effused-pulvinate stroma in surface view; B: Stromatal surface in close-up showing the ostiolar discs and the greyish coating; C, D: Stroma in vertical section (C broken, D sectioned) showing the perithecia, the carbonaceous crust and the subperithecial tissue E: Ostiolar discs in close-up showing their notched edges; F, G: Asci in black Pelikan ink and water respectively, showing the short stipes and the arrangement of ascospores inside the ascus; H: Ascal apical apparatus in Melzer's reagent; I: Brown stromatal waxy granules and blackish carbonaceous fragments from the subsurface, in water; J, K: KOH-extractable pigments after 1 min and 20 min incubation respectively; L-N: Ascospores in PVA-lactophenol showing the black spot next to the attenuated end (arrows). Scale bars: A = 5 mm; B = 2 mm; C, D = 1 mm; E = 0.5 mm; F, G = 20 µm; H, L = 5 µm; I, M, N = 10 µm.

its variety in a phylogenetic tree based on α-actin sequences (Hsieh *et al*., 2005), suggesting affinities between those *Annulohypoxylon* spp. with a similar deviating ascospore morphology.

Annulohypoxylon leucadendri was described from the fynbos vegetation in South Africa and was said to be similar to *A. leptascum* var. *macrosporum*, just differing by larger ascospores 24–27 × 6–7.5 µm (Marincowitz *et al*., 2008). According to the protologue, *A. leucadendri* features small black pulvinate stromata 1–3 mm diam lacking KOH-extractable pigments, with ostiolar discs 0.6–0.8 mm diam and therefore cannot be confused with *A. leptascum* var. *macrosporum* that in contrast features largely effused stromata with green KOH-extractable pigments and much smaller ostiolar discs. Furthermore, the ascospores of *A. leucadendri* are brown and have a short central germ slit 3–4 µm long, unlike *A. leptascum* var. *macrosporum* in which ascospores are pale yellowish brown and have the germ slit, when visible, originating from one end.

Annulohypoxylon* cf. *moriforme (Henn.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh, *Mycologia*, 97(4): 859 (2005) – Plates 5–6, table 1.

Stromata glomerate, pulvinate or effused-pulvinate, confluent or separate, with conspicuous perithecial contours ⅓ to ½ exposed, 1.4–25 mm long × 1–13 mm wide × 0.8–1 mm thick; outermost coating dark mouse grey (118, oac902 to 903), olivaceous brown in places, leaving a dull black and roughened squamulose surface; texture carbonaceous, with subsurface composed of dull olivaceous yellow granules mixed with dark brown to black carbonaceous tissue forming a thick layer above and around the perithecia, releasing greenish olivaceous (90, oac859) KOH-extractable pigments within 1 min incubation, evolving to dark greenish grey (856, oac83) upon prolonged incubation; subperithecial tissue 0.25–0.4 mm thick, greyish brown to blackish. **Perithecia** spherical, 0.5–0.6 mm diam. **Ostioles** conic-papillate, encircled with a flattened *truncatum*-type disc 0.33–0.38 mm diam with notched edges.

Asci cylindrical, with eight uniseriate ascospores, 95–100 µm total length, the spore-bearing parts 65–70 × 4.5–5 µm, the stipes 27–32 µm long, with a discoid apical apparatus 0.6–0.7 × 1.7–1.8 µm, bluing in Melzer's reagent. **Paraphyses** filiform, abundant in places. **Ascospores** (7.6–) 7.9–8.8 (–9.3) × (2.7–) 3–3.6 (–3.9), Q = (2.1–) 2.3–2.8 (–3.2); N = 60 (Me = 8.4 × 3.3 µm; Qe = 2.5), ellipsoid-inequilateral with narrowly rounded ends, brown, with a straight germ slit spore-length; perispore dehiscent in 10% KOH, smooth, with a thickening on the dorsal side at ca. ⅓ spore length; epispore smooth.

Asexual morph on the natural substrate not seen. Asexual morph in culture on OA nodulisporium-like, based on material from Taiwan and USA (Hawaii) (Ju & Rogers, 1996).

Specimens examined: French Guiana: Sinnamary, Paracou, CIRAD field station, plot 9, lowland rainforest, dead corticated branch, 23 Jun. 2012, *leg*. J. Fournier, GYJF 12137 (LIP); *ibid*., vic. of the field station, dead corticated branch, 23 Jun. 2012, *leg*. J. Fournier, GYJF 12158 (LIP); *ibid*., 23 Jun. 2012, *leg*. J. Fournier, GYJF 12166 (LIP); *ibid*., Guyaflux plot, dead corticated branch, associated with weathered stromata of *Annulohypoxylon stygium*, 24 Jun. 2012, *leg*. J. Fournier, GYJF 12181 (LIP); *ibid*., path to the field station, dead corticated branch, 25 Jun. 2012, *leg*. J. Fournier, GYJF 12204 (LIP).

Known distribution: Pantropical.

Discussion: As delimited by Ju & Rogers (1996), *A. moriforme* is a pantropical taxon characterized by dull black stromata with an olivaceous tone when young, varying from glomerate to effused-pulvinate, with greenish olivaceous or dull green KOH-extractable pigments, by *truncatum*-type ostiolar discs 0.2–0.4 mm diam, by short- to long-stipitate asci with or without an apical apparatus bluing in Melzer's reagent, and by ascospores 6–9 × 2.5–4 µm with a germ slit spore-length and a perispore more or less easily dehiscent in 10% KOH. Collections of *Annulohypoxylon* fitting in this concept are commonly encountered in the tropics but they frequently slightly deviate from each other, making their identification to *A. moriforme* tricky.

The above description is based on the collection GYJF 12181 which fits well the above concept of *A. moriforme*. It is morphologically very similar to GYJF 12137 and GYJF 12158 but features larger ascospores averaging 8.4 × 3.3 µm vs. 7.2 × 3 µm and 7.7 × 3.3 µm respectively (Table 1). The collections GYJF 12166 and GYJF 10204 feature young stromata with an olivaceous brown coating reported as typical of *A. moriforme* by Ju & Rogers (1996). The former primarily differs from GYJF 12181 and from typical *A. moriforme* as defined by these authors by more broadly conical ostioles and a periconiella-like asexual morph associated with the stromata. On the other hand, the latter differs by a slightly shiny subsurface composed of brownish orange granules yielding fugacious hazel KOH-extractable pigments before turning olivaceous after 1–2 min incubation; however, its nodulisporium-like asexual morph present around young stromata conforms to that of *A. moriforme*.

The five studied collections, originating from a very restricted area, have many traits in common and could be indeed regarded as representing the same species. Their differences reported and illustrated here suggest either that *A. moriforme* is a fairly variable taxon or that collections conforming to *A. moriforme* possibly represent different taxa that cannot be unequivocally separated based on morphology only. For this reason we tentatively refer the material we collected to *A. moriforme*. A revision of the status of *A. moriforme*, including epitytification based on material collected in its original location (Samoa, Polynesia), should involve material widely sampled in tropics and be based on cultural, molecular and chemotaxonomic grounds to test the validity of the morphological differences observed within this taxon as currently conceived.

Confusions of *A. moriforme* with *A. nitens* (Ces.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh may occur when the stromata of the latter do not exhibit their typical shiny black subsurface owing to the partially persistent outermost coating. The *bovei*-type ostiolar discs with sharp rims

Table 1 – Ascospore dimensions in five collections of *A.* cf. *moriforme* from the same limited area, compared with the values reported by Ju & Rogers (1996).

	Ascospore measurements with extreme values in parentheses	Q = quotient l/w N = number of measurements	Mean values
GYJF 12137	(6.5–).7–7.6(–7.7) × (2.4–)2.7–3.2(–3.4) µm	Q = (2.1–)2.2–2.7(–2.9); N = 60	Me = 7.2 × 3 µm; Qe = 2.4
GYJF12158	(6.6–)7.3–8.1(–8.9) × (2.9–)3–3.6(–3.8) µm	Q = (2–)2.1–2.5(–2.8); N = 65	Me = 7.7 × 3.3 µm; Qe = 2.3
GYJF 12166	(6.9–)7.1–7.7(–8.1) × (2.7–)2.9–3.3(–3.5) µm	Q = (2.1–)2.2–2.5(–2.7); N = 60	Me = 7.4 × 3.1 µm; Qe = 2.4
GYJF 12181	(7.6–)7.9–8.8(–9.3) × (2.7–)3–3.6(–3.9) µm	Q = (2.1–)2.3–2.8(–3.2); N = 60	Me = 8.4 × 3.3 µm; Qe = 2.5
GYJF 12204	(6.5–)6.8–7.7(–8) × (2.7–)3.1–3.5(–3.7) µm	Q = (1.9–)2–2.4(–3); N = 60	Me = 7.2 × 3.3 µm; Qe = 2.2
cumulated values from above	(6.5–)6.7–8.8(–9.3) × (2.4–)2.7–3.6(–3.9) µm	Q = (1.9–)2–2.8(–3.2); N = 305	Me = 7.6 × 3.2 µm; Qe = 2.4
Ju & Rogers (1996)	6–9 × 2.5–4 µm	–	Me = 7.5 × 3.3 µm; Qe = 2.2

Plate 5 – *Annulohypoxylon* cf. *moriforme*

GYJF 12181. A: Effused-pulvinate stroma; B: Stromatal waxy granules mixed with carbonaceous tissue, in water; C, D: KOH-extractable pigments after 1 min and 20 min incubation respectively; E: Glomerate stromata; F: Few-perithecia stroma with olivaceous brown coating; G: Stromatal surface in close-up showing the ostiolar discs and the slightly exposed perithecial contours; H: Ostiolar disc in close-up; I, J: Stromata in vertical section showing the perithecia and the carbonaceous interperithecial tissue; K: Ascospores in 10% KOH showing the dehiscent perispore with a thickening on the dorsal side; L, M: Immature and mature asci in black Pelikan ink and 1% SDS respectively; N: Ascal apical apparati in Melzer's reagent; O, P: Ascospores in Melzer's reagent and 10% KOH respectively, showing the germ slit; P: Ascospores in 1% SDS. Scale bars: A, E = 5 mm; B, Q = 10 µm; F, G = 1 mm; H = 0.2 mm; I, J = 0.5 mm; K, N-P = 5 µm; L, M = 20 µm.

