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New species of *Hypoxylon* from western Europe and Ethiopia

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Abstract — Three new species of *Hypoxylon* are described from France, Portugal, and the United Kingdom based on new combinations of teleomorphic morphology. *Hypoxylon fuscoides* is related to *H. fuscum* but differs in having purple pigments. *Hypoxylon lusitanicum* is similar to *H. perforatum* but differs in having orange stromatal pigments. *Hypoxylon gibriacense* features glomerulate stromata and resembles the American *H. shearii* but has discoid ostiolar areas and different ascospores. In this context, *H. addis*, collected from Ethiopia, is also newly described because it appears morphologically similar to *H. gibriacense*. Their secondary metabolite profiles, as inferred from high performance liquid chromatography coupled with diode array detection and mass spectrometric detection (HPLC-DAD/MS), confirm their uniqueness as compared to related species. Lecanoric acid (widely distributed in lichenized ascomycetes) is revealed to as the major stromatal metabolite of *H. addis* and is for the first time reported as present in a xylariaceous species. A new key to European *Hypoxylon* species is provided.

Key words —*Xylariaceae,* chemotaxonomy, systematics, pyrenomycetes

Introduction

Hypoxylon Bull. has traditionally comprised the largest genus of family *Xylariaceae* with (fide Index Fungorum) over 1100 epithets associated with the generic name. The revision by Ju & Rogers (1996) introduced new species concepts based on a combination of teleomorphic and anamorphic characters

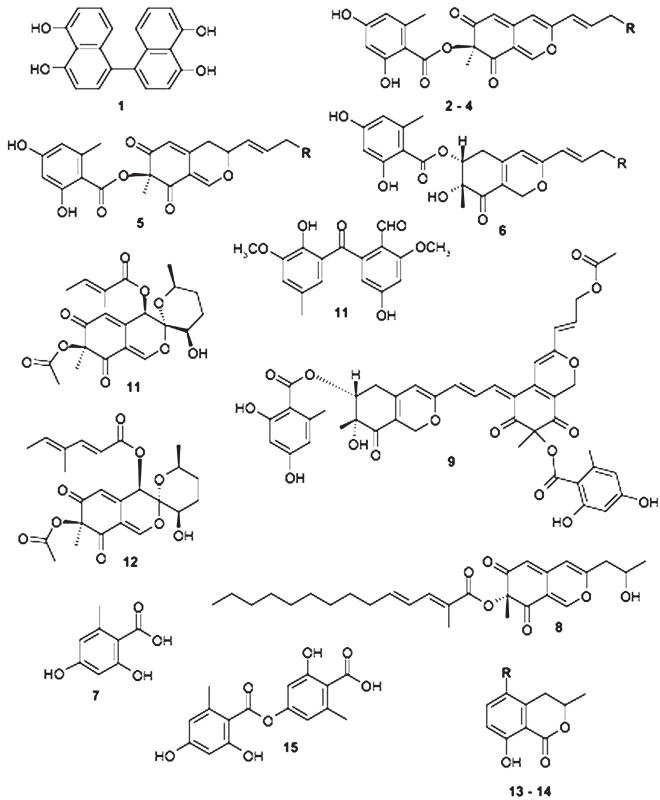

Fig. 1. Chemical structures of characteristic pigments and other secondary metabolites of *Hypoxylon* and allied genera, detected in this study by HPLC. **1**: Binaphthalene tetrol (BNT); **2**: Mitorubrin (R = H); **3:** Mitorubrinol (R = OH); **4**: Mitorubrinol acetate (R = $OCCH_3$); **5**: Hypomiltin (R = $OCCH_3$); **6:** Rubiginosin A (R = $OCCH_3$); **7**: Orsellinic acid; **8**: Rubiginosin C; **9**: Rutilin A; **10**: Daldinal A; **11**: Daldinin C; **12**: Daldinin E; **13:** Mellein.(R = H) **14**: 5-Methylmellein (R = CH_3); **15:** Lecanoric acid..

in conjunction with chemotaxonomy (i.e., stromatal pigment colors in 10% KOH). *Hypoxylon* was thus restricted to stromatic pyrenomycetes with an essentially homogenous stromatal context and *Nodulisporium*-like anamorphs. After erection of the genus *Annulohypoxylon* Y.M. Ju et al. (Hsieh et al. 2005) for sect. *Annulata* of *Hypoxylon* sensu Ju & Rogers (1996), *Hypoxylon* s. str. is now restricted to their sect. *Hypoxylon*.

