

Hypoxylon flavocreameum sp. nov. (Xylariaceae), a new species from Mayotte (France Outre-Mer), with notes on some other *Hypoxylon* spp. collected in this island

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Summary: During a survey of mycobiota in the island of Mayotte situated in the Comores archipelago, a remarkable *Hypoxylon* with unusual off-white to pale yellowish stromata was collected by one of us (MP). Besides its striking colour it is characterized by small orbicular effused-pulvinate stromata (< 6 mm diam), with conspicuous perithecial contours, papillate ostioles and subsurface with yellow waxy granules yielding orange brown pigments in 10% KOH. Upon microscopical observation, its asci appear devoid of an apical apparatus, thus not reacting with iodine, and its ascospores have a transversely striated perispore dehiscent in 10% KOH. This combination of characters is compared with the *Hypoxylon* spp. appearing morphologically the most closely related and this comparative study is summarized in a table. As this *Hypoxylon* features a set of characters deviating from known species, it is described as new, as *H. flavocreameum*. A culture derived from single spore isolation was obtained and described. The ex-type culture was deposited at the CBS Fungal Biodiversity Center (CBS, Utrecht, the Netherlands). All attempts to obtain the asexual state on various media remained unsuccessful. The few other species of *Hypoxylon* collected during this survey are listed and briefly commented upon. The rarely recorded *H. lividipigmentum* is illustrated in colour.

Keywords: Ascomycota, Comores archipelago, Indian Ocean, Mozambique channel, pyrenomycetes, saproxylic, taxonomy, Xylariales.

Résumé : au cours d'une étude des mycobiotes de l'île de Mayotte (archipel des Comores) un *Hypoxylon* remarquable par l'inhabituelle couleur blanchâtre à jaunâtre pâle des stromas a été récolté par l'un d'entre nous (MP). En plus de sa couleur particulière il est caractérisé par des stromas orbiculaires de petite taille (< 6 mm de diamètre), étalés-pulvins, à surface bosselée par les périthèces, des ostioles papillés et des granules jaunes sous la surface du stroma qui libèrent un pigment brun orange dans la potasse à 10%. L'observation microscopique révèle des asques dépourvus d'appareil apical et donc ne réagissant pas à l'iode et les ascospores ont une périspore striée transversalement, déhiscente dans la potasse à 10%. Cette combinaison de caractères est comparée aux espèces d'*Hypoxylon* qui apparaissent les plus proches morphologiquement et cette étude comparative est résumée dans un tableau. Comme cet *Hypoxylon* présente un ensemble de caractères morphologiques qui ne correspond à aucune autre espèce connue, il est décrit comme une espèce nouvelle, *H. flavocreameum*. Une culture obtenue à partir de l'isolation d'une seule spore a été observée et décrite, et la culture a été déposée au CBS Fungal Biodiversity Center (CBS, Utrecht, Pays-Bas). Le stade asexué n'a pas pu être obtenu malgré l'essai de milieux de culture variés. Les quelques autres espèces d'*Hypoxylon* récoltées pendant cette étude sont répertoriées et brièvement commentées. *Hypoxylon lividipigmentum*, une espèce rarement signalée, est illustré en couleur.

Mots-clés : Ascomycota, archipel des Comores, Océan Indien, canal du Mozambique, pyrénomycètes, saproxylique, taxinomie, Xylariales.

Introduction

Mayotte is one of the four major islands of the Comores archipelago. Mayotte itself comprises the main island "Grande Terre" and five smaller islets. The archipelago is located in the Mozambique channel to the north west of Madagascar. The islands are of volcanic origin, encircled by coral reefs and enjoy a tropical marine climate with high local variations depending on altitude and relief. Mayotte is culminating at 660 m and is globally warmer and drier than the other islands, with average temperatures between 21 and 28° C and annual rainfall varying from 900 mm to 2300 mm (D.A.A.F., 2012). There is no active volcano in Mayotte. Unlike the other islands it is under French administration. It underwent a rapid development of its population during the last twenty years, reaching a population density around 550 / km², resulting in degradation and decrease of the forest and disturbances of most of the biotopes (D.A.A.F., 2012). Moreover, many exotic invasive plants threaten the local flora which is well known due to the major work of VOELTSKOW (1917), completed by floristic inventories carried out in Mayotte in 1995-1997 (PASCAL, 2002). Aside from two surveys carried out in 2010 and 2013 by members of the Muséum national d'histoire naturelle (Paris), resulting in the publication of a new taxon (DUHEM & BUYCK, 2011), the mycobiota of Mayotte have not been further investigated.

In this context one of us (MP) undertook sampling on a large scale in various fungal groups since 2011, which resulted in a great deal of interesting records including rare or undescribed species (VAN VOOREN & PELISSIER, 2014; JEANNEROT & PELISSIER, in prep.).