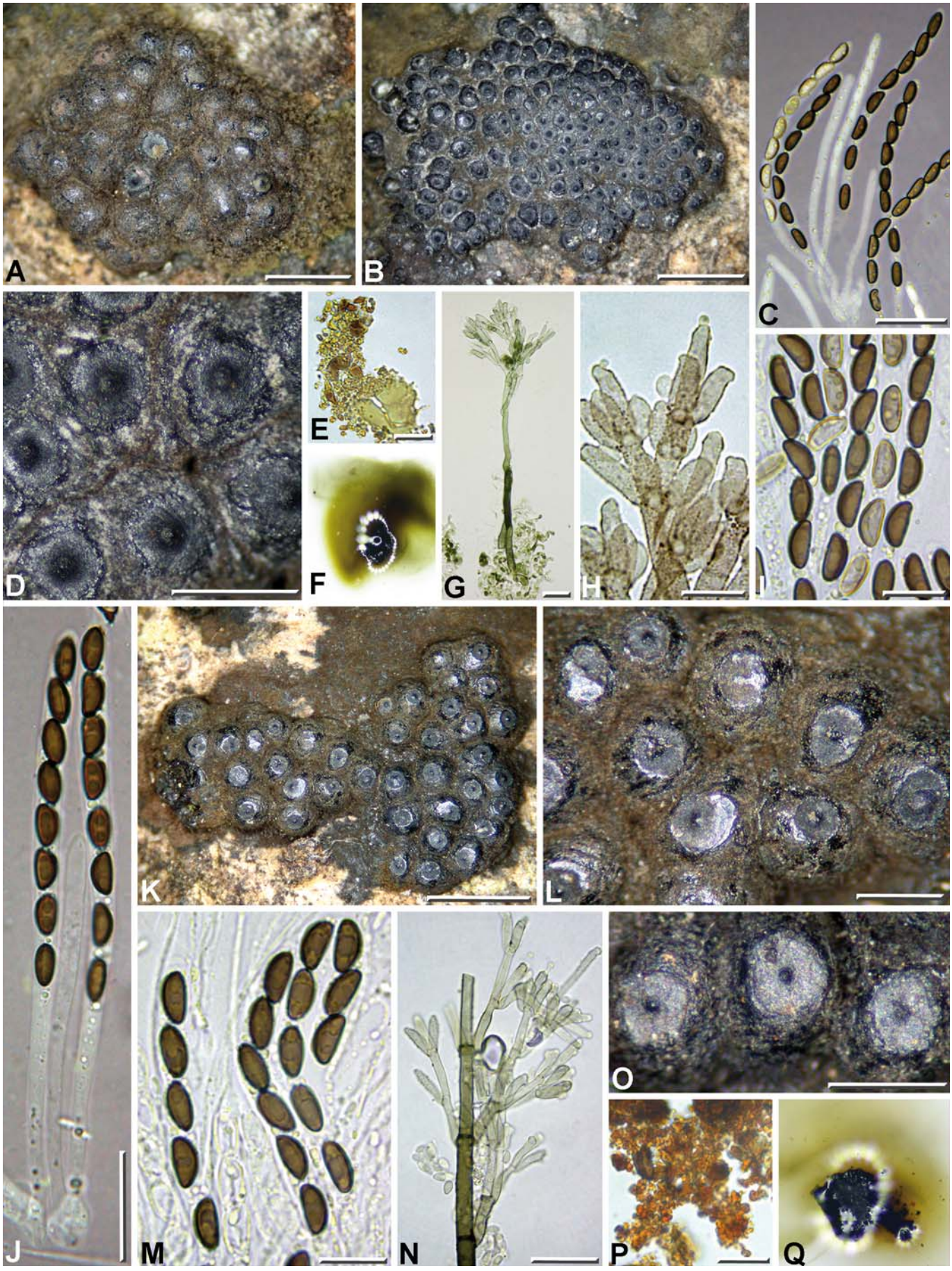

Plate 6 – *Annulohypoxylon* cf. *moriforme*

A-I: GYJF 12166; J-Q: GYJF 12204. A, K: Effused-pulvinate young stromata with olivaceous brown coating; B: Mature stroma; C, J: Immature and mature asci in black Pelikan ink; D, L, O: Stromatal surface in close-up showing the ostiolar discs and the perithecial contours; E, P: Stromatal waxy granules, in water; F, Q: KOH-extractable pigments after 1 min incubation; G, N: Conidiophores and conidiogenous structures, in 3 % KOH; H: Periconiella-like conidiogenous structure, in 3% SDS; I, M: Ascospores in 1% SDS. Scale bars: A = 1 mm; B, K = 2 mm; C, J, N, G = 20 µm; D, L, O = 0.5 mm; E, H, I, M, P = 10 µm.

of *A. nitens* help distinguish it from *A. moriforme* characterized by *truncatum*-type discs with notched rims. In practice, assigning the ostiolar discs to a morphological type without observing their dehiscence is by no means unequivocal and may lead to confusions between *A. moriforme* and *A. nitens*.

Annulohypoxylon elevatidiscum, known from Taiwan, resembles *A. moriforme* by its dull black stromata with *truncatum*-type ostiolar discs, greenish olivaceous KOH-extractable pigments and a nodulisporium-like asexual morph in culture; it is distinguished from *A. moriforme* by its smaller and convex ostiolar discs 0.2–0.3 mm diam and larger ascospores 8–10 × 3.5–4.5 µm (Ju *et al*., 2004).

Annulohypoxylon annulatum (Schwein. : Fr.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh and *A. truncatum* (Schwein. : Fr.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh likewise feature dull black stromata with *truncatum*-type ostiolar discs and greenish olivaceous to dull green KOH-extractable pigments. They both primarily differ from *A. moriforme* in their host affiliation to *Quercus* and larger, darker brown ascospores respectively 7.5-10.5 × 3.5–5 µm and 8–10.5 × 4–5 µm.

Finally, *A. moriforme* var. *macrosporum* Hladki & A.I. Romero, was reported from Argentina with larger and darker ascospores 9–10.5 × 4–5.5 µm (Hladki & Romero, 2009a), which obviously differs from our collections from French Guiana.

Annulohyopoxylon nouraguense J. Fourn. & Lechat, *sp. nov.* –MycoBank MB815538 – Plates 7–9, table 2.

Diagnosis: Differs from the most resembling species *A. purpureonitens* by KOH-extractable pigments evolving from vinaceous grey to hazel after prolonged incubation vs. livid violet evolving to vinaceous purple and slightly shorter, more broadly ellipsoid ascospores 6.2–8.1 × 2.6 –3.6 µm vs. 7.9–9 × 3–3.6 µm.

Holotype: French Guiana: Régina, Nouragues natural reserve, Inselberg camp, plot H19, primary rainforest, dead corticated branch, 18 Jun. 2012, *leg*. J. Fournier, GYJF 12057 (LIP).

Etymology: After the location Nouragues where this species was commonly encountered.

Stromata in small clusters to widely effused, with inconspicuous to ¾ exposed perithecial contours, perithecioid in places, 4–70 mm long × 3–36 mm wide × 0.8–1 mm thick, associated with wide dark vinaceous (82, oac523) sterile primordial areas or on bare bark; outermost coating dark vinaceous (82, oac523) to blackish brown, persistent or cracking and flaking off into large scales, leaving a dull black and roughened or smooth and shiny black surface; shiny crust 40–70 µm thick, brittle, completely encasing the perithecia and spreading around the base of perithecioid stromata and uniting them, primarily composed of dull yellow brown waxy granules in a loose carbonaceous matrix, releasing vinaceous grey (116, oac627) to vinaceous purple (101, oac401) KOH-extractable pigments, gradually becoming pale vinaceous (85, oac521) and eventually turning pale hazel (88, oac799) after 30 min incubation; subperithecial tissue 0.1–0.3 mm thick, blackish. **Perithecia** spherical, 0.6–0.7 mm diam. **Ostioles** finely papillate, encircled with a flattened disc 0.3–0.4 mm diam with slightly notched edges, possibly *bovei*-type.

Asci cylindrical, with eight uniseriate ascospores, originating from short contorted denticulate ascogenous hyphae 3–3.5 µm wide, 100–120 µm total length, the spore-bearing parts 60–68 × 4.5–5 µm, the stipes 34–56 µm long, with a discoid apical apparatus 0.6–0.8 × 1.4–1.7 µm, bluing in Melzer's reagent. **Paraphyses** filiform, embedded in mucilage. **Ascospores** (5.9–) 6.2–8.1(–8.6) × (2.4–) 2.6–3.6 (–3.8) µm, Q = (1.7–)1.9–2.6(–2.8); N = 495 (Me = 7.3 × 3.2 µm; Qe = 2.3), ellipsoid slightly inequilateral with narrowly to broadly rounded ends, pale to medium brown, with a straight germ slit spore-length; perispore dehiscent in 10% KOH, smooth, with a thickening on the dorsal side; epispore smooth.

Asexual morph on the natural substrate: Present in places on mature stromata, forming honey (64, oac847) to fawn (87, oac646) tufts arising from a loose vinaceous brown hyphal tissue; conidiophores upright, non-septate, 70–110 µm high, 4–6 µm wide, brown, coarsely roughened; conidiogenous structure periconiella-like, conidiogenous cells 10–20 × 2.4–3.7 µm, roughened, conidia ellipsoid, 5–6 × 2.5–3 µm, hyaline, smooth.

