

New, rarely recorded and unsettled species of *Hypoxylon* (*Xylariaceae*) from French Guiana

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Summary: In June 2012, a field trip carried out over two weeks in two different localities in French Guiana resulted in a collection of 256 specimens of pyrenomycetes, mostly *Xylariaceae*. Out of them 42 specimens of *Hypoxylon* were recorded, several of them could not be equated to known species. Specimens of *Hypoxylon* collected by C. Lechat during previous collecting trips in French Guiana in 2008 and 2010 are likewise included in this survey. As a result of the thorough morphological study of these specimens 13 taxa are presented herein, including six new species, *H. aureolimbatum*, *H. lepieurianum*, *H. nudum*, *H. paracouense*, *H. rhombisporum* and *H. verruciperisporium* that are described, illustrated and commented upon. *H. aureolimbatum* strikingly resembles the temperate species *H. subticinense* but besides its different geographical distribution it deviates in the combination of morphological differences of stromata, asci, ascospores and asexual morph associated on natural substrate; *H. lepieurianum* is segregated from *Hypoxylon* spp. with similar external morphology based on small equilateral ascospores with broadly rounded ends, a perispore indehiscent in KOH and a faint short germ slit; *H. nudum* is unique in having a stroma reduced to a black basal tissue and lacking stromatal pigments; *Hypoxylon paracouense* resembles *H. duranii* but deviates by different KOH-extractable pigments and smaller ascospores with a sigmoid germ slit; *H. rhombisporum* is diagnosed based on frequently rhomboid ascospores with laterally ridged epispore and several further morphological differences from the closely related *H. rectangulosporum*; *H. verruciperisporium* is unique in having a verrucose perispore. Four rarely recorded species are likewise described and illustrated: *H. aeruginosum*, *H. musceum* and *H. trugodes* that are recorded from French Guiana for the first time, and *H. fusoidesporum* that was only known from two collections in French Guiana and Guyana. Finally, two collection deviating respectively from *H. cinnabarinum* and *H. hypomiltum* and possibly different are described, for which no formal taxonomic decision can be made until the natural range of variations encountered in these taxa is clearly understood; furthermore, three specimens are tentatively referred to *H. dieckmannii*, a pantropical taxon that appears to be a complex of closely related species. The holotypes of *H. duranii*, *H. musceum* and *H. rectangulosporum* that were studied for comparison are likewise illustrated. A dichotomous key the *Hypoxylon* spp. known from French Guiana is provided. In conclusion, the use of new morphological characters to segregate *Hypoxylon* spp. is suggested and commented.

Keywords: Ascomycota, *Hypoxylodeae*, Nouragues natural reserve, Paracou, pyrenomycetes, saproxylic, taxonomy, tropical mycology, type studies, *Xylariales*.

Résumé : lors d'une expédition sur le terrain qui s'est déroulée pendant deux semaines en juin 2012 en Guyane dans deux localités différentes, 256 spécimens de pyrénomycètes ont été récoltés, pour la plupart des Xylariacées. Parmi eux 42 récoltes de *Hypoxylon* furent enregistrées, dont plusieurs ont posé des problèmes d'identification. Après une étude morphologique approfondie de ces récoltes, 13 taxons sont présentés ici dont six espèces nouvelles, *H. aureolimbatum*, *H. lepieurianum*, *H. nudum*, *H. paracouense*, *H. rhombisporum* et *H. verruciperisporium* qui sont décrites, illustrées et commentées. *H. aureolimbatum* ressemble de façon frappante à *H. subticinense*, une espèce tempérée, mais à part son origine géographique différente il s'en distingue aussi par la combinaison de différences morphologiques des stromas, des ascus, des ascospores et de la forme asexuée associée sur le substrat naturel; *H. lepieurianum* se distingue des autres espèces de *Hypoxylon* morphologiquement similaires par des ascospores équilatérales à extrémités obtuses, à périspore non déhiscente dans la potasse et à sillon germinatif peu visible et plus court que la spore; *H. nudum* est unique à cause d'un stroma réduit à un tissu stromatique noir présent à la base des périthèces, dépourvu de pigments stromatiques; *H. paracouense* ressemble à *H. duranii* mais s'en distingue par des pigments extraits par la potasse différents et des ascospores plus petites pourvues d'un sillon germinatif sigmoïde; *H. rhombisporum* se reconnaît principalement à des ascospores souvent losangiques, à épispore pourvue de côtes latérales, ainsi qu'à plusieurs autres caractères morphologiques qui le distinguent de l'espèce la plus proche *H. rectangulosporum*; *H. verruciperisporium* est unique par sa périspore verruqueuse. Quatre autres espèces rarement signalées sont également décrites et illustrées: *H. aeruginosum*, *H. musceum* et *H. trugodes* qui sont signalées de Guyane pour la première fois, et *H. fusoidesporum* qui n'était connu que de deux récoltes en Guyane française et au Guyana. Enfin une récolte proche de *H. hypomiltum* mais peut être différente est décrite, pour laquelle aucune décision taxinomique ne peut être proposée tant que les limites des variations naturelles de ce taxon ne sont pas clairement fixées; en outre, trois récoltes sont provisoirement imputées à *H. dieckmannii*, une espèce mal définie qui apparaît être un complexe d'espèces proches. Les holotypes de *H. duranii*, *H. musceum* et *H. rectangulosporum* qui ont été examinés à titre de comparaison sont également illustrés. Une clé dichotomique des espèces de *Hypoxylon* connues de Guyane est proposée. En conclusion, l'utilisation de caractères morphologiques nouveaux est suggérée et commentée en vue de faciliter la détermination des espèces de ce genre.

Mots-clés : Ascomycota, études de types, *Hypoxylodeae*, mycologie tropicale, Paracou, pyrénomycètes, réserve naturelle des Nouragues, saproxyliques, taxinomie, *Xylariales*.

Introduction

French Guiana is a French territory in the northwestern part of the South American continent, the surface of which is occupied at 96% by rainforests spreading over 8 million hectares that are regarded as among the best preserved in the Amazonian basin (ONF, <http://www.onf.fr/guyane/@@index.html>).

The *Xylariaceae*, as most of Ascomycota, are known to display an especially high diversity in the tropics and in this respect French Guiana can be expected to be a biodiversity hotspot. The xylariaceous genus *Hypoxylon* Bull. is one of the most diverse in the family, that has been monographed twice in the 20th century by MILLER (1961) and by JU & ROGERS (1996).

The first investigations on the *Xylariaceae* in French Guiana date back to the 19th century with the material collected by F.M.R.

Leprieur during the years 1837–1839 and described by MONTAGNE (1840). The generic concept of *Hypoxylo* was much wider than currently delimited but two species named by Montagne based on material collected in French Guiana are still valid: *H. hypomiltum* Mont. and *H. monticulosum* Mont. (JU & ROGERS, 1996). Two specimens regarded by Montagne as *H. hypomiltum* were further segregated and accommodated in *H. anthochroum* Berk. & Broome (Leprieur 371 “junior”) and *H. pelliculosum* Petch (Leprieur 371), and *H. vinosum* Mont. (Leprieur 442) was regarded as a synonym of *H. fuscum* (Pers. : Fr.) Fr. by JU & ROGERS (1996).

In their check-list of Fungi from French Guiana, COURTECUISE *et al.* (1996) reported 11 species of *Hypoxylo* *sensu* MILLER (1961) but most of them were later moved to *Annulohypoxylo* Y.-M. Ju, J.D. Rogers & H.-M. Hsieh (HSIEH *et al.*, 2005), *Camillea* Fr. (LÆSSØE *et al.*, 1989), *Kretzschmaria* Fr. (ROGERS & JU, 1998) and *Stilbohypoxylo* Henn. (ROGERS & JU, 1997).

The status of the only remaining species cited in the check-list, *H. sclerophaeum* Berk. & M.A. Curtis, is questionable since in their revision of the genus, JU & ROGERS (1996) segregated eight different taxa formerly accommodated under this name *sensu* MILLER (1961). The five collections of *H. sclerophaeum* reported in the check-list were made by H. Jacquemin in the late 1970’s and early 1980’s, at a time when Miller’s concepts were still prevailing. They are a part of 250 collections housed in CAY herbarium (Cayenne, French Guiana) but have unfortunately not been critically revised (COURTECUISE *et al.*, 1996).

Further contributions to the knowledge of *Hypoxylo* in French Guiana were those of G.J. Samuels and collaborators in their 1986 and 1987 forays during which the 10 following species were collected and identified according to the current taxonomic concepts viz.: *H. dieckmannii* Theiss., *H. erythrostroma* J.H. Mill., *H. fendleri* Berk. ex Cooke, *H. fusoideosporum* Y.-M. Ju & J.D. Rogers, *H. investiens* (Schwein.) M.A. Curtis, *H. munkii* Whalley, Hammelev & Talig., *H. rickii* Y.-M. Ju & J.D. Rogers, *H. rubellum* Penz. & Sacc., *H. samuelsii* Y.-M. Ju & J.D. Rogers and *H. symphyon* A. Möller (JU & ROGERS, 1996).

More recently, *H. lechatii* J. Fourn. & M. Stadler was described from French Guiana based on material collected by C. Lechat in 2008 and recollected by J. Fournier in 2012 (KUHNERT *et al.*, 2014b). A further taxon that can be finally added is *H. pulicicidum* J. Fourn., Polishook & Bills, based on an endophytic strain originating from French Guiana shown to correspond with the sexual morph described from Martinique (BILLS *et al.*, 2012), bringing up the number of known species of *Hypoxylo* in French Guiana to 17. The sexual morph of *H. pulicicidum* has not yet been recorded from French Guiana, perhaps because it is difficult to distinguish morphologically from *H. investiens*.

In the frame of an ongoing research program carried out by Labex CEBA in French Guiana, mainly aiming at the evaluation of the biodiversity and the interactions between plants and other organisms in Amazonian forests, a collecting trip was organized for mycologists in late June 2012 by the CNRS Laboratory EDB (Toulouse) and UMR EcoFog (Kourou). The first week was devoted to collecting fungi in the Nouragues natural reserve, 4° 4’ 24” N, 52° 44’ 1” W, around Inselberg camp, in primary rainforest with altitude ranging from 100 to 400 m. The second week was spent at Paracou CIRAD field station, 5°16’ 54” N, 52°54’ 44” W in lowland rainforest with altitude averaging 32 m. In both locations the average annual rainfall is 3,000 mm and this period of the year corresponds to the end of the rain season.

During these two weeks we (JF and occasionally CL) collected 256 specimens of pyrenomycetes, mostly *Xylariaceae*, out of which the best represented genera were *Xylaria* Hill ex Schrank (76 samples), *Camillea* (52 samples), *Hypoxylo* (42 samples) and *Annulohypoxylo* (28 samples). The present study deals with the specimens of *Hypoxylo* encountered during this short period of time, along with some specimens collected by C. Lechat over previous collecting trips in 2008 and 2010.

Rapidly, it appeared that the two widespread pantropical species *H. hypomiltum* and *H. monticulosum* were largely prevailing. Interestingly, as mentioned above, both were described by MONTAGNE (1840), suggesting they were, as expected, already widespread at this time. Aside from the common pantropical *H. fendleri* that was collected twice in 2012 but already known from several collections from French Guiana made by Samuels in 1987, three common pantropical taxa were collected in 2008 and 2010 by CL (unpublished data, collecting details listed at the end of taxonomic part), viz.: *H. haematostroma* Mont., *H. investiens* and *H. lenormandii* Berk. & Curtis, the former and the latter reported here for the first time from French Guiana. All other collections turned out to be either rarely recorded taxa unknown from French Guiana or undescribed taxa, which prompted this study.

As a result, we propose below six new species that could be segregated from known species based on distinctive morphological traits, viz.: *H. aureolimbatum* sp. nov., *H. leprieurianum* sp. nov., *H. nudum* sp. nov., *H. paracouense* sp. nov., *H. rhombisporum* sp. nov. and *H. verruciperisporium* sp. nov., along with four species not or rarely recorded in French Guiana viz.: *H. aeruginosum* J.H. Mill., *H. fusoideosporum*, *H. musceum* J.D. Rogers and *H. trugodes* Berk. & Broome. Furthermore, three collections that could not be safely assigned to a species name are described and illustrated until a formal decision can be made. One fits in the current concept of *H. cinnabarinum* (Henn.) Y.-M. Ju & J.D. Rogers but it is suspected that this taxon is a complex of closely related cryptic species, the second is closely related to but deviating from *H. hypomiltum* by a purplish stromatal surface and olivaceous stromatal pigments, the last one keys out to *H. dieckmannii* Theiss. but with reservations since the concept of this taxon appears unsettled.

Finally, to facilitate further studies on *Hypoxylo* in French Guiana and, more broadly, in the Neotropics, a dichotomous key to the species known from French Guiana is proposed.

Material and methods

The observations were carried out on dry material rehydrated in water. Measurements of asci and ascospores were made in water and ascospores measurements processed with the free software Piximetre 5.2 (<http://ach.log.free.fr/Piximetre/>). In the formula given by this software the values into brackets represent the extreme values (20%) that are not taken into account for the calculation, N represents the number of ascospores measured, Q the quotient length/width, Me the mean values of length × width and Qe the mean value of quotient length/width. The amyloid reaction of the ascus apical apparatus was tested by adding a drop of Melzer’s reagent to a water mount of centrum contents. Microscopic observation of the asci and the ascogenous hyphae was carried out after 1 min in 1% SDS and mouting in blue or black Waterman inks, chlorazol black, black Pelikan ink or India ink; in case of ascospores with germ slit morphology difficult to make out, the ascospores were mounted in PVA-lactophenol and observed after 48 h incubation; the asexual morph structures were observed in 1% SDS. Measurements of perithecia, asci and ascal apical apparatus are recorded as height × width.

The pigments released by the outer stromatal crust were observed through the stereomicroscope by adding a small fragment of this tissue to a drop of 10% KOH on a glass slide placed on a white sheet of paper and recorded within 1 min and after a further incubation time of 20–30 min. To be consistent with previous works on *Hypoxylo* and especially with JU & ROGERS (1996), the colours of stromata and pigments in KOH were coded according to Rayner’s mycological chart (RAYNER, 1970). As the access to this chart is difficult to many mycologists, colours are coded in parallel according to Online Auction colour chart which is easily available at a very affordable price, can be consulted online and offers a much wider palette of nuances (as oacxxx).

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

a: JU & ROGERS (1996); b: this paper; c: KUHNERT *et al.* (2014).

1	Ascospores with perispore dehiscent in 10% KOH	2
1	Ascospores with perispore not dehiscent in 10% KOH	19*
*Species with perispore rarely dehiscent in KOH or dehiscent with difficulty are dealt with in both parts of the key.		
2	KOH-extractable pigments yellow orange, orange red or orange brown	3
2	KOH-extractable pigments different or absent	12
3	Ascospores with oblique or sigmoid germ slit	4
3	Ascospores with straight germ slit	7
4	Germ slit less than spore-length, stromatal surface olivaceous to isabelline	<i>H. musceum</i> (a, b)
4	Germ slit almost spore-length to spore-length, stromatal surface without olivaceous tones	5
5	Stromata rosellinoid to glomerate; KOH-extractable pigments dark brick; ascospores 11–13 × 4.8–5.8 µm	<i>H. lenormandii</i> (a)
5	Stromata effused to effused pulvinate; KOH-extractable pigments orange	6
6	Stromatal surface greyish sepia to fawn, ascospores 7.5–8.5 × 3.2–3.8 µm with coarsely striated perispore	<i>H. erythrostroma</i> (a)
6	Stromatal surface vinaceous with orange tone, ascospores 9.3–10.5 × 4–5 µm with faintly striated perispore	<i>H. fendleri</i> (a)
7	Ascospores 13–16.6 × 6.3–7.5 µm	<i>H. haematostroma</i> (a)
7	Ascospores less than 11 µm long	8
8	Ascospores equilateral with a short often oblique germ slit	<i>H. hypomiltum</i> (a)
8	Ascospores inequilateral with a germ slit spore-length	9
9	Perithecia tubular to long tubular	10
9	Perithecia spherical	11
10	Stromatal surface rust or sienna; KOH-extractable pigments orange, ascospores 7.3–8.1 × 3.2–3.5 µm	<i>H. rickii</i> (a)
10	Stromatal surface vinaceous to purplish; KOH-extractable pigments luteous to cinnamon; ascospores 6.8–7.4 × 3.2–3.7 µm	<i>H. trugodes</i> (a, b)
11	Stromata glomerate; ascospores 9–11 × 4.5–5.5 µm with coarsely striated perispore	<i>H. pelliculosum</i> (a)
11	Stromata effused; ascospores 8.5–9.3 × 3.8–4.4 µm with warted perispore	<i>H. verruciperisporium</i> sp. nov. (b)
12	KOH-extractable pigments absent at maturity	13
12	KOH-extractable pigments present at maturity	15
13	Stromata black with papillate ostioles, ascospores with germ slit on the convex side	14
13	Stromata white to brown vinaceous, ostioles umbilicate, ascospores 6.5–7.5 × 3.5–4 µm with germ slit on the flattened side	<i>H. munkii</i> (a)
14	Stromata effused-pulvinate, carbonaceous, black at maturity, rust when young and then with purple KOH-extractable pigments, ascospores 7.2–8.5 × 3–3.5 µm with a sigmoid germ slit	<i>H. monticulosum</i> (a)
14	Stromata highly reduced, perithecia rosellinoid, leathery, ascospores 8.5–9.2 × 3.4–3.7 µm with a straight germ slit	<i>H. nudum</i> sp. nov. (b)
15	KOH-extractable pigments olivaceous	16
15	KOH-extractable pigments greenish grey or amber	18
16	Perithecia long tubular; ascospores 8.5–10 × 4–4.5 µm with straight germ slit spore-length	<i>H. lechatii</i> (c)
16	Perithecia spherical to obovoid; ascospores with different germ slit morphology	17
17	Perithecia 0.4–0.5 mm diam; ascospores equilateral, 7.1–7.9 × 3.2–3.6 µm with short often oblique germ slit	<i>H. cf. hypomiltum</i> (b)
17	Perithecia 0.1–0.3 mm diam; ascospores inequilateral 8–20 × 4–8 µm with sigmoid germ slit spore-length	<i>H. fuscum</i> (a)
18	KOH-extractable pigments greenish grey; ascospores 10–12 × 5–5.5 µm with straight germ slit	<i>H. anthochroum</i> (a)
18	KOH-extractable pigments amber, becoming vinaceous upon prolonged incubation; ascospores 7.5–8.2 × 3.6–4.1 µm with sigmoid germ slit	<i>H. paracouense</i> sp. nov. (b)
19	KOH-extractable pigments orange or sienna	20
19	KOH-extractable pigments different or absent	26
20	Ascospores inequilateral, 23–27 × 11–13.5 µm	<i>H. fusoidesporum</i> (a, b)
20	Ascospores equilateral less than 20 µm long	21
21	Ascospores 18–21 × 7.5–8.5 µm with acute ends and epispore with reticulate rims; on bamboo	<i>H. rubellum</i> (a)
21	Ascospores less than 12 µm long with obtuse ends and smooth epispore; on wood	22
22	Stromata with tubular to long tubular perithecia	23
22	Stromata with spherical or obovoid perithecia	24
23	Stromatal surface ochreous to cinnamon, stromata 2.5–5.7 mm thick, ascospores 10.8–12.3 × 5.9–6.8 µm	<i>H. cf. cinnabarium</i> (b)
23	Stromatal surface dark vinaceous, stromata 0.8–2.5 mm thick, ascospores 7.3–9 × 2.8–3.3 µm	<i>H. samuelsii</i> (a)
24	Ascospores with narrowly rounded to acute ends, with a short often oblique germ slit	<i>H. hypomiltum</i> (a)
24	Ascospores with broadly rounded ends, with a germ slit parallel to the sides	25
25	Stromata with an orange yellow fimbriate margin when young; ascospores broadly ellipsoid, dark brown, 9.7–11.2 × 5.9–6.8 µm	<i>H. aureolimbatum</i> sp. nov. (b)
25	Stromata with concolourous, undifferentiated margin; ascospores oblong, lighter brown, 7.1–8.1 × 3.2–3.8 µm	<i>H. lepreurianum</i> sp. nov. (b)
26	Stromata highly reduced, perithecia rosellinoid, black, ascospores 8.5–9.2 × 3.4–3.7 µm with rarely dehiscent perispore	<i>H. nudum</i> sp. nov. (b)
26	Stromata effused-pulvinate or peltate, with coloured surface	27
27	Stromata peltate, 7–13 mm thick, ascospores 9.5–12 × 4–5 µm, highly variable in shape with germ slit frequently on the flattened side when inequilateral	<i>H. symphyon</i> (a)
27	Stromata effused-pulvinate, not over 1.5 mm thick	28
28	Stromatal surface cyan blue	<i>H. aeruginosum</i> (a, b)
28	Stromata surface in shades of brown, purplish or vinaceous	29
29	Ascospores often rhomboid, with longitudinally ridged epispore, 6.3–7.4 × 3.2–3.8 µm	<i>H. rhombisporum</i> sp. nov. (b)
29	Ascospores ellipsoid with smooth epispore	30
30	Stromatal surface purplish, KOH-extractable pigments dull green or olivaceous	31
30	Stromata surface pale to dark brown, KOH-extractable pigments ochreous, fawn, greyish sepia or absent	32
31	KOH-extractable pigments dull green, perithecia tubular, ascospores with broadly rounded ends	<i>H. investiens</i> (a)
31	KOH-extractable pigments olivaceous, perithecia spherical to obovoid, ascospores with narrowly rounded to acute ends	<i>H. cf. hypomiltum</i> (b)
32	Stromatal surface brown vinaceous to chestnut, KOH-extractable pigments greyish sepia or absent, ascospores 6.5–10 × 3.5–4 µm	<i>H. dieckmannii</i> (a)
32	Stromatal surface dark brick to fawn, KOH-extractable pigments ochreous to fawn, ascospores 6.7–7.6 × 3.3–3.8 µm	<i>H. cf. dieckmannii</i> (b)

Photomicrographs were taken with a Nikon Coolpix 995 digital camera either directly mounted on a stand or, for higher magnifications, through the eyepiece of an Olympus SZ60 stereomicroscope, by the means of a 30 mm diameter adapter. Photomicrographs were taken with the same camera mounted on the trinocular port of a Leitz Orthoplan microscope. The digitalised photographs were processed with Adobe Photoshop Elements 10 and the figures assembled with the same software.

The holotype material and paratypes were deposited in LIP (University of Lille, France) and duplicates are kept in the personal herbarium of JF. Initials JF and CL/CLL refer to Jacques Fournier and Christian Lechat respectively. Nomenclature follows MycoBank.

Taxonomy

Hypoxyton aeruginosum J.H. Mill., *Mycologia*, 25 (4): 321(1933). Plate 1

Stromata effused-pulvinate, rounded to orbicular, often coalescent, with inconspicuous to conspicuous perithecial contours, 3–19 mm long × 3–13 mm wide × 0.4–0.45 mm thick; surface verdigris (74, oac217) to cyan blue (26, oac260) or sky blue (25, oac218), when young covered by a honey (64, oac847) to cinnamon (62, oac715) woolly coating consisting of sterile contorted or diverticulate hyphae, remaining present at margins of mature stromata, possibly the remnants of the asexual morph; mature stromata with a persistent white pruina on surface; bluish green granules present beneath surface and interspersed in the black slightly carbonaceous interperithecial tissue, turning blue in 10% KOH, yielding dilute pale vinaceous (85, oac515) KOH-extractable pigments after several minutes incubation; subperithecial tissue 0.1–0.15 mm thick, formed by a thin pale bluish green layer seated on a thin black carbonaceous layer. **Perithecia** obovoid, 0.25–0.3 × 0.2 mm. **Ostioles** umbilicate, appearing as black dots on stromatal surface or inconspicuous.