We present here a recently collected and distinctive species of *Hypoxylon* Bull. that appears to be new to science based on morpho-

logical comparison with related tropical taxa. The six other species of *Hypoxylon* collected and identified during this survey are listed and briefly commented upon.

Material and methods

Methods of evaluation of morphological characters follow FOURNIER (2014), including colour charts. As the asci appeared to lack an apical apparatus the absence of reaction to iodine was controlled in Melzer's reagent and Lugol's solution, directly or after a pretreatment in 3% KOH.

Cultures were grown on Difco Oatmeal Agar supplemented by 5mg/l of streptomycin in Petri dishes 5 cm diam and incubated at 25°C. A mass of ascospores and asci was removed from a rehydrated perithecialium with a fine needle and placed in a drop of sterile water that was stirred with a sterile needle to distribute the elements on the slide. A part of the drop containing some ascospores was taken with a sterile micropipette and placed on the medium. After 24 h, a single germinating ascospore was transferred with a fine insect pin directly to the same medium.

Taxonomy

Hypoxylon flavocreameum J. Fourn., Péliissier & Lechat, sp. nov. — MB 809874. Figures 1-3

Holotype: Mayotte: Trevani, trail from Mayco to Trevani, below Kangani pass, ca. 100 m elev., S 12° 44' 34.0" E 45° 10' 59.5", corticated branch of *Mangifera indica*, 10 Mar. 2014, Maurice Péliissier, MP 2014-100 (duplicate CLL 14017) (LIP, ex-type culture CBS 138643).