Specimens examined: French Guiana: Régina, Nouragues natural reserve, Inselberg camp, rainforest, on bark, 16 Jun. 2012, *leg*. J. Fournier, GYJF 12006 (LIP, paratype); *ibid*., plot H 19, on bark, 18 Jun. 2012, *leg*. J. Fournier, GYJF 12047 (LIP, paratype); *ibid*., trail 11, on bark, 19 Jun. 2012, *leg*. J. Fournier, GYJF 12072 (LIP, paratype); *ibid*., edge of the camp, on bark, 20 Jun. 2012, *leg*. J. Fournier, GYJF 12101 (LIP, paratype); *ibid*., trail to Inselberg, on bark, 20 Jun. 2012, *leg*. J. Fournier, GYJF 12107 (LIP, paratype); *ibid*., on bark, 19 Jun. 2012, *leg*. J. Fournier, GYJF 12109 (LIP, paratype); *ibid*., edge of the camp, on bark, 20 Jun. 2012, *leg*. J. Fournier, GYJF 12111 (LIP, paratype); *ibid*., trail to Pararé, 0.5 km from the camp, on bark, 21 Jun. 2012, *leg*. J. Fournier, GYJF 12120 (LIP, paratype).

Specimens of *A. purpureonitens* examined: Brazil: Serra Araca, corticated wood, 10–13 Mar. 1984, *leg*. G.J. Samuels, G.J. Samuels 808 (WSP 69635, isotype). Mexico: Chiapas state, Ocosingo municipality, Ejido Boca de Chajul, wood, 29 May 1988, *leg*. F. San Martín, San Martin-862 (JDR).

Known distribution: French Guiana.

Discussion: Stromata of an *Annulohypoxylon* with a dark vinaceous coating gradually flaking off, frequently revealing a shiny black subsurface and yielding vinaceous grey pigments in 10% KOH were repeatedly collected in the vicinity of the Inselberg camp in the Nouragues natural reserve. Based on the combination of these two characters it was provisionally assigned to *A. purpureonitens* (Y.-M. Ju & J.D. Rogers) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh (Ju & Rogers, 1996). Upon further examination, the nine collections of this *Annulohypoxylon* appeared to feature a stromatal surface varying from dull black to shiny black with more or less developed scaly outermost coating but were shown to all have KOH-extractable pigments evolving from vinaceous grey to pale vinaceous and strikingly turning hazel after 20–30 min incubation.

Annulohypoxylon purpureonitens is defined as having the same sexual morph morphology as *A. nitens* (Ces.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh, from which it only differs by purple to vinaceous KOH-extractable pigments vs. greenish olivaceous in *A. nitens*. As *A. nitens* is described as having ostiolar discs 0.2–0.5 mm diam of the *bovei*-type and ascospores 6.5–10 × 3–4.5 µm, our collections appeared to fit in this concept with the exception of the type of ostiolar disc which is often tricky to evaluate. The *bovei*-type refers to the mode of dehiscence of the ostiolar disc in *A. bovei* Speg. (Y.-M. Ju, J.D. Rogers & H.-M. Hsieh) in which the disc dehisces abruptly, in one piece, leaving sharp edges to the disc. In contrast, the *truncatum*-type refers to *A. truncatum* (Schwein. : Fr.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh in which the disc flakes off gradually, usually leaving notched edges to the disc (Ju & Rogers, 1996). In our experience this character is difficult to appraise unless the dehiscence of the discs can be directly observed on young stromata, which occurs rarely. No ostiolar disc was observed dehiscing in the nine collections examined and while they seemed in most case to correspond better to the *bovei*-type, many occurrences of discs with more or less notched edges made it difficult to interpret.

The isotype material of *A. purpureonitens*, kindly sent by Prof. Rogers, along with a collection from Mexico assigned to this name were examined for comparison with the Guianese collections. Like in our collections, the stroma of *A. purpureonitens* features a dark vinaceous coating, barely to strongly exposed perithecial contours, a shiny, weakly carbonaceous subsurface crust appearing in places where the outermost coating is cracked and flaked off and similar

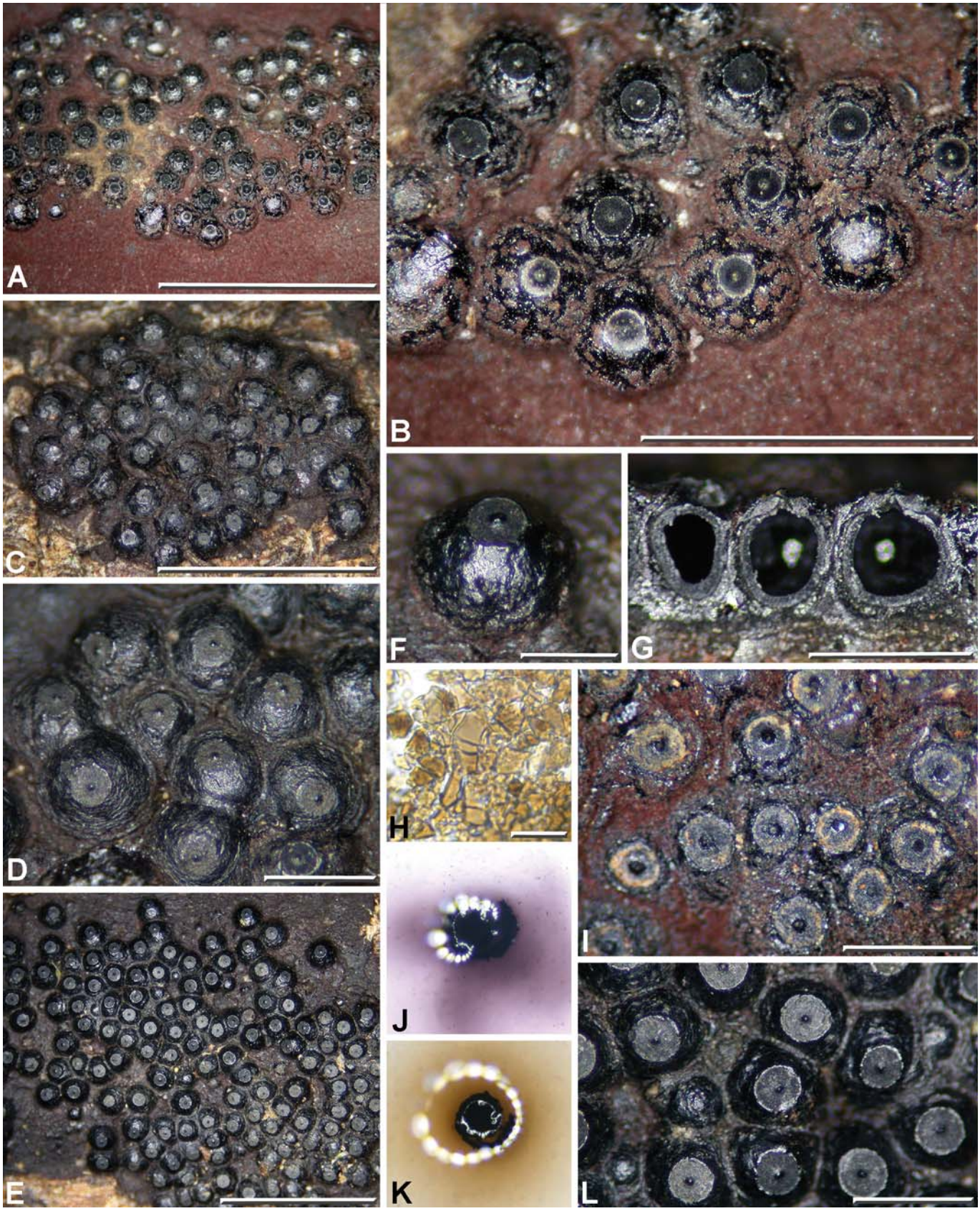

Plate 7 – *Annulohyopoxylon nouraguense*

A, B: GYJF 12072; C, E-L: Holotype GYJF 12057; D: GYJF 12107. A, C, E: Stromata in surface view; B: Perithecioid stromata in close-up showing the thick reddish brown scales and the shiny subsurface; D: Stromatal surface in close-up showing half-exposed perithecial contours and dull black roughened surface; F: Isolated stroma with shiny black surface; G: Stroma in vertical section; H: Waxy stromatal granules in water; I: Close-up on stromatal surface showing the faintly exposed perithecial contours, the thick reddish brown coating and the ostiolar discs; J, K: KOH-extractable pigments after 1 min and 30 min incubation respectively; L: Close-up on stromatal surface showing half-exposed perithecial contours and shiny black surface. Scale bars: A, C, E = 5 mm; B = 2 mm; D, G, I, L = 1 mm; F = 0.5 mm; H = 10 µm.

yellow brown to orange brown stromatal granules encasing the perithecia. However, it primarily differs by dense livid purple KOH-extractable pigments that fade to pale vinaceous grey upon prolonged incubation and do not become hazel.

It also deviates from the Guianese collections in having stromata up to 2 mm thick with blackish subperithecial tissue 1 mm thick, slightly smaller ostiolar discs 0.3–0.33 mm diam, larger ascospores 7.9–9 × 3–3.6 µm and a nodulisporium-like asexual morph associated at margins of the stroma. The stroma is not in ascigerous condition, thus the morphology of asci could not be recorded. Based on the different KOH-extractable pigments and the above morphological differences, the new species *A. nouraguense* is proposed to accommodate the Guianese collections.