Asci cylindrical with eight obliquely uniseriate ascospores, the spore-bearing parts 65–76 × 6–7.5 µm, the stipes 25–38 µm long, with a discoid apical apparatus 0.6–0.8 × 2–2.2 µm, bluing in Melzer's reagent. **Ascospores** (7.5–) 8.1–9.5 (–9.9) × (3.4–) 4.0–4.6 (–4.8) µm; Q = (1.6–) 1.8–2.3 (–2.8); N = 54 (Me = 8.6 × 4.3 µm; Qe = 2.0), ellipsoid-equilateral with broadly rounded ends, pale olivaceous brown, with a straight germ slit ca. ½ spore-length; perispore indehiscent in 10% KOH; epispore smooth, that of immature ascospores turning bluish grey in 10% KOH.

Asexual morph on natural substrate not seen.

Specimens examined: FRENCH GUIANA: Sinnamary, Paracou, edge of parking area of CIRAD field station, on a corticated branch, possibly on old remnants of a Corticiaceae, 26 Jun. 2012, *leg.* J. Fournier, GYJF 12236 (LIP); *ibid.*, on primordia of *Hypoxyton* sp. on a corticated branch, 27 Jun. 2012, *leg.* J. Fournier, GYJF 12245 (LIP).

Distribution: Ecuador, French Guiana, Guadeloupe, Guyana, Mexico, Russia, Uganda.

Discussion: This outstanding *Hypoxyton* is characterized by effused-pulvinate greenish blue stromata with a carbonaceous interior and bluish green granules that change colour in 10% KOH (under the microscope) but take a long time to give faint pigments in a drop of 10% KOH, and by small equilateral ascospores with a straight germ slit and an indehiscent perispore. The occasionally faintly striated epispore reported by Ju & ROGERS (1996) was not made out here.

It is impossible to assess whether the dark brown tissue on which stromata of GYJF 12236 develop is of fungal origin but in GYJF 12245 the stromata of *H. aeruginosum* clearly invade primordia of a *Hypoxyton* sp. with orange KOH-extractable pigments. This supports a strong suspicion of a fungicolous life style already suggested by the type collection (LÆSSØE *et al.*, 2010).

The collection GYJF 12236 illustrated here was cultured and sequenced and designated as epitype of *H. aeruginosum* (KUHNER *et al.*, 2014).

Hypoxyton aureolimbatus J. Fourn. & Lechat *sp. nov.* – MycoBank MB811911. Plates 2-3

Diagnosis: Differs from *Hypoxyton subticinense* by a tropical distribution, subglobose perithecia vs. tubular, short-stipitate asci, larger ascospores 9.7–11.2 × 5.9–6.8 vs. 8–10 × 4–5 µm and a nodulisporium-like to periconiella-like asexual morph vs. virgariella-like.

Holotype: FRENCH GUIANA: Sinnamary, Saint Elie track, lowland rainforest, dead corticated branch, 22 Apr. 2010, *leg.* C. Lechat, CLL 10008 (LIP).

Etymology: From Latin *aureus* = gold colour for the yellowish orange stromatal margin of young stromata and Latin *limbatus* = fringed.

Stromata effused-applanate, orbicular to irregularly ellipsoid, with slightly exposed perithecial contours, 10–43 mm long × 8–28 mm wide × 0.4–0.65 mm thick, lined when young by a wide luteous (12, oac810) sterile fimbriate margin, turning sienna (8, oac664); surface fulvous (43, oac706) turning dark brick (60, oac637), pruinose-fluffy, slightly uneven; orange red granules forming a thick waxy layer above and between perithecia, with sienna (8, oac664) KOH-extractable pigments, barely changing upon prolonged incubation; interperithecial tissue greyish brown, subperithecial tissue woody, black, homogeneous, 0.15–0.35 mm thick. **Perithecia** subglobose 0.25–0.3 mm diam. **Ostioles** umbilicate, blackish.

Asci cylindrical, originating from long ascogenous hyphae in unilateral spicate arrangement, with eight obliquely uniseriate ascospores, the spore-bearing parts 61–72 (–78) × 7–7.5 µm, the stipes 34–52 (–75) µm long, with a discoid apical apparatus 0.8–1.2 × 2.5–2.8 µm bluing in Melzer's reagent. **Paraphyses** filiform, septate, 4–6 µm wide at base, tapering above asci. **Ascospores** (9.3–) 9.7–11.2 (–11.6) × (5.4–) 5.9–6.8 (–7.0) µm; Q = (1.4–) 1.5–1.8 (–2.1); N = 50 (Me = 10.4 × 6.3 µm; Qe = 1.6), broadly ellipsoid nearly equilateral with most frequently narrowly rounded ends, brown to dark brown, with a conspicuous straight germ slit spore-length; perispore indehiscent and immature ascospores turning bluish grey in 10% KOH; epispore smooth.

Asexual morph on natural substrate occurring prior to stromata, forming greyish sepia (106, oac640) to isabelline (65, oac820) silky to fluffy colonies on bark with scattered orange patches; conidiogenous structure nodulisporium-like to periconiella-like, yellowish, roughened, with conidiogenous cells 10–16 (–22) × 3.5–4.5 µm and conidia ellipsoid, pale yellowish, smooth, 4.5–5.5 × 2.7–3.2 µm.

Distribution: French Guiana.

Discussion: *Hypoxyton aureolimbatus* is morphologically reminiscent of the temperate species *H. subticinense* Y.-M. Ju & J.D. Rogers (Ju & ROGERS, 1996) in stromatal and ascospore morphology. Both share orange brown effused stromata with yellow to orange fimbriate margins when young and orange KOH-extractable pigments, a thick black subperithecial tissue and equilateral ascospores with a straight germ slit spore-length and a perispore indehiscent in 10% KOH. However, besides its different geographical origin, it deviates from *H. subticinense* in having thinner stromata with subglobose perithecia, asci with stipes less than to as long as the spore-bearing parts and more broadly ellipsoid ascospores 9.7–11.2 × 5.9–6.8 vs. 8–10 × 4–5 µm. Moreover the asexual morph found at the vicinity of the stromata displays a nodulisporium-like to periconiella-like branching pattern as compared to the virgariella-like branching pattern reported for *H. subticinense* by Ju & ROGERS (1996). However, the latter observation was based on cultures on artificial medium (OA)

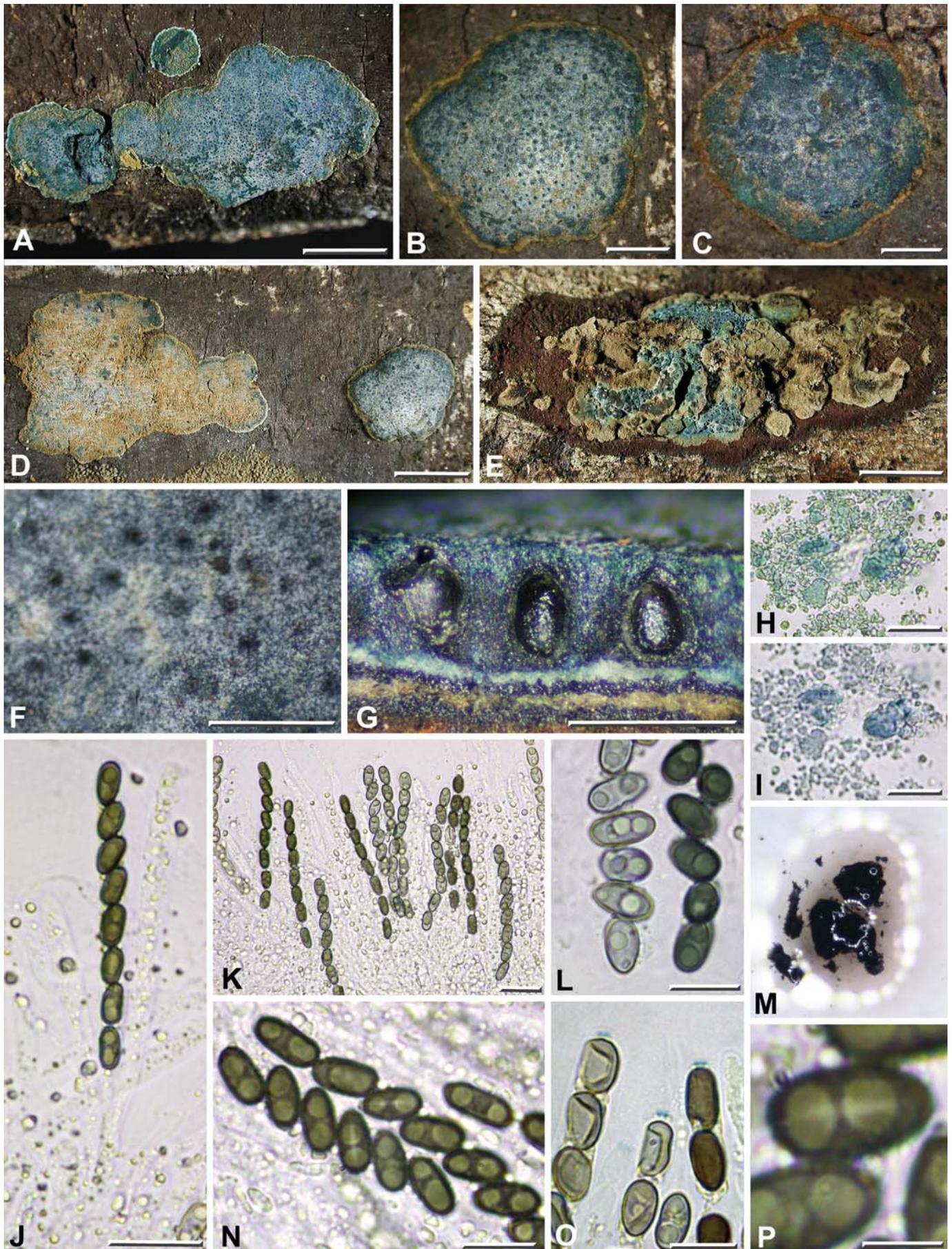


Plate 1 – *Hypoxylon aeruginosum*

A-D, F-P: GYJF 12236; E: GYJF 12245. A-C: Mature stromata; D, E: Maturing stromata; F: Stromatal surface in close-up showing the whitish pruina and the black ostioles; G: Stroma in vertical section showing the perithecia encased in carbonaceous tissue mixed with bluish waxy granules; H: Waxy stromatal granules observed in water under the microscope; I: Waxy stromatal granules after addition of 10% KOH; J: Mature ascus in water; K: Mature and immature asci in water; L: Ascospores in 10% KOH; M: KOH-extractable pigments after 5–10 min incubation; N: Ascospores in water; O: Ascical apical apparatus in Melzer's reagent; P: Ascospore in water showing the germ slit. Scale bars: A-E = 5 mm; B = 2 mm; C = 1 mm; F, G = 0.5 mm; J, K = 20 µm; H, I, L, N, O = 10 µm; P = 5 µm.

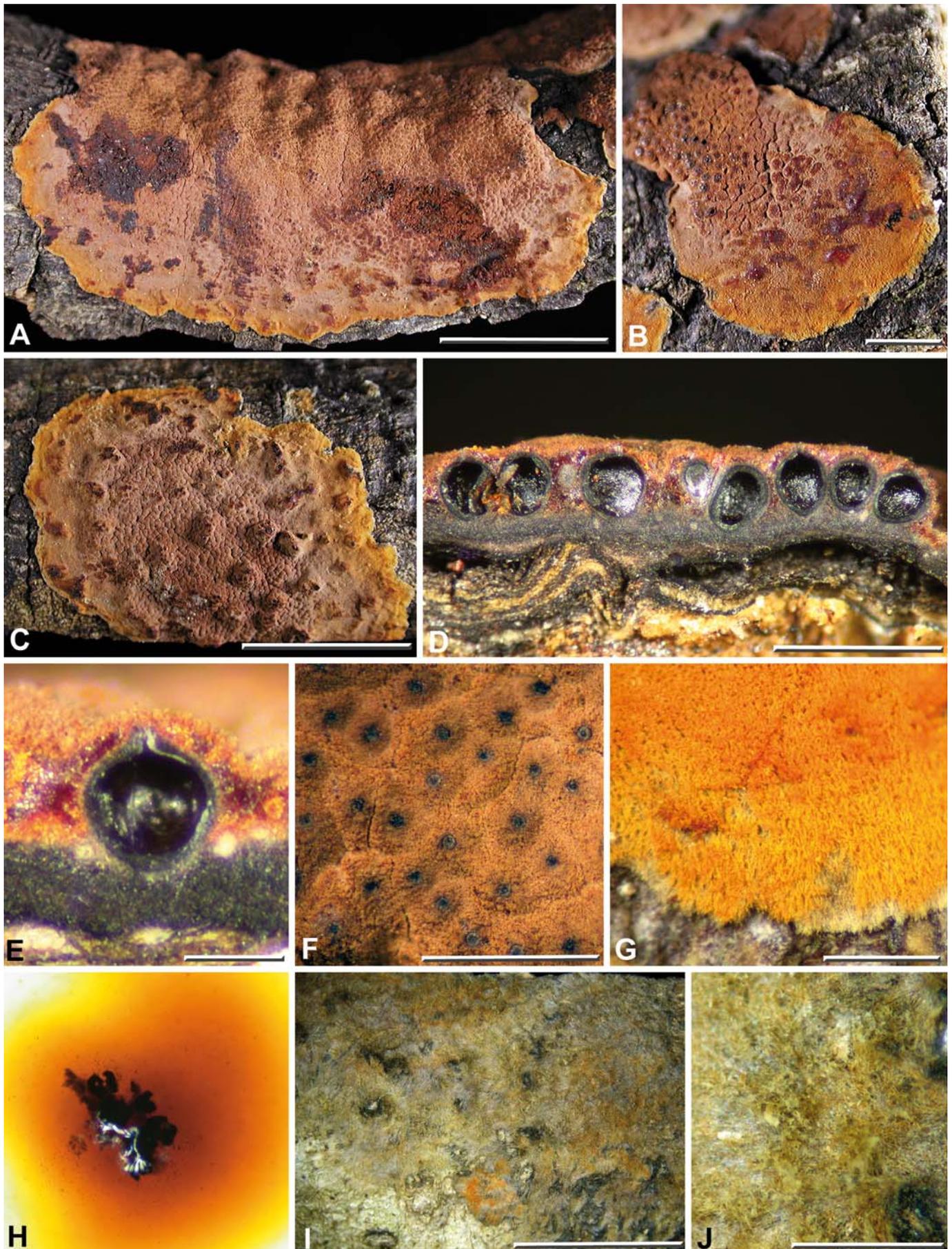


Plate 2 – *Hypoxylon aureolimbatum*. Holotype CLL 10008

A-C: Effused stromata on bark; D: Stroma in vertical section showing the perithecia; E: Stroma in vertical section showing a perithecium embedded in abundant waxy granules contrasting with the black basal layer; F: Stromatal surface in close-up showing the ostioles; G: Yellow-orange fimbriate sterile margin of a young stroma; H: KOH-extractable pigments after 20 min incubation; I: Asexual morph spreading on bark, with orange marks; J: Close-up on the asexual morph showing tufts of conidiophores. Scale bars: A, C, I = 10 mm; B = 2 mm; D, F, G, J = 1 mm; E = 0.2 mm.

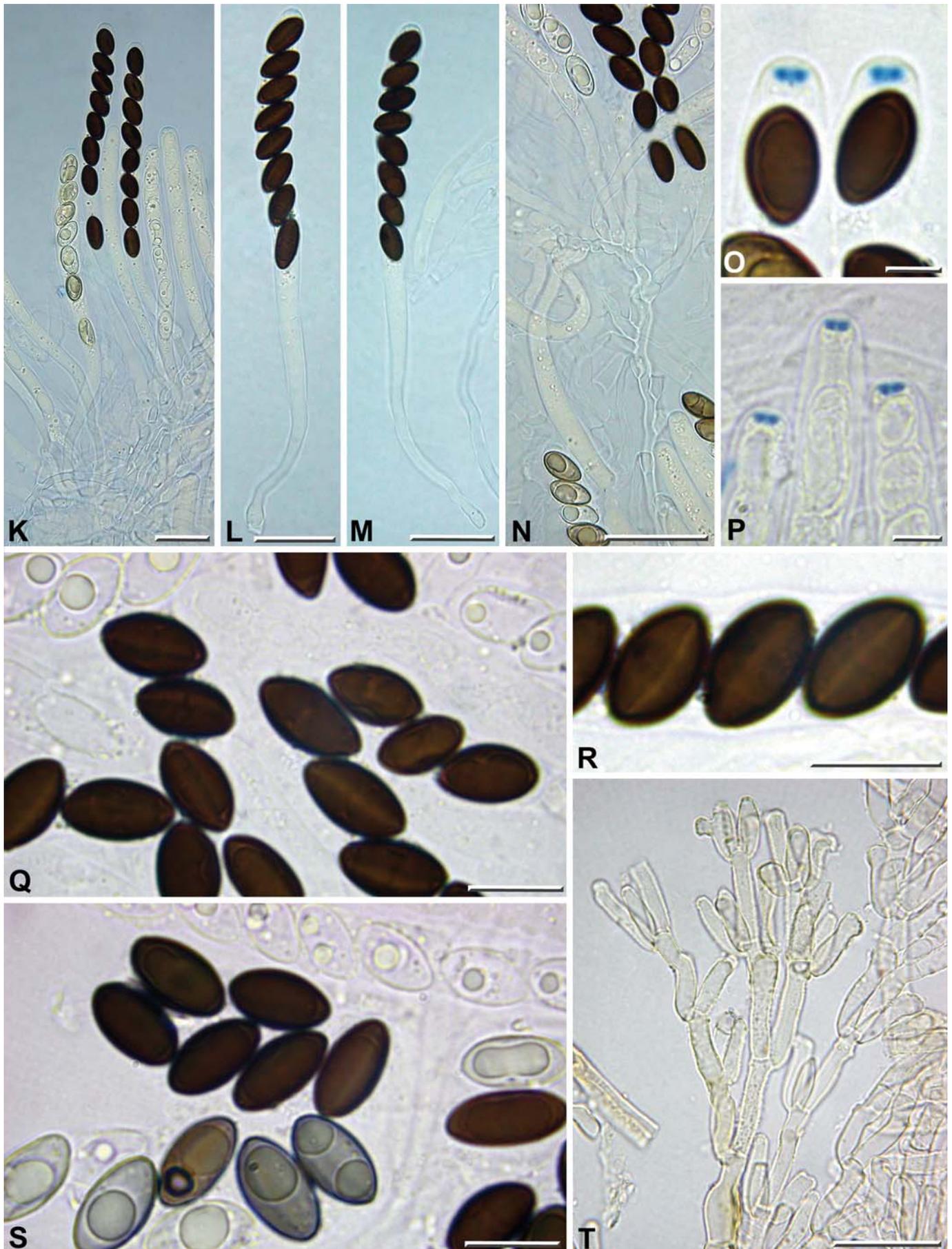


Plate 3 – *Hypoxylon aureolimbatum*. Holotype CLL 10008

K-N: Immature and mature asci in blue Waterman ink with 1% SDS, arising from ascogenous hyphae visible in K and N; O, P: Ascus apical apparatuses of mature and immature asci bluing in Melzer's reagent; Q: Ascospores in water; R: Ascospores in water showing the germ slit; S: Ascospores in 10% KOH showing the absence of dehiscent perispores and the bluish grey discolouration of immature ascospores; T: Nodulisporium- to periconiella-like asexual morph in 1% SDS. Scale bars: K-N, T = 20 µm; Q-S = 10 µm; O, P = 5 µm.

and should be compared with the asexual morph of *H. aureolimbatum* obtained under similar conditions.

Hypoxylon subticinense is likewise morphologically very similar to the temperate taxon *H. ticinense* L.E. Petrini which is readily recognized based on smaller ascospores $5\text{--}7 \times 2.5\text{--}3.5 \mu\text{m}$ that are inequilateral and possess a perispore dehiscent in 10% KOH. Ascospore morphology appears to be the most reliable way to discriminate these three taxa.

Interestingly *H. aureolimbatum* was included (as *H. cf. subticinense*) in a phylogenetic study comparing the sequences of ITS and β -tubulin loci of 57 temperate and tropical *Hypoxylon* spp. (KUHNER *et al.*, 2014a). Sequences of *H. ticinense* were included in this study but unfortunately sequences of *H. subticinense* were not available for comparison. However, the two genes used in this phylogenetic study taken separately often gave incongruent results and did not appear to reliably resolve the relationships within *Hypoxylon* and with the most closely related genera in the *Hypoxyloideae* (KUHNER *et al.*, 2014a). For instance *H. aureolimbatum* appeared basal to other *Hypoxylon* spp. and very distant from *H. ticinense* in the ITS-based phylogeny while they appeared more closely related in the β -tubulin-based phylogeny.

Hypoxylon lepieurianum sp. nov. (this paper) appears to be the most closely related tropical taxon to *H. aureolimbatum*. *H. lepieurianum* is mainly distinguished from *H. aureolimbatum* by having smaller, paler brown, more narrowly ellipsoid ascospores (Me = $7.7 \times 3.5 \mu\text{m}$; Qe = 2.2 vs. Me = $10.4 \times 6.3 \mu\text{m}$; Qe = 1.6), with an inconspicuous, often less than spore-length germ slit.

Hypoxylon cf. H. cinnabarinum (Henn.) Y.-M. Ju & J.D. Rogers, *Mycol. Mem.*, 20: 99 (1996). Plates 4–5

Stromata irregularly effused-pulvinate, 37–65 mm long \times 21–40 wide \times 2.5–5.7 mm thick, with sloping margins; surface pruinose, ochreous (44, oac756) to cinnamon (62, oac715), exposing the dark brick (60, oac637) subsurface layer when bruised, uneven but perithecial contours barely exposed; subsurface granules yellow and orange when observed in water, forming a conspicuous orange red crust above the perithecia, yielding dense orange (7, oac629) pigments in 10% KOH, not notably changing colour upon prolonged incubation; subperithecial tissue massive, 1.5–4.5 mm thick, woody, whitish to light grey around and beneath the base of perithecia, dark grey below, interspersed with light grey and orange vertically oriented streaks. **Perithecia** tubular to long tubular, 0.85–1 \times 0.4–0.5 mm. **Ostioles** umbilicate, fringed with white material forming small discs 80–100 μm diam.

Asci cylindrical, originating from long ascogenous hyphae in unilateral spicate arrangement, with eight obliquely uniseriate ascospores, mature asci fragmentary, the spore-bearing parts 72–82 \times 9–11 μm , the stipes up to 135 μm long when immature, with a discoid apical apparatus 0.5–0.8 \times 2.5–3 μm , bluing in Melzer's reagent. **Ascospores** (10.1–) 10.8–12.3 (–13.3) \times (5.3–) 5.9–6.8 (–7.3) μm , Q = (1.5–) 1.7–2.1 (–2.2); N = 50 (Me = $11.5 \times 6.4 \mu\text{m}$; Qe = 1.8), ellipsoid nearly equilateral to slightly inequilateral with narrowly rounded to acute, less frequently broadly rounded ends, dark brown to blackish brown, with a conspicuous straight germ slit spore-length; perispore indehiscent and immature ascospores turning greenish in 10% KOH; epispore smooth.

Asexual morph on natural substrate not seen.