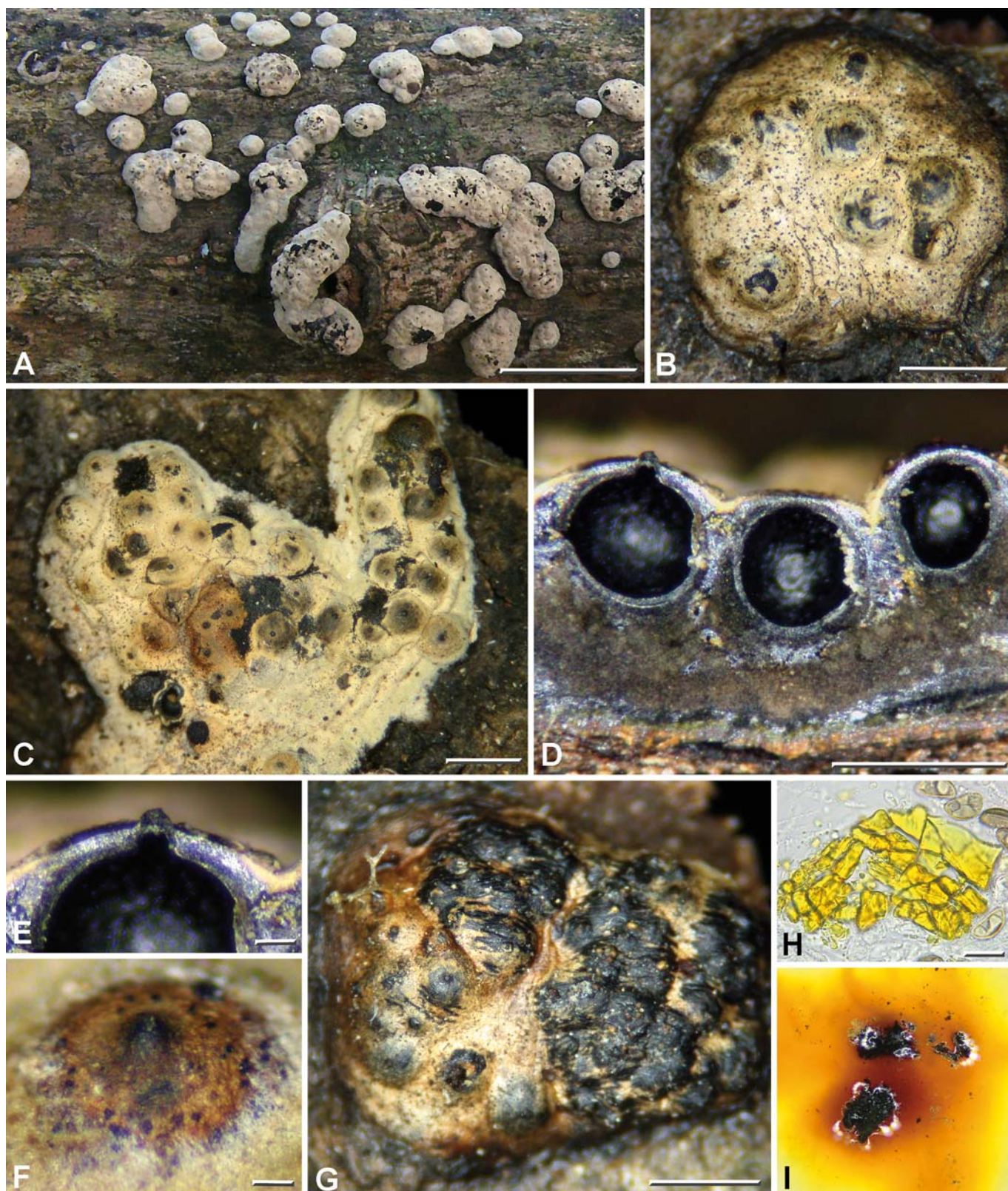


Fig. 1. — *Hypoxylon flavocremeum* holotype

A: Stromata in situ at fresh state; B, C: Mature stromata in dry condition; D: Vertical section of a stroma showing the perithecia, the yellow subsurface granules and the subperithecial sterile tissue; E, F: Papillate ostioles in close up; G: Overmature stroma; H: Waxy granules observed in water; I: KOH-extractable pigments after 1 mn incubation. Scale bars: A: 1 cm; B, C, G: 1 mm; D: 0.5 mm; E, F: 100 µm; H: 10 µm.

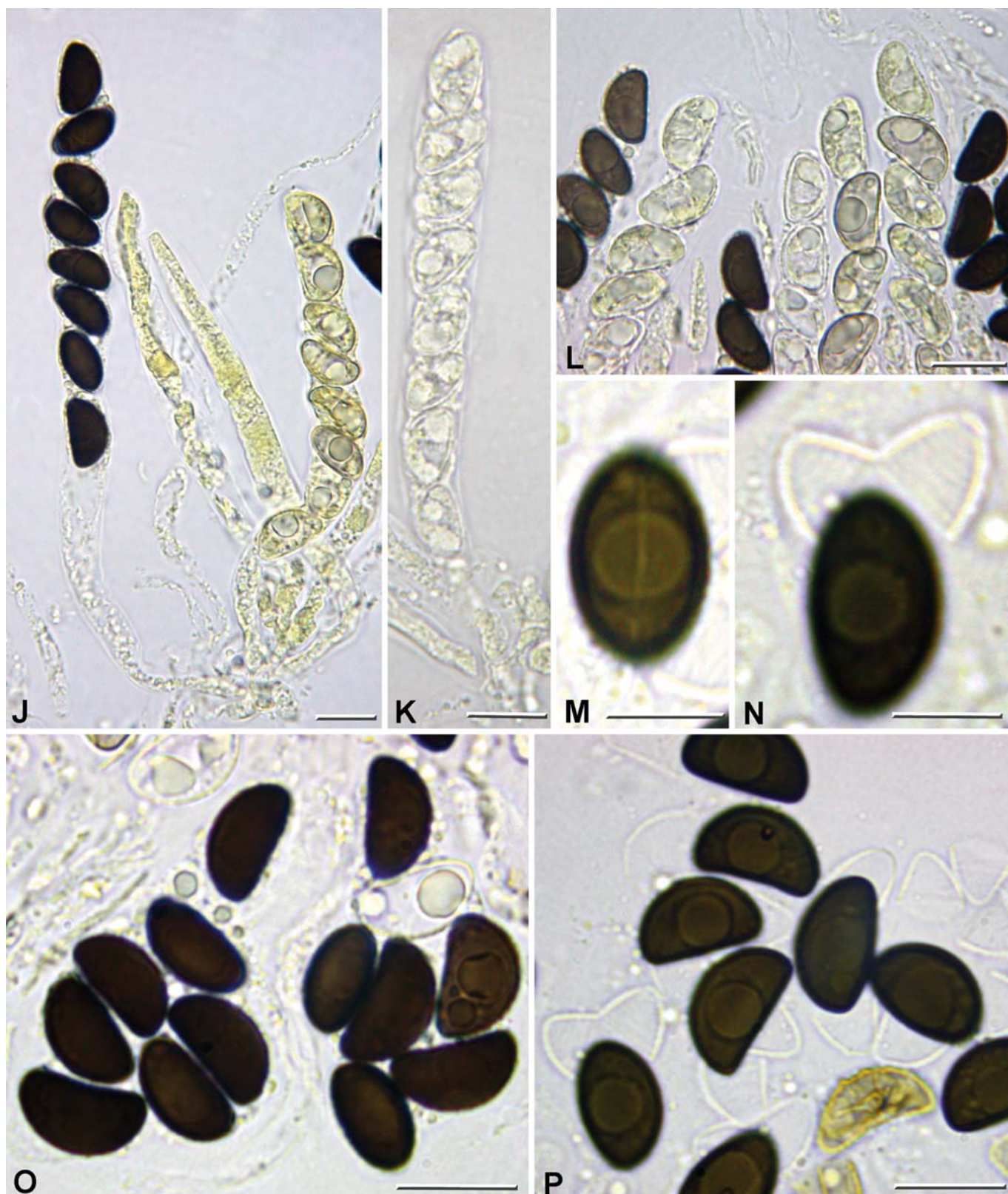


Fig. 2. — *Hypoxylon flavocrellum* holotype

J: Mature and immature asci in Lugol's solution; K, L: Mature and immature asci in Melzer's reagent showing no amyloid reaction; M: Ascospore in 10% KOH with focus on convex side showing the faint germ slit; N: Ascospore in 10% KOH with dehiscent ornamented perispore; O: Ascospores in water; P: Ascospores in 10% KOH showing dehiscent perispores. Scale bars: J-L, O, P: 10 µm; M, N: 5 µm.

Diagnosis: Differs from known species of *Hypoxylon* with dull white, yellowish or pale brown stromata by its small orbicular effused stromata with papillate ostioles, yellow stromatal waxy granules yielding sienna pigments in 10% KOH, asci devoid of apical apparatus and ascospores $9.5\text{--}10.9 \times 5.0\text{--}5.9\ \mu\text{m}$ with conspicuously transversely striated dehiscent perispores.

Etymology: The epithet *flavocremeum* (from Latin *flavus* = yellow and *cremum* = cream coloured) refers to its unusual pale yellowish colour.

Stromata effused-pulvinate, typically orbicular with a thinly effused fugacious margin, $2.8\text{--}6\ \text{mm}$ diam $\times$ $0.6\text{--}1\ \text{mm}$ thick, turning irregularly elongate when adjacent stromata become coalescent and then up to $10 \times 6\ \text{mm}$, with moderately to strongly exposed perithecial contours (A–C); surface dull white (oac900) at fresh state (A), turning pale buff (45) (oac805) upon drying (B), pruinose, fawn (87) (oac825) and eventually black when overmature (G), with subsurface layer dark orange brown appearing first around the ostioles and forming darker spots (B, C), composed of amorphous waxy granules that appear yellow when bruised and when observed in water (H) and extend downwards between the perithecial walls (D), yielding sienna (8) (oac706) pigments in 10% KOH (I) with a yellow halo, not changing colour after 30 min of incubation; subperithecial tissue $0.1\text{--}0.5\ \text{mm}$ thick, greyish brown, pithy, lined by a thin black basal layer (D). **Perithecia** spherical, $0.45\text{--}0.5\ \text{mm}$ diam (D). **Ostioles** appearing obscure or umbilicate on young stromata but turning out to be bluntly papillate, black, as the pruina wears off (E, F).

Asexual state on the natural substrate not seen.

Asci cylindrical, fragile, with eight obliquely uniseriate ascospores, $90\text{--}110\ \mu\text{m}$ total length, the spore-bearing parts $60\text{--}72\ \mu\text{m}$ long $\times$ $7\text{--}9\ \mu\text{m}$ broad, the stipes $18\text{--}45\ \mu\text{m}$ long, apical apparatus absent, not bluing in Melzer's reagent nor in Lugol's solution (J–L). Paraphyses sparse, filiform, minutely guttulate. **Ascospores** (8.8) $9.5\text{--}10.9$ (11.5) $\times$ (4.8) $5.0\text{--}5.9$ (6.1) μm , $Q = (1.6)$ $1.7\text{--}2.1$ (2.3); $N = 60$ ($Me = 10.2 \times 5.4\ \mu\text{m}$; $Qe = 1.9$), ellipsoid strongly inequilateral with most often broadly rounded ends (O), at times slightly ventrally concave, dark brown, with a faint germ slit spore-length on the convex side, straight, parallel to the sides (M); perispore dehiscent in 10% KOH (P), transversely striated (N); epispore smooth.

Culture (Fig. 3): After two weeks at 25°C on OA, colony $2\text{--}2.5\ \text{cm}$ diam, greyish in the centre, white at margin, diffusing a brownish coloration in the medium. Reverse brown in the centre, buff at margin. After four weeks colony reaching the edges of the Petri dish, white to pale greyish, becoming pale brownish in the centre with pale brown droplets. Reverse dark brown to nearly black. No con-

diogenous structures observed after up to two months of cultivation.

Other specimens examined: Mayotte: Dzoumogné, vicinity of the hospital on the way to the lake, S $12^\circ 42' 56.8''$ E $45^\circ 06' 24.0''$, corticated branch, 23 Feb. 2014, Maurice Pélissier, MP 2014-081 (LIP, paratype); Vanilla trail from Coconi to Chiconi, S $12^\circ 50' 13.7''$ E $45^\circ 07' 15.9''$, corticated branch of presumably *Mangifera indica*, 13 Apr. 2014, Maurice Pélissier, MP 2014-142 (LIP, paratype).

Discussion: *Hypoxylon flavocremeum* is characterized by the combination of small orbicular pulvinate stromata with strongly exposed perithecial contours and a dull white to yellowish cream surface, yellow waxy subsurface granules yielding brownish orange pigments in KOH and papillate ostioles. Its most distinctive microscopic characters are the asci lacking an apical apparatus and thus without amyloid reaction in iodine and its inequilateral ascospores with a faint straight germ slit spore-length and a fairly conspicuously ornamented dehiscent perispore.

As its pale stromatal colour is the most striking character it must be first compared with *Hypoxylon subalbum* J.D. Rogers, Y.-M. Ju & Hsieh (Ju *et al.*, 2004), a species from Costa Rica that features dull white pulvinate stromata and ascospores $11\text{--}12 \times 6\text{--}6.5\ \mu\text{m}$ with a strongly ornamented perispore. *Hypoxylon subalbum* differs from *H. flavocremeum* in having a black ostiolar region with an umbilicate ostiole, in lacking coloured stromatal granules and KOH-extractable pigments and by its ascospores having a short germ slit. It is unknown whether an ascial apical apparatus is present since asci of *H. subalbum* were not documented in the original description.

Known *Hypoxylon* spp. that roughly resemble *H. flavocremeum* by small glomerate or pulvinate stromata with pale brown surface, KOH-extractable pigments in shades of orange, asci lacking an apical apparatus and ascospores with striated dehiscent perispores are *H. addis* J. Fourn., M. Stadler & U. Lindem., *H. gibriacense* J. Fourn., M. Stadler & Gardiennet (FOURNIER *et al.*, 2010), *H. notatum* Berk. & M.A. Curtis, *H. shearii* Y.-M. Ju & J.D. Rogers (Ju & ROGERS, 1996) and its variety *minor* San Martín, Y.-M. Ju & J.D. Rogers (SAN MARTÍN *et al.*, 1999). They differ from *H. flavocremeum* in having umbilicate ostioles that are surrounded by a wide flattened disc except in *H. notatum* where this disc is lacking. Moreover their ascospore size range is over that of *H. flavocremeum*, with the exception of *H. shearii* var. *minor* that is smaller (Table 1).

Hypoxylon ulmophilum Lar. N. Vasilyeva (VASILYEVA, 1998) is a poorly documented taxon from Far East Russia that should be considered too since it features greyish sepia glomerate stromata and asci devoid of apical apparatus. Ju *et al.* (2004) who revised the holotype reported that it was lacking asci and referred it to *H. notatum*, a

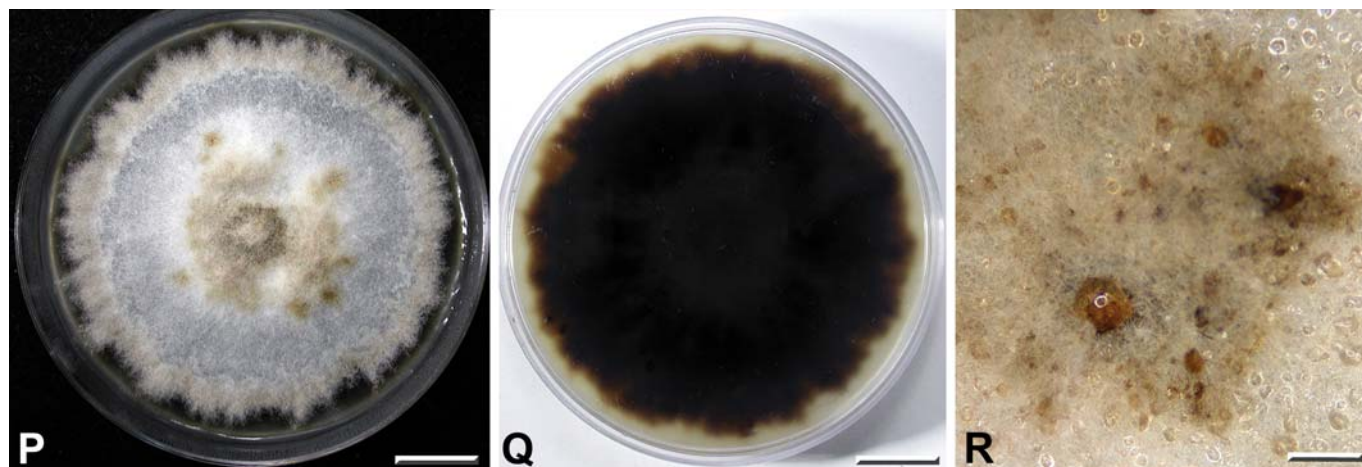


Fig. 3. — *Hypoxylon flavocremeum* ex type culture CBS 138643

Culture on Difco Oatmeal agar after 4 weeks on 5 cm Petri dish. P: Surface; Q: Reverse; R: Close up on darker central spots showing pale brown sterile mycelium bearing droplets. Scale bars: P, Q: 1 cm; R: 1 mm.

taxon that can be readily distinguished from *H. flavocreumeum* as seen above.

The pantropical *H. erythrostroma* J.H. Miller resembles *H. flavocreumeum* by its greyish sepia, effused-pulvinate, often orbicular stromata with sometimes finely papillate ostioles. However it differs in having red stromatal granules yielding orange red pigments in 10% KOH and smaller ascospores with a sigmoid germ slit.

Another pantropical taxon to consider is *H. lenormandii* Berk. & M.A. Curtis that frequently features greyish sepia, rosellinioid stromata and sienna KOH-extractable pigments. It differs mainly in having umbilicate ostioles, asci with an amyloid apical apparatus and larger ascospores with a sigmoid germ slit and a faintly ornamented perispore.

Hypoxylon flavoargillaceum J.H. Miller, known from Colombia and Venezuela, roughly resembles *H. flavocreumeum* by its fawn or hazel glomerate to pulvinate stromata but deviates by yellow to amber KOH-extractable pigments, umbilicate ostioles, asci possessing an

amyloid apical apparatus and larger ascospores with an often slightly sigmoid germ slit (JU & ROGERS, 1996).

Lastly, two species of *Hypoxylon* that feature small glomerate to pulvinate stromata with a pale surface and exposed perithecial contours should be compared with *H. flavocreumeum*. *Hypoxylon musceum* J.D. Rogers (ROGERS, 1981), pantropical, differs mainly in its olivaceous stromatal surface, umbilicate ostioles, asci with amyloid apical apparatus and ascospores with a short and sigmoid or oblique germ slit. *Hypoxylon isabellinum* J. Fourn., Kuhnert & M. Stadler (KUHNERT *et al.*, 2014), known from Martinique, is different from *H. flavocreumeum* by having dull yellowish subsurface granules yielding pale isabelline pigments in 10% KOH and by having umbilicate ostioles, asci with an amyloid apical apparatus and ascospores with a more strongly ornamented perispore.

The differential teleomorphic characters among *Hypoxylon* spp. with pale brown glomerate stromata including *H. flavocreumeum* are summarized in Table 1.

	Stromatal shape	Surface colour	KOH	Perithecia mm diam	Ostioles	Apical apparatus	Ascospores µm	Germ slit	Perispore
<i>H. flavocreumeum</i>	effused pulvinate, orbicular	dull white to pale buff	sienna	0.45–0.5 contours exposed	papillate	absent I-	9.5–10.9 × 5.0–5.9	long, straight, faint	striated +
<i>H. addis</i>	glomerate	vinaceous buff to dark brick	luteous to orange	0.5–0.55 contours exposed	umbilicate discoid	absent I-	13–16.5 × 6–7.7	long, straight	faintly striated
<i>H. erythrostroma</i>	pulvinate, often orbicular	greyish sepia	orange red	0.2–0.4 contours faintly exposed	flush with surface or slightly papillate	I+	7.5–9.5 × 3–4.5	long sigmoid	striated ++
<i>H. flavoargillaceum</i>	glomerate to pulvinate	fawn or hazel	yellow, amber	0.3–0.4 contours exposed or not	umbilicate	I+	11–16 × 6–7	long, straight to slightly sigmoid	faintly striated
<i>H. gibriacense</i>	glomerate	greyish sepia	amber to sienna	0.4–0.5 contours exposed	umbilicate discoid	absent I-	11.5–13 × 6–6.8	long, straight	striated +
<i>H. isabellinum</i>	glomerate	hazel	pale isabelline	0.3–0.4 contours exposed	umbilicate	I +	9.5–12 × 5–6	long, straight, faint	striated ++
<i>H. lenormandii</i>	glomerate to effused pulvinate or rosellinioid	greyish sepia to brown vinaceous	sienna	0.3–0.5, often rosellinioid	umbilicate	I+	9.5–15 × 4–6.5	long, slightly sigmoid	faintly striated
<i>H. musceum</i>	glomerate	olivaceous	yellow to orange	0.2–0.4 contours exposed	umbilicate	I +	9–13 × 4.5–6	short, sigmoid to oblique	faintly striated
<i>H. notatum</i>	glomerate to pulvinate	sepia to brown vinaceous	yellow	0.3–0.5 contours exposed	umbilicate	absent I-	12–16 × 6–7.5	long straight	faintly striated
<i>H. shearii</i>	glomerate to pulvinate	buff to fawn	luteous	0.4–0.7 contours exposed	umbilicate discoid	absent I-	12–14 × 5.5–6.5	long straight	striated ++
<i>H. shearii</i> var. <i>minor</i>	glomerate to pulvinate	buff to fawn	luteous	0.4–0.7 contours exposed	umbilicate discoid	absent I-	7–8 × 3.5–4	long straight	striated ++
<i>H. subalbum</i>	pulvinate	dull white	absent	0.5 contours barely exposed	umbilicate	nd	11–12 × 6–6.5	short straight	striated ++
<i>H. ulmophilum</i>	glomerate	buff to greyish sepia	orange	0.2–0.4 contours exposed	umbilicate	I+	nd	nd	nd

Table 1. — Synoptic table summarizing the differential characters among *Hypoxylon* spp. morphologically resembling *H. flavocreumeum*. I+ or I- refer to the reaction of the apical apparatus to iodine, respectively bluing or not bluing. nd is the abbreviation for not documented.

Further species of *Hypoxylon* recorded in Mayotte Island

H. erythrostroma J.H. Miller

Foot of Choungi Mt., S 12° 57' 29.9" E 45° 07' 47.3", corticated branchlet, 8 May 2014, Maurice Pélissier, MP 2014-161.

H. fendleri Berk. ex Cooke

Kangani, brook downstream the dam, S 12° 46' 26.4" E 45° 08' 41.8" dead blackened wood, 16 Jun. 2011, Maurice Pélissier, MP 2011-73 ; Benara Mt. crest, S 12° 51' 56.8" E 45° 09' 20.8", dead wood, 22 Mar. 2013, Maurice Pélissier, MP 2013-111 ; Koungou, downstream Kan-

gani brook, S 12° 44' 48.0" E 45° 11' 21.3", dead blackened wood, 22 Mar. 2014, Maurice Pélissier, MP 2014-125.

H. haematostroma Mont.

Coconi, road to Ongojou, S 12° 50' 15.73" E 45° 09' 02.69", dead wood, 25 Jun. 2011, Maurice Pélissier, MP 2011-61 ; Ngouja, S 12° 57' 47.67" E 45° 05' 14.15", dead corticated branch of *Crescendia cujele*, 15 Apr. 2012, Maurice Pélissier, MP 2012-142 ; Moutsamoudou, trail around Saziley, S 12° 58' 19.4" E 45° 10' 44.5", dead branch of *Tamarindus indica*, 1 Sept. 2012, Maurice Pélissier, MP 2012-235.

H. lenormandii Berk. & M.A. Curtis

Coconi, banks of the brook, S 12° 49' 52.2" E 45° 08' 09.2", dead wood, 31 Mar. 2012, Maurice Pélissier, MP 2012-96.

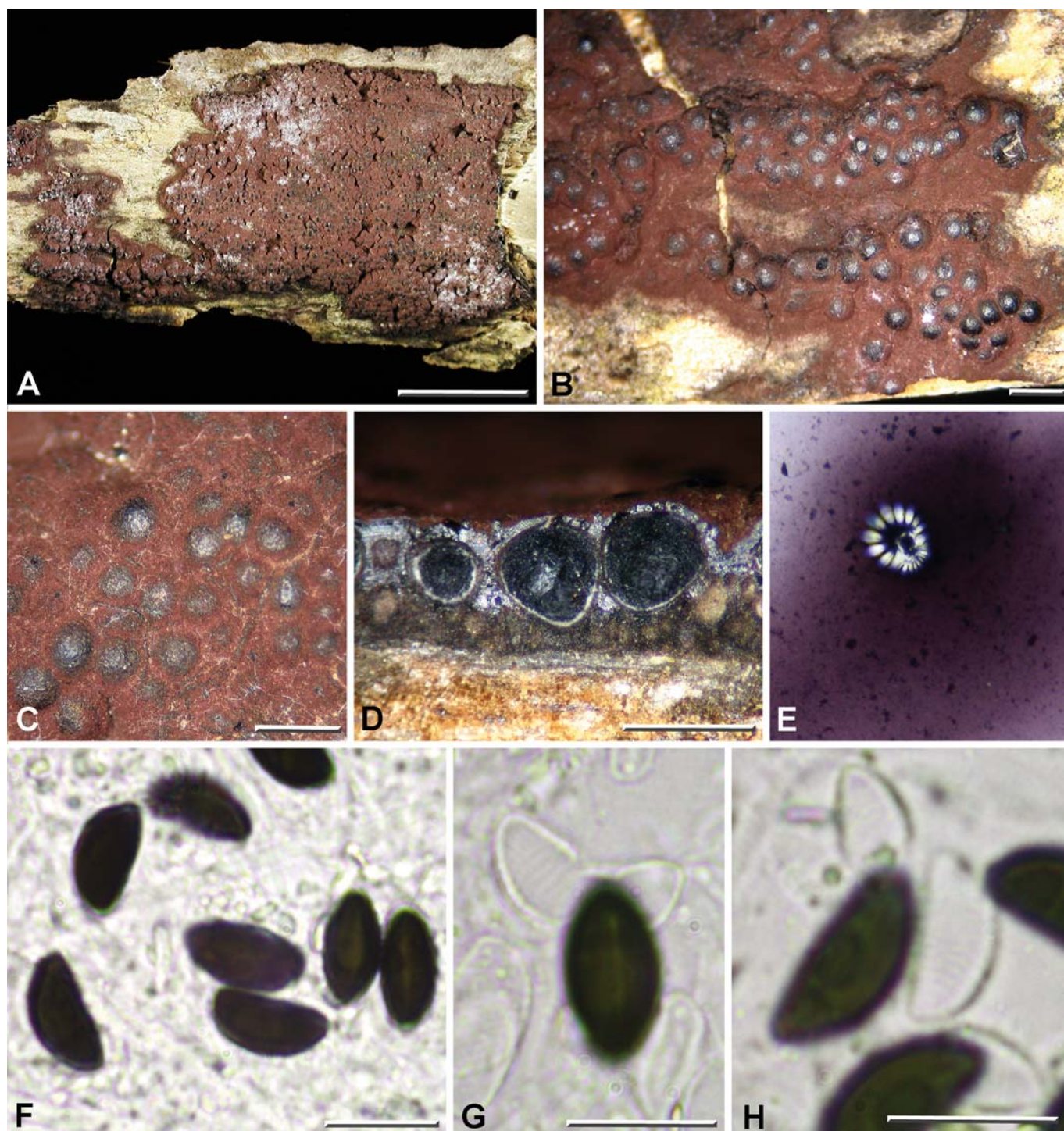


Fig. 4. — *Hypoxylon lividipigmentum* MP 2011-70

A : Stroma ; B, C : Stromatal surface in close-up to show the perithecial contours ; D : Stroma in vertical section showing the perithecia under the waxy crust ; E : KOH-extractable pigments ; F : Ascospores in water ; G, H : Ascospores in 10% KOH showing the striated dehiscence of the perispores and partially the germ slit (G). Scale bars : A : 1 cm ; B, C : 1 mm ; D : 0.5 mm ; F-H : 10 μ m.

H. lividipigmentum San Martín, Y.-M. Ju & J.D. Rogers
Kangani, upstream the brook, S 12° 44' 29.31" E 45° 11' 30.09", corticated branch, 5 Jul. 2011, Maurice Péliissier, MP 2011-70.

H. monticulosum Mont.
Kangani, Kangani brook, S 12° 44' 34.50" E 45° 11' 26.54", corticated wood, 3 Jul. 2012, Maurice Péliissier, MP 2012-200.

Collections of *H. erythrostroma*, *H. fendleri*, *H. haematostroma*, *H. lenormandii* and *H. monticulosum* were typical and conformed to the species concepts delineated by Ju & ROGERS (1996). These five common species have a well-documented pantropical distribution but have not been reported from this region of the world yet.

In contrast to the above species, *H. lividipigmentum* is a rarely recorded taxon so far reported from Mexico and Venezuela (Ju & ROGERS, 1996) and from Panama (CARMONA *et al.*, 2009) only. By extending its distribution to the West Indian Ocean, the present record shows that it is not restricted to neotropics. *Hypoxylon lividipigmentum* (Fig. 4) is characterized by reddish brown thinly effused stromata with spherical perithecia and reddish brown stromatal granules yielding dense purple pigments in 10% KOH. Only a small number of *Hypoxylon* spp. feature such dense purple pigments (Ju & ROGERS, 1996; Ju *et al.*, 2004). Microscopically, its ascospores are dark brown, inequilateral, 10–13.5 × 4.5–6 µm, with a straight germ slit spore-length and transversely striated perispores dehiscing in 10% KOH (Ju & ROGERS, 1996). Ascospores in the collection from Mayotte are 9.5–11 × 4.5–5.1 µm (Me = 10.3 × 4.8 µm, N = 36), i.e. in the lower range of dimensions given by Ju & ROGERS (1996) for this taxon but all other characters conform well with *H. lividipigmentum*.

Additional material of several *Hypoxylon* spp. was collected but unfortunately either immature and sterile or overmature and depauperate and consequently could not be identified. This preliminary survey introducing a new and a rare species of *Hypoxylon* from Mayotte, along with further unpublished data on *Annulohypoxylon* Y.-M. Ju, J.D. Rogers & H.-M. Hsieh, *Camillea* Fr., *Daldinia* Ces. & De Not., *Kretzschmaria* Fr., *Rosellinia* De Not., *Whalleya* J.D. Rogers, Y.-M. Ju, & San Martín and *Xylaria* Hill ex Schrank suggests that more extensive studies should reveal a rich diversity of xylariaceous fungi in this part of the world.

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