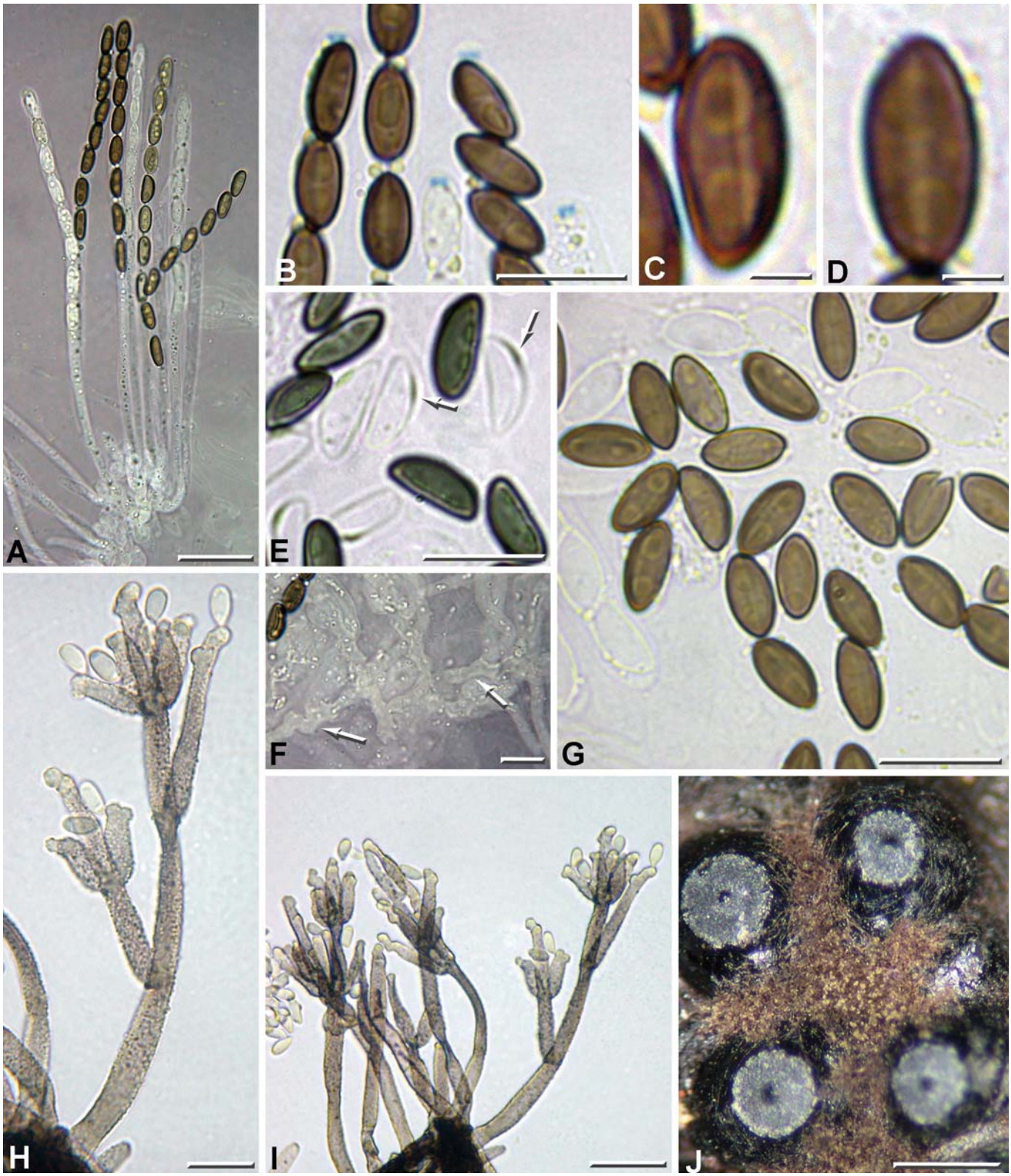

Plate 8 – *Annulohyopoxylon nouraguense*

Holotype GYJF 12057. A: Immature and mature asci in black Pelikan ink; B: Ascal apical apparati of mature and immature asci bluing in Melzer's reagent; C, D: Ascospores in dorsal view in water, showing the germ slit; E: Ascospores in 10% KOH showing the dehiscent perispore with a thickening on the dorsal side (arrows); F: Spiny ascogenous hyphae, in black Pelikan ink (arrows); G: Ascospores in water; H, I: Conidiophores, conidiogenous structure and conidia, in 1% SDS; J: Tufts of conidiophores on stromatal surface. Scale bars: A, I = 20 µm; B, E-H = 10 µm; C, D = 2 µm; J = 0.5 mm.

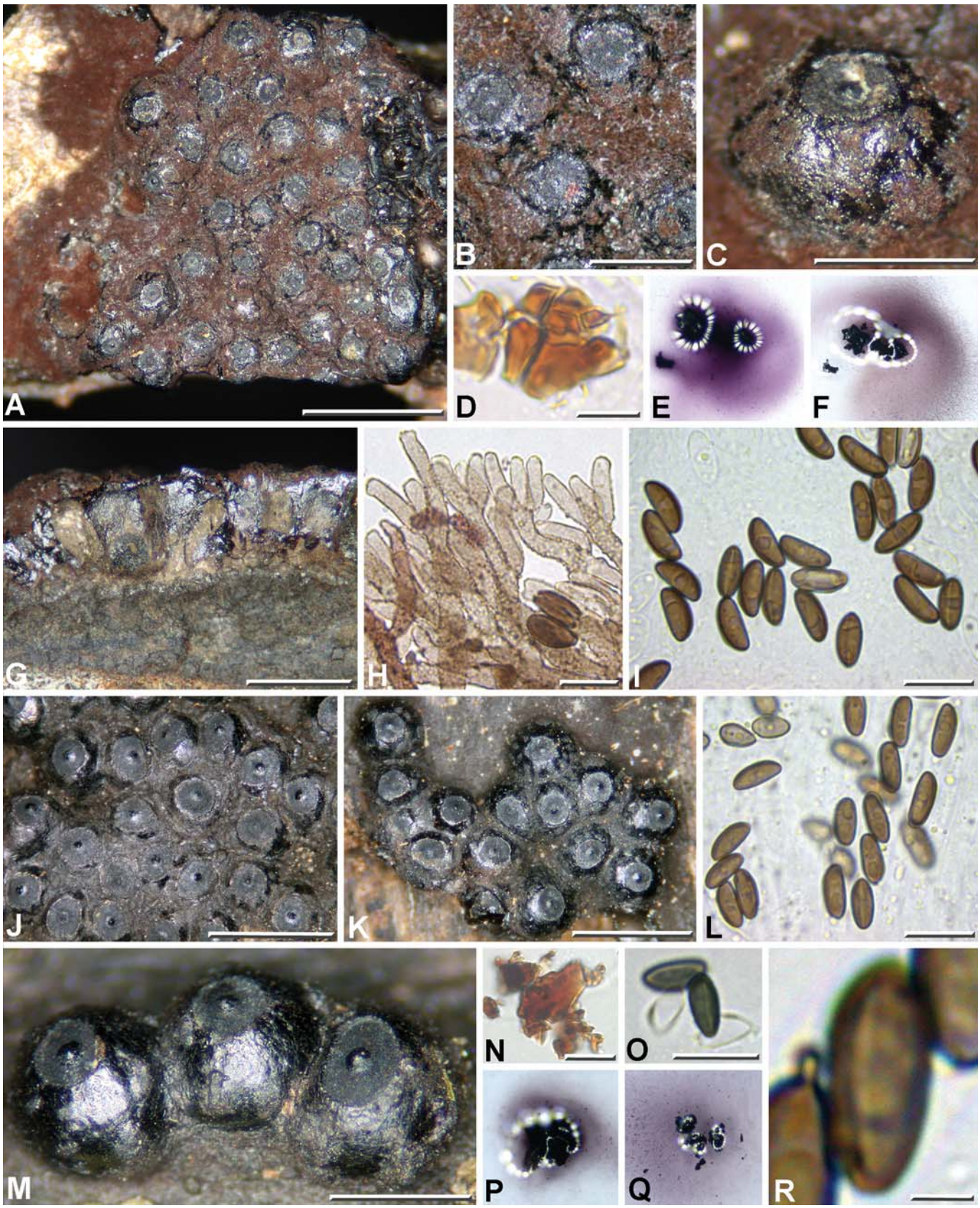

Plate 9 – *Annulohyopoxylon purpureonitens*

A-I: Isotype WSP 69635; J-R: San Martin-862. A, J, K: Stromata in surface view; B, C: Stromatal surface in close-up showing the thick reddish brown coating and the ostiolar discs; D, N: Waxy stromatal granules in water; E, F: KOH-extractable pigments after 1 and 30 min incubation respectively; G: Stroma in vertical section (broken), showing the thick subperithecial tissue; H: Nodulisporium-like conidiogenous stucture, in 1% SDS; I, L: Ascospores in water; M: Perithecioid stromata with shiny surface and blackish scales; O: Ascospores in 10% KOH showing the dehiscent perispore with a thickening on the dorsal side; P, Q: KOH-extractable pigments after 1 and 30 min incubation respectively; R: Ascospore in dorsal view in water, showing the germ slit. Scale bars: A, J, K = 2 mm; B, C = 0.5 mm; D, H, I, L, N, O = 10 µm; G, M = 1 mm; R = 2 µm.

Table 2 – Ascospore dimensions in nine collections of *A. nouraguense*, compared with those of the isotype of *H. purpureonitens* and *H. purpureonitens* SM-862.

	Ascospore measurements with extreme values in parentheses	Q = quotient l/w N = number of measurements	Mean values
A. nouraguense GYJF 12006	(6.2–)6.4–7(–7.2) × (2.4–)2.6–3.1(–3.4) µm	Q = (2–)2.1–2.6(–2.7); N = 50	Me = 6.7 × 2.9 µm; Qe = 2.3
A. nouraguense GYJF 12047	(6.3–)6.6–7.4(–7.7) × (2.8–)2.9–3.3(–3.4) µm	Q = (2–)2.1–2.5(–2.6); N = 50	Me = 7 × 3 µm; Qe = 2.3
A. nouraguense GYJF 12057	(6.8–)6.9–7.7(–7.9) × (2.9–)3.1–3.5(–3.9) µm	Q = (1.9–)2–2.4(–2.5); N = 50	Me = 7.2 × 3.3 µm; Qe = 2.2
A. nouraguense GYJF 12072	(7–)7.2–8(–8.4) × (2.6–)3–3.5(–3.7) µm	Q = (2.1–)2.2–2.6(–2.8); N = 60	Me = 7.6 × 3.2 µm; Qe = 2.4
A. nouraguense GYJF 12101	(6.5–)6.9–7.9(–8.4) × (2.8–)3–3.4(–3.6) µm	Q = (1.9–)2.1–2.5(–2.7); N = 50	Me = 7.4 × 3.2 µm; Qe = 2.3
A. nouraguense GYJF 12107	(7.2–)7.6–8.1(–8.5) × (2.9–)3.1–3.6(–3.8) µm	Q = (2–)2.2–2.5(–2.7); N = 60	Me = 7.8 × 3.3 µm; Qe = 2.4
A. nouraguense GYJF 12109	(7.2–)7.3–8(–8.5) × (2.8–)3–3.5(–3.7) µm	Q = (2.1–)2.2–2.6(–2.7); N = 50	Me = 7.7 × 3.2 µm; Qe = 2.4
A. nouraguense GYJF 12111	(5.9–)6.2–7.2(–7.6) × (2.7–)3–3.5(–3.6) µm	Q = (1.7–)1.9–2.4(–2.6); N = 50	Me = 6.7 × 3.2 µm; Qe = 2.1
A. nouraguense GYJF 12120	(7.2–)7.4–8.1(–8.6) × (2.9–)3.1–3.5(–3.8) µm	Q = (2.1–)2.2–2.5(–2.8); N = 55	Me = 7.8 × 3.3 µm; Qe = 2.4
WSP 69635 isotype of *A. purpureonitens*	(7.3–)7.9–9(–9.8) × (2.7–)3–3.6(–3.7) µm	Q = (2.2–)2.3–2.8(–3.6); N = 60	Me = 8.5 × 3.3 µm; Qe = 2.6
A. purpureonitens SM 862	(6.2–)6.7–7.8(–8.4) × (2.5–)3–3.4(–3.8) µm	Q = (1.8–)2–2.5(–3); N = 60	Me = 7.3 × 3.2 µm; Qe = 2.3

The collection San Martin-862 from Mexico deviates from the isotype of *A. purpureonitens* by vinaceous grey KOH-extractable pigments, larger ostiolar discs 0.5–0.6 mm diam and smaller ascospores 6.7–7.8 × 3–3.4 µm. It resembles *A. nouraguense* in KOH-extractable pigments and ascospores dimensions but differs in lacking the delayed hazel reaction in KOH, in having larger ostiolar discs and in having long-stipitate asci with stipes up to 120 µm long.

The taxonomic status of the collection San Martin-862 appears intermediate between *A. nouraguense* and *A. purpureonitens* and would require further studies based on more material.

It is noteworthy that *A. nouraguense* was commonly collected around the Inselberg camp, located in primary rainforest with altitude ranging from 100 to 400 m while it was not encountered in the lowland rainforest of Paracou explored during the following week. This suggests an ecological preference that needs to be confirmed by further sampling in the Guianese forests.

Annulohypoxylon purpureopigmentum Jad. Pereira, J.D. Rogers & J.L. Bezerra, *Mycologia*, 102(1): 250 (2010) – Plate 10.

Stromata glomerate and few-peritheciate, 1.5–2.5 mm diam, confluent into irregularly effused-pulvinate stromata 5–24 mm long × 3–5 mm wide × 0.8–1.2 mm thick, with ⅓ to strongly exposed perithecial contours; surface even, matt, with persistent pruinose outermost coating rust (39, oac644) to umber (9, oac734), turning blackish brown at maturity; texture carbonaceous around the perithecia, with subsurface composed of pale orange brown granules yielding dense livid purple (81, oac439) KOH-extractable pigments within 1 min incubation, fading to pale vinaceous grey (115, oac569) upon prolonged incubation; subperithecial tissue 0.2–0.5 mm thick, brown to blackish. **Perithecia** subspherical, 0.5–0.6 mm diam. **Ostioles** conic-papillate, encircled with a flattened *truncatum*-type disc 0.20–0.25 mm diam with sharp rims.

Asci cylindrical, with eight uniseriate ascospores, 85–105 µm total length, the spore-bearing parts 46–52 × 3.5–4 µm, the stipes 30–58 µm long, with a discoid apical apparatus 0.6–0.9 × 1.5–1.7 µm, bluing in Melzer's reagent; swollen, moniliform, contorted hyphae 3–5 µm wide are present in the subhymenium, possibly ascogenous. **Paraphyses** filiform, inconspicuous. **Ascospores** (5.8–) 6.1–6.8 (–7.4) × (2.3–) 2.6–2.9 (–3.1) µm, Q = (2.1–) 2.2–2.5 (–2.9); N = 65 (Me = 6.4 × 2.8 µm; Qe = 2.3), ellipsoid slightly inequilateral with narrowly to broadly rounded ends, brown, with a straight germ slit almost spore-length; perispore dehiscent in 10% KOH, smooth, with a thickening on the dorsal side at ca. ⅓ spore length; epispore smooth.

Asexual morph on the natural substrate not seen.

Specimens examined: French Guiana: Sinnamary, Paracou, CIRAD field station, Guyaflux plot, lowland rainforest, dead corticated branch, 24 Jun. 2012, *leg*. J. Fournier, GYJF 12178 (LIP). French West Indies: Martinique: Case-Pilote, Morne-Rose, mesophilic rainforest, dead corticated and decorticated branch, associated with depauperate stromata of *Annulohypoxylon* sp., 1 Sept. 2010, MJF 10309 (LIP); Saint-Joseph, Coeur-Bouliki forest, hygrophilic rainforest, on dead corticated branchlets in a Mahogany plantation, 26 Aug. 2007, *leg* J. Fournier, MJF 07109 (LIP).

Known distribution: Brazil, French Guiana, Martinique.

Discussion: *Annulohypoxylon purpureopigmentum* was recently described from Brazil and its morphological differences with other *Annulohypoxylon* spp. with purplish KOH-extractable pigments were discussed in detail by Pereira *et al.* (2010). It is indeed a distinctive species, readily recognized based on its small, often glomerate stromata with conspicuous perithecial contours, with livid purple KOH-extractable pigments and small black ostiolar discs contrasting with the dull brown persistent pruinose surface.

The ostiolar discs were reported as of the *bovei*-type in the original description and they indeed seem to correspond to this configuration by their sharp and regular rims. However, the partly immature state of our collection made it possible to observe the dehiscence of many ostiolar discs. It appeared that they result from the outwardly flaking off of the tissue surrounding the ostioles,

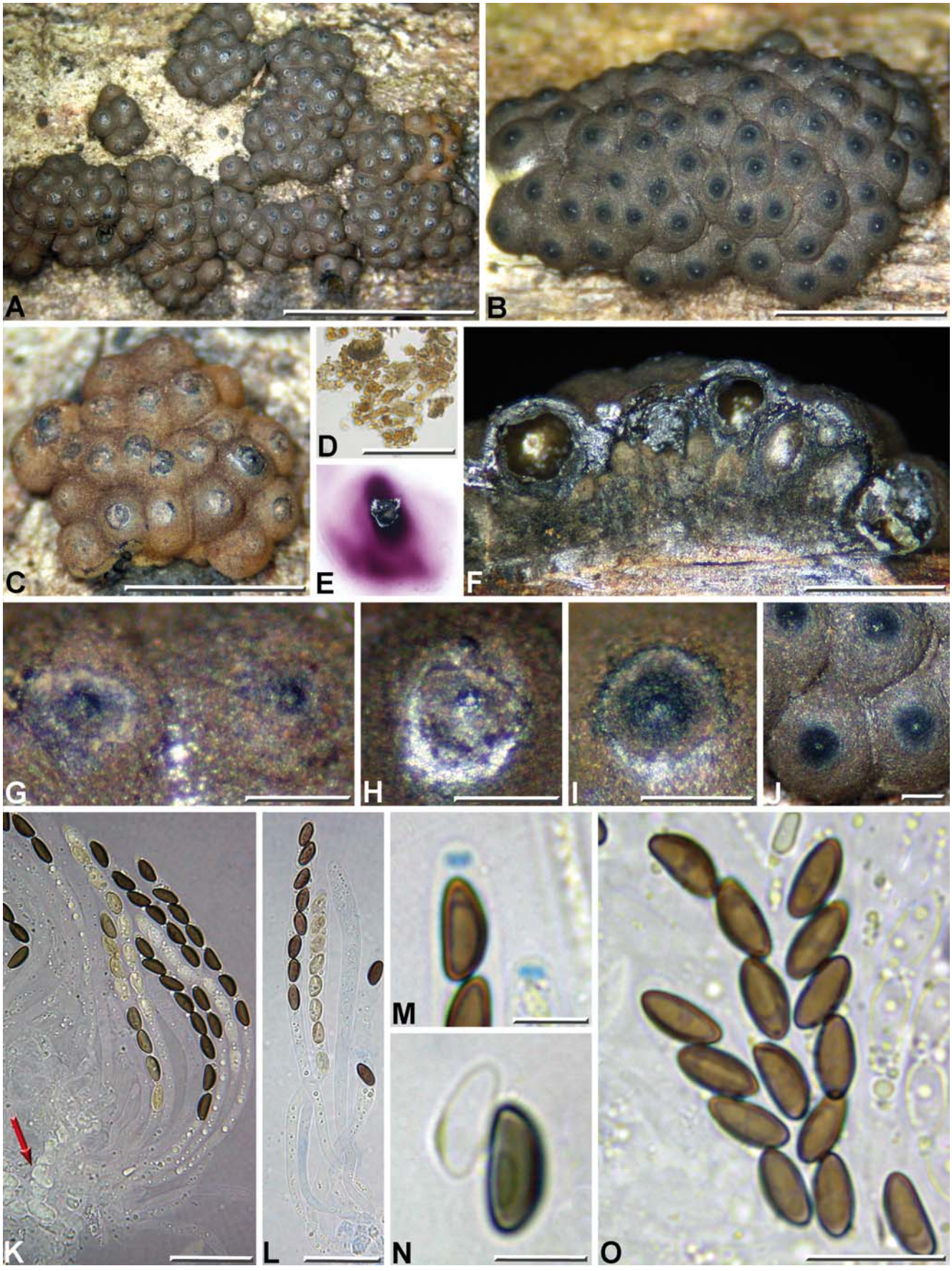

Plate 10 – *Annulohypoxylon purpureopigmentum*

GYJF 12178. A: Glomerate mature and immature stromata; B: Effused-pulvinate mature stroma; C: Immature stroma; D: Stromatal waxy granules from subsurface, in water; E: KOH-extractable pigments after 1 min incubation; F: Stroma in vertical section showing the perithecia encased by carbonaceous tissue; G, H: Close-up on two adjacent ostioles of an immature stroma, showing the outermost stromatal tissue flaking off outwardly from the ostiole (*truncatum*-type); I, J: Close-ups on ostiolar discs after the dehiscence of the discs; K: Mature and immature asci in black Pelikan ink, with a swollen hypha of the subhymenium (arrow); L: Mature and immature asci in blue Waterman ink diluted in 1% SDS; M: Ascal apical apparati in Melzer's reagent; N: Ascospore in 10% KOH showing the dehiscent perispore with a thickening on the dorsal side; O: Ascospores in 1% SDS, some showing a germ slit. Scale bars: A = 5 mm; B, C = 2 mm; D, O: 10 µm; F = 1 mm; G-J = 0.2 mm; K, L = 20 µm; M, N = 5 µm.

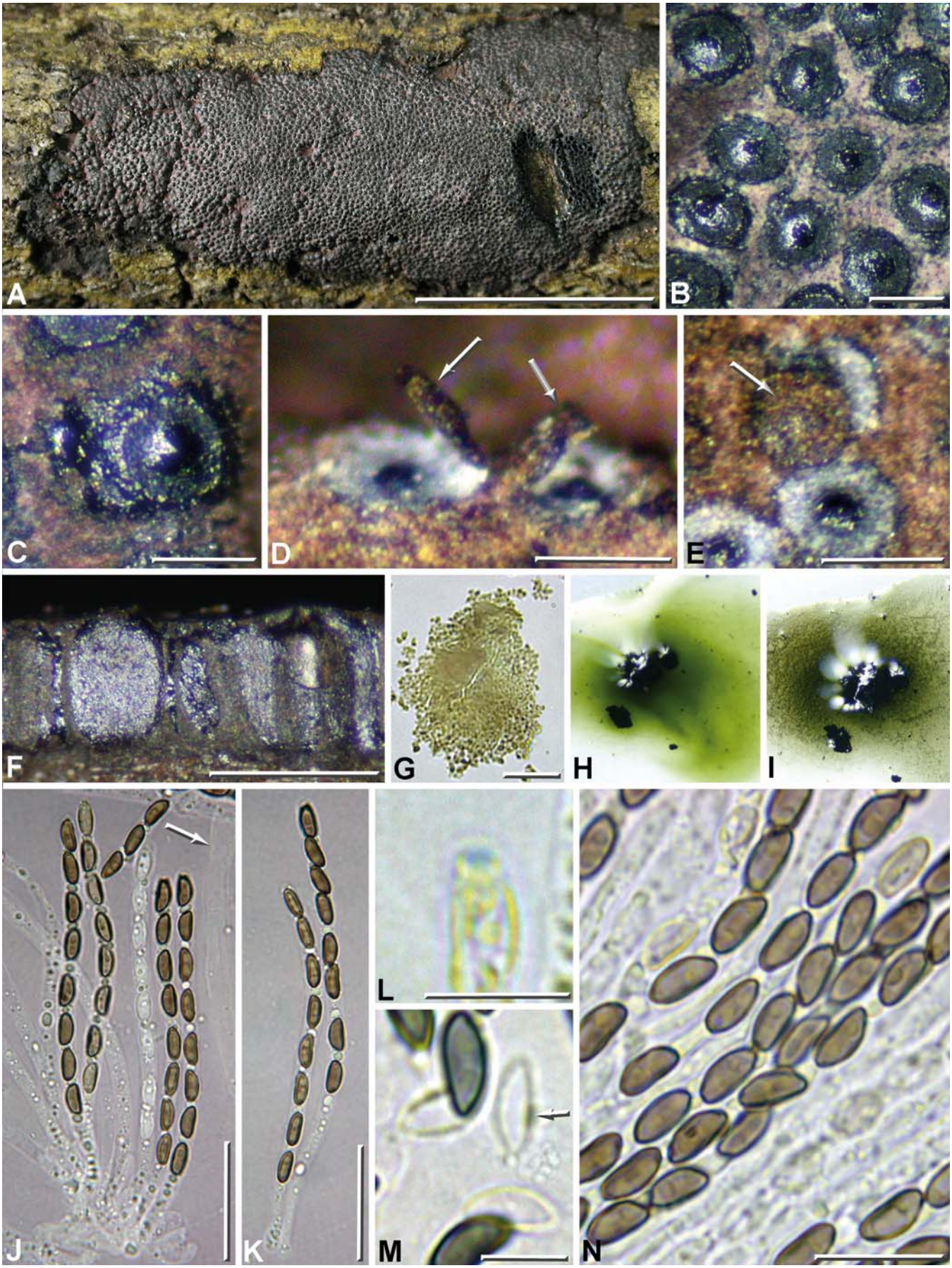

Plate 11 – *Annulohypoxylon stygium*

A-C, F-N: GYJF 12104; D, E: GYJF 12010. A: Mature stroma; B: Stromatal surface in close-up showing the shiny black ostiolar discs; C: Close-up on an ostiolar disc showing the shiny black exposed surface; D, E: Lifted ostiolar discs (arrows); F: Stroma in vertical section showing the perithecia encased by a thin carbonaceous tissue; G: Stromatal waxy granules from subsurface, in water; H, I: KOH-extractable pigments after 1 min and 30 min incubation respectively; J, K: Mature and immature asci and a wide paraphysis (arrow) in black Pelikan ink; L: Apical apparatus of an immature ascus in Melzer's reagent; M: Ascospores in 10% KOH showing the dehiscent perispore with a thickening on the ventral side (arrow); N: Ascospores in 1% SDS. Scale bars: A = 10 mm; B-E = 0.2 mm; F = 0.5 mm; G, N = 10 µm; J-K = 20 µm; L, M = 5 µm.

which corresponds to the *truncatum*-type ostiolar discs, not to the *bovei*-type in which the discs dehisce abruptly in one piece (Ju & Rogers, 1996).

Ascospores dimensions of our collection are at the lower range given in the protologue viz.: 6.1–6.8 × 2.6–2.9 µm vs. 6.5–8(–10) × 3–3.5(–4.5) µm. Ascospores of two collections of *A. purpureopigmentum* from Martinique (JF, unpublished data) were measured and both gave an average of 6.6 × 2.8 µm, thus closer to the Guianese material than to the Brazilian original material. As these values slightly overlap and are not correlated with other morphological differences we feel justified to consider this collection from French Guiana as *A. purpureopigmentum*.

The stromata of *A. purpureopigmentum* are small and dull brown and thus easily overlooked but our records from French Guiana and Martinique suggest it is likely to have a wider distribution. Further collections of this species should help better appraise the variation range in ascospores dimensions.

Annulohypoxylon stygium (Lév.) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh, *Mycologia*, 97(4): 861 (2005) – Plate 11.

Stromata effused pulvinate, 6–27 mm long × 6–10 mm wide × 0.4–0.6 mm thick, with inconspicuous to faintly exposed perithecial contours, rarely strongly exposed to perithecioid and then shiny black; surface with a fugacious dark brick (60, oac637) pruinose outermost coating, sometimes with a faint purplish tone, blackish with age; texture weakly carbonaceous, with subsurface composed of olivaceous buff (89, oac891) granules yielding dark herbage green (69, oac54) KOH-extractable pigments within 1 min incubation, darkening upon prolonged incubation; subperithecial tissue inconspicuous. **Perithecia** obovoid to tubular, 0.34–0.4 × 0.13–0.18 mm. **Ostioles** broadly conic-papillate, encircled with a shiny black, slightly convex *bovei*-type disc 0.15–0.2 mm diam with most often irregular rims.

Asci cylindrical, with eight uniseriate ascospores, 60–78 µm total length, the spore-bearing parts 48–52 × 3–3.5 µm, the stipes 11–16 (–28) µm long, with a minute discoid apical apparatus faintly or not bluing in Melzer's reagent. **Paraphyses** sparse, simple to furcate, 5–6 µm wide at base, tapering to 2–3 µm above asci. **Ascospores** (4.9–) 5.1–5.8 (–6.3) × (2–) 2.2–2.5 (–2.7) µm, Q = (2.1–) 2.2–2.5 (–2.7); N = 60 (Me = 5.5 × 2.3 µm; Qe = 2.3), ellipsoid-inequilateral with narrowly to broadly rounded ends, pale brown, with a very inconspicuous straight germ slit almost spore-length on the ventral side; perispore dehiscent in 10% KOH, smooth, with a thickening on the ventral side at ca. ⅓ spore length; epispore smooth.

Asexual morph on the natural substrate not seen. Asexual morph in culture on OA periconiella-like, based on material from Taiwan (Ju & Rogers, 1996).

Specimens examined: French Guiana: Matoury, garden of the CNRS lodge, dead corticated branchlet on the ground, 16 Jun. 2012, *leg*. J. Fournier, GYJF 12010 (LIP); Régina, Nouragues natural reserve, Inselberg camp, primary rainforest, plot H 20, dead corticated branch, 17 Jun. 2012, *leg*. J. Fournier, GYJF 12035–2 (LIP); *ibid*., way up to the Inselberg on a sun-exposed slope, dead corticated branch, 20 Jun. 2012, *leg*. J. Fournier, GYJF 12104 (LIP); *ibid*., trail to Pararé, 0.5 km from the camp, dead corticated branch, 21 Jun. 2012, *leg*. J. Fournier, GYJF 12132 (LIP); Sinnamary, Paracou, CIRAD field station, lowland rainforest, dead corticated branch, 23 Jun. 2012, *leg*. J. Fournier, GYJF 12136 (LIP); *ibid*., plot 11, dead corticated branch, 23 Jun. 2012, *leg*. J. Fournier, GYJF 12142 (LIP); *ibid*., vicinity of the station, dead corticated branch, 23 Jun. 2012, *leg*. J. Fournier, GYJF 12160 (LIP); *ibid*., path to the station, dead corticated branch, 25 Jun. 2012, *leg*. J. Fournier, GYJF 12205 (LIP); *ibid*., dead corticated branch, 27 Jun. 2012, *leg*. J. Fournier, GYJF 12249 (LIP).

Known distribution: Pantropical.

Discussion: *Annulohypoxylon stygium* is a widespread pantropical taxon easily set apart from other *Annulohypoxylon* spp. by the combination of small ostiolar discs < 0.2 mm diam and small perithecia, green KOH-extractable pigments, small ascospores 5–6 × 2–3 µm with a very inconspicuous germ slit on the ventral side and a perispore dehiscent in 10% KOH. Its ostiolar discs are said to be of the *truncatum*-type (Ju & Rogers, 1996), which is understandable based on the often irregular rims of the discs but the study of a barely mature and well-preserved stroma allowed to observe in detail their dehiscence. Instead of flaking off like in the typical *truncatum*-type, they appeared to lift and to dehisce in one piece in the way of a lid or an opercle, just like in *A. bovei*. Therefore the ostiolar discs of *A. stygium* should be termed of the *bovei*-type.

Freshly exposed ostiolar discs of *Annulohypoxylon* spp. appear coated with a white substance known to be involved in the dehiscence by swelling (Tsuneda & Arita, 1984; Ju & Rogers, 1996). This white substance usually vanishes rapidly but in some circumstances may remain on the ostiolar discs, giving the stromatal surface an unusual, at times misleading appearance.

The morphologically very similar *A. atroroseum* (J.D. Rogers) Y.-M. Ju, J.D. Rogers & H.-M. Hsieh, known from Gabon and Taiwan (Ju & Rogers, 1996), was segregated from *A. stygium* based on a vinaceous grey stromatal surface and a nodulisporium-like asexual morph in culture on OA (Rogers, 1981). We did not encounter stromata with definitely vinaceous grey surface in French Guiana but we frequently observed a faint rosy tone on the typically brown outermost coating of *A. stygium*. In absence of data on the asexual morph in culture, the distinction between *A. atroroseum* and *A. stygium* remains difficult.

Annulohypoxylon stygium was frequently collected during this short field trip and equally distributed in both stations sampled. It apparently shares with *H. monticulosum* Mont. a similar ecological preference for the canopy where they both develop on small dying branches. Both are usually found in good condition on such branches recently fallen from the canopy while stromata collected on dead branches having been lying for long on the ground often are depauperate. However, field observations of young stromata on dead rotting wood show that this species is also capable of growing on dead wood following the primary colonization.

Miller (1961) reported the presence of *A. stygium* in French Guiana based on a collection made by Leprieur, as *Hypoxylon annulatum* var. *depressum* Fr., but no other mention of a collection from French Guiana referable to *A. stygium* was found in literature.

Hypoxylon puiggarii Speg. from Brazil was synonymized to *A. stygium* (as *H. stygium*) by Miller (1961) but regarded by Ju & Rogers (1996) as a different taxon similar to *H. subeffusum* Speg., which has a closer affinity with *A. truncatum* than with *A. stygium*. This was confirmed after examination of the holotypes by Hladki & Romero (2009b) who synonymized *H. puiggarii* and *H. subeffusum* and recombined them in *A. subeffusum* (Speg.) Hladki & A.I. Romero.

Annulohypoxylon subnitens J. Fourn. & Lechat, *sp. nov.* – MycoBank MB815539 – Plate 12.

Diagnosis: Differs from *Annulohypoxylon nitens* by smaller ostiolar discs, pale greyish yellow stromatal granules and inconspicuous to pale olivaceous grey KOH-extractable pigments.

Holotype: French Guiana: Régina, Nouragues natural reserve, Inselberg camp, primary rainforest, way up to the Inselberg on a sun-exposed slope, dead corticated branch, 20 Jun. 2012, *leg*. J. Fournier, GYJF 12106 (LIP).

Etymology: Refers to the stromata resembling those of *A. nitens*.

Stromata irregularly effused, with strongly exposed perithecial contours, 7–24 mm long × 3–11 mm wide × 1 mm thick, with widely

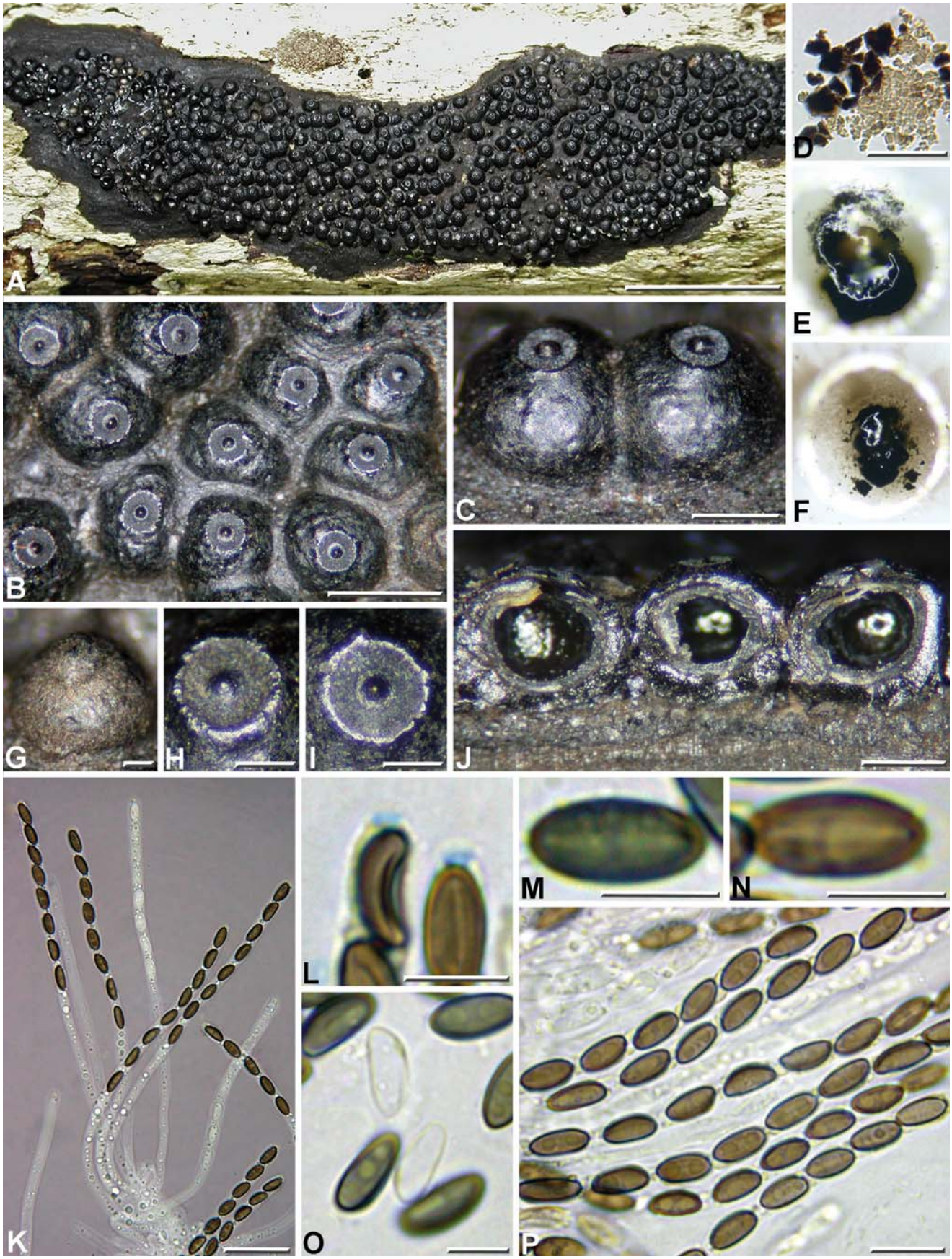

Plate 12 – *Annulohypoxylon subnitens*

GYJF 12106. A: Effused stroma with shiny surface and effused sterile black margin; B: Stromatal surface showing the ostiolar discs and the half-exposed perithecial contours; C: Close-up on two adjacent strongly exposed perithecial contours showing the shiny black and slightly roughened surface; D: Stromatal waxy granules mixed with carbonaceous fragments, in water; E, F: KOH-extractable pigments after 1 min and 45 min incubation respectively; G: Immature isolated stroma showing the apex prior to the dehiscence of the ostiolar disc; H, I: Close-up on ostiolar discs, respectively slightly concave to flat, showing the sharp rims and the broadly conical papilla; J: Stroma in vertical section showing the perithecia encased by carbonaceous tissue; K: Immature and mature asci in black Pelikan ink; L: Ascal apical apparati in Melzer's reagent; M, N: Ascospores in dorsal view showing the germ slit, in 10% KOH and 1% SDS respectively; O: Ascospores in 10% KOH showing the dehiscent perispore with a thickening on the dorsal side; P: Ascospores in 1% SDS. Scale bars: A = 10 mm; B = 1 mm; C, J = 0.5 mm; D, P: 10 µm; G-I = 0.2 mm; K = 20 µm; L-O = 5 µm.

effused black sterile margins; surface somewhat shiny black, with outermost coating dark grey to dull black, forming small persistent scales roughening the shiny black subsurface; texture carbonaceous, with subsurface composed of pale greyish yellow granules mixed with dark brown to black carbonaceous tissue, forming a thick layer above and around the perithecia, releasing inconspicuous to faint pale olivaceous grey (120, oac877) KOH-extractable pigments within 2–3 min incubation, evolving to pale greyish sepia (106, oac841) upon prolonged incubation; subperithecial tissue 0.2–0.3 mm thick, black, barely differentiated from the blackened underlying bark tissue. **Perithecia** subspherical, 0.6–0.7 mm diam. **Ostioles** broadly conic-papillate, encircled with a flattened or rarely slightly concave *bovei*-type disc 0.30–0.33 mm diam with sharp to notched rims.

Asci cylindrical, with eight uniseriate ascospores, 125–130 µm total length, the spore-bearing parts 58–62 × 4 µm, the stipes 65–72 µm long, with a discoid apical apparatus 0.6–0.8 × 1.5–1.7 µm, bluing in Melzer's reagent. **Paraphyses** filiform, abundant in places. **Ascospores** (6.7–) 7–7.7 (–7.9) × (2.3–) 2.6–3.2 (–3.3) µm, Q = (2.1–) 2.3–2.8 (–3.2); N = 65 (Me = 7.4 × 2.9 µm; Qe = 2.6), ellipsoid slightly inequilateral with broadly rounded ends, brown, with a straight germ slit almost spore-length; perispore dehiscent in 10% KOH, smooth, with a thickening on the dorsal side at ca. ⅓ spore length; epispore smooth.

Asexual morph on the natural substrate not seen.

Specimens of *A. nitens* examined: French West Indies: Guadeloupe: Le Gosier, Grand-Bois, Montête, xerophilic to mesophilic forest, dead corticated branch, Sept. 2004, *leg*. C. Lechat, CLL 2328 (LIP). Martinique: Prêcheur, Anse Couleuvre, coastal mesophilic forest, dead corticated branch, 23 Aug. 2007, *leg*. J. Fournier, MJF 07040 (LIP). P. R. China: Hainan, Wuhzi mountain, ca. 700 m elevation, rainforest, dead decorticated wood, Aug. 2007, *leg*. Y. Zhang, ZY 07046 (JF).

Known distribution: French Guiana.

Discussion: *Annulohypoxylon* spp. with a stromatal surface more or less shiny black, *bovei*-type ostiolar discs and greenish olivaceous KOH-extractable pigments were referred to *A. nitens* by Ju & Rogers (1996), a taxon formerly synonymized with *H. truncatum sensu* Miller (1961). *Annulohypoxylon purpureonitens*, though morphologically very similar to *A. nitens*, was segregated based on different purplish KOH-extractable pigments (Ju & Rogers, 1996). *Annulohypoxylon nouraguense* sp. nov., resembling *A. purpureonitens* but deviating in having different stromatal granules and paler purplish KOH-extractable pigments evolving to hazel upon prolonged incubation was likewise segregated primarily on these coloured reactions in relation with possibly different stromatal metabolites (this paper).

A similar species from Thailand, *A. bahnphadengense*, was segregated from *A. nitens* based on greyish sepia KOH-extractable pigments that were shown to correspond to a peculiar HPLC profile including naphthalene derivatives and lacking the typical daldinone and truncatone derivatives responsible for the greenish olivaceous KOH-extractable pigments encountered in *A. nitens* (Fournier *et al*., 2010). From these examples, the chemotaxonomic approach often appears as the most reliable way to discriminate species morphologically related to *A. nitens* since it is based on more stable characters than stromatal morphology only.

Our new taxon is likewise strikingly reminiscent of *A. nitens*, with which it shares a black stromatal surface gradually becoming shiny, *bovei*-type ostiolar discs and ascospores 6.5–10 × 3–4.5 µm (Ju & Rogers, 1996). However, instead of having the typical greenish olivaceous KOH-extractable pigments encountered in *A. nitens*, those occurring in *A. subnitens* are consistently inconspicuous, turning faint olivaceous grey within several minutes and evolving to pale greyish sepia after 45–60 min incubation.

The type of *A. nitens*, originating from Malaysia (Borneo) was not examined for comparison during this study but two specimens from Martinique and one from Hainan conforming to the concept of this taxon delimited by Ju & Rogers (1996) were studied as to their stromatal granules and their KOH-extractable pigments. They all feature typical greenish olivaceous KOH-extractable pigments evolving to pale olivaceous grey and their stromatal granules are olivaceous yellow. The greyish yellow stromatal granules detected in *A. subnitens* are paler and lack the olivaceous tone present in the specimens of *A. nitens* we examined. This difference supports the presence of likely different compounds responsible for a different colour reaction in 10% KOH and thus supports the segregation of *A. subnitens*.

In addition ostiolar discs of *A. subnitens* average smaller than those of the above specimens of *A. nitens* (0.30–0.33 vs. 0.4-0.5 mm diam).

Conclusion

The relatively limited material of *Annulohypoxylon* collected during this short field trip cannot reflect the species richness that can likely be expected from the forests of French Guiana. However, the present survey increases the number of *Annulohypoxylon* spp. known from French Guiana from two to seven, including as new records a species discovered in Brazil, several collections referable to the pantropical *A. moriforme* and three new species.

In absence of distinctive ascospore characters in most *Annulohypoxylon* spp., their segregation primarily relies on stromatal morphology, including ostiolar disc morphology, colour of the outermost coating at early stages and KOH-extractable pigments.

Ostiolar discs offer a good taxonomic marker as to their diameter which is fairly consistent within a given species. They were assigned to two different types according to the way they dehisce from the stroma (Ju & Rogers, 1996). These authors recognized the *bovei*-type and the *truncatum*-type in reference to what occurs in *A. bovei* and in *A. truncatum*. In the former type the disc dehisces abruptly, in one piece, leaving a sharp rim, while in the latter the disc dehisces gradually by outwardly extending fragmentation of the stromatal tissue around the ostiole, leaving a more irregular rim. In practice, assigning the ostiolar discs of a stroma of *Annulohypoxylon* to one of these types is often delicate owing to variations in the morphology of their rims, unless the discs can be observed during their dehiscence. This is only possible on young, barely mature stromata, during a very limited period of time and we had the opportunity to observe this phenomenon in only three collections. The formation of the ostiolar discs of *A. fulvum* appeared to occur according to the *truncatum*-type, in agreement with the notched edges of the resulting discs (Plate 2). Ostiolar discs in *A. purpureopigmentum* were referred to the *bovei*-type (Pereira *et al*., 2010), in agreement with their overall morphology, but we observed a dehiscence of the *truncatum*-type in all the dehiscing discs (Plate 10). In contrast, the ostiolar discs of *A. stygium*, that have fairly irregular edges and are referred to the *truncatum*-type (Ju & Rogers, 1996) were shown to have an unexpected dehiscence of the *bovei*-type (Plate 11). These observations show that the type of ostiolar disc cannot be safely inferred from the appearance of its rims and necessitates the observation of the dehiscence that occurs only on young stromata during a limited period of time, rendering this character often difficult to appraise. For instance, we failed to observe dehiscing ostiolar discs in the nine collections of *A. nouraguense* while most of them featured fairly young stromata.

One can also interpret the discrepancies between the putative ostiolar type and the mode of dehiscence reported above as the result of the influence of environmental factors, which would reduce the taxonomic value of this character. More thorough observations of the mode of dehiscence of the ostiolar discs of *Annulohypoxylon* spp. are needed to assess the validity of this differential character.

By the wide range of their variations in stromatal morphology, the nine collections of *H. nouraguense* reported and illustrated here show well how most morphological characters may vary with the age of the stromata and the influence of other unknown factors is

difficult to evaluate. The degree of conspicuousness of the perithecial contours and the shininess of the stromal surface that are the most visible features are also the most variable and therefore the less informative. However, the colour of the stromatal surface at early stages appears consistent in a given species and is a reliable differential character as long as it has not vanished over time.

Despite their variable stromatal morphology, the nine collections of *A. nouraguense* were recognized as the same species based on their distinctive KOH-extractable pigments gradually evolving from purple to hazel, a feature that was not observed in the isotype of *A. purpureonitens*, its closest relative. The observation of the subsurface waxy granules under the microscope, combined with the KOH-extractable pigments and their evolution over at least 20–30 min appears in many case more discriminant than the other stromatal characters except the ostiolar discs diameter.

The most difficult group and perhaps the best represented includes species resembling *A. moriforme* and *A. nitens* that have similar green KOH-extractable pigments, similar olivaceous stromatal granules and pale to medium brown ascospores rarely over 9 µm long. The delimitation of these species is a challenge that should be addressed with other approaches than classical morphological characterization.

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