Two new species of Hypoxylon (Xylariaceae) from French Central Pyrénées occurring on Fagus sylvatica

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Two new species of *Hypoxylon* (*Xylariaceae*) from French Central Pyrenees occurring on *Fagus sylvatica*

Jacques FOURNIER

Ascomycete.org, 6 (3) : 53-60.
Juin 2014
Mise en ligne le xx/06/2014

Summary: In the course of an extensive survey of *Xylariaceae* in the French Central Pyrenees over the last fifteen years two undescribed species of *Hypoxylon* were recently discovered, both on *Fagus sylvatica*. Based on the combination of remarkable ecological and morphological characters that does not match known taxa, both species are recognized as new to science. *Hypoxylon cazenavei* is distinctive in having brown vinaceous effused-pulvinate stromata turning fuscous, with tubular to lanceolate perithecia and yellow brown KOH-extractable pigments. *Hypoxylon invadens* is diagnosed on its occurrence on aborted primordia of *H. fragiforme*, its fulvous stromata soon turning shiny black with strongly exposed perithecial contours, slightly carbonaceous subsurface and yellow brown KOH-extractable pigments.
Keywords: Ariège, Ascomycota, fungicolous, Hautes Pyrénées, *Hypoxylon fragiforme*, pyrenomycetes, saproxylic, taxonomy, *Xylariales*.

Résumé : au cours d'une étude approfondie des *Xylariaceae* présentes dans les Pyrénées centrales françaises entreprise depuis une quinzaine d'années, deux espèces d'*Hypoxylon* non décrites ont été récemment découvertes sur *Fagus sylvatica*. En se fondant sur les caractères écologiques et morphologiques remarquables dont la combinaison ne correspond à aucune espèce connue, les deux espèces sont reconnues comme nouvelles pour la science. *Hypoxylon cazenavei* est reconnaissable à ses stromas brun vineux étalés à pulvinés qui deviennent noirâtres, dont les périthèces sont tubulaires à lancéolés et qui libèrent un pigment jaune brunâtre dans la potasse à 10 %. Les caractères distinctifs d'*Hypoxylon invadens* sont sa présence sur des primordia avortés d'*Hypoxylon fragiforme*, ses stromas de couleur fauve aux contours bosselés évoluant rapidement vers un noir brillant, la texture légèrement carbonacée de la surface des stromas et le pigment de couleur jaune brunâtre qu'ils libèrent dans la potasse à 10 %.
Mots-clés : Ariège, Ascomycota, fongicole, Hautes Pyrénées, *Hypoxylon fragiforme*, pyrénomycètes, saproxylique, taxinomie, *Xylariales*.

Introduction

According to its modern and emended concept (Ju & Rogers, 1996; Hsieh *et al.*, 2005) the genus *Hypoxylon* Bull. is restricted to xylariaceous Ascomycota that have hemispherical to effused stromata with a coloured surface at least in early stages of development, that possess ostiolate perithecia lying under a crust of coloured waxy granules releasing pigments in 10% KOH, a solid and homogeneous dark-coloured tissue beneath the perithecia, ascospores with perispores usually dehiscent in 10% KOH and germ slit on the convex side when ascospores are inequilateral, and are associated with a nodulisporium-like asexual state. The most closely related genera are *Daldinia* Ces. & De Not. from which *Hypoxylon* differs mainly in lacking concentrically arranged internal layers and *Annulohypoxylon* Y.-M. Ju, J. D. Rogers & H.-M. Hsieh which differs in having often carbonaceous stromata with papillate ostioles encircled by a disc and in having perispores that, when dehiscent, possess a thickening on the same side as the germ slit. Though this delimitation practically works well, a few troublesome exceptions exist, especially in tropical taxa and even phylogenetic studies do not always clearly support the delimitation of these three genera (Sánchez-Ballesteros *et al.*, 2000; Hsieh *et al.*, 2005; Triebel *et al*., 2005; Peláez *et al*., 2008; Kuhnert *et al.*, 2014; Stadler *et al.*, 2014).

Since the masterful world monograph of *Hypoxylon* published by Ju & Rogers (1996) that gave a strong impetus to the study of this genus worldwide, the number of species reported from Europe increased notably by the addition of three new species from Norway *H. liviae* Granmo (Granmo, 1999), *H. porphyreum* Granmo and *H. salicicola* Granmo (Granmo, 2001), the record of North American species newly reported from Europe including *H. crocopeplum* Berk. & M. A. Curtis, *H. submonticulosum* Y.-M. Ju & J. D. Rogers (Fournier & Magni, 2002), *H. fuscopurpureum* (Schwein. : Fr.) Curtis (Mühlbauer *et al.*, 2002) and the tropical species *H. carneum* Petch (Fournier & Magni, 2002; Quang *et al.*, 2006), followed by the addition of *H. petriniae* M. Stadler & J. Fourn. (Stadler *et al.*, 2004), a new species segregated from the *H. rubiginosum* (Pers. : Fr.) Fr. complex. More recently, three further European species were proposed as new to science, i.e. *H. fuscoides* J. Fourn., P. Leroy, M. Stadler & Roy Anderson, *H. lusitanicum* J. Fourn., M. Stadler & Priou and *H. gibriacense* J. Fourn., M. Stadler & Gardiennet (Fournier *et al.*, 2010). The two species from Canary Islands described by Stadler *et al.* (2008) are not taken into account since these islands cannot be regarded geographically as European. After moving the species formerly placed in section *Annulata s.* Ju & Rogers (1996) to *Annulohypoxylon* (Hsieh *et al.*, 2005) and adding the newly described taxa the number of *Hypoxylon* spp. recorded from Europe so far is 28, out of which 21 species are known from France, mostly on the northern slopes of the Pyrenees under Atlantic ocean influence, ranging from the Basque Country to Ariège.