Specimens examined: BRAZIL: 1908, J. Rick, decorticated wood, as *H. perforatum* by J. Rick, as *H. crocopleum* by J.H. Miller (BPI 11328). FRENCH GUIANA: Maripasoula, Saül, rainforest, "Gros Arbres" path, on a dead corticated branch, 3 May 2008, *leg.* C. Lechat, CLL 8081 (LIP).

Distribution: French Guiana, Guadeloupe, Martinique.

Discussion: This *Hypoxylon* is distinctive by its thick pulvinate stromata approaching 6 mm thick with ochreous to pale orange sur-

face, long tubular perithecia with the base seated on a thin layer of pale grey tissue, a thick blackish grey subperithecial tissue with vertical pale grey and orange streaks, combined with dark brown almost equilateral ascospores with frequently acute ends, a conspicuous straight germ slit and a perispore indehiscent in 10% KOH. This set of characters keys out to *H. cinnabarinum* in Ju & ROGERS's keys (1996). However, in the protologue of the type of *H. cinnabarinum* (as *Nummularia cinnabarina*) in HENNINGS (1897) its author reports subglobose perithecia and ascospores $8\text{--}10 \times 4\text{--}5 \mu\text{m}$, while Ju & ROGERS (1996) reported tubular to long tubular perithecia and ascospores $9.5\text{--}14 \times 5\text{--}7.5 \mu\text{m}$. On the other hand, another specimen collected by Rick in Brazil (BPI 11328) included in Ju & ROGERS's concept of *H. cinnabarinum* (1996) was re-examined (Plate 6) and found to have stromata ca. 0.6 mm thick with small obovoid perithecia and ascospores often citriform, (12.1–) 13.8–15.8 (–16.5) \times (6.2–) 7–8.1 (–8.3) μm , Q = (1.5–) 1.8–2.2 (–2.3); N = 31 (Me = $14.8 \times 7.4 \mu\text{m}$; Qe = 2). Moreover, it differs from the guianese *Hypoxylon* by darker reddish brown stromata with dark orange brown stromatal granules.

All the specimens cited by Ju & ROGERS (1996) have ascospores with perispore indehiscent in 10% KOH, except a collection from New Zealand (Chatham Islands) that conforms to their concept of *H. cinnabarinum*, even in culture morphology, but deviates in having perispores dehiscent in 10% KOH. Even though our Guianese *Hypoxylon* can fit in the species concept of *H. cinnabarinum* delimited by Ju & ROGERS (1996), the above observations suggest that this concept might comprise several different taxa or one taxon with a wide range of morphological variations that needs to be carefully evaluated. Molecular data showing that two collections referred to *H. cinnabarinum* from Mexico and Puerto Rico cluster only distantly in the same subclade also suggest that different species might be involved (HSIEH *et al.*, 2005). For the time being we prefer to regard our Guianese collection as closely related to *H. cinnabarinum* but possibly different.

Two strikingly similar collections from Guadeloupe and Martinique can be referred to the same taxon (unpublished results), which suggests a neotropical distribution.

Hypoxylon cf. H. dieckmannii Theiss., *Ann. Mycol.*, 6: 346 (1908). Plate 7

Stromata irregularly effused-pulvinate with most often inconspicuous perithecial contours, 6–53 mm long \times 6–16 mm wide \times 0.4–0.6 mm thick; surface dark brick (60, oac636) to fawn (87, oac645), pruinose; dull yellowish granules forming a thin waxy layer just beneath surface, yielding ochreous (44, oac756) KOH-extractable pigments turning fawn (87, oac645) after a prolonged incubation over 20 min; interperithecial tissue black, carbonaceous, brittle, devoid of coloured granules, subperithecial tissue woody, black, 0.1–0.3 mm thick. **Perithecia** spherical to obovoid, 0.25–0.3 \times 0.2–0.25 mm. **Ostioles** umbilicate, at times surrounded by a narrow disc of white powdery substance.

Asci cylindrical with eight obliquely uniseriate ascospores, the spore-bearing parts 48–54 \times 6–6.5 μm , the stipes 40–62 μm long, originating in spicate arrangement from long ascogenous hyphae, with a discoid to wedge-shaped apical apparatus 0.5–0.8 \times 1.5–2 μm , bluing in Melzer's reagent. **Paraphyses** sparse, filiform. Ascospores (6.5–) 6.7–7.6 (–8.1) \times (3.0–) 3.3–3.8 (–4.1) μm ; Q = (1.7–) 1.8–2.2 (–2.4); N = 40 (Me = $7.1 \times 3.5 \mu\text{m}$; Qe = 2.0), ellipsoid-equilateral with broadly rounded ends, medium brown, with a straight germ slit slightly less than spore-length to almost spore-length; perispore indehiscent in 10% KOH; epispore smooth.

Asexual morph on natural substrate not seen.

Specimens examined: FRENCH GUIANA: Sinnamary, Saint-Elie, botanical trail, lowland rainforest, corticated branch, 26 Jun. 2012, *leg.* J. Fournier, GYJF 12229 (LIP); *ibid.*, GYJF 1225-2 (LIP); Paracou,

trail to CIRAD field station, lowland rainforest, corticated branch, 25 Jun. 2012, leg. J. Fournier, GYJF 12196 (LIP).

Distribution: French Guiana, Neotropics, Taiwan.

Discussion: The *Hypoxylon* described above is characterized by effused-pulvinate pale reddish brown stromata with small perithecia encased in partly carbonaceous tissue and yellowish waxy gra-

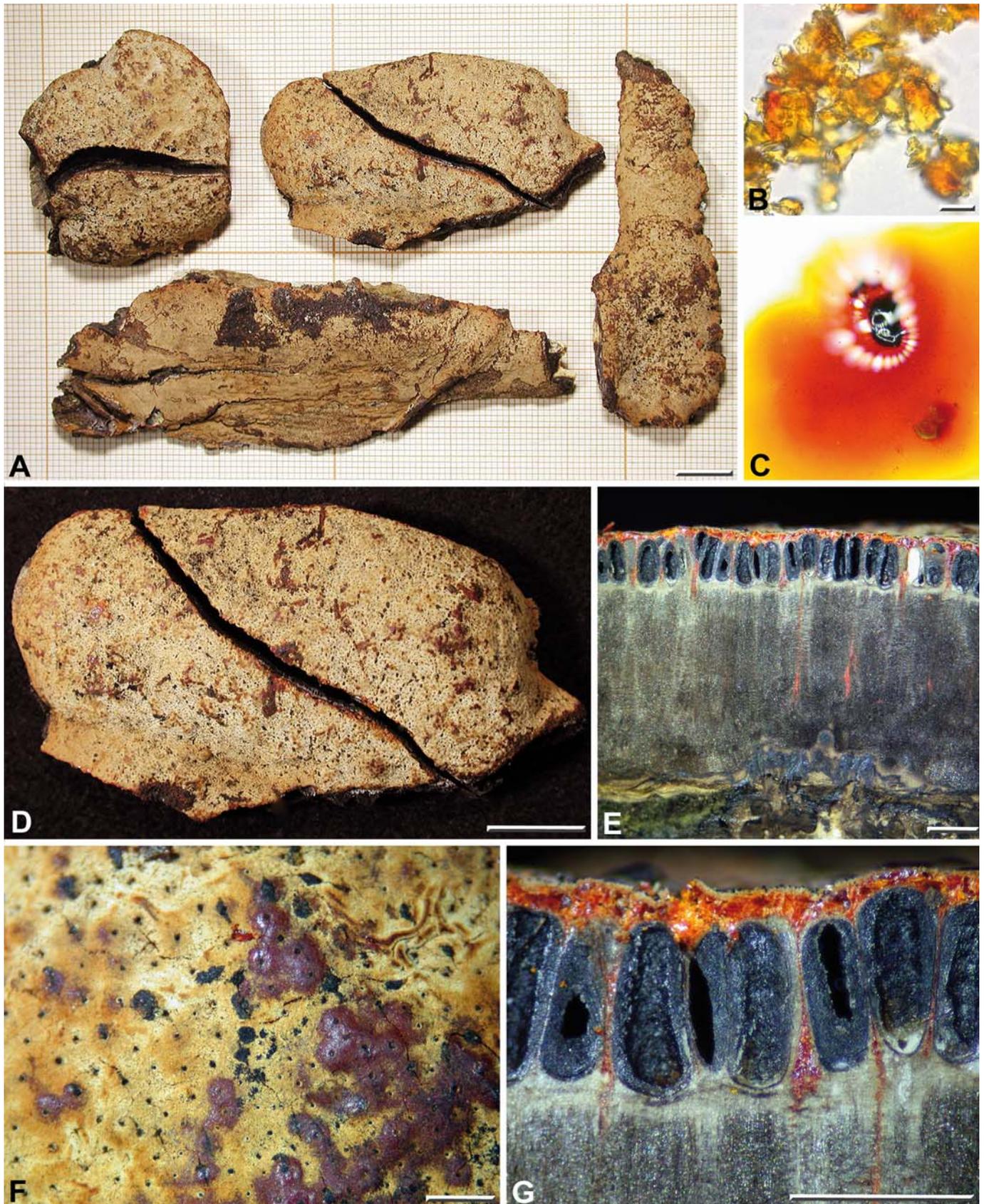


Plate 4 – *Hypoxylon* sp. cf. *H. cinnabarinum*. CLL 8081

A, D: Stromata in surface view; B: Stromatal granules observed in water; C: KOH-extractable pigments (1 min incubation); E: Stroma in vertical section showing the perithecia, the subsurface orange granules and the thick subperithecial tissue; F: Stromatal surface in close up showing the ostioles, the pruinose surface and the reddish brown subsurface; G: Close-up on the perithecial layer in vertical section. Scale bars: A, D = 10 mm; B = 10 μ m; E, F, G = 1 mm.

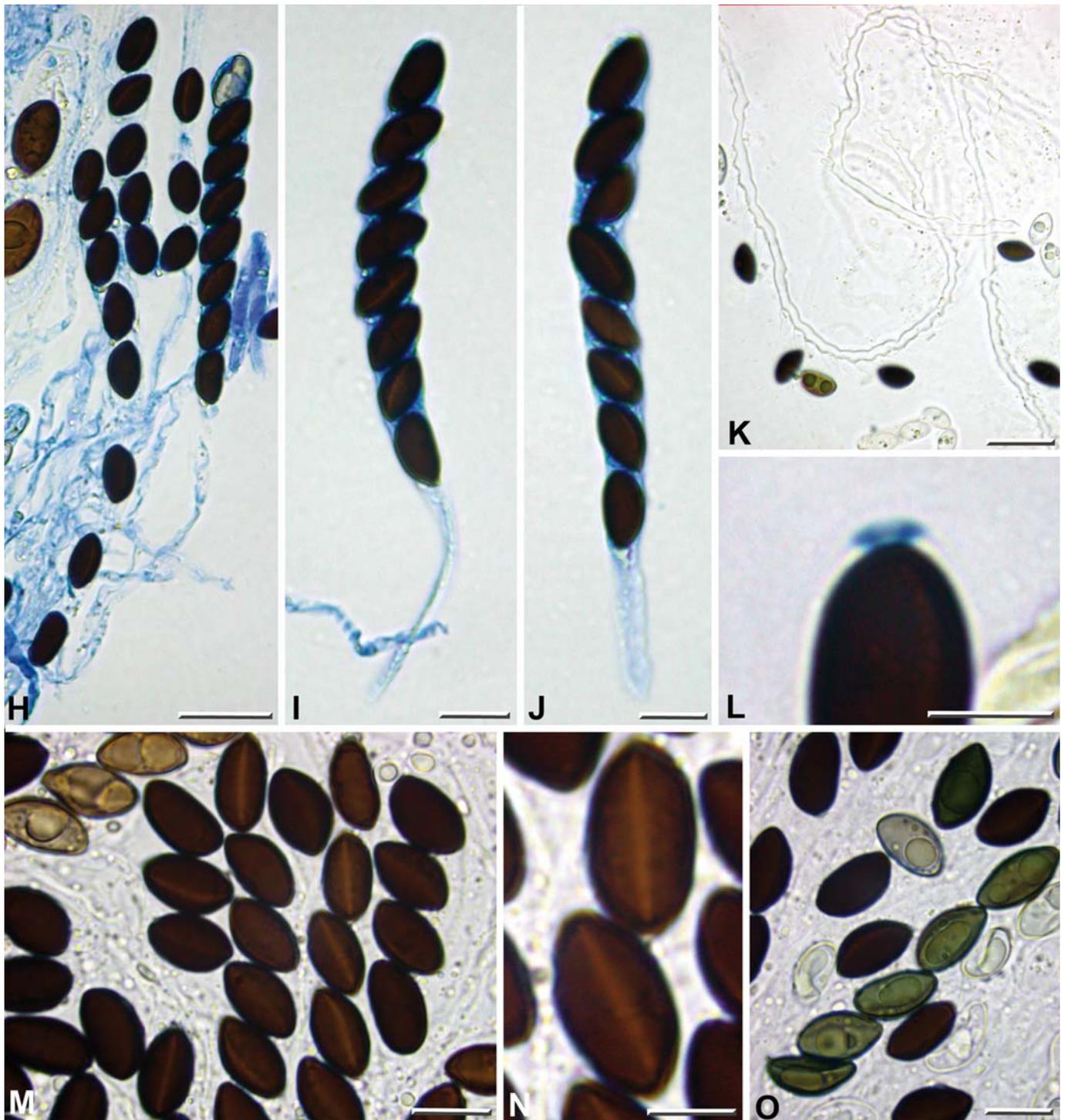


Plate 5 – *Hypoxylon* sp. cf. *H. cinnabarinum*. CLL 8081

H-J: Mature asci with broken stipes, in blue Waterman ink with 1% SDS; K: Long ascogenous hyphae in 1% SDS; L: Ascular apical apparatus of a mature ascus bluing in Melzer's reagent; M: Ascospores in water; N: Ascospores in water showing the germ slit; O: Ascospores in 10% KOH showing the absence of dehiscent perispores and the greenish discoloration of immature ascospores. Scale bars: H, K = 20 μ m; I, J, M, O = 10 μ m; L, N = 5 μ m.

nules beneath surface yielding yellow brown pigments in 10% KOH, combined with small equilateral ascospores with broadly rounded ends, with a straight germ slit and a perispore indehiscent in 10% KOH. This set of characters, except the carbonaceous tissue that has not been reported by JU & ROGERS (1996), conforms fairly well to *H. gilbertsonii* Y.-M. Ju & J.D. Rogers, a species so far only known from Hawaii.

However, as kindly communicated by Dr. Yu-Ming Ju (Taiwan), its ITS sequence matches very closely that of a collection from Martinique (CLL 5531) and one from Taiwan (HAST 90112602), both tentatively regarded as *H. dieckmannii* by Dr. Yu-Ming Ju. On the other hand, the ITS sequences of the two collections of *H. dieckmannii*

from Mexico and Taiwan used in the phylogenetic study of *Hypoxylon* (HSIEH *et al.*, 2005), appeared to strongly deviate from the three above specimens (Yu-Ming Ju, pers. comm. Nov. 2014). This, added to the fact that some variations in stromatal texture, waxy granules and KOH-extractable pigments occur among these collections, suggests that the current concept of *H. dieckmannii* might encompass several closely related but different species. Until the type collection from Brazil is re-evaluated based on freshly collected material originating from the same region it is preferable to regard *H. dieckmannii* as a complex of species to which our collections from French Guiana can be tentatively referred.

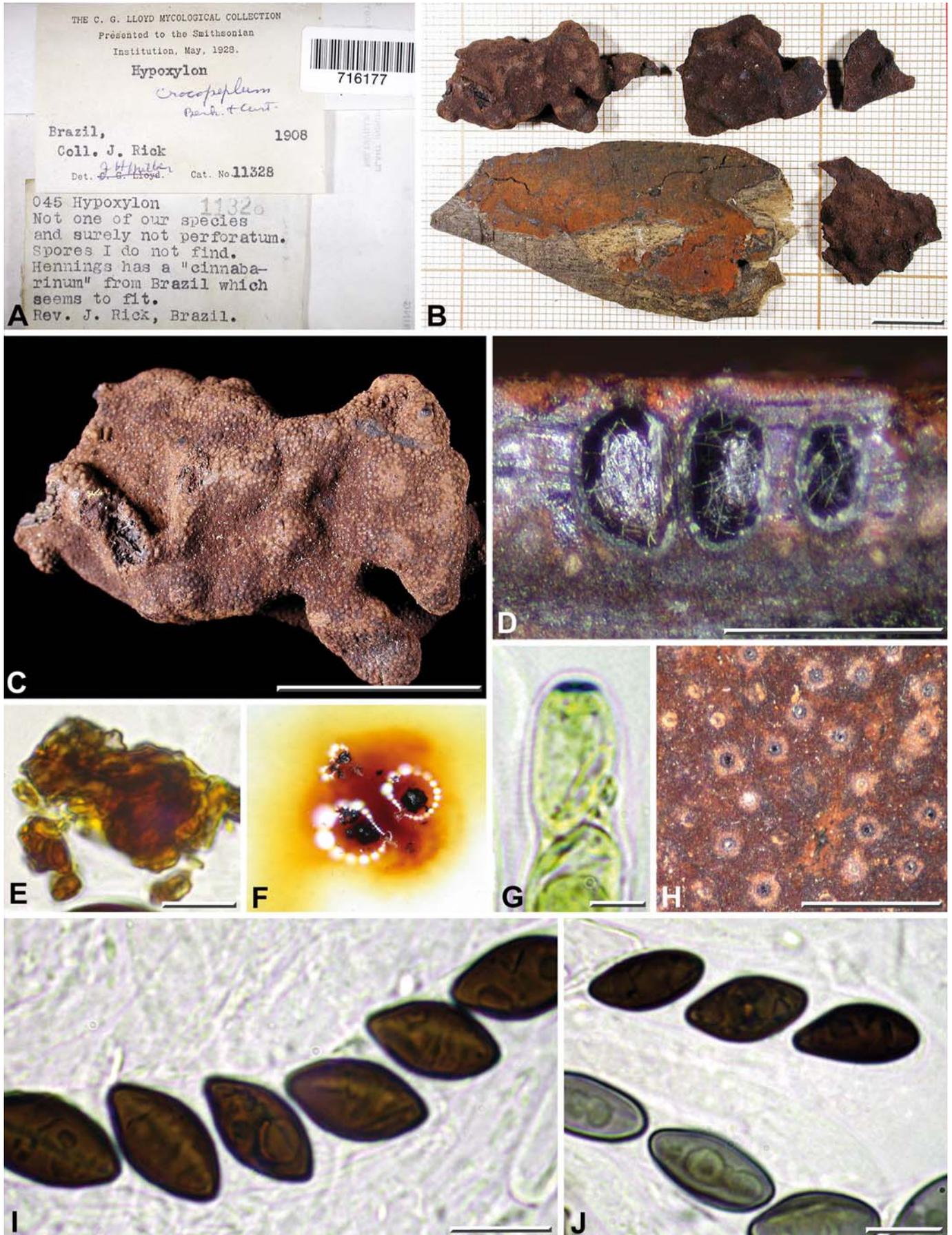


Plate 6 – *Hypoxylon cinnabarinum*. Rick 1908, BPI 11328

A: Herbarium label; B, C: Stromata in surface view; D: Stroma in vertical section showing the perithecia and the subsurface granules; E: Stromatal granules observed in water; F: KOH-extractable pigments; G: Ascus apical apparatus of a mature ascus in Melzer's reagent; H: Stromatal surface in close-up showing the ostioles; I: Ascospores in water showing the germ slits; J: Ascospores in 10% KOH showing the absence of dehiscent perispores and the greyish green discolouration of immature ascospores. Scale bars: B, C = 10 mm; D = 5 mm; E, I, J = 10 μ m; G = 5 μ m; H = 1 mm.

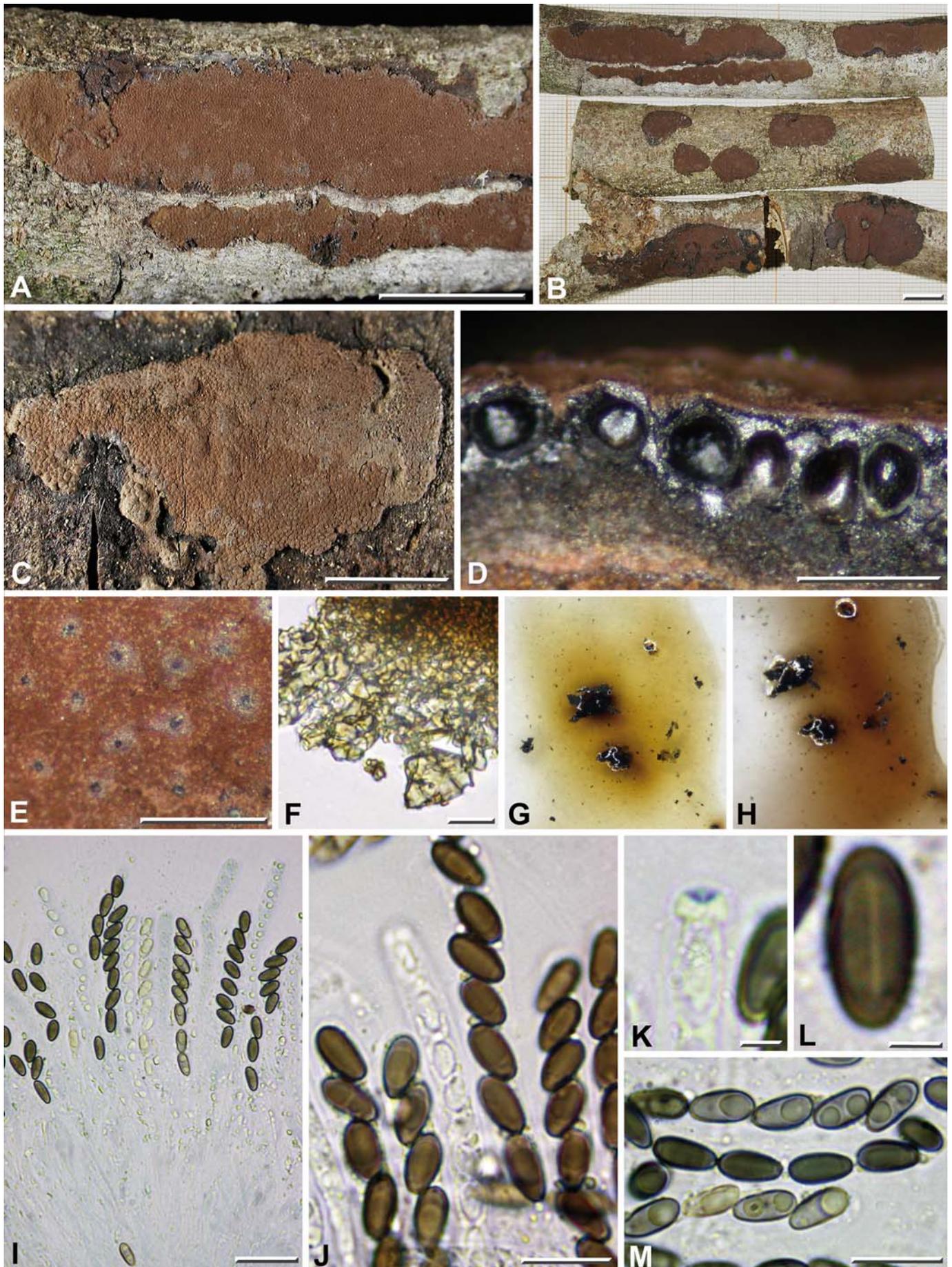


Plate 7 – *Hypoxylon* cf. *H. dieckmannii*. GYJF 12229

A-C: Stromata effused on bark; D: Stroma in vertical section showing the perithecia encased in carbonaceous tissue; E: Stromatal surface in close-up showing the ostioles; F: Waxy stromatal granules in water; G, H: KOH-extractable pigments, respectively after 1 min and 20 min incubation; I: Asci in chlorazol black; J: Ascospores in water; K: Ascical apical apparatus in Melzer's reagent; L: Ascospores in dorsal view showing the germ slit; M: Ascospores in 10% KOH showing the absence of dehiscent perispores. Scale bars: A, B = 10 mm; C = 5 mm; D, E = 0.5 mm; F, J, M = 10 µm; I = 20 µm; K, L = 2 µm.