In past years, we have studied several thousands of herbarium specimens and fresh material of *Hypoxylon* spp. from around the world. In addition to the characters deemed diagnostically important by Ju & Rogers (1996), we

studied secondary metabolite profiles recorded by high performance liquid chromatography coupled with diode array detection and mass spectrometry (HPLC-DAD/MS; cf. Hellwig et al. 2005, Stadler et al. 2001, 2004, 2008). Such HPLC profiles have proved quite valuable, because the production of secondary metabolites was largely found to be consistent in a given species, with the characteristic stromatal metabolites remaining stable even in ancient specimens collected up to 200 years previously. Due to this work, a comprehensive matrix of chemical and morphological data has become available that facilitates substantially the recognition of new taxa. On the other hand, novel biologically active compounds with potential utility were often encountered in rare species of *Hypoxylon*, as exemplified by the discoveries of rutilins (Quang et al. 2005) and carneic acids (Quang et al. 2006).

The current paper describes four new species of *Hypoxylon* from Western Europe and Eastern Africa that deviate significantly from all described taxa with respect to their morphological and chemotaxonomic traits.

Materials and methods

Teleomorphic structures were microscopically observed in water (to study ascospore morphology), in Melzer´s reagent (to test for amyloid ascal apical structures), in Chlorazol black (to measure ascal stipes), and in 10% KOH (to test for perispore dehiscence). In cases of apparent absence or lack of reactivity of ascal apical structures in Melzer's reagent, a pretreatment by 3% KOH was attempted. Ascospores were measured in water at 1000x magnification. KOH-extractable pigments were obtained as described in Ju & Rogers (1996). Color codes follow Rayner (1970). Ascospores were photographed in water or 10% KOH. Anamorphic structures were observed microscopically in water at 400–1000× magnification using phase contrast.

Cultures were obtained from ascospores prepared from perithecial contents on yeast-malt glucose (YMG) medium supplemented by antibiotics (Stadler et al. 2008). For morphological studies, the cultures were grown YMG and Difco Oatmeal agar (OA).

HPLC analyses of stromatal methanolic extracts were carried out according to Stadler et al. (2008) in two different gradients, using UV-visual detection (HPLC-UV/Vis) with diode array detection (DAD) and mass spectrometric detection (HPLC-MS) in both the positive and negative electrospray ionisation (ESI) mode. Secondary metabolites were identified by matching their retention times (Rt), HPLC-DAD and HPLC-ESI-MS spectra with external or internal standards of pure compounds that had been obtained previously. HPLC data of extracts and pure compounds from previous studies on *Xylariaceae* (Hellwig et al. 2005, Bitzer et al. 2007, Stadler et al. 2004, 2008) were also used for comparison. Cultures were propagated on YMG medium, and their extracts analyzed on the occurrence of secondary metabolites as described by Bitzer et al. (2008). Some compounds that were detected in the new taxa described herein or in morphologically similar species are depicted in Fig. 1. The trivial names of these compounds have been assigned a **bold** number in the legend of Fig. 1, to which they are referred in the taxonomic part.

Hypoxylon fuscoides J. Fourn., P. Leroy, M. Stadler & Roy Anderson, **sp. nov.**

MycoBank MB 516748 Figs 2–4

A Hypoxylon fuscum *differt granulis violaceis ad vinaceis in KOH dissolutis; a* Hypoxylon rosieri *differt ascosporibus ellipsoideo-inequilaterales, apicibus angustatis, 9.5–12.5 × 5–6 µm µm, riminibus germinativis sigmoideis praeditae. Status anamorphosis ad genero* Virgariella *similis.*

Type: FRANCE, Vosges, Forêt de Rambervilliers, on bark of *Betula pendula* (*Betulaceae*), 7.X.2003, Paul Leroy, PL 03142B (**Holotype** – LIP; culture in MUCL 52670 and CBS 126418).