As it is usually the case in most fungal genera, the genus *Hypoxylon* is overrepresented by a few widespread species that are well known but may encompass cryptic species like in the *H. fuscum* or *H. rubiginosum* complexes. Besides these common and well known species, most species of *Hypoxylon* are rarely recorded or even not yet recognized as distinct species by many mycologists. The main reasons for which many species of *Hypoxylon* are overlooked are the small size and dull colours of stromata, the resemblance with common species, a strict host specificity for rare hosts but often specific ecological requirements that are rarely met or occur in micro-habitats that are not often explored by mycologists. The two new taxa described and discussed herein give a good example of the potential richness of a fungal genus when extensive field work is carried out in favourable environments. The number of *Hypoxylon* spp. in Europe is thus now raised up to 30.

Material and methods

The observations were carried out on dry material. 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.

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 minute and after a further incubation time of 20-30 min. To be consistent with previous works on *Hypoxylon* 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 OAC colour chart (http://www.onlineauctioncolorchart.com/) which is easily available at a very affordable price, can be consulted online and offers a much wider palette of nuances (as oacxxx).

Cultures were carried out at room temperature and daylight illumination on yeast malt glucose (YMG) agar supplemented by antibiotics and Difco Oatmeal agar (OA) plates kindly provided by Dr. M. Stadler (Intermed Discovery, Dortmund). Microscopic observation of the asexual state structures was performed in 1% SDS.

Photomacrographs 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 University of Lille (LIP) and duplicates are kept in the personal herbarium of JF.

Taxonomy

Hypoxylon cazenavei J. Fourn., *sp. nov.* — MB808737.
Figures 1-2

Holotype: France, Ariège, Rimont, Grand Bois, Combe Fourcade, 42°57′45.64″ N, 1°17′38.04″ E, mixed beech forest, ca. 700 m asl, 27 Sept. 2011, on bark of a rotting trunk of *Fagus sylvatica*, associated with old weathered stromata of *Annulohypoxylon cohaerens*, *leg*. J. Fournier, JF 11128 (LIP).

Diagnosis: Differs from known temperate species of *Hypoxylon* with purplish brown stromata by its long tubular to lanceolate perithecia and orange yellow stromatal waxy granules yielding cinnamon pigments in 10% KOH.

Etymology: For Robert Cazenave (Séméac), the French mycologist who collected this taxon first.

Stromata corticolous, erumpent through the periderm, widely effused-pulvinate with rounded to sinuous margins (A-C), at times orbicular (D), 0.5–65 mm long × 0.4–25 mm wide × 0.9–1.3 mm thick, with barely exposed perithecial contours, or pulvinate (E, F), 1–7 mm diam ×1.5–1.8 mm thick, with most often more strongly exposed perithecial contours (G), rarely on wood (C); surface pruinose, brown vinaceous (84) (oac635) to dark vinaceous grey (116) (oac525), turning fuscous black (104) to black when overmature; waxy granules forming a thin orange yellow crust beneath the surface (I-L), appearing honey (64) (oac812) in water (M), yielding ochreous (44) (oac715) to cinnamon (62) (oac714) pigments in 10% KOH (N), turning vinaceous (57) (oac615) after 20–30 mn of incubation (O); subperithecial tissue blackish, woody, homogeneous, 0.2–1 mm thick. **Perithecia** tubular to lanceolate, 0.7–1 mm high × 0.15–0.2 mm diam. **Ostioles** typically umbilicate (G), rarely appearing obscurely papillate in older stromata (H).

Asci cylindrical (P, Q), with eight obliquely uniseriate ascospores, 140–190 µm total length, the spore-bearing parts 60–68 µm long × 6.5–7.5 µm wide, the stipes 70–130 µm long, originating from long ascogenous hyphae in unilateral spicate arrangement (S), with apical apparatus discoid to slightly wedge-shaped, 0.6–0.8 µm high, 2.2–2.5 µm wide, bluing in Melzer's reagent (T). **Paraphyses** copious, up to 6 µm wide, with conspicuous refractive content (R). **Ascospores** (7.8) 8.3–9.9 (10.5) × (4.0) 4.1–4.8 (4.9) µm, Q = (1.6) 1.8–2.3 (2.5); N = 60 (Me = 9.1 × 4.4 µm; Qe = 2.1), ellipsoid-inequilateral with narrowly to broadly rounded ends, at times slightly ventrally concave, dark brown (U), with a narrow straight germ slit sporelength on the convex side (W, X); some asci feature significantly larger ascospores up to 13–14 × 6–7 µm that were not taken into account for measurements; perispore dehiscent in 10% KOH, fairly thick, smooth by L. M. (V); epispore smooth.

Asexual state on natural substrate not seen. **Culture** attempt on YMG agar plates was unsuccessful.

Other specimens examined (paratypes): FRANCE: Ariège, Rimont, Grand Bois, Combe Fourcade, 42°57′45.64″ N, 1°17′38.04″ E, mixed beech forest, ca. 700 m asl, 20 Oct. 2012, on bark of a rotting trunk of *Fagus sylvatica*, *leg.* J. Fournier, JF 12111 (LIP); Rimont, Grand Bois, Maury, 42°57′48.01″ N, 1°17′53.23″ E, mixed beech forest, ca. 750 m asl, 20 Sept. 2013, on bark of a rotting trunk of *Fagus sylvatica*, *leg*. J. Fournier, JF 13175 (LIP); Rimont, banks of Maury brook next to Pladellac, 560 m asl, 42°58′28.56″ N, 1°17′29.93″ E, mixed riparian beech forest, 21 Mar. 2014, on branches of *Fagus sylvatica*, associated with or growing over weathered stromata of *Annulohypoxylon cohaerens*, *Diatrype decorticata* and *Hypoxylon fragiforme*, *leg*. J. Fournier, JF 14014 (overmature) (LIP); Hautes Pyrénées, Castillon, Le Luz, 380 m asl, 43° 5′56.69″ N, 0°13′4.41″ E, beech forest, on corticated branchlets of *Fagus sylvatica*, 1.5–2 cm diam., 9 Apr. 2011, *leg*. Robert Cazenave, JF 11027 (overmature) (LIP).

Discussion: *Hypoxylon cazenavei* is recognized as a new taxon among the known temperate species by the combination of its purplish brown stromata with orange yellow waxy granules yielding brownish yellow pigments in 10% KOH and long tubular to lanceolate perithecia. The latter character is distinctive since it is only encountered in *H. macrocarpum* Pouzar, a taxon that mainly deviates from *H. cazenavei* in its greyish brown KOH-extractable pigments, more narrowly ellipsoid ascospores (Q averaging 2.2–2.6) and the strong aromatic odour of the colonized substrate (Pouzar, 1978).

Other comparable temperate species with purplish brown stromata and yellowish to olivaceous KOH-extractable pigments are those of the *H. fuscum* (Pers. : Fr.) Fr. complex including *H. porphyreum* Granmo. They can be readily distinguished from *H. cazenavei* by their significantly smaller subglobose perithecia and the sigmoid germ slits of their ascospores. Based on its purplish brown effused stromata and olivaceous to greenish grey KOH-extractable pigments, *H. fuscopurpureum*, known from North America and from Germany, may be likewise considered but it clearly deviates by its larger piriform ascospores with perispore indehiscent in 10% KOH.

If the comparative study of *H. cazenavei* is extended to tropical species, it appears to have similarities with *H. anthochroum* Berk. & Broome as broadly circumscribed by Ju & Rogers (1996) and *H. trugodes* Berk. & Broome. Examination of the type specimen of *H. anthochroum* from Sri Lanka (Stadler *et al.*, 2008) revealed that its stromata possess small subglobose perithecia and reddish brown waxy granules yielding greenish grey pigments in 10% KOH and that its ascospores have a conspicuously striated perispore, all characters differing from those encountered in *H. cazenavei*.

Interestingly the resemblance with the pantropical *H. trugodes* is more striking since this species likewise features long tubular perithecia associated with yellow waxy granules and brownish yellow KOH-extractable pigments and its ascospores are in the same size range, with a smooth perispore. However the stromata of *H. trugodes* are more purplish, the waxy granules are not restricted to a thin crust above the perithecia but are distributed all around the perithecia and even more densely around their base and the pig-

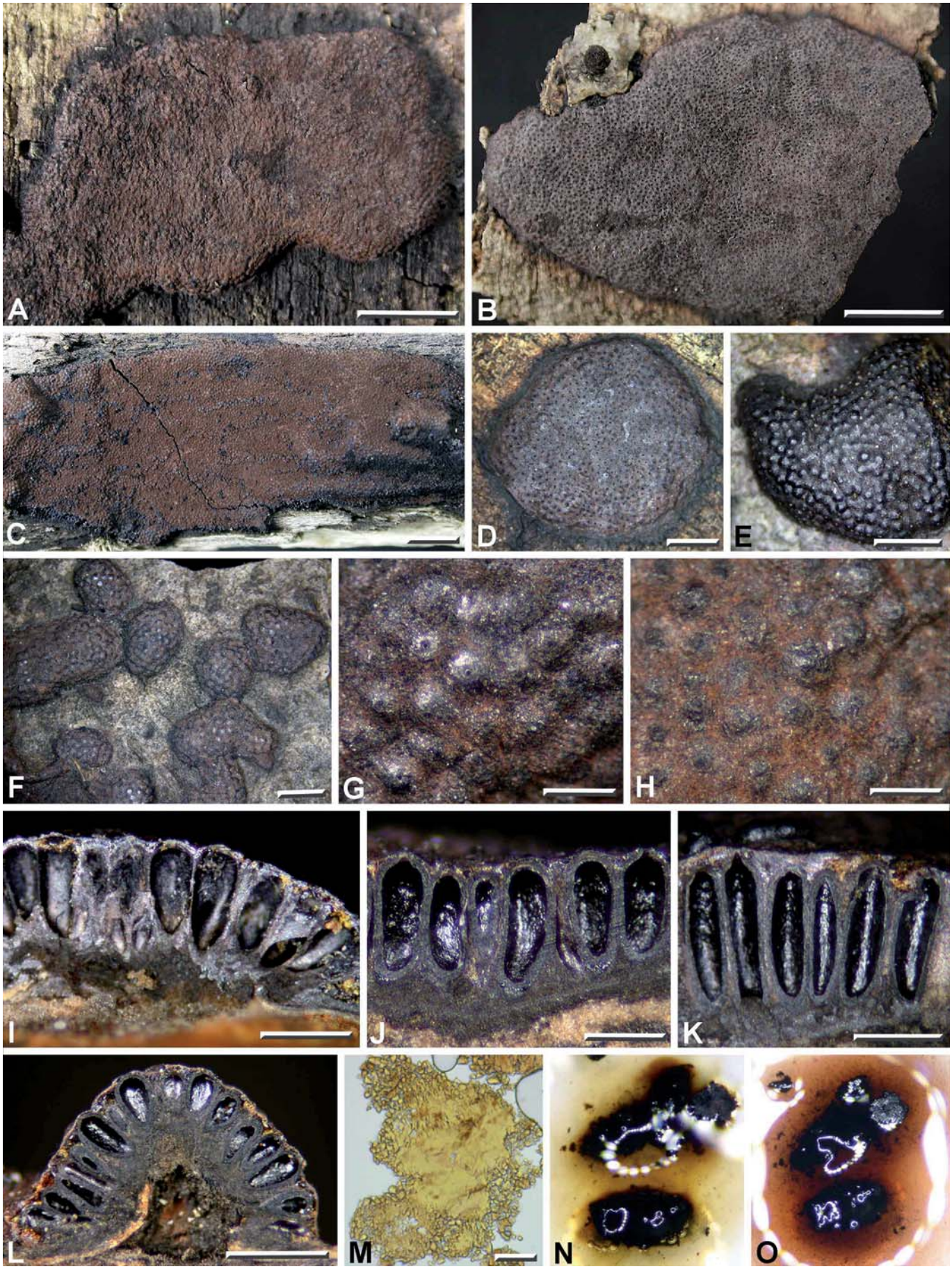

Fig. 1. – *Hypoxylon cazenavei*. A, F, G, J, L-N: Holotype JF 11128; B, D, K: JF 13175; C, H: JF 12111; E, I: JF 11027. A, B, D, F: Mature stromata on bark; C: Mature stroma on wood; E: Overmature stroma; G, H: Stromatal surface in close up with perithecial contours and ostioles; I-L: Stromata in vertical section showing the perithecia and the superficial layer of orange yellow waxy granules; M: Stromatal granules observed in water; N: KOH-extractable pigments after 1 min of incubation; O: KOH-extractable pigments after 30 min of incubation.
Scale bars: A-C: 5 mm; D-F: 2 mm; G, H, J, K: 0.5 mm; I, L: 1 mm; M: 50 µm.

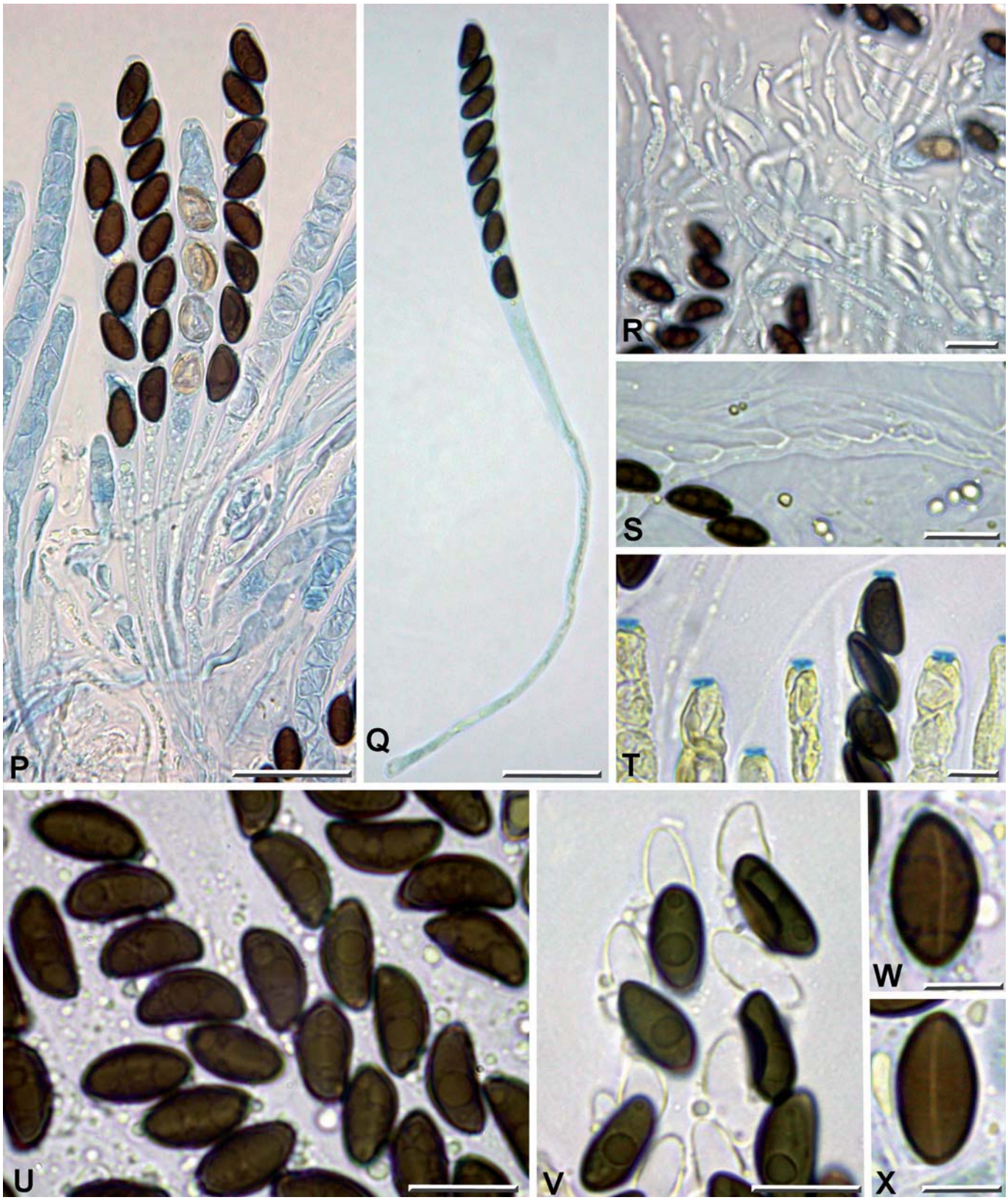

Fig. 2. – *Hypoxylon cazenavei*. Holotype JF 11128. P: Immature and immature asci in dilute black Waterman ink with 1% SDS; Q: Long-stipitate ascus in chlorazol black with 1% SDS; R: Paraphyses with refractive content in dilute black Waterman ink with 1% SDS; S: Ascogenous hypha with asci arising in spicate arrangement, in dilute black Waterman ink with 1% SDS; T: Ascal apical apparati in Melzer's reagent; U: Ascospores in water; V: Ascospores with perispores dehiscing in 10% KOH; W, X: Ascospores with thin germ slit, in dilute black Waterman ink with 1% SDS.
Scale bars: P, Q: 20 µm; R, S, U, V: 10 µm; T, W, X: 5 µm.

ments observed in 10% KOH do not turn vinaceous after a prolonged incubation, which most likely reflects the presence of different compounds in the stromatal granules. The stromata of *H. trugodes* were reported by Hellwig *et al.* (2005) to contain a derivative of hypomiltin as prevailing metabolite and species known to contain hypomiltin like *H. intermedium* (Schwein.: Fr.) Y.-M. Ju & J. D. Rogers and *H. perforatum* (Schwein.: Fr.) Fr. have KOH-extractable pigments that do not turn vinaceous upon prolonged incubation.

Hypoxylon cazenavei is so far known from five collections in four different sites on the northern slopes of the Pyrenees, all on *Fagus*. This suggests a strong host preference, if not host specificity for *Fagus* but it is premature to draw any conclusion until further records of this species help define more accurately its ecology and distribution.

Mature fertile stromata were all collected in autumn, while stromata collected in March-April were largely overmature, which suggests a seasonality to confirm on the basis of a wider sampling.

The fact that it has been overlooked thus far may be due to its rarity but is more likely the result of the lack of striking colours and the overall habit recalling old stromata of *Biscogniauxia nummularia* (Bull.: Fr.) Kuntze and *Annulohypoxylon cohaerens* (Pers.: Fr.) Y.M. Ju, J.D. Rogers & H.M. Hsieh that are common saprobes of *Fagus*.

Hypoxylon invadens J. Fourn., *sp. nov.* — MB808739.
Figures 3-4

Holotype: France, Ariège, Rimont, road D18a, ca. 1.5 km from the village in a riparian mixed deciduous forest, ca. 500 m asl, 42°59′15.35″ N, 1°17′28.25″ E, on bark of a dead branch of *Fagus sylvatica* in the leaf litter, on aborted primordia of *Hypoxylon fragiforme*, 15 Nov. 2011, *leg*. J. Fournier, JF 11167 (LIP).

Diagnosis: Differs from other known species of *Hypoxylon* by the unique combination of fulvous stromata with strongly exposed perithecial contours that soon turn shiny black, with a slightly carbonaceous subsurface, yellowish green waxy granules yielding cinnamon pigments in 10% KOH and habitat restricted to primordia of *Hypoxylon fragiforme*.

Etymology: The epithet *invadens* (Latin *invadere* = to invade) refers to the fungicolous habitat.

Stromata appearing glomerate (A-E) with perithecial contours strongly exposed, 1,8-4 mm diam × 0.9–1.1 mm thick, developing on aborted primordia of *Hypoxylon fragiforme* (Pers. : Fr.) J. Kickx f.; the base of the stromata is seated on the surface of hemispherical primordia that can be seen in vertical section as an orange brown layer (F, G, arrowheads); first a fulvous (43) (oac706) pruina, composed of incrusted hyphae and greenish yellow granules spreads over primordia and around their base (C, D), from which the perithecial contours later emerge (D, E); surface pruinose, with fulvous coating rapidly worn off revealing a shiny black subsurface but remaining between perithecial contours; subsurface slightly carbonaceous, with a thin layer of waxy granules that appear greyish yellow-green (68) (oac727) in water (I), yielding cinnamon (62) (oac714) pigments in 10% KOH (J) turning fulvous (43) (oac706) with a faint vinaceous (57) (oac615) tinge after 30 mn of incubation (K); the tissue below the perithecial layer blackish brown, woody, homogeneous, 0.25–0.4 mm thick. **Perithecia** subglobose to obovoid, 0.6–0.8 mm high × 0.5 mm diam. (F, G). **Ostioles** umbilicate, typically surrounded by a dull orange brown slightly raised disc 120–150 µm diam. (H).

Asci cylindrical, with eight obliquely uniseriate ascospores, 125–220 µm total length, the spore-bearing parts 74–83 µm long × 7–8 µm broad, the stipes 47–143 µm long, originating from long ascogenous hyphae in spicate arrangement (I, M, arrowheads), with apical apparatus discoid to wedge-shaped, 1–1.5 µm high × 2.5 µm broad, bluing in Melzer's reagent (O). **Paraphyses** copious, filiform, 1–1.5µm diam, with scattered small refractive guttules (N). **Ascospores** (9.6) 10.3–12.0 (14.3) × (4.4) 4.7–5.8 (6.3) µm, Q = (1.8) 1.9–2.3 (2.6); N = 60 (Me = 11.2 × 5.3 µm; Qe = 2.1), ellipsoid-inequilateral with narrowly rounded ends, brown to dark brown (P), with a narrow and inconspicuous straight germ slit spore-length on the convex side (Q); perispore dehiscent in KOH, smooth by L.M. (R); epispore smooth.

Asexual state on natural substrate not seen. **Culture** on Oatmeal agar 7 cm diam. in 25 days (S), first whitish to tan with pale orange brown pigments diffusing in the medium, velvety, zonate, centre hazel (88) (oac799) with brown droplets, turning isabelline (65) (oac820) and pistachio green (92) (oac875) toward periphery, margin narrow, white. Reverse dark orange brown. Strong odour, complex, a mixture of vanilla and pharmaceutical drug. Sporulating region restricted to the hazel-coloured centre. Conidiogenous structure sporothrix- (T) to virgariella-like (U, V), hyaline to pale brown. Conidiogenous cells roughened, 25–40 × 2.5–3 µm, conidia ovoid, hyaline, smooth, 6–7.5 × 4.5–5 µm.

Other specimen examined: France, Ariège, Rimont, road D18a, ca. 1.5 km from the village in a riparian mixed deciduous forest, ca. 500 m asl, 42°59′15.35″ N, 1°17′28.25″ E, on bark of a dead branch of *Fagus sylvatica* in the leaf litter, on aborted primordia of *Hypoxylon fragiforme*, 3 Oct. 2013, J. Fournier, JF 13181 (LIP) (paratype).

Discussion: In the field, the first striking character of *H. invadens* is the unusual shiny black surface of its mature stromata with strongly exposed perithecial contours. Among temperate species of *Hypoxylon*, only *H. macrocarpum* is known to sometimes feature such a shiny stromatal surface but it deviates in having stromata in shades of purple with greyish brown KOH-extractable pigments, tubular perithecia and narrower ascospores (averaging 10.2 × 4.2 µm, Q = 2.2–2.6). Moreover the wood colonized by *H. macrocarpum* releases a strong and typical aromatic odour at fresh state that lacks in *H. invadens* and in almost all species of *Hypoxylon* (Pouzar, 1978).

The stromata of *H. invadens* appear hemispherical-glomerate but closer examination and cross-section reveal that its stromata spread over aborted primordia of *H. fragiforme* that give them their hemispherical habit. One might regard the tissue beneath the stromata as a part of the stromata themselves since it is sterile. The orange layer on which they take place is composed of orange granules that yield dark orange pigments in 10% KOH typical of H. fragiforme and different from those of *H. invadens*. Moreover, the stromata of *Hypoxylon* feature waxy granules beneath the surface, sometimes between the perithecia, but these granules never form a continuous layer beneath the sterile subperithecial tissue.

The plentiful material collected shows the fungus at all stages of development. It first colonizes entirely the primordia as a thin fulvous pruinose tissue that is also present around the base but does not develop on the surrounding bare bark or wood. Mature stromata develop from this fulvous tissue but often do not spread over the whole primordium, leaving fulvous tissue persistent in sterile zones uniting the separate ascomata. Based on these observations and a second collection featuring exactly the same configuration it seems appropriate to regard this *Hypoxylon* as fungicolous, a very distinctive feature rarely encountered in the genus and even in the family *Xylariaceae*.

Hypoxylon parasiticum Ellis & Everh. ined. was found growing on the stromata of a depauperate *Hypoxylon* sp. and regarded as parasitic by its authors who however did not published it formally. The specimen was revised by Y.-M. Ju & J.D. Rogers who assessed it was *H. jecorinum* Berk. & Ravenel growing on *H. duranii* J. D Rogers (Ju & Rogers, 1996), both lignicolous species known from southern USA and Central and South America. Stromata of lignicolous *Hypoxylon* spp. can occasionally occur on stromata of other xylariaceous fungi just by extending on a substrate previously colonized by another species but this can be regarded as fortuitous without involving a parasitic lifestyle since the same stromata are usually present on the surrounding wood or bark. *Hypoxylon aeruginosum* Miller is the only

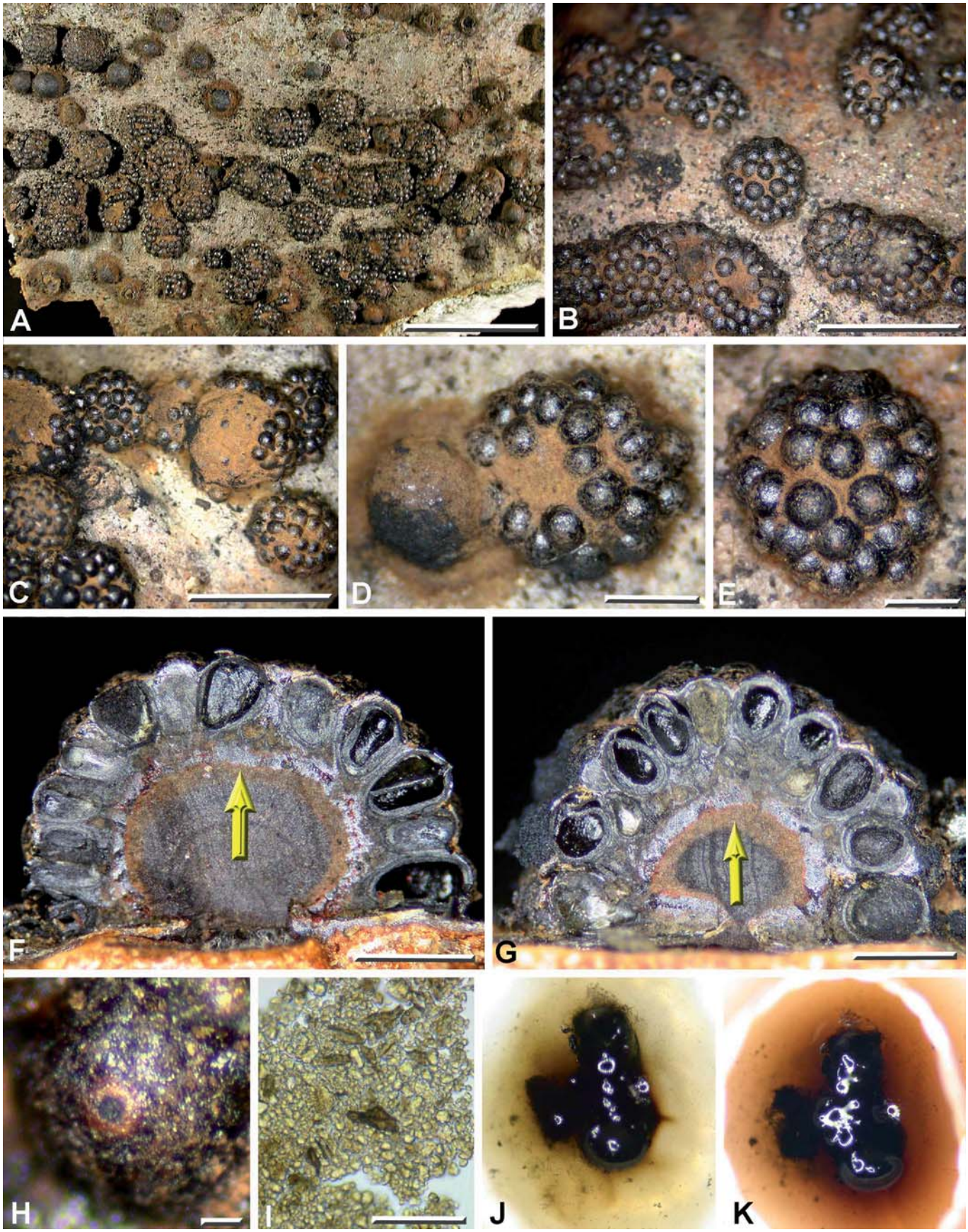

Fig. 3. – *Hypoxylon invadens*. Holotype JF 11167. A: Stromata on bark; B-E: Stromata in close up, spreading more or less completely over the primordia of H. fragiforme; F, G: Stromata in vertical section showing the underlying primordia on which the stromata of *H. invadens* develop, with arrowheads on the outermost orange layer of the primordia; H: Ostiole in close up; I: Stromatal granules observed in water; J: KOH-extractable pigments after 1 min of incubation; K: KOH-extractable pigments after 30 min of incubation.
Scale bars: A: 10 mm; B, C: 5 mm; D-G: 1 mm; H: 100 µm; I:10 µm.

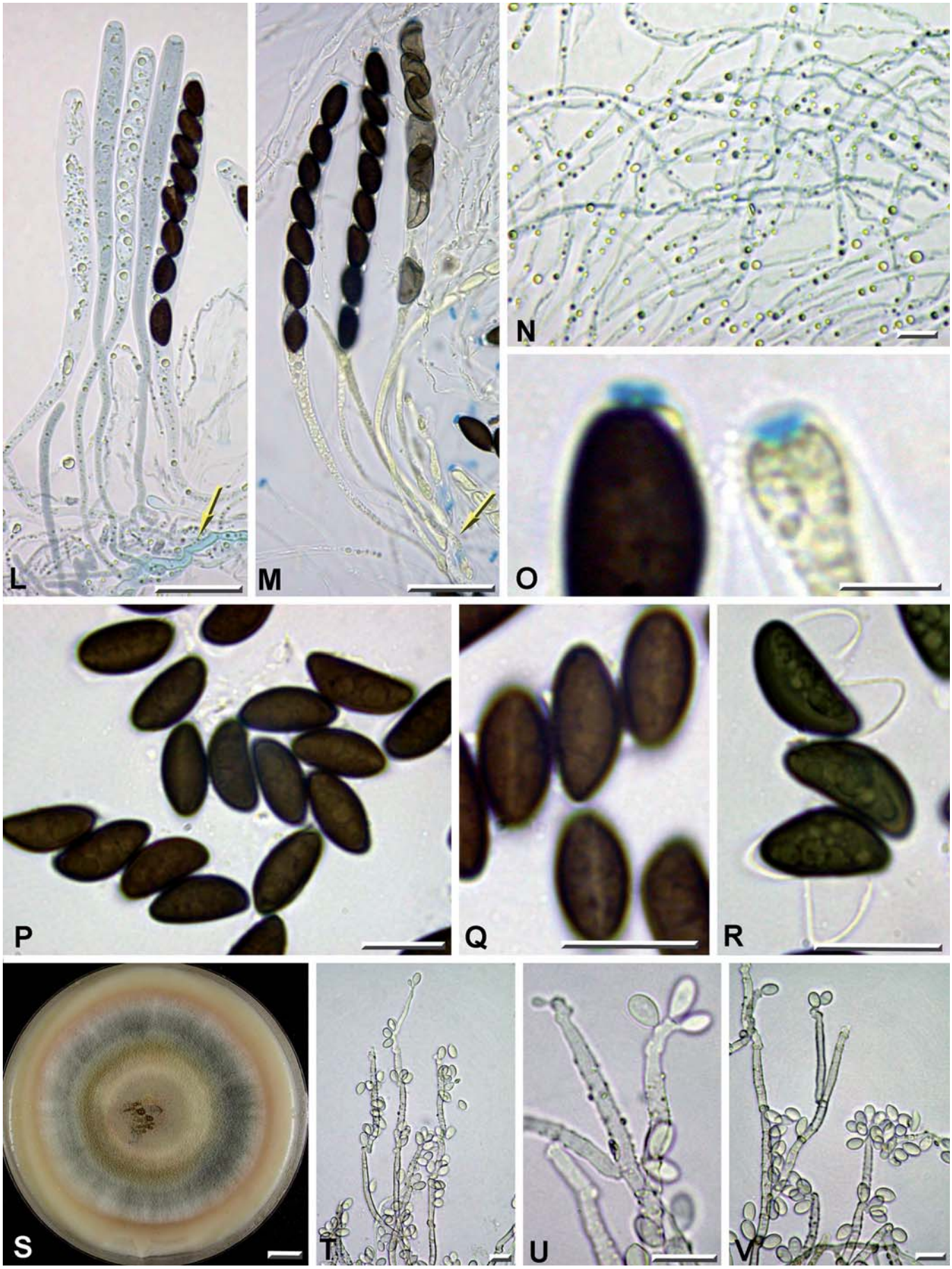

Fig. 4. – *Hypoxylon invadens*. Holotype JF 11167. L: Immature and mature asci arising from long ascogenous hyphae (arrowhead) in chlorazol black; M: Asci arising from long ascogenous hyphae (arrowhead) in Melzer's reagent; N: Paraphyses in chlorazol black; O: Ascal apical apparati in Melzer's reagent; P: Ascospores in water; Q: Ascospores in water with focus on convex side showing germ slits; R: Ascospores with perispores dehiscing in 10% KOH; S: Culture on OA after 25 days; T: sporothrix-like anamorph in 1% SDS; U, V: virgariella-like anamorph in 1% SDS. Scale bars: L, M: 20 µm; N, O: 5 µm; P-R, T-V: 10 µm; S: 1 cm.

known species of *Hypoxylon* that has been repeatedly found growing on stromata of other *Hypoxylon* spp. (Læssøe *et al.*, 2010). It is a predominantly neotropical taxon well characterized by cyan blue stromata (Ju & Rogers, 1996), a colour shared with the xylariaceous genus *Chlorostroma* A.N. Miller, Lar. N. Vassiljeva & J.D. Rogers (Miller *et al.*, 2007) that includes *C. subcubisporum* A.N. Miller, Lar. N. Vassiljeva & J.D. Rogers and *C. cyaninum* Læssøe, Srikitikulchai & J. Fourn. (Læssøe *et al*., 2010). Interestingly both species of *Chlorostroma* were reported to parasitize stromata of *Hypoxylon* and thus appear to have strong affinities with *H. aeruginosum* but their stromatal colour and overall morphology clearly set them apart from *H. invadens*.

Aside from its parasitic lifestyle and its shiny black stromata, further morphological traits help recognize *H. invadens* as a new taxon. The greyish yellow-green stromatal waxy granules recall those encountered in the species of the *H. fuscum* complex but instead of olivaceous their KOH-extractable pigments differ in being cinnamon and turning slightly vinaceous after prolonged incubation. The barely raised orange brown discs present around ostioles at maturity are likewise distinctive. They might suggest affinities with the closely related genus *Annulohypoxylon* but in this genus the ostiolar discs, when present, result from a loss of carbonaceous tissue around a papillate ostiole and ascospores differ in that their dehiscing perispores typically have a thickening on the same side as the germ slit at a position of 1/3 ascospore length (Hsieh *et al.*, 2005).

Moreover, the culture of *H. invadens* differs from cultures of most *Hypoxylon* spp. in being conspicuously zonate and in yielding volatile compounds. It is noteworthy that this peculiar odour was not recorded in the wood underlying the stromata of *H. invadens*, unlike what is observed in *H. macrocarpum* and *H. rutilum* Tul. & C. Tul., which supports the assumption that H. invadens is not lignicolous.

Hypoxylon invadens is so far known from only two collections in the same site. Despite that its host *H. fragiforme* is extremely common in beech forests and an extensive search on dead branches of *Fagus sylvatica* in similar habitats has been made since its discovery, it has not been collected again. *Hypoxylon invadens* may be a rare taxon but additional records are required to confirm its host-specificity, to evaluate its distribution and to better understand its ecology.

Acknowledgments

I gratefully acknowledge Prof. Jack Rogers (USA) and Dr. Yu-Ming Ju (Taiwan) for their presubmission reviews and their helpful comments, corrections and suggestions. I thank Dr. Marc Stadler (Dortmund) for having provided the YMG and OA plates for the cultures and Robert Cazenave (Séméac) for having collected the original material of *Hypoxylon cazenavei*.

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