Hypoxylon fusioideosporum Y.-M. Ju & J.D. Rogers, *Mycol. Mem.*, 20: 124 (1996). Plate 8

Stromata effused-applanate with moderately to conspicuously exposed perithecial contours, 29–60 mm long × 16–20 mm wide × 0.7–0.8 mm thick; surface dark brick (60, oac 638), pruinose; orange yellow granules forming a thick waxy layer above and around perithecia, yielding dense orange (7, oac 629) KOH-extractable pigments, turning sienna (8, oac 664) upon prolonged incubation; subperithecial tissue dark brown, inconspicuous. **Perithecia** spherical, 0.5–0.6 mm diam. **Ostioles** discoid to raised-discoid, concolourous, 170–200 µm diam, with umbilicate opening.

Asci cylindrical with eight but often less obliquely uniseriate ascospores, the spore-bearing parts 150–160 × 14–16 µm, the stipes 75–80 µm long, recorded on immature asci because the stipes are usually broken at maturity, with a discoid to lens-shaped apical apparatus 1.8–2 × 5–6 µm, bluing in Melzer's reagent. **Paraphyses** moniliform, 12–17 µm wide at base, tapering to 2–2.5 µm above asci, copious. **Ascospores** (22.1–) 23.1–27.1 (–30.6) × (9.8–) 11.1–13.5 (–14.2) µm; Q = (1.6–) 1.9–2.4 (–2.7); N = 52 (Me = 25 × 12.3 µm; Qe = 2.1), ellipsoid-inequilateral with acute ends varying from navicular to almost citriform, the ends often slightly pinched, blackish, with a short straight to slightly oblique germ slit 8–12 µm long, difficult to make out on mature ascospores; perispore indehiscent in 10% KOH; episporium smooth.

Asexual morph on natural substrate not seen.

Specimens examined: FRENCH GUIANA: Saül, Monts La Fumée, dry primary forest, 03°60'N, 53°20'W, ca. 400 m elev., corticated wood, Feb. 1986, *leg.* G.J. Samuels, WSP 69619 (isotype of *H. fusioideosporum*); Sinnamary, Paracou, CIRAD field station, plot 12, lowland rainforest, corticated branch, 23 Jun. 2012, *leg.* G. Gruhn, GYJF 12150 (LIP); *ibid.*, trail to the station, corticated branch, 27 Jun. 2012, *leg.* J. Fournier, GYJF 12248 (LIP) (not in ascigerous condition).

Distribution: French Guiana, Guyana.

Discussion: *Hypoxylon fusioideosporum* externally resembles many *Hypoxylon* spp. with stromatal surface in shades of orange brown but it is readily recognized by its large blackish inequilateral ascospores with acute ends, perispore indehiscent in 10% KOH and short germ slit. The only two species with inequilateral fusoid ascospores with perispore indehiscent in 10% KOH that reach 20 µm long are *H. californicum* Ellis & Everh. and *H. rubellum* Penz. & Sacc. (Ju & ROGERS, 1996). Both differ in having ascospores with ornamented episporium and averaging smaller in size, respectively 17–22 × 7.5–9 µm with episporium with short striae, and 16.5–21 × 7–8 µm with episporium with reticulate rims.

Species of *Hypoxylon* with ascospores over 20 µm long are few and all can be readily distinguished from *H. fusioideosporum*. *Hypoxylon chionostomum* (Speg.) Speg. has blackish brown ascospores 25–31 × 14–18 µm, perithecia 1–1.4 mm diam and lacks KOH-extractable pigments. *Hypoxylon megalosporum* Speg. and *H. umbilicatum* Speg., that most likely represent the same taxon, differ from *H. fusioideosporum* by olivaceous KOH-extractable pigment and much larger ascospores 30–40 × 19–25 µm. *Hypoxylon vogesiacum* (Pers.) Sacc. is a temperate taxon with purplish stromata and faint purple KOH-extractable pigments, light brown to brown ascospores 20–25 × 8–10 µm with a spore-length germ slit (Ju & ROGERS, 1996). The specimens that we collected conform well to the isotype of *H. fusioideosporum* we examined. They just deviate in lacking the vinaceous tinge of the stromatal surface of the isotype and in having more spherical perithecia.

Hypoxylon cf. H. hypomiltum Mont., *Ann. Sci. Nat., Bot., ser. II*, 13: 356 (1840). Plate 9

Stromata effused-pulvinate with slightly lobed margins, with barely exposed perithecial contours, 10–18 mm long × 5–13 mm wide

× 1–1.1 mm thick (a depauperate overmature stroma is over 80 mm long); surface dark livid (80, oac 397), pruinose, slightly uneven and mottled; dull olivaceous yellow granules interspersed in a matrix of dark brown hyphal rods 2–4 µm wide, forming a thick brittle layer above and between perithecia, shiny black in section, with greyish sepia (106, oac 838) to olivaceous (48, oac 868) KOH-extractable pigments, turning olivaceous upon prolonged incubation; subperithecial tissue blackish, 0.2–0.3 mm thick. **Perithecia** subglobose 0.4–0.5 mm diam. **Ostioles** deeply umbilicate, inconspicuous.

Asci cylindrical, with eight obliquely uniseriate ascospores, the spore-bearing parts 51–57 × 6–7 µm, the stipes 90–140 µm long, originating from short sinuous ascogenous hyphae, with a discoid apical apparatus 0.8–1 × 2–2.5 µm, faintly bluing in Melzer's reagent.

Paraphyses filiform, copious, with small oily guttules. **Ascospores** (6.3–) 7.1–7.9 (–8.2) × (2.9–) 3.2–3.6 (–3.8) µm; Q = (1.8–) 2.0–2.4 (–2.6); N = 60 (Me = 7.5 × 3.4 µm; Qe = 2.2), ellipsoid-equilateral with narrowly rounded to acute ends, often fusiform, brown, with a straight to slightly oblique germ slit most often much less than spore length; perispore rarely dehiscent in 10% KOH, smooth to faintly striated; episporium smooth.

Asexual morph on natural substrate not seen.

Specimens examined: FRENCH GUIANA: Sinnamary, Paracou, CIRAD field station, lowland rainforest, trail 11, dead corticated branch, 23 Jun. 2012, *leg.* J. Fournier, GYJF 12143 (LIP); *ibid.*, trail to CIRAD field station, dead corticated branch, 23 Jun. 2012, *leg.* J. Fournier, GYJF 12159 (LIP).

Distribution: French Guiana, Guadeloupe, Martinique.

Discussion: Aside from the very common and ubiquitous *H. monticulosum*, the most frequent species of *Hypoxylon* collected in both sites during this field work was by far *H. hypomiltum*, with 12 records out of 43. *Hypoxylon hypomiltum* as circumscribed by Ju & ROGERS (1996) appears to be fairly variable in stromatal surface colour ranging from brown vinaceous to sepia, in KOH-extractable pigments ranging from luteous to amber or citrine and perithecia ranging from obovoid to tubular. In contrast all collections referred to this name share the same distinctive ascospore morphology, viz. small equilateral fusoid ascospores 7–9 × 3–4 µm with narrowly rounded to acute ends, a perispore dehiscent in 10% KOH and a short, often oblique or slightly sigmoid germ slit. While stromata of most collections of *H. hypomiltum* made in Guadeloupe, Martinique and French Guiana that we studied yielded luteous to cinnamon pigments in 10% KOH, six of them deviated in featuring greenish to olivaceous pigments, in correlation with a purplish to violaceous surface. Such a difference in stromatal pigments is usually regarded as discriminant within the genus *Hypoxylon* and would suggest the segregation of a distinct taxon. However, we refrain to do so until a large number of collections of *H. hypomiltum s. l.* is revised based on chemotaxonomic, cultural and molecular investigations to assess whether they support the morphological variability observed in this taxon.

The reaction to iodine of the ascus apical apparatus is likewise a character that appears to vary from positive to negative within collections with orange or olivaceous KOH-extractable pigments but could not be correlated with other morphological characters. Ascospores of *H. hypomiltum* are reported to have the perispore dehiscent in 10% KOH (Ju & ROGERS, 1996) but this character is sometimes difficult to interpret when the reaction only occurs after 5–15 min incubation on a limited number of ascospores. Again, this character of delayed reaction of ascospores to KOH could not be correlated with other features.

During our attempts to find a correlation between the stromatal granules as observed in water under the microscope and the pigments released in 10% KOH we failed to find a visible difference but we noticed a character common to all specimens of *H. hypomiltum s. l.*, regardless of their reaction in KOH. When a fragment of the blackish tissue lying above and around the perithecia is crushed in water the olivaceous yellow stromatal granules appear interspersed

in a matrix of dark brown to blackish thick-walled hyphae, giving this tissue a somehow carbonaceous texture differing from the waxy texture that is usually encountered in stromata of *Hypoxyylon*. We regard this easily observed character as a signature of *H. hypomiltum* or maybe the complex of species currently merged under this name.

Hypoxyylon lepriurianum J. Fourn. & Lechat *sp. nov.* – MycoBank MB811912. Plate 10

Diagnosis: Differs from all known species of *Hypoxyylon* with an orange stromatal surface and orange stromatal pigments in having equilateral ascospores with broadly rounded ends averaging $7.7 \times 3.5 \mu\text{m}$ with a perispore not dehiscent in 10% KOH, and in having an inconspicuous germ slit less than spore-length.

Holotype: FRENCH GUIANA: Sinnamary, Paracou, CIRAD field station, lowland rainforest, plot 3, dead corticated branch, 24 Jun. 2012, *leg.* G. Gruhn, GYJF 12180 (LIP).

Etymology: In honour of the naturalist F.R.M. Leprieur (1799–1870), for his invaluable contribution to the knowledge of the *Xylariaceae* in French Guiana.

Stroma irregularly effused-applanate with, in places, effused concolourous sterile margin, with inconspicuous to slightly exposed perithecial contours, 32 mm long \times 18 mm wide \times 0.5–0.6 mm thick; surface sienna (8, oac657), pruinose, slightly uneven; bright yellow and orange granules forming a thick waxy layer above and slightly extending downwards between the perithecia, with orange (7, oac629) KOH-extractable pigments; subperithecial tissue inconspicuous, blackish. **Perithecia** obovoid $0.38\text{--}0.45 \times 0.25\text{--}0.35 \text{ mm}$. **Ostioles** umbilicate, black, surrounded by white material forming a small disc $60\text{--}80 \mu\text{m}$ diam.

Asci cylindrical, with eight uniseriate ascospores, the spore-bearing parts $60\text{--}72 \times 5.5\text{--}6 \mu\text{m}$, the stipes $45\text{--}76 \mu\text{m}$ long, originating in unilateral spicate arrangement from long contorted ascogenous hyphae, with a discoid to wedge-shaped apical apparatus $0.5\text{--}0.8 \times 1.8\text{--}2.5 \mu\text{m}$, bluing in Melzer's reagent. **Ascospores** (6.9–) $7.1\text{--}8.1$ (-8.5) \times (3.2–) $3.3\text{--}3.8$ (-4.1) μm ; $Q = (1.9\text{--}) 2.0\text{--}2.4$ (-2.5); $N = 50$ ($Me = 7.7 \times 3.5 \mu\text{m}$; $Qe = 2.2$), ellipsoid-equilateral with broadly rounded ends, frequently oblong with parallel sides, medium brown, with a blurred, inconspicuous straight germ slit slightly less than spore length; perispore indehiscent in 10% KOH; episporium smooth.

Asexual morph on natural substrate not seen.

Distribution: French Guiana.

Discussion: Numerous *Hypoxyylon* spp. resemble the present species in having effused stromata in shades of orange, with orange KOH-extractable pigments. However, most of them, unlike *H. lepriurianum*, feature ellipsoid-inequilateral ascospores with a perispore dehiscent in 10% KOH. These characters usually are easy to observe and are consistent within a given taxon, the reason why they were used as key characters by JU & ROGERS (1996) in their dichotomous keys.

The most resembling species is undoubtedly *H. subtucinense* Y.-M. Ju & J.D. Rogers which differs in having a temperate distribution, a more massive black tissue $0.1\text{--}0.3 \text{ mm}$ thick beneath the perithecia and larger ascospores $8\text{--}10 \times 4\text{--}5 \mu\text{m}$ with narrowly rounded ends and a conspicuous germ slit spore-length (JU & ROGERS, 1996). Before maturity, the stromata of *H. subtucinense* are typically fringed by a yellow, often fimbriate margin that vanishes at maturity. We did not encounter immature stromata of *H. lepriurianum* but the orange sterile tissue present at the margin of the mature stroma studied here suggests that such a yellow marginal tissue may have existed. *Hypoxyylon aureolimbatum* *sp. nov.* (this paper) should be considered for comparison as it also shares similar features with *H. lepriurianum*. They are mainly distinguished based on the smaller, paler brown, more narrowly ellipsoid ascospores of *H. lepriurianum* ($Me = 7.7 \times 3.5 \mu\text{m}$; $Qe = 2.2$ vs. $Me = 10.4 \times 6.3 \mu\text{m}$; $Qe = 1.6$), with an inconspicuous, often less than spore-length germ slit.

Among the known tropical taxa, *H. cinnabarinum* differs from *H. lepriurianum* by its often much thicker stromata up to 2.8 mm thick, with often tubular perithecia and larger ascospores $9.5\text{--}14 \times 5\text{--}7.5 \mu\text{m}$ with darker brown wall, more narrowly rounded ends and a more conspicuous germ slit. See comments on *Hypoxyylon cf. cinnabarinum* above.

Hypoxyylon musceum J.D. Rogers, *Canad. J. Bot.*, 59: 1363 (1981). Plates 11–12

Stromata glomerate, with conspicuous perithecial contours, few-peritheciate, $1\text{--}1.2 \text{ mm}$ diam \times $0.4\text{--}0.6 \text{ mm}$ thick, separate to coalesce into larger pulvinate stromata to $2.4 \times 1.7 \mu\text{m}$; surface isabelline (65, oac820), pruinose, shiny black where the pruina is worn off; dull orange brown and yellowish granules forming a waxy layer beneath surface and around perithecia, yielding sienna (8, oac630) KOH-extractable pigments; subperithecial tissue blackish brown, inconspicuous. **Perithecia** spherical, $0.25\text{--}0.3 \text{ mm}$ diam. **Ostioles** umbilicate, surrounded by a conspicuous disc of white substance $50\text{--}70 \mu\text{m}$ diam.

Asci cylindrical, short-stipitate, with eight obliquely uniseriate ascospores, the spore-bearing parts $59\text{--}68 \times 6\text{--}6.5 \mu\text{m}$, the stipes $18\text{--}32 \mu\text{m}$ long, with a discoid apical apparatus $0.8 \times 2\text{--}2.5 \mu\text{m}$, bluing in Melzer's reagent. **Paraphyses** copious, ribbon-like, $3\text{--}4.5 \mu\text{m}$ wide. **Ascospores** (8.9–) $9.2\text{--}10.5$ (-10.9) \times (4.2–) $4.6\text{--}5.3$ (-5.6) μm ; $Q = (1.8\text{--}) 1.8\text{--}2.2$ (-2.4); $N = 52$ ($Me = 9.9 \times 5 \mu\text{m}$; $Qe = 2$), ellipsoid slightly inequilateral with narrowly to broadly rounded ends, dark brown, with a straight to slightly oblique or slightly sigmoid germ slit less than spore length, fairly conspicuous; perispore dehiscent in 10% KOH, conspicuously striated; episporium smooth.

Asexual morph on natural substrate not seen.

Specimens examined: FRENCH GUIANA: Sinnamary, Paracou, trail to CIRAD field station, lowland rainforest, on a corticated twig (possibly a liana), 27 Jun. 2012, *leg.* J. Fournier, GYJF 12250 (LIP). FRENCH WEST INDIES: Guadeloupe, Basse Terre, Petit Bourg, forest trail of Jules, corticated branch, associated with *H. aeruginosum*, 1 Sept. 2004, *leg.* C. Lechat, CLL 2262–2 (LIP). GABON: near Libreville, corticated twigs, 16 Jun. 1979, *leg.* G. Gilles, G-81 (WSP 64843, Holotype).

Distribution: Gabon, Neotropics, Taiwan.

Discussion: *Hypoxyylon musceum* is a rarely reported taxon. It is diagnosed by the small glomerate stromata with an olivaceous surface and yellow to orange KOH-extractable pigments, combined with ellipsoid-inequilateral ascospores $9\text{--}13 \times 4.5\text{--}6 \mu\text{m}$ with a short slightly sigmoid to oblique germ slit and a perispore dehiscent in 10% KOH (ROGERS, 1981; JU & ROGERS, 1996). Since its original description (ROGERS, 1981) this distinctive taxon has been recorded in the Neotropics and Taiwan (JU & ROGERS, 1996), expanding the range of morphological variations accepted for this taxon. Slight differences occur between the African type collection and material from French West Indies (CLL 2232) and French Guiana as to ascospore dimensions and ornamentation of the perispores. Examination of the holotype (Plate 15) revealed ascospores averaging $8.7 \times 4 \mu\text{m}$ with narrowly rounded to acute ends and thin, smooth perispore (C-E) while the material from the Neotropics features slightly larger ascospores with a darker wall and more broadly rounded ends, and a more conspicuously striated perispore. The germ slits reported as sigmoid in the original description are also frequently oblique and barely sigmoid, like in our collections.

Though the ornamentation of the perispores appears consistent within a species and is a good taxonomic marker that proved useful to segregate species in the closely related genus *Daldinia* Ces. & De Not. (STADLER *et al.*, 2002; VAN DER GUCHT, 1993), it is still unknown whether these morphological differences reflect a complex of closely related species or merely intraspecific variations in relation with

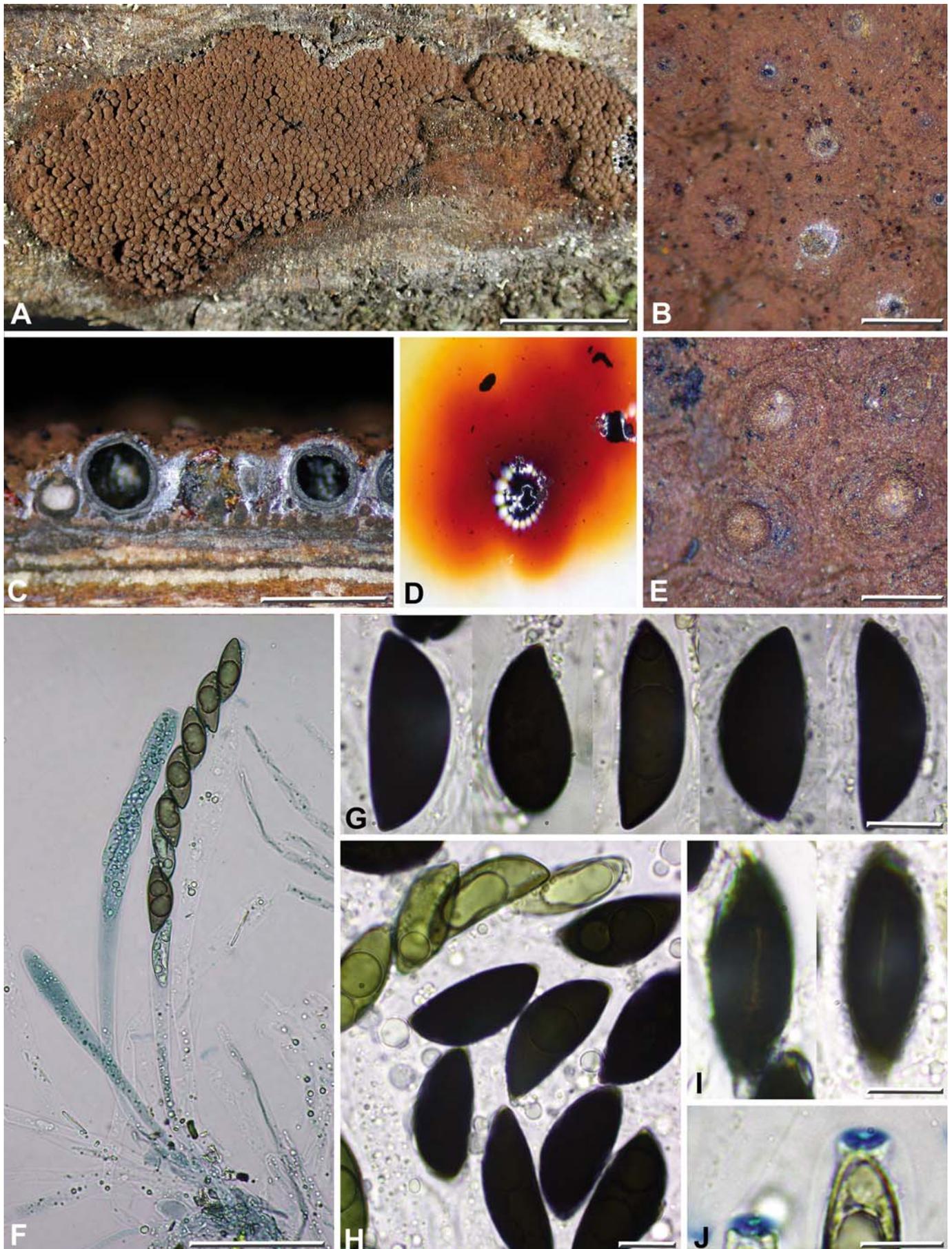


Plate 8 – *Hypoxylon fusioideosporum*

A-D, F-J: GYJF 12150; E: GYJF 12248. A: Stromata effused on bark; B, E: Stromatal surface in close-up showing the ostiolar discoid structures; C: Stroma in vertical section showing the perithecia; D: KOH-extractable pigments (1 min); F: Immature ascus in black Waterman ink with 1% SDS; G: Ascospores in water, in side view; H: Ascospores in 10% KOH showing the absence of dehiscent perispores and the green discoloration of immature ascospores; I: Ascospores in dorsal view showing the short germ slit; J: Ascus apical apparatus in Melzer's reagent. Scale bars: A = 10 mm; C = 1 mm; B, E = 0.5 mm; F = 50 μm; G-J = 10 μm.



Plate 9 – *Hypoxylon* cf. *H. hypomiltum*. GYJF 12143

A, B: Stromata on bark; C, D: KOH-extractable pigments (C after 1 min, D after 20 min incubation); E: Stroma in vertical section (broken) showing the perithecia; F: Waxy stromatal granules mixed with dark brown hyphal rods in water; G: Mature long-stipitate ascus in chlorazol black with 1% SDS; H: Ascospores in 10% KOH showing the germ slits; I, J: Ascospores in Melzer's reagent showing the germ slits; K: Ascospore in 10% KOH showing the dehiscent perispore; L: Ascus apical apparatus in Melzer's reagent; M: Ascospores in water. Scale bars: A = 10 mm; B = 5 mm; E = 1 mm; F, G = 20 μ m; H-L = 5 μ m; M = 10 μ m.

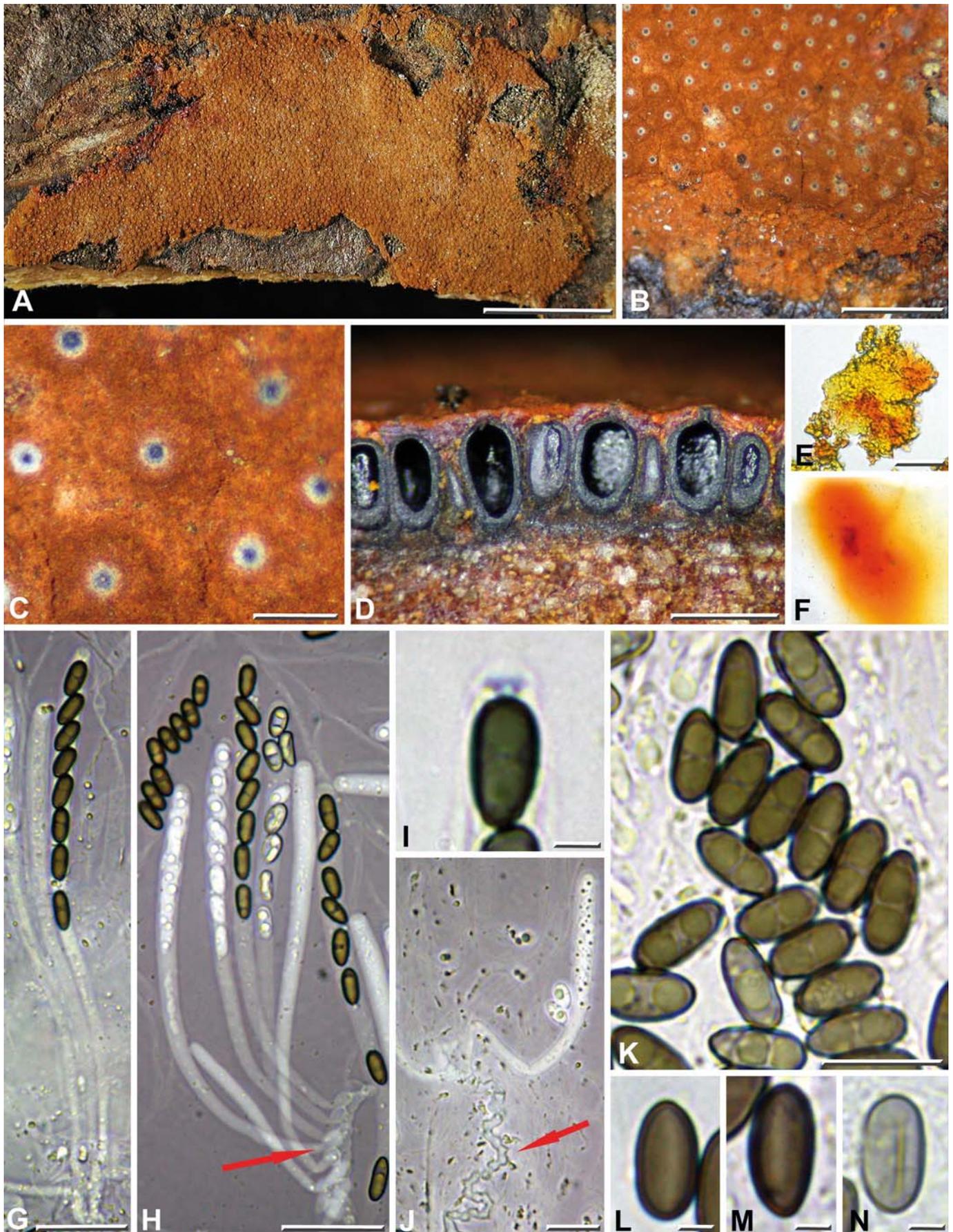


Plate 10 – *Hypoxylon leprieurianum*. Holotype GYJF 12180

A: Stroma on bark; B: Stroma in close-up showing the effused sterile margin; C: Stromatal surface in close-up showing the ostioles; D: Stroma in vertical section showing the perithecia beneath the layer of orange waxy granules; E: Stromatal waxy granules observed in water; F: KOH-extractable pigments within 1 min incubation; G, H: Mature and immature asci in black Pelikan ink, showing the unilateral spiculate arrangement of ascial stipes on an ascogenous hypha (red arrow); I: Ascial apical apparatus in Melzer's reagent; J: Immature ascus in black Pelikan ink originating from a contorted ascogenous hypha bearing secession scars giving its spinulose outline (red arrow); K: Ascospores in water; L-N: Ascospores in PVA-lactophenol showing a blurred germ slit less than spore-length, best seen on an immature ascospore (N). Scale bars: A = 10 mm; B = 1 mm; C = 0.2 mm; D = 0.5 mm; E, G, H, J = 20 μ m; I, L-N = 2 μ m; K = 10 μ m.

geographical distribution; therefore we tentatively regard this material from French Guiana as *H. musceum*.

The species most resembling *H. musceum* is *H. isabellinum* J. Fourn., Kuhnert & M. Stadler (KUHNERT *et al.*, 2014a), which primarily differs by its pale greenish yellow KOH-extractable pigments and its more strongly inequilateral ascospores with a straight germ slit spore-length.

Hypoxylon nudum J. Fourn. & Lechat *sp. nov.* – MycoBank MB811913. Plates 13–14

Diagnosis: Differs from all known species of *Hypoxylon* by lacking stromatic tissue around the ascomata.

Holotype: FRENCH GUIANA: Régina, Nouragues natural reserve, Inselberg camp, trail to Pararé, ca. 1 km from the camp, rainforest, on bark of unidentified dead standing trunk, 21 Jun. 2012, *leg.* J. Fournier, GYJF 12134 (LIP).

Etymology: From Latin *nudus* = naked, for the lack of stromatic coating around the ascomata.

Stroma lacking or reduced to a black basal tissue. **Ascomata** perithecioid, densely gregarious and spreading over a large area ca. 20 cm long, separate or in loose contact, not fused together, subglobose to depressed-spherical, 0.5–0.6 × 0.65–0.85 mm, attached to a black tissue irregularly distributed on the host surface by small tubercles or connectives up to 0.6 mm high; surface dull black, finely roughened; perithecial wall slightly carbonaceous, brittle, pseudo-parenchymatous, two-layered: inner layer 12–15 µm thick, olivaceous brown, composed of flattened thin-walled to thick-walled cells, outer layer 30–35 (–45) µm thick, dark brown, with small cells protruding outwardly, composed of small, angular, heavily melanized often opaque cells extending into the inner layer forming short columns of dark brown cells; waxy granules absent; no pigments released in 10% KOH; basal black tissue composed of thick-walled dark brown angular cells and hyphae, coated with dark brown thick-walled hyphae 2.5–4 µm wide, smooth to most often roughened. **Ostioles** finely conic-papillate, black.

Asci cylindrical, with eight obliquely uniseriate ascospores, 135–165 µm total length, the spore-bearing parts 60–68 × 5–5.5 µm, the stipes 70–100 µm long, originating from spiny ascogenous hyphae 2–3 µm wide, with a discoid to wedge-shaped apical apparatus 0.8–1 × 2–2.5 µm, bluing in Melzer's reagent. **Paraphyses** filiform, copious, containing minute oily guttules. **Subhymenium** containing angular white crystals 2.5–4 µm in greatest dimension, not dissolving in 10% KOH, in places agglomerated into clusters 10–18 µm diam. **Ascospores** (7.9–) 8.5–9.2 (–9.4) × (3.2–) 3.4–3.7 (–4.0) µm; Q = (2.2–) 2.3–2.6 (–2.7); N = 50 (Me = 8.9 × 3.6 µm; Qe = 2.5), ellipsoid-inequilateral with narrowly rounded to subacute ends, medium brown, with a straight germ slit spore-length on the convex side, fairly conspicuous; perispore indehiscent in 10% KOH but occasionally dehiscent after a prolonged incubation, smooth; epispore smooth.

Asexual morph on natural substrate not seen.

Distribution: French Guiana.

Discussion: Externally this fungus resembles a black and glabrous sordariaceous fungus but microscopical observation reveals asci with an amyloid apical apparatus and brown one-celled ascospores typical of the *Xylariaceae*. Furthermore, ascospores with a germ slit on the convex side and a perispore occasionally dehiscent in 10% KOH strongly suggest affinities with *Hypoxylon*. Many species of *Hypoxylon* feature asci arising in spicate arrangement from elongate ascogenous hyphae, a pattern not observed in related xylariaceous genera (personal observations). The presence in the hymenium of this fungus of such ascogenous hyphae, featuring an unusual spiny outline, can be regarded as a proof of being a good *Hypoxylon* (see *H. lepreurianum*, plate 10, fig. J for a similar configuration). It is no-

teworthy that colourless crystals are present in the subhymenium and the hymenium, a feature unknown from other *Hypoxylon* spp. except in *H. rhombisporum* sp. nov. (this paper). These crystals are easily made out in black Pelikan ink by contrast but are very inconspicuous in water, which may account for the fact they have been overlooked in some other species.

All known species of *Hypoxylon* feature a more or less developed stromatic tissue consisting of prosenchymatous tissue forming an outer pruina and a layer of waxy granules above and sometimes around the perithecia. The present *Hypoxylon* is distinctive in lacking the outer pruina and waxy granules and consequently lacks KOH-extractable pigments. Thin sections of the ascomatal wall mounted in heated chloral-lactophenol reveal that the wall anatomy is that of a sordariaceous fungus with a typical two-layered peridium. This sets it clearly apart from species with often rosellinoid stromata like *H. lenormandii* Berk. & M.A. Curtis in which perithecia are encased in a continuous crust of waxy granules and a thin hyphal pruinose external coating usually uniting adjacent stromata (Ju & ROGERS, 1996). Externally, the most resembling species is *H. cypraeisporum* J. Fourn. & Lechat (FOURNIER & LECHAT, 2015a) in which ascomata are likewise black and rosellinoid, but this species differs from *H. nudum* in having perithecia encased by a crust of waxy granules yielding purple pigments in 10% KOH and ascospores with a pitted epispore.

Despite the lack in *H. nudum* of the stromatic tissue surrounding the perithecia typically encountered in *Hypoxylon*, the black prosenchymatous tissue on which perithecia are seated may be considered as a stromatic structure homologous with the usually blackish tissue underlying the perithecia in typical stromata.

The separate and naked superficial perithecia lacking waxy granules and stromatic coating give to this species a very peculiar status within *Hypoxylon* and even *Xylariaceae*. It can be questioned whether this absence of stroma corresponds to a primitive stage of evolution, which would make it an ancestor of typically stromatic *Hypoxylon* species, or less likely represents a more advanced evolutionary step. It is even unknown whether the absence or presence of stromatal tissue reflects an evolutionary trend (Ju, pers. comm.)

Hypoxylon paracouense J. Fourn. & Lechat *sp. nov.* – MycoBank MB811914. Plates 15–16

Diagnosis: Differs from *Hypoxylon duranii* by amber to fawn KOH-extractable pigments turning vinaceous and smaller ascospores 7.5–8.2 × 3.6–4.1 µm with a sigmoid germ slit vs. 10.5–12.3 × 4.9–5.8 µm with a straight germ slit.

Holotype: FRENCH GUIANA: Sinnamary, Paracou, lowland rainforest, CIRAD field station, edge of parking area, on dead corticated branch ca. 2 cm diam, 25 Jun. 2012, *leg.* J. Fournier, GYJF 12185 (LIP).

Etymology: After Paracou, the location where the holotype comes from.

Stromata effused-applanate, with inconspicuous to slightly exposed perithecial contours, 8–28 mm long × 5–9 mm wide × 0.4–0.5 mm thick, lined by a narrow dark brick (60, oac637) sterile pruinose margin; surface dark vinaceous (82, oac523), pruinose, slightly uneven; yellowish and pale pinkish brown granules forming a thick waxy layer above and between perithecia, with amber (47, oac852) to fawn (87, oac645) KOH-extractable pigments, turning dilute livid vinaceous (83, oac513) after 20 min incubation; subperithecial tissue inconspicuous. **Perithecia** subglobose 0.25–0.3 mm diam. **Ostioles** umbilicate, inconspicuous, at times fringed with white material forming a small disc.

Asci cylindrical, originating in bundles from short ascogenous hyphae, with eight obliquely uniseriate ascospores, the spore-bearing parts 53–58 × 6–7 µm, the stipes 35–45 µm long, with a discoid apical apparatus 0.5–0.8 × 1.8–2 µm faintly bluing in Melzer's reagent. **Ascospores** (7.2–) 7.5–8.2 (–8.5) × (3.5–) 3.6–4.1 (–4.3) µm; Q = (1.8–) 1.9–2.2 (–2.4); N = 50 (Me = 7.9 × 3.8 µm; Qe = 2.1), ellipsoid strongly

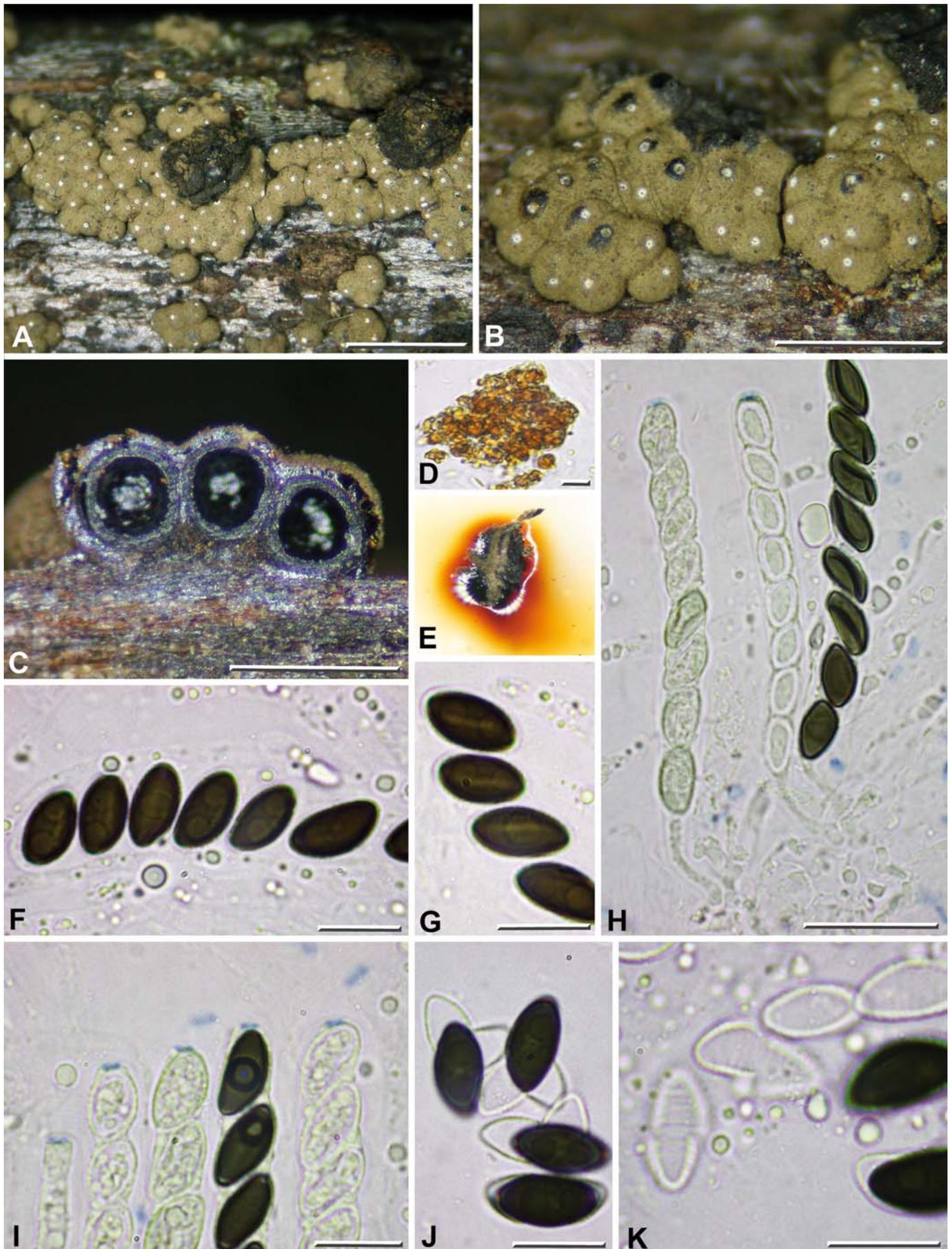


Plate 11 – *Hypoxylon musceum*. GYJF 12150

A, B: Glomerate stromata, associated with gall-like formations; C: Stroma in vertical section showing the perithecia; D: Stromatal granules in water; E: KOH-extractable pigments (1 min incubation); F, G: Ascospores in water, with germ slits visible on G; H: Immature and mature asci in Melzer's reagent; I: Ascial apical apparatus in Melzer's reagent; J, K: Ascospores in 10% KOH showing the striated dehiscent perispores. Scale bars: A, B = 2 mm; C = 0.5 mm; D, F, G, I-K = 10 μ m; H = 20 μ m.

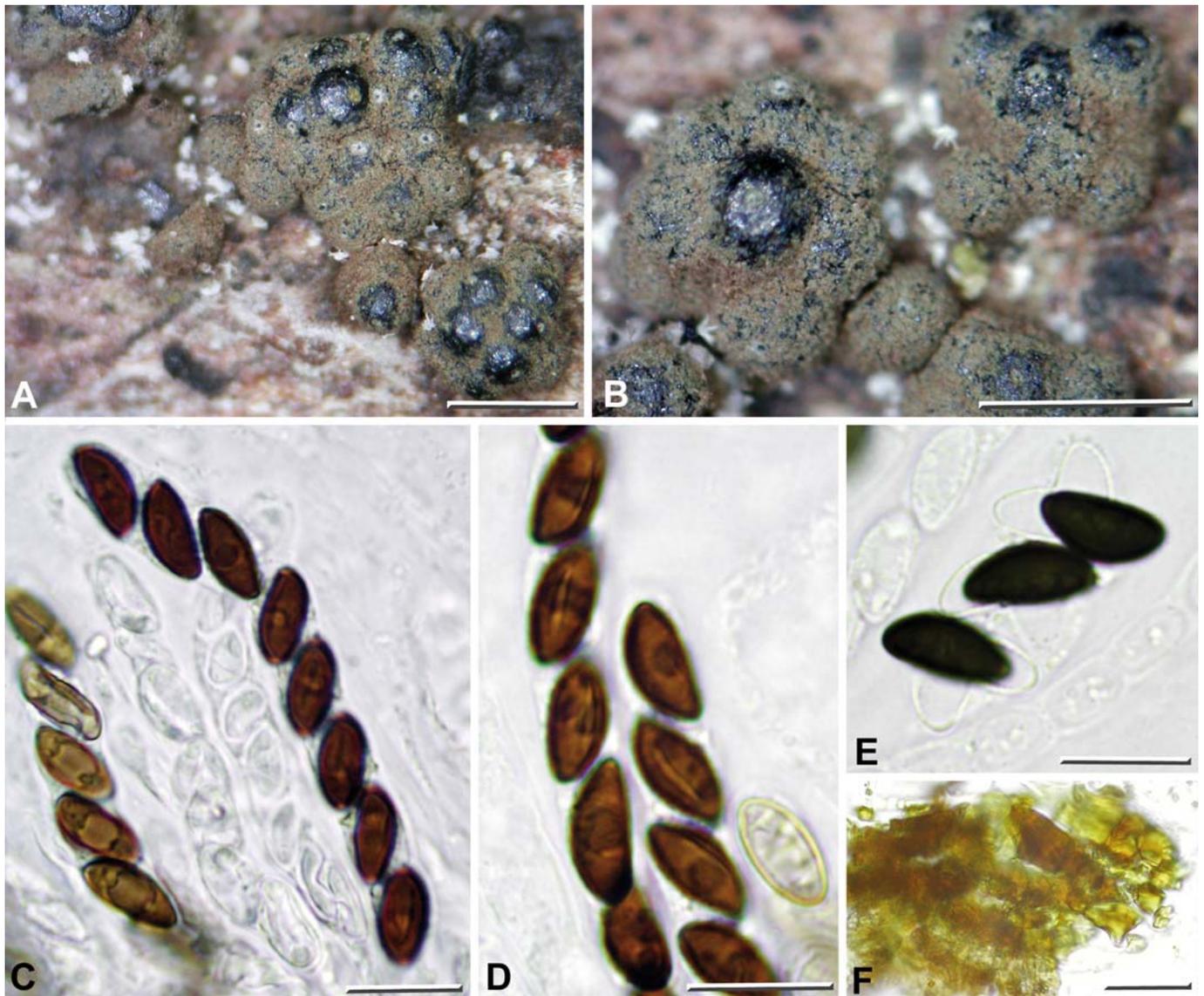


Plate 12 – *Hypoxylon musceum*. Holotype WSP 64843

A,B: Glomerate stromata; C, D: Ascospores in water, with germ slits visible on D; E: Ascospores in 10% KOH showing the smooth dehiscent perispores; F; Stromatal granules in water. Scale bars: A, B = 1 mm; C-F = 10 μ m.

inequilateral with narrowly rounded ends, often ventrally concave, dark brown, with a faint sigmoid germ slit spore-length on the convex side; perispore dehiscent in 10% KOH, conspicuously striated; episore smooth.

Asexual morph on natural substrate not seen.

Distribution: French Guiana.

Other species examined: MEXICO: Yucatán, Yucatán Peninsula, on ground of Chichén Itzá ruins, corticated wood of *Acacia?*, Nov. 1984, leg. R. Durán (WSP 67597, holotype of *H. duranii*).

Discussion: When run through Ju & Rogers' key (Ju & ROGERS, 1996), this fungus readily keys out to *H. duranii* based on stromata with purplish brown surface and olivaceous KOH-extractable pigments, combined with strongly inequilateral ascospores with coarsely striated dehiscent perispore (ROGERS, 1985). However, examination of the holotype of *H. duranii* (Plate 17) showed that it deviates in several respects from our fungus. While the stromata of *H. duranii* are glomerate to pulvinate with sepia (63, oac833) KOH-extractable pigments slightly fading after prolonged incubation, those of the *Hypoxylon* species from French Guiana appear effused-applanate and yield amber to fawn pigments in KOH that turn livid vinaceous after a while. Moreover the ascospores of the latter are significantly smaller than those of *H. duranii* ($7.5\text{--}8.2 \times 3.6\text{--}4.1$ vs. $10.5\text{--}12.3 \times 4.9\text{--}5.8$ μ m) and they have a sigmoid germ slit vs.

straight in *H. duranii*. Another differential character, more difficult to make out, is the presence in *H. duranii* of long ascogenous hyphae from which asci arise in spicate arrangement, while in our fungus the asci arise in bundles from short ascogenous hyphae. For all these reasons we recognize the *Hypoxylon* from French Guiana as a distinct taxon from *H. duranii*. The segregation of *H. paracouense* suggests that Ju & Rogers' concept of *H. duranii* (Ju & ROGERS, 1996) possibly encompasses several closely related species accounting for the wide ascospore size range, the varying germ slit morphology and the KOH-extractable pigments ranging from amber to isabelline reported by these authors. Variable metabolic profiles reported by KUHNERT *et al.* (2014a) from different collections listed as *H. duranii* by Ju & ROGERS (1996) likewise suggest that *H. duranii* is a complex of related taxa.

Hypoxylon griseobrunneum (B.S. Mehrotra) J. Fourn., Kuhnert & M. Stadler was likewise recently segregated from the *H. duranii* complex (KUHNERT *et al.*, 2014a) and indeed it resembles *H. paracouense* by several characters of which the most striking is the yellowish brown extractable-pigments that turn vinaceous after a prolonged incubation. This may suggest a similar metabolic profile but *H. griseobrunneum* morphologically differs from *H. paracouense* in having thicker stromata with tubular perithecia, long ascogenous hyphae and less strongly inequilateral ascospores with a straight germ slit.

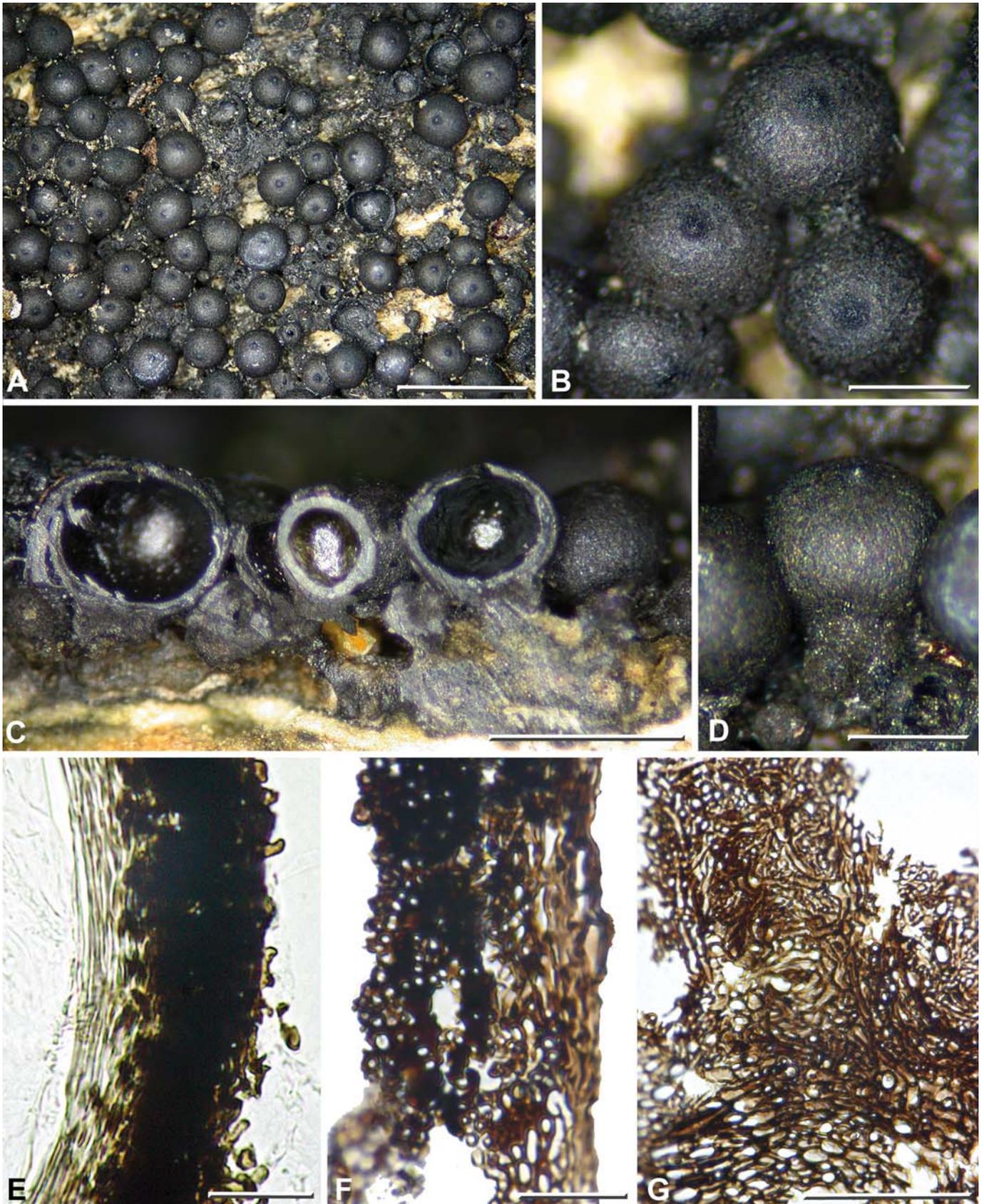


Plate 13 – *Hypoxylon nudum*. Holotype GYJF 12134

A: Ascomata superficial on bark; B: Ascomata in close up showing the finely roughened wall and the papillate ostioles; C: Ascomata in vertical section showing the basal stromatic tissue; D: Ascomata in side view showing the papillate ostiole and a pseudostipe; E, F: Ascomatal wall in chloreal lactophenol, showing the two-layered structure and the small protruding cells; G: Prosenchymatous and pseudoparenchymatous basal stroma in chloreal lactophenol. Scale bars: A = 2 mm; B, D = 0.5 mm; C = 1 mm; E, F = 20 μ m; G = 50 μ m.

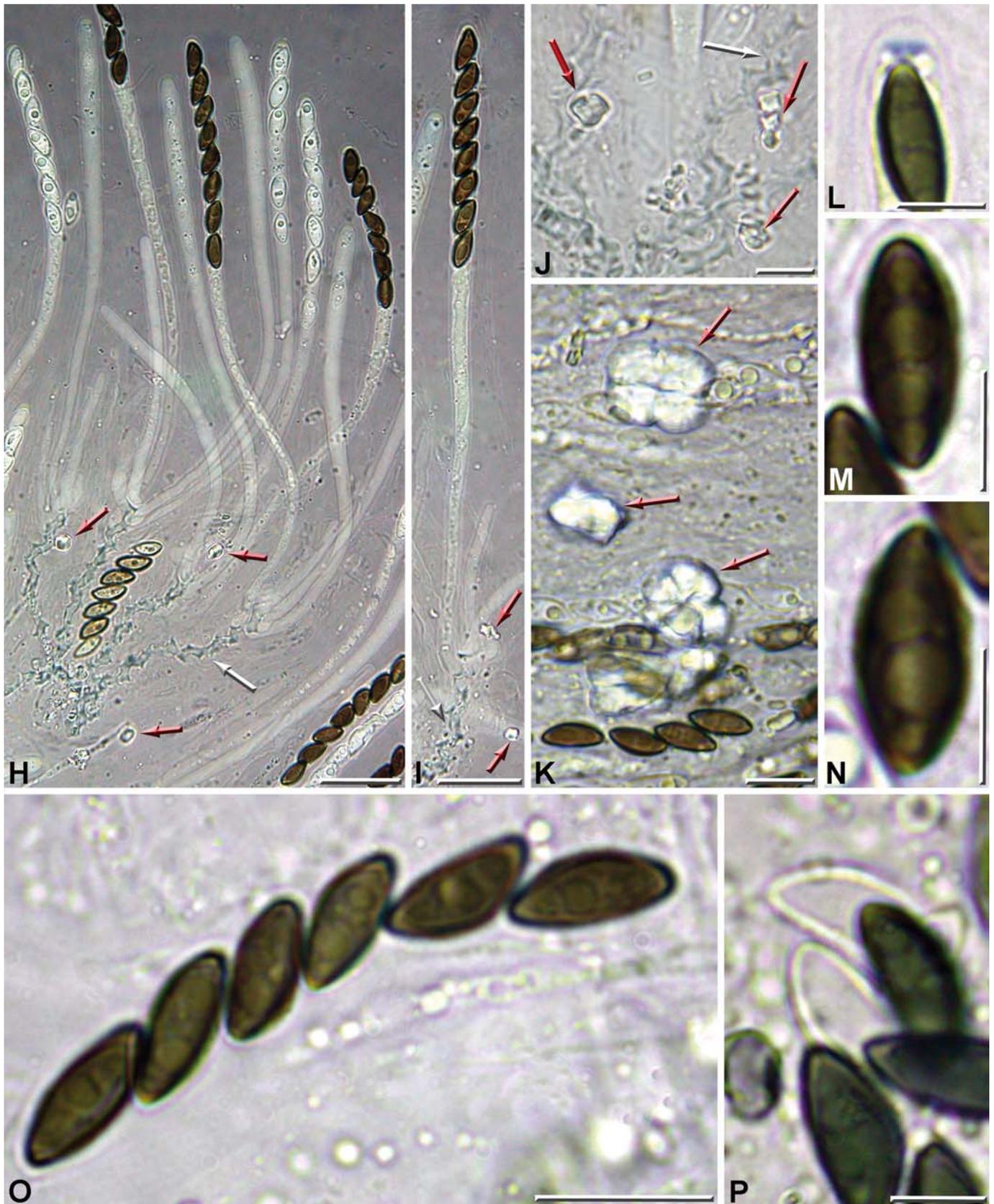


Plate 14 – *Hypoxylon nudum*. Holotype GYJF 12134

H, I: Immature and mature asci in black Pelikan ink, showing the spiny ascogenous hyphae (white arrows) and scattered crystals (red arrows); J: Subhymenium with spiny ascogenous hyphae (white arrows) and scattered crystals (red arrows), in black Pelikan ink; K: Agglomerated crystals in the hymenium (red arrows), in black Pelikan ink; L: Apical apparatus in Melzer's reagent; M, N: Ascospores in dorsal view in water showing the germ slits; O: Ascospores in water; P: Ascospores in 10% KOH with dehiscent perispores. Scale bars: H, I = 20 μ m; J, K, O = 10 μ m; L, M, N, P = 5 μ m.

Hypoxylon rhombisporum J. Fourn. & Lechat *sp. nov.* – MycoBank MB811915. Plates 18–19

Diagnosis: Differs from the most closely related *Hypoxylon rectangularulosporum* by a dark purplish brown stromatal surface, pinkish brown stromatal granules yielding dilute yellowish brown pigments in 10% KOH that turn vinaceous after prolonged incubation, tubular perithecia and frequently rhomboid ascospores averaging $6.8 \times 3.4 \mu\text{m}$.

Holotype: FRENCH GUIANA: Régina, Nouragues natural reserve, Inselberg camp, trail to Pararé, ca. 1 km from the camp, rainforest, on bark of unidentified dead standing trunk, 21 Jun. 2012, *leg.* J. Fournier, GYJF 12123 (LIP).

Etymology: For the angular, frequently rhomboid ascospores.

Stromata effused-applanate on blackened bark with abrupt, sinuous margins, with inconspicuous perithecial contours, 8–35 mm long \times 11–15 mm wide \times 0.7–1 mm thick; surface dark brown vinaceous (84, oac523), pruinose, slightly uneven; subsurface reddish brown, with waxy granules appearing pinkish brown to reddish

brown in water, forming a thick layer above perithecia and extending downwards between them, yielding dilute ochreous (44, oac709) KOH-extractable pigments turning livid vinaceous (83, oac506) after 20 min incubation; subperithecial tissue blackish brown, not clearly differentiated from the underlying wood. **Perithecia** tubular to long tubular, $0.6\text{--}0.7 \times 0.2\text{--}0.3 \text{ mm}$. **Ostioles** umbilicate, fringed with white powdery material.

Asci cylindrical, with eight uniseriate ascospores, the spore-bearing parts $53\text{--}59 \times 5 \mu\text{m}$, the stipes $90\text{--}110 \mu\text{m}$ long, originating in spicate arrangement from long ascogenous hyphae, with a discoid to wedge-shaped apical apparatus $0.8\text{--}1 \times 1.5\text{--}1.8 \mu\text{m}$, bluing in Melzer's reagent. **Cuboid crystals** interspersed in the subhymenium $6\text{--}7.5 \mu\text{m}$ on a side with pyramidal upper face, appearing translucent white, often aggregated in large clusters, undissolved in 10% KOH or in lactic acid. **Paraphyses** filiform, copious, filled with minute oily guttules. **Ascospores** $(6.1\text{--}) 6.3\text{--}7.4 (-8.8) \times (3.0\text{--}) 3.2\text{--}3.8 (-3.9) \mu\text{m}$; $Q = (1.7\text{--})1.74\text{--}2.3 (-3.0)$; $N = 60$ ($Me = 6.8 \times 3.4 \mu\text{m}$; $Qe = 2.0$), rectangular to oblong at both ends of the ascus, with most middle ascospores often distinctly rhomboid, brown, with a conspicuous straight germ slit almost spore-length; perispore indehiscent

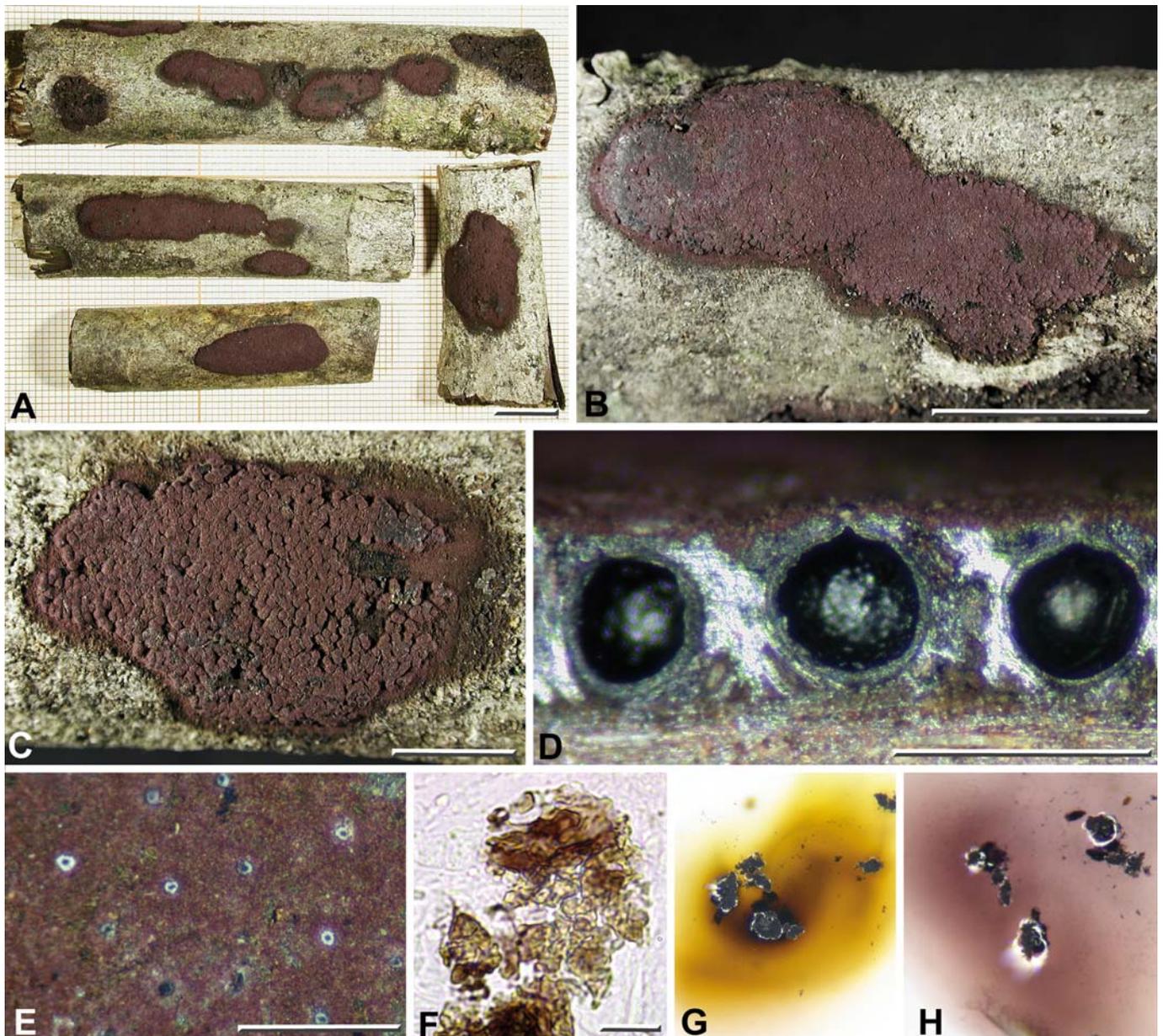


Plate 15 – *Hypoxylon paracouense*. Holotype GYJF 12185

A-C: Effused stromata on bark; D: Stroma in vertical section showing the perithecia; E: Stromatal surface in close-up showing the ostioles; F: Stromatal waxy granules observed in water; G, H: KOH-extractable pigments, respectively after 1 min and 20 min incubation. Scale bars: A, B = 10 mm; C = 5 mm; D, E = 0.5 mm; F = 10 μm .

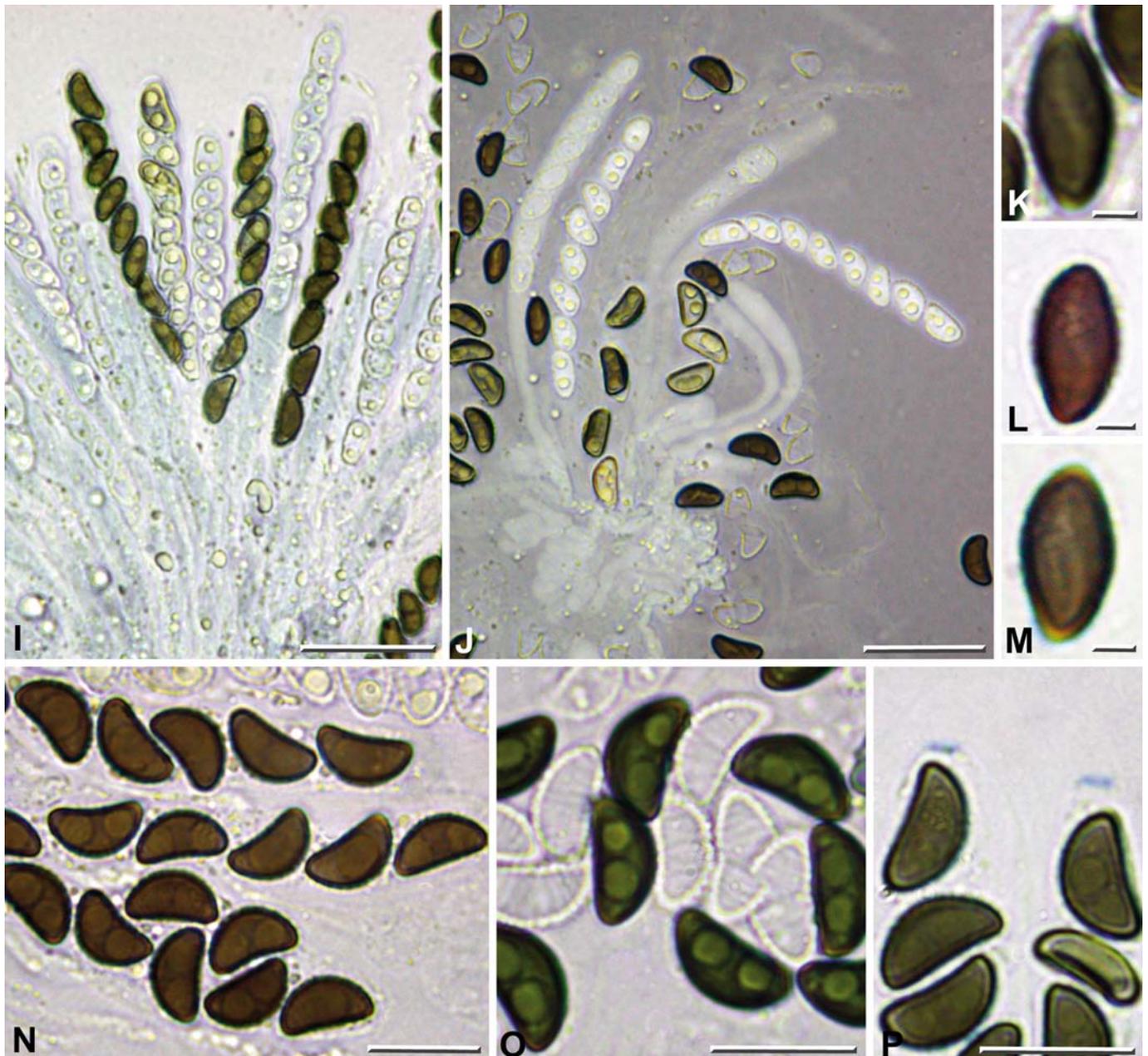


Plate 16 – *Hypoxylon paracouense*. Holotype GYJF 12185

I: Mature asci in chlorazol black; J: Immature asci in black Pelikan ink showing the short ascogenous hyphae; K-M: Ascospores with sigmoid germ slit (K in Melzer's reagent, L, M in chloral-lactophenol); N: Ascospores in water; O: Ascospores in 10% KOH showing the ornamented dehiscent perispores; P: Ascular apical apparatus in Melzer's reagent. Scale bars: I, J = 20 μ m; K-M = 2 μ m; N-P = 10 μ m

in 10% KOH; episporium with two parallel ridges on each side, that of immature nonpigmented ascospores turning bluish grey in KOH.

Asexual morph on natural substrate not seen.

Distribution: French Guiana.

Other species examined: GUYANA: Cuyuni-Mazaruni region VII; Mazaruni subregion VII-2, Chi-Chi Mt. Range, 4 km W of Chi-Chi falls, S bank of the river, Im baimadai, 450–550 m elev., on wood, 17–18 Feb. 1987, leg. G.J. Samuels *et al.*, 4627A (NY, Holotype of *H. rectangulosporum*) (Plate 20).

Discussion: This *Hypoxylon* is distinctive mainly because of its angular ascospores with ridged walls. This unusual feature is known only from *H. rectangulosporum* Y.-M. Ju, J.D. Rogers & Samuels, with which it likely has strong affinities. *Hypoxylon rectangulosporum* is known from only one collection in Guyana and was given a detailed description by its authors who stressed the peculiar ascospore morphology with the help of SEM (scanning electron microscopy) (ROGERS *et al.*, 1992). In order to evaluate thoroughly the status of our

find from French Guiana, the holotype of *H. rectangulosporum* was examined and its features are illustrated in Plate 20. Based on the following measurements of ascospores from the holotype of *H. rectangulosporum*, (6–) 6.4–8 (–9.4) \times (3.7–) 4–5.1 (–5.5) μ m; Q = (1.1–) 1.3–1.9 (–2.3); N = 60 (Me = 7.2 \times 4.7 μ m; Qe = 1.6) it appears that although similar in length the ascospores of our fungus differ in being significantly narrower, which is reflected by a larger quotient Qe averaging 2 vs. 1.6. Moreover, unlike in *H. rectangulosporum* where the ascospores are consistently rectangular to barrel-shaped, those of our fungus are frequently rhomboid, especially in the mid-part of the ascus, their episporium has less prominent ridges and a more conspicuous germ slit. As the variation of ascospore morphology of *H. rectangulosporum* is poorly known because only one collection has ever been made, further morphological traits were compared between the two collections.

While the stromatal surface of *H. rectangulosporum* is pale yellow brown (ochreous 44) with subsurface composed of dull yellow waxy granules, that of our *Hypoxylon* differs in being dark purplish brown

with a reddish brown subsurface layer composed of pale pinkish and reddish brown waxy granules. Accordingly, their KOH extractable pigments differ in being pale luteous with a faint orange tinge in *H. rectangulosporum*, turning slightly darker upon prolonged incubation, while those of our *Hypoxylon* are first also in shades of luteous but turn livid vinaceous after 20 min incubation. Moreover, the perithecia of our fungus appear more tubular than those of

H. rectangulosporum, a character that should be evaluated in more specimens to assess whether it is significant or not.

The differential characters discussed above justify our *Hypoxylon* to be recognized as a distinct species from *H. rectangulosporum*.

The question of the presence or absence of a germ slit in ascospores of *H. rectangulosporum* was commented by ROGERS *et al.* (1992) and they concluded to the lack of germ slits, based on SEM

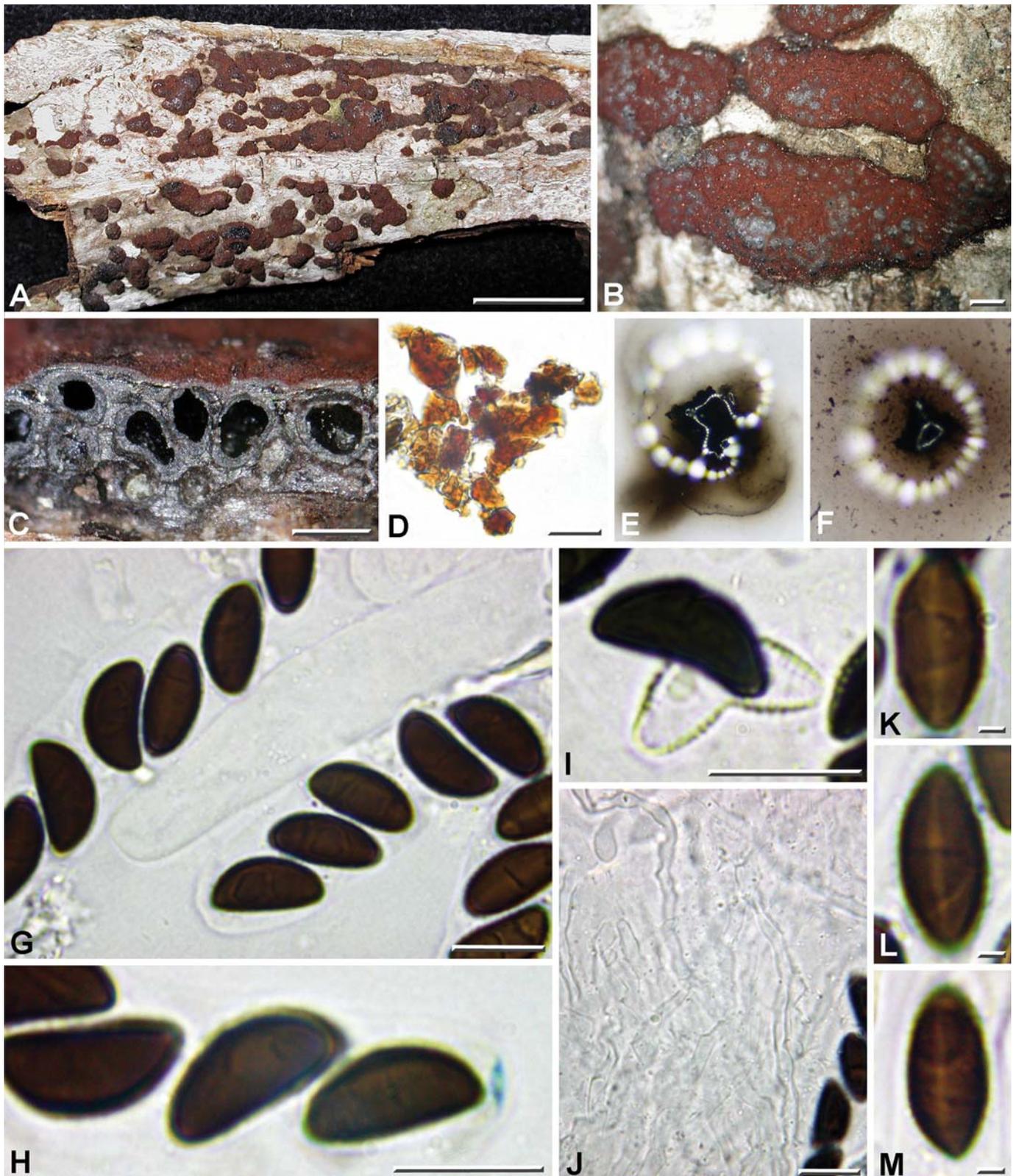


Plate 17 – *Hypoxylon duranii*. Holotype WSP 67597

A, B: Pulvinate stromata on bark; C: Stroma in vertical section; D: Stromatal granules in water; E, F: KOH-extractable pigments after 1 min and 20 min incubation respectively; G: Ascospores in water; H: Ascus apical apparatus in Melzer's reagent; I: Ascospore in 10% KOH with ornamented dehiscent perispore; J: Long ascogenous hyphae; K-M: Ascospores in water showing the straight germ slit. Scale bars: A = 10 mm; B = 1 mm; C = 0.5 mm; D, G-I = 10 µm; J-L = 2 µm.

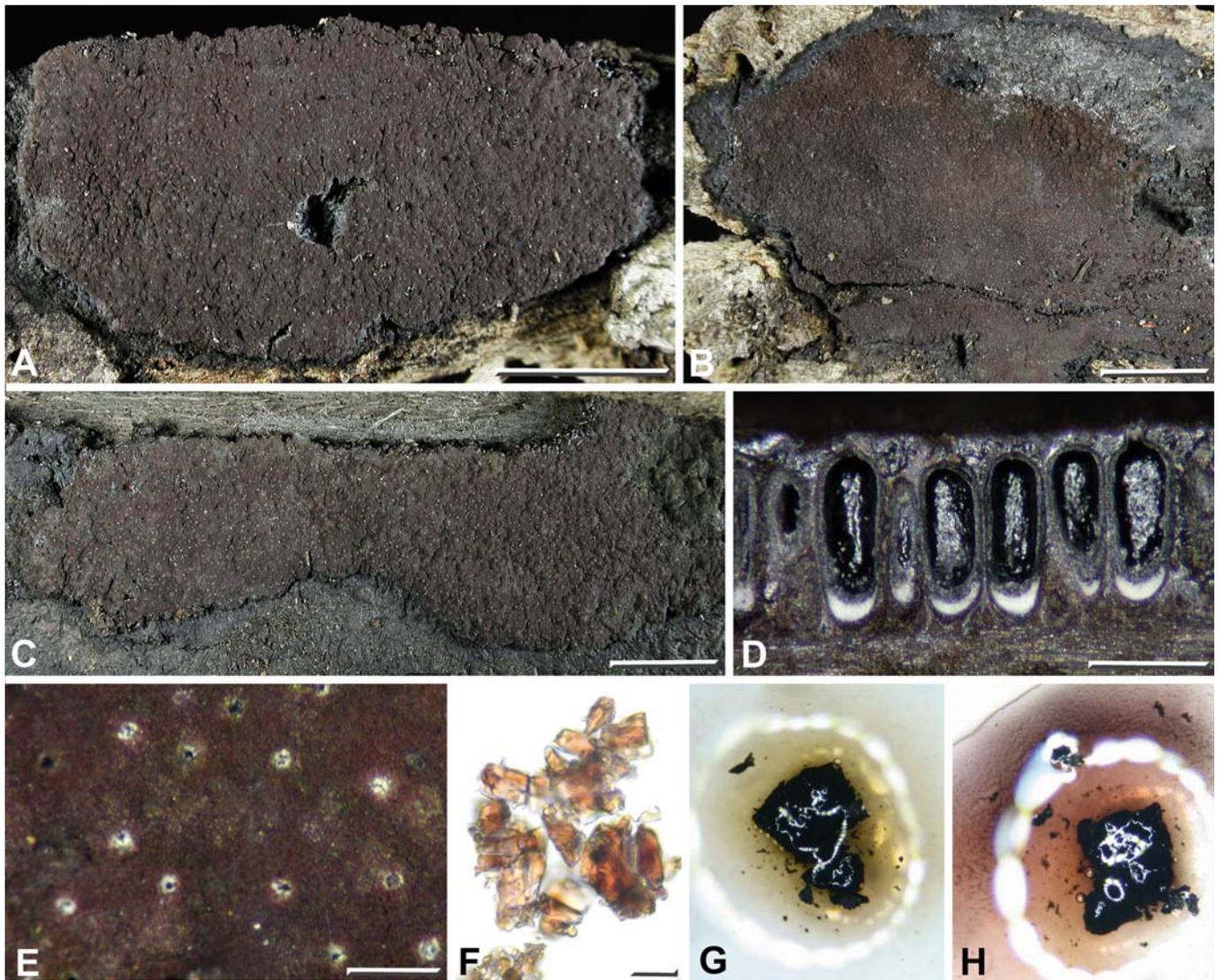


Plate 18 – *Hypoxylon rhombisporum*. Holotype GYJF 12123

A-C: Various effused stromata; D: Stroma in vertical section showing the perithecia; E: Stromatal surface in close-up showing the ostioles fringed with white material; F: Subsurface granules in water; G, H: KOH-extractable pigments after 1 min and 20 min incubation respectively. Scale bars: A-C = 5 mm; E = 0.2 mm; F = 10 μ m.

observations. The presence of the prominent ridges makes the observations on mature pigmented ascospores difficult but paler lines present in valleys between ridges that are visible on immatures ascospores (Plate 20, fig. J) suggest the possible presence of a germ slit. In contrast *H. rhombisporum* features a conspicuous germ slit easily distinguished from the lateral ridges.

ROGERS *et al.* (1992) reported and illustrated the presence of geniculate paraphyses in *H. rectangulosporum* (Fig. 4). These elements most likely are long ascogenous hyphae from which asci arise in spicate arrangement, leaving spiny scars responsible for their peculiar outline. Such ascogenous hyphae were indeed found in the hymenium of *H. rectangulosporum* upon examination of the holotype and were also observed in *H. rhombisporum*. Though usually unnoticed they occur in most *Hypoxylon* spp., their absence being more a diagnostic character than their presence.

Finally, the presence of crystals in the subhymenium of this collection appears to be a consistent feature that might be a significant taxonomic marker since it has not been shown from other *Hypoxylon* spp., except the morphologically very different *H. nudum* (this paper). Of course the presence of crystals in the subhymenium and its consistency in a given species should be investigated on a larger scale before it becomes a reliable differential character.

Hypoxylon trugodes Berk. & Broome, *J. Linn. Soc.*, 14: 122 (1873). Plate 21

Stromata irregularly effused-pulvinate with lobed margins, with inconspicuous perithecial contours, 10–48 mm long \times 3–16 mm wide \times 0.6–0.9 mm thick, often coalescent; surface dark vinaceous (82, oac523), pruinose, slightly uneven; yellow to ochraceous granules forming a thick waxy layer above and between perithecia and unusually well developed around the base of perithecia, with fugacious luteous (12, oac810) KOH-extractable pigments, soon turning cinnamon (62, oac715), not notably changing upon prolonged incubation; subperithecial tissue inconspicuous. **Perithecia** long tubular to more rarely obovoid-elongate, 0.5–0.7 \times 0.25–0.3 μ m. **Ostioles** umbilicate, inconspicuous.

Asci cylindrical, with eight obliquely uniseriate ascospores, the spore-bearing parts 55–60 \times 5–6.5 μ m, the stipes 60–95 μ m long, originating in unilateral spicate arrangement from long ascogenous hyphae, with a discoid apical apparatus 0.8–1 \times 1.8–2 μ m, bluing in Melzer's reagent. **Ascospores** (7.0–) 7.3–8.1 (–8.7) \times (2.9–) 3.2–3.5 (–3.8) μ m; Q = (2.0–) 2.2–2.5 (–2.7); N = 60 (Me = 7.7 \times 3.3 μ m; Qe = 2.3), ellipsoid-inequilateral with narrowly rounded ends, frequently slightly ventrally concave, medium to dark brown, with a straight germ slit spore-length on the convex side; perispore dehiscent in 10% KOH, smooth; epispore smooth.

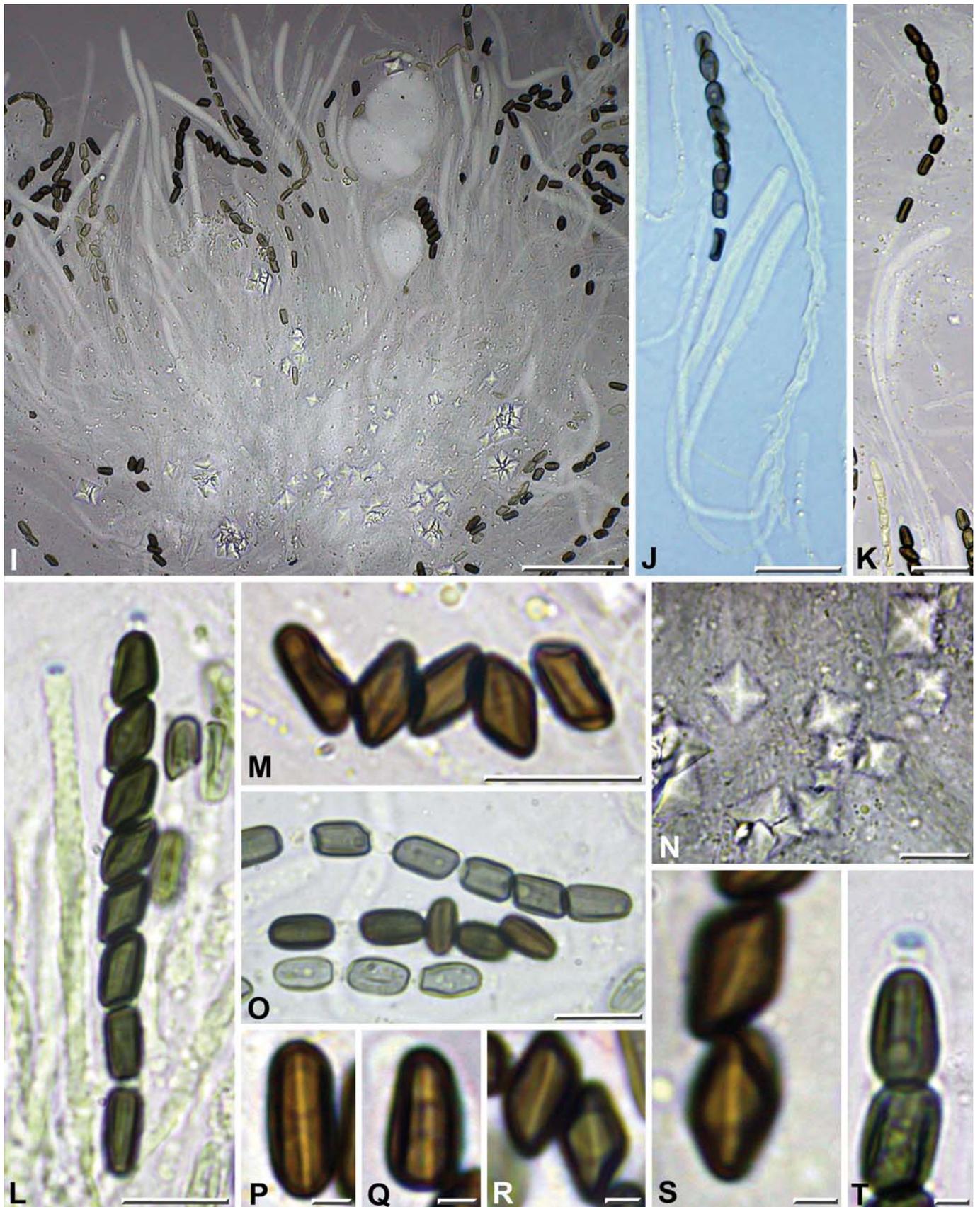


Plate 19 – *Hypoxylon rhombisporum*. Holotype GYJF 12123

I: Hymenium in black Pelikan ink showing immature and mature asci with crystals in the subhymenium; J: Immature and mature asci in dilute blue Waterman ink in 1% SDS, showing ascial stipes arising from a long ascogenous hypha; K: Mature long-stipitate ascus in black Pelikan ink; L: Ascus in Melzer's reagent showing the ascospores arrangement and the apical apparatus; M: Oblong and rhomboid ascospores in water; N: Subhymenial crystals in black Pelikan ink; O: Ascospores in 10% KOH showing the absence of dehiscent perispores and the immature ascospores turning bluish grey; P-S: Ascospores in water showing the germ slits; T: Ascus apex in Melzer's reagent showing the apical apparatus. Scale bars: I = 50 µm; J, K = 20 µm; L-O = 10 µm; P-T = 2 µm.

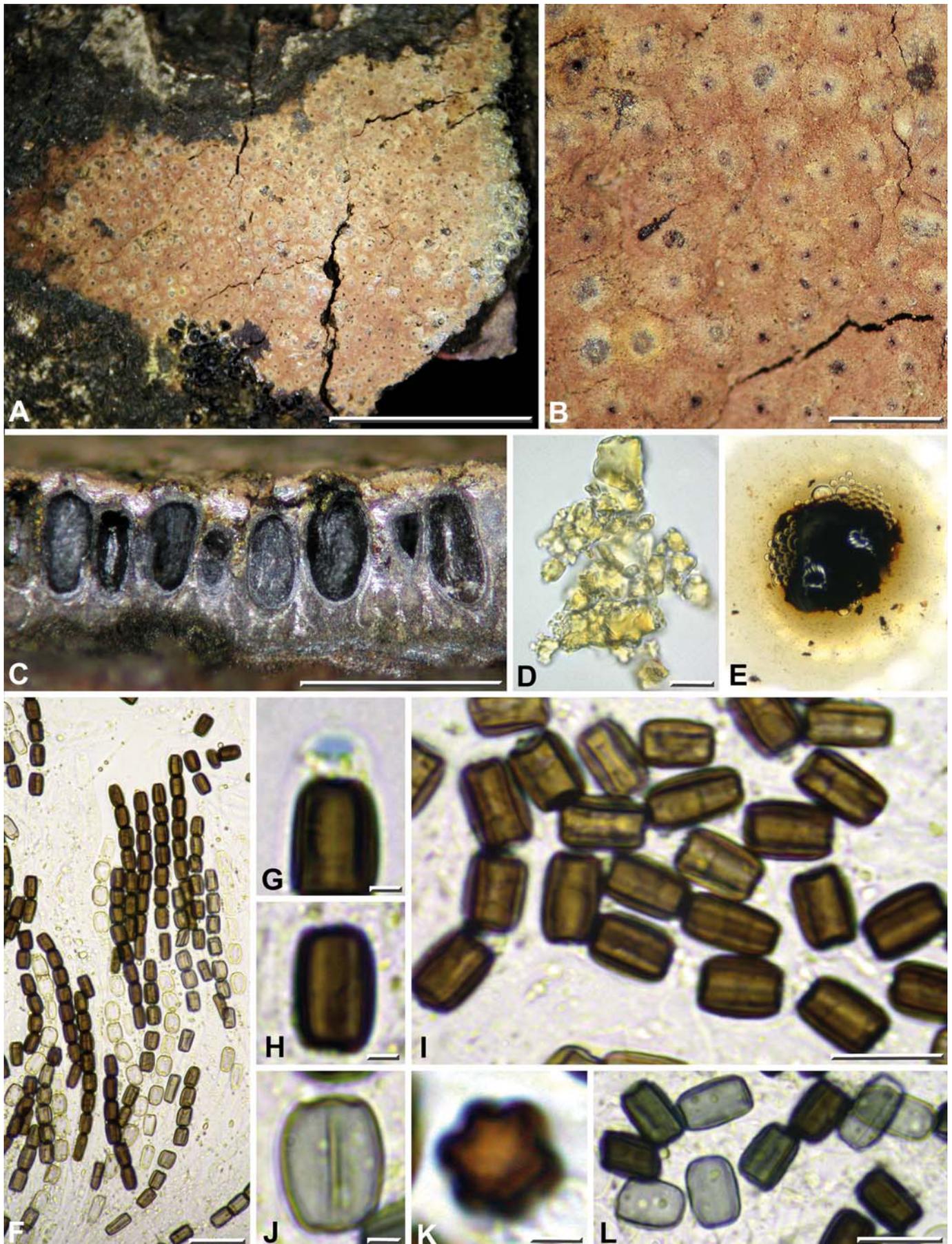


Plate 20 – *Hypoxylon rectangulosporum*. Holotype NY 4627A

A: Fragment of a stroma; B: Stromatal surface in close-up showing the faintly exposed perithecial contours and the ostioles; C: Stroma in vertical section showing the perithecia and the yellow subsurface; D: Subsurface granules in water; E: KOH-extractable pigments within 1 min incubation; F: Asci in 1% SDS; G: Ascal apical apparatus in Melzer's reagent; H, I: Ascospores in water; J: Immature ascospore in 10% KOH showing a supposed germ slit; K: Ascospore in polar view showing the lateral ridges; L: Ascospores in 10% KOH showing the absence of dehiscence of the perispore and the bluish grey colour of immature ascospores. Scale bars: A = 5 mm; B, C = 1 mm; D, I, L = 10 μ m; F = 20 μ m; G, H, J, K = 2 μ m.

Asexual morph on natural substrate not seen.

Specimens examined: FRENCH GUIANA: Régina, Nouragues natural reserve, Inselberg camp, west trail ca. 1 km from the camp, rainforest, bark of unidentified dead standing trunk, 18 Jun. 2012, *leg.* J. Fournier, GYJF 12043 (LIP) (illustrated here); *ibid.*, trail H 19, bark of a dead trunk, 17 Jun. 2012, *leg.* C. Lechat, GYJF 12022 (LIP). Sinnamary, Paracou, CIRAD field station, trail to the station, dead corticated branch, 25 Jun. 2012, *leg.* J. Fournier, GYJF 12189 (LIP).

Distribution: Neotropics, southeast Asia.

Discussion: *Hypoxylon trugodes* is characterized by effused stromata with purplish brown surface, long tubular perithecia embedded in an abundant waxy tissue composed of yellow granules that yield orange yellow pigments in 10% KOH, combined with strongly inequilateral ascospores less than 10 µm long with a straight germ slit and a smooth dehiscent perispore.

The holotype is from Sri Lanka and the few collections cited by JU & ROGERS (1996) originate from Singapore, Taiwan and Hawaii. The present records of *H. trugodes*, along with a collection from Panama (CARMONA *et al.*, 2009, as *H. anthochroum*), extend its known distribution to the Neotropics. If the stromatal granules and their KOH reaction are not investigated *H. trugodes* can be easily confused with other *Hypoxylon* spp. with similar purplish brown stromatal surface, of which *H. fendleri* Berk. ex Cooke is the most common. *Hypoxylon fendleri* differs from *H. trugodes* by obovoid perithecia, orange stromatal granules and KOH-extractable pigments and ascospores with a sigmoid germ slit.

Hypoxylon verruciperisporium J. Fourn. & Lechat *sp. nov.* – MycoBank MB811916. Plates 22–23

Diagnosis: Differs from all known species of *Hypoxylon* by a verrucose perispore.

Holotype: FRENCH GUIANA: Sinnamary, Paracou, CIRAD field station, lowland rainforest, edge of parking area, dead corticated branch, 26 Jun. 2012, *leg.* J. Fournier, GYJF 12239 (LIP).

Etymology: From Latin *verruca* = wart and *perisporium* = perispore, for the unusual ornamentation on the perispore.

Stromata irregularly effused-applanate with frequently indented margins, with slightly exposed to half-exposed perithecial contours, 3–35 mm long × 2–17 mm wide × 0.3–0.45 mm thick; surface dark brick (60, oac637) with faint vinaceous shade in places or dark vinaceous (83, oac524), pruinose, slightly uneven, at times with an effused scarlet (5, oac628) margin; subsurface scarlet, appearing upon bruising or section, composed of dark red granules forming a thick waxy layer above and between perithecia and especially abundant at the edges of the stromata, with dense orange (7, oac629) KOH-extractable pigments, not notably changing upon prolonged incubation; subperithecial tissue inconspicuous, blackish. **Perithecia** subglobose 0.20–0.25 mm diam or obovoid 0.35 × 0.25–0.3 mm. **Ostioles** umbilicate, inconspicuous.

Asci cylindrical, short-stipitate, with eight obliquely uniseriate ascospores, the spore-bearing parts 59–70 × 7–7.5 µm, the stipes 14–28 µm long, originating from short sinuous ascogenous hyphae, with a discoid apical apparatus 0.5–0.8 × 1.8–2 µm, bluing in Melzer's reagent. **Paraphyses** 6–8 µm wide and slightly constricted at septa at base, tapering above asci, faintly guttulate. **Ascospores** (8.0–) 8.5–9.3 (–9.9) × (3.6–) 3.8–4.4 (–4.6) µm; Q = (1.9–) 2.0–2.4 (–2.5); N = 50 (Me = 8.9 × 4.1 µm; Qe = 2.2), ellipsoid-inequilateral with most often narrowly rounded ends, at times slightly ventrally concave, brown to dark brown, with a faint straight germ slit spore-length; perispore dehiscent in 10% KOH, verrucose, with warts evenly distributed, separate, also visible on immature ascospores; epispore smooth.

Asexual morph on natural substrate: Present in places at margins of stromata in GYJF 12239, forming scattered honey (64, oac847)

tufts, with a sporothrix- to virgariella-like pattern of branching. Conidiogenous cells pale brown, 13.5–22 × 2.5–3 µm, finely roughened; conidia ovoid, 3.5–4.5 × 2.2–3 µm, yellowish, smooth to faintly roughened.

Other specimen examined: FRENCH GUIANA: Régina, Nouragues natural reserve, Inselberg camp, rainforest, edge of the dropping zone, dead corticated branch, 20 Jun. 2012, *leg.* J. Fournier & C. Lechat, GYJF 12110 (LIP), paratype.

Discussion: *Hypoxylon verruciperisporium* is readily set apart from all known species of *Hypoxylon* by the verrucose ornamentation of its perispores. Indeed in *Hypoxylon*, like in *Daldinia* one of its most closely related genus, the epispore is typically and consistently smooth or transversely striated (JU & ROGERS, 1996). Within the *Hypoxylodeae*, warty ascospores are known only in the genus *Camillea* Fr. subgenus *Jongiella* (M. Morelet) Læssøe, J.D. Rogers & Whalley represented by *C. obularia* (Fr.) Læssøe, J.D. Rogers & Lodge and its allies *C. selangorensis* M.A. Whalley & A.J.S. Whalley, *C. signata* (S.C. Jong & C.R. Benj.) Læssøe, J.D. Rogers & Whalley and *C. verruculosa* J.D. Rogers, Læssøe & Lodge (LÆSSØE *et al.*, 1989; ROGERS *et al.*, 1991; WHALLEY *et al.*, 1996). However, *Camillea* is clearly different from *Hypoxylon* in having carbonaceous bipartite stromata lacking stromatal pigments, asci with a massive apical apparatus, variously ornamented ascospores lacking a perispore dehiscent in 10% KOH and a different asexual morph (LÆSSØE *et al.*, 1989; JU & ROGERS, 1996).

Another noteworthy character of *H. verruciperisporium* is the presence of abundant dark red stromatal granules.

Hypoxylon erythrostroma J.H. Mill. is a pantropical taxon the stromata of which sometimes exhibit a red subsurface when bruised but its waxy stromatal granules are yellow when observed in water, not red like in *H. verruciperisporium*. *H. erythrostroma* also differs from *H. verruciperisporium* by thicker stromata 0.7–1 mm thick with ostioles frequently slightly papillate, long-stipitate asci and ascospores with a sigmoid germ slit and a conspicuously transversely striated perispore. Moreover, *H. erythrostroma* was reported to produce a nodulisporium-like asexual morph in culture (JU & ROGERS, 1996).

Two temperate species likewise feature red stromatal granules when sectioned or bruised, viz.: *H. julianii* L.E. Petrini and *H. rutilum* Tul. & C.Tul. The former has similar reddish waxy granules when observed in water but mainly differs from *H. verruciperisporium* by significantly larger ascospores 14.5–18 × 6–7.5 µm while the latter has orange waxy granules when observed in water, thicker stromata up to 1.5 mm thick, smaller perithecia 0.1–0.2 mm diam and papillate ostioles. Moreover they both lack a warty ornamentation on the perispore.

The two collections presented here under the name *H. verruciperisporium* are fairly different in stromatal surface colour and perithecial shape and were first regarded as different. However, upon closer examination they appeared to share the two highly diagnostic characters of scarlet stromatal granules and verrucose perispore, along with very similar ascospore dimensions in GYJF 12110: (7.9–) 8.1–9.1 (–9.5) × (3.4–) 3.7–4.3 (–4.6) µm; Q = (2.0–) 2.1–2.3 (–2.6); N = 50 (Me = 8.6 × 4.0 µm; Qe = 2.2). It can be expected that further records of this taxon will exhibit stromatal characters intermediate between the two collections reported here. *H. verruciperisporium* is the only of the six new species that was found in both sites during this study.

*Further *Hypoxylon* spp. collected in 2008 and 2010 by C. Lechat in French Guiana.

Hypoxylon haematostroma: Macouria, 06 May 2008, CLL 8100 (LIP); Cayenne, Matoury, La Mirande path, dead wood, 11 May 2008, *leg.* C. Lechat, CLL 8150 (LIP).

Hypoxylon investiens: Sinnamary, Saint-Elie track, dead corticated wood, 22 Apr. 2010, *leg.* C. Lechat, CLLG 10011 (LIP).

Hypoxylon lenormandii: Maripasoula, Saül, "Gros Arbres" path, dead corticated wood, 3 May 2008, *leg.* C. Lechat, CLL 8077 (LIP).

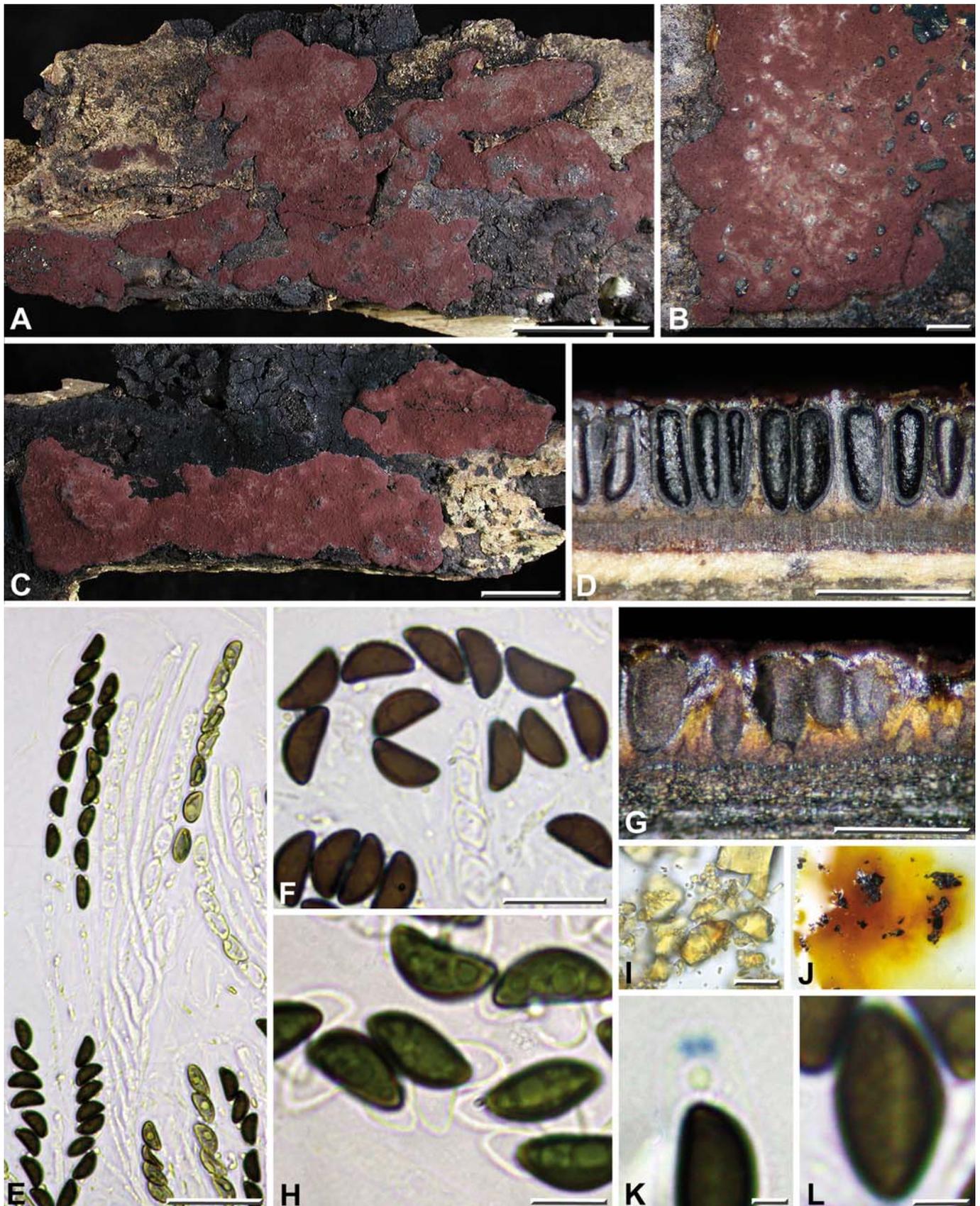


Plate 21 – *Hypoxylon trugodes*. GYJF 12043

A, C: Stromata effused on bark; B: Stromatal surface in close-up; D: Stroma in vertical section showing the perithecia; E: Immature and mature asci arising from a long ascogenous hypha, in 1% SDS; F: Ascospores in water; G: Stroma in vertical section (broken) showing the yellow granules completely surrounding the perithecia and densely distributed at the base; H: Ascospores in 10% KOH showing the dehiscence perispores; I: Stromatal granules in water; J: KOH-extractable pigments (1 min incubation); K: Ascospore in Melzer's reagent; L: Ascospore in dorsal view showing the germ slit. Scale bars: A, C = 10 mm; B, D, G = 1 mm; E = 20 μ m; F, I = 10 μ m; H = 5 μ m; K, L = 2 μ m.

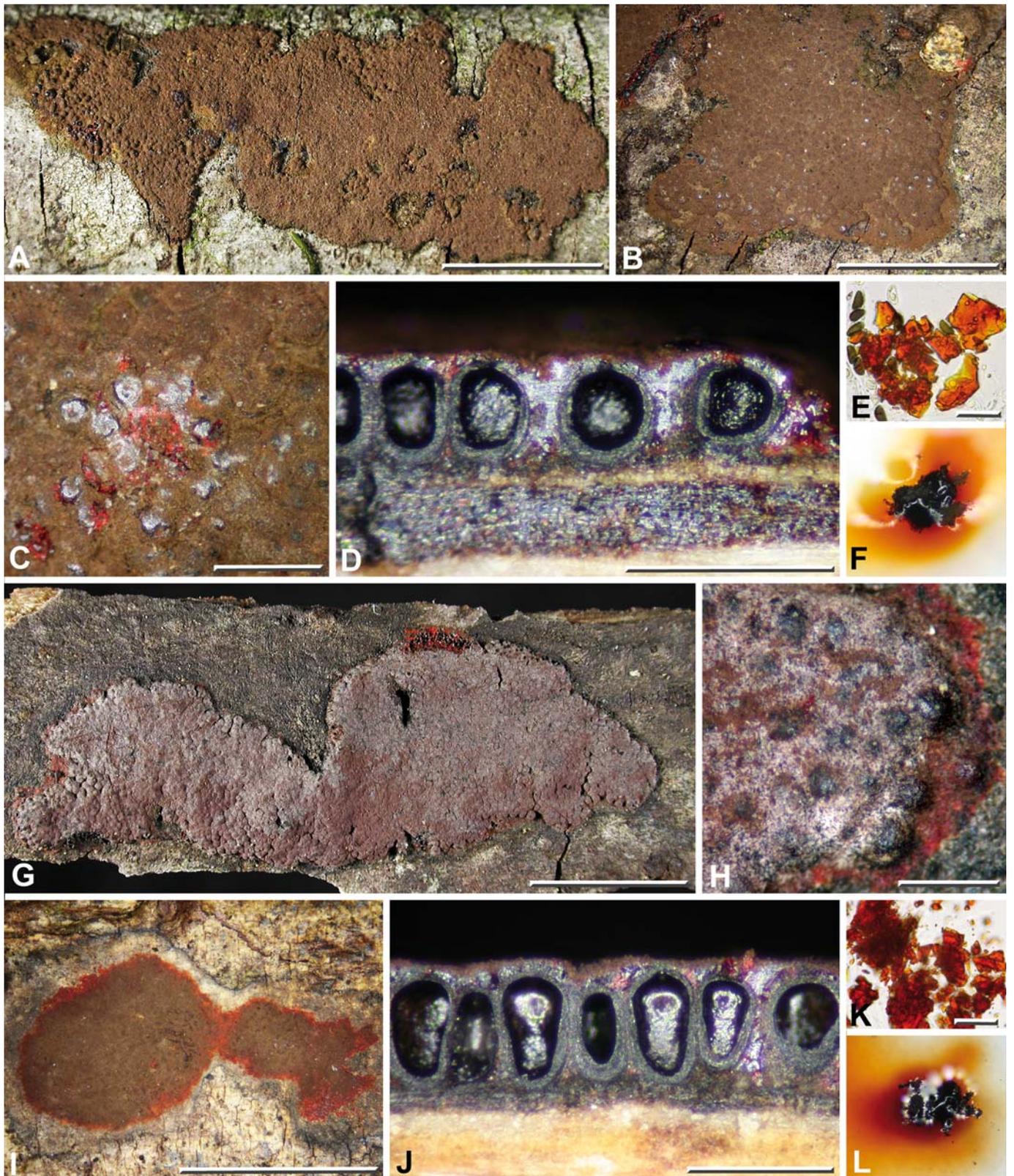


Plate 22 – *Hypoxylon verruciperisporium*

A-F: Holotype GYJF 12239; G-L: Paratype GYJF 12110. A, B, G, I: Stromata effused on bark; C, H: Stromatal surface in close-up showing the red subsurface or margin; D, J: Stromata in vertical section showing the perithecia and the red waxy granules; E, K: Stromatal granules in water; F, L: KOH-extractable pigments (1 min incubation). Scale bars: A, G = 10 mm; B, I = 5 mm; C, D, H, J = 0.5 mm; E, K = 20 μ m.

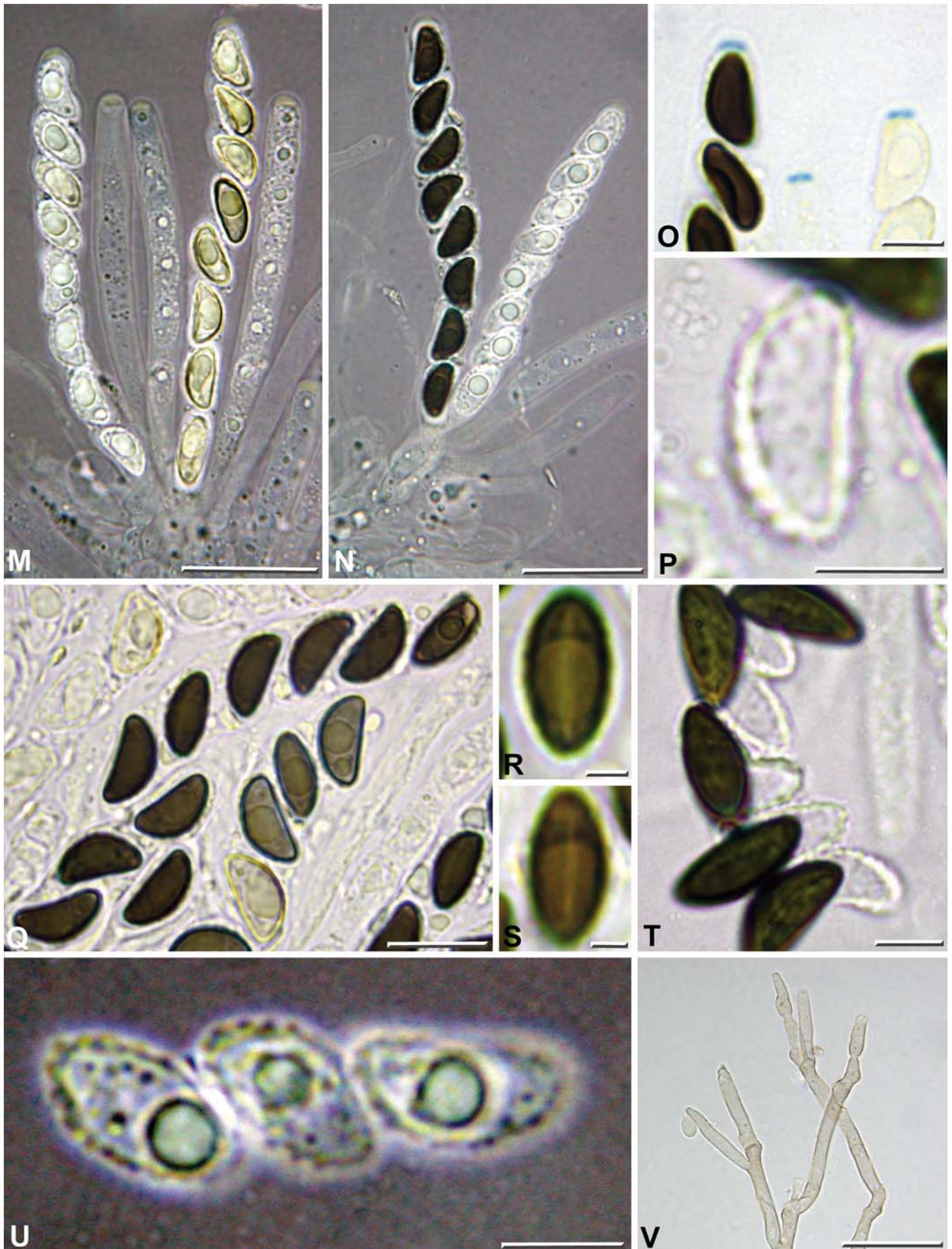


Plate 23 – *Hypoxylon verruciperisporium*. Holotype GYJF 12239

M, N: immature and mature asci in black Pelikan ink, showing the short stipes; O: Ascular apical apparatus in Melzer's reagent; P, T: Verrucose perispores dehiscent in 10% KOH; Q: Ascospores in water; R, S: Ascospores in dorsal view showing the germ slit (in black ink); U: Immature ascospores in black Pelikan ink showing the verrucose ornamentation on the perispores; V: Conidiogenous cells and one conidium of the asexual morph, in 1% SDS. Scale bars: M, N, V = 20 µm; O, P, T, U = 5 µm; Q = 10 µm; R, S = 2 µm.

Conclusion

By the addition of six new species and five species so far unrecorded, the number of *Hypoxylon* spp. known from French Guiana raises from 17 to 28. Such a result obtained after a limited time of sampling strongly suggests that more extensive field work should reveal an amazing diversity within this genus in this part of the world.

As the number of known species is increasing and can be expected to keep on increasing in the future if field work is carried out, the morphological characterization of *Hypoxylon* spp. has to rely on as many stable features as possible when it comes to discriminate closely related species. This is why the search for new informative characters is necessary to support taxonomic decisions.

The use by JU & ROGERS (1996) of new discriminant characters such as KOH-extractable pigments, dehiscence and ornamentation of the perispores, accurate morphology of the ascus apical apparatus and ascospore germ slit, combined with the morphology of cultures on artificial media and that of the asexual morph allowed them to revise MILLER'S (1961) concepts and to successfully segregate many species formerly lumped under the same name. Based on a much wider set of differential characters, the recognition of new taxa became much easier and safer.

The stromatal metabolites responsible for the coloured reactions in KOH were further investigated by means of HPLC coupled with diode array detection and mass spectrometric detection, and this method was extended to the metabolites produced in culture. This chemotaxonomic approach, backing morphological phenotypic studies, proved efficient to support the segregation of many taxa of *Hypoxyloideae* (STADLER *et al.*, 2001; HELLWIG *et al.*, 2005; MÜHLBAUER *et al.*, 2002; STADLER *et al.*, 2004; STADLER *et al.*, 2008; STADLER & FOURNIER, 2006). However, implementing this method is out of range for most taxonomists, which prompted, in parallel, the search for other approaches to study the stromatal metabolites of *Hypoxylon* and their pigments released in 10% KOH.

When a section of a stroma is observed with the naked eye or under a stereomicroscope, the subsurface crust of waxy granules appears most often dark-coloured and is consequently often recorded as dark brown or blackish. When the stromatal surface is crumbled instead of being sectioned the granules often appear more colourful but it is when they are crushed in a drop of water and observed under the microscope that the wide range of their colour variations can be discovered and evaluated. The colour of the granules in water is highly consistent for a given taxon and it is postulated that this colour is correlated with specific compounds. They may appear of uniform colour but sometimes appear as a mixture of several differently coloured types of granules. The addition of a drop of 10% KOH to the edge of the slide allows to observe how the granules dissolve and release pigments, even when the reaction is fugacious and would escape observation with the naked eye. Interestingly, when the granules have dissolved in KOH one can observe that they were fixed on a hyphal matrix composed of thin-walled often moniliform hyphae that are likely specialized in the production of the chemical compounds. Microscopic observation of the carbonaceous tissue enclosing perithecia in some species of *Hypoxylon* may also reveal coloured granules that otherwise would be overlooked. The routine observation of the stromatal granules lead us to discover in *H. hypomiltum* the presence of dark hyphal rods interspersed within the crust of olivaceous yellow granules, giving it a subcarbonaceous texture and making a highly discriminant character.

The stromata of *Hypoxylon* in good condition usually are coated by a thin pruinose layer that can also be interestingly submitted to microscopic observation in water and in 10% KOH. This tissue appears composed of coiled hyphae encrusted with coloured granules that likewise release small amounts of pigments and change colour in 10% KOH. This reaction is most often masked by the more colourful reaction of the stromatal granules but may be informative in

some cases. It cannot unfortunately be applied to old stromata the pruina of which has worn off.

The pigments released in 10% KOH by the stromatal granules usually appear within 1 min incubation and they remain stable or just fade after 20–30 min. In some cases they strikingly evolve with time and then they give a precious discriminant character. A good example of the utility of combining the observation of stromatal granules in water and to observe the delayed KOH reaction is given by the comparative study of *H. rhombisporum* and *H. rectangulosporum* in this survey. While both have similar ochreous KOH-extractable pigments, they can be separated based on differently coloured stromatal granules when observed in water and pigments that become vinaceous in the former while they remain unchanged in the latter. A similar delayed reaction also occurs in *H. paracouense*, supporting its segregation from *H. duranii*.

The ascogenesis is another character that deserves to be recorded. The asci of most *Hypoxylon* spp. arise from long ascogenous hyphae in spicate, often unilateral arrangement, which gives the ascogenous hyphae a more or less spiny or saw edge-like outline when asci are detached. These hyphae are present in the subhymenium and can be straight to contorted. More rarely the ascogenous hyphae are short and strongly contorted in a way that makes them difficult to be made out. In this case, asci arise in bundles and provide a differential character that may be useful since it is consistent for a given taxon.

Long-stipitate asci and ascogenous hyphae are more easily seen on fresh specimens and observations on dry material are facilitated by mounting them in 1% SDS. The use of stains is often needed to record the length of ascus stipes and to study the ascogenesis; in our experience good results can be obtained with chlorazol black or blue or black inks diluted in 1% SDS or with Congo red followed by mounting in 10% KOH. Alternatively, mounting in black Pelikan ink material previously rehydrated in 1% SDS usually provides a good contrast suitable for photomicrographs and allows a better observation of the paraphyses.

To summarize our observations on the characterization of stromata of *Hypoxylon* and to provide further differential characters, we suggest to routinely observe the stromatal granules in water under the microscope, to record the pigments released in 10% KOH immediately after the contact but also after 20–30 min incubation and to study the ascogenesis with the help of stains when water mounts do not give accurate images.

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