Etymology: Latin, for its strong resemblance to *Hypoxylon fuscum*

Stromata (Fig. 2) erumpent from bark, pulvinate, slightly constricted at base, gregarious, separate to coalescent, 1.4–3 mm diam × 0.8–1.4 mm thick; surface pruinose, Brown Vinaceous (84), pruina made up of red brown granules turning bluish green in 10% KOH, slightly uneven, with perithecial contours not exposed, with a thick layer of yellowish waxy granules beneath the surface turning colorless in 10% KOH, the whole stroma yielding Vinaceous Purple (101) pigments in 10% KOH; the tissue beneath the perithecia 0.5–1.2 µm thick, greyish brown with blackish marks, soft-textured. Perithecia subglobose to obovoid, rarely slightly tubular, 0.32–0.38 mm high × 0.13–0.22 mm diam. Ostioles umbilicate, inconspicuous. Asci (Fig. 3) cylindrical, short-stipitate, 8-spored, readily deliquescent, 100–120 µm total length, the spore bearing-parts 70–84 µm long × 7–8 µm broad, the stipes 24–42 µm long, with a discoid apical ring 0.5–0.8 µm high × 3–3.4 µm broad, bluing in Melzer's reagent. Paraphyses filiform, septate. Ascospores (Fig. 3) 9.5–12.5 × 5–6 µm (M = 11 × 5.4 µm, n = 30) ellipsoid slightly inequilateral with narrowly rounded to acute ends, brown, smooth, with a conspicuous spore- length sigmoid germ slit, swelling rapidly in water; perispore dehiscent in 10% KOH, thin-walled, with faint transverse striae. Epispore smooth.

Cultures and anamorph: Colonies on OA covering Petri dish in 2–3 weeks, at first white, becoming Hazel (88), velvety, azonate, with diffuse margins; reverse remaining uncolored. Sporulating regions in patches, vinaceous buff (86). Conidiogenous structure referable to the *Virgariella*-like branching pattern as defined by Ju & Rogers (1996), hyaline, smooth to finely roughened. Conidiogenous cells (Fig. 4) hyaline, smooth, 8–14(–25) × 2.5–4 µm, often arranged repetitively at the tips of the conidiophores (Fig. 4), so that up to ten conidiogenous cells are produced in succession. Conidia hyaline, smooth, ellipsoid, 5–7 × 2.5–3 µm.

Secondary metabolites: HPLC profiling (Fig. 5a) revealed that this species differs from all morphochemotypes of *H. fuscum,* we have hitherto studied, regardless from which host plants their stromata have been encountered, in lacking daldinins C, E and F and daldinal A. The lack of these pigments clearly

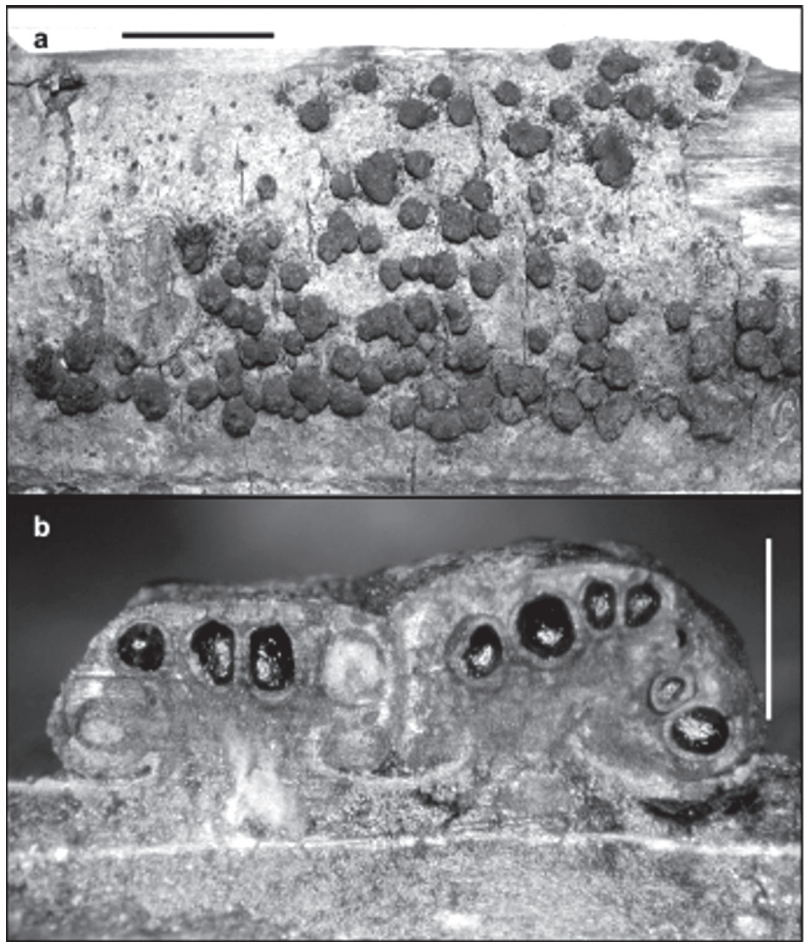

Fig. 2. Stromata of *Hypoxylon fuscoides*, from holotype (PL 03142B). a. Stromatal habit on the natural substrate. b. Section through stroma, showing the ruptured periderm and perithecial arrangement. Scale bars: a. 1cm, b: 1 mm.

accounts for the pigments in KOH being purple, rather than olivaceous brown. Binaphthalenes (in particular binaphthalene tetrol, BNT) were found to be the prevailing stromatal metabolites. The cultures produced 5-methylmellein (**14**) as major component in YMG medium.

Further specimens examined: **UNITED KINGDOM**, **Northern Ireland**. **Vice-county H37 (Armagh),** Oxford Island (J045620), on bark of fallen branches of *Alnus incana* (*Betulaceae*), 18.X.2007. R. Anderson. **Vice-county H38 (Down),** Belfast, Belvoir Forest, (J333693), on bark of fallen branches of *Alnus incana*, 5.II.2008. R. Anderson (K, culture in MUCL 52423).

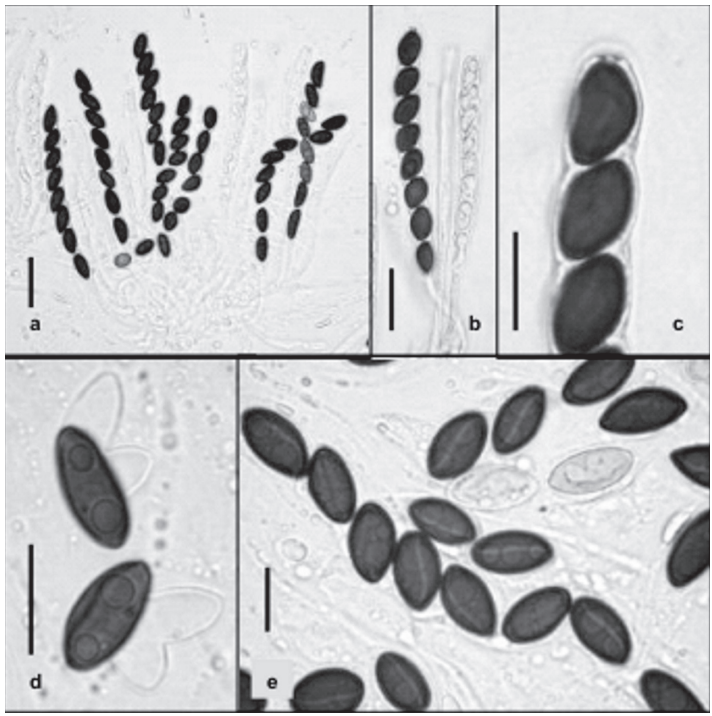

Fig. 3. Microscopic characteristics of *Hypoxylon fuscoides*, from paratype (JF 09347; K). a. Asci in water. b, c. Asci in Melzer's reagent, c showing amyloid apical apparatus. d Ascospores in KOH, showing dehiscent perispore. e. Ascospores in water, showing the sigmoid germ slits. Scale bars: a, b: 20 µm, c, d, e: 10 µm

Comments: *Hypoxylon fuscoides,* already mentioned on a website dedicated to fungal taxonomy (Fournier & Magni 2004) and by Anderson (2008), is not distinguishable from *H. fuscum* in the field. Despite the fact that these species share many morphological features, the new taxon can be readily separated by its reaction in KOH and its smaller ascospores with more acute ends.

Hypoxylon fuscum as currently conceived (Petrini & Müller 1986, Ju & Rogers 1996, Granmo 1999) features a very wide ascospore size range that is not clearly correlated with other morphological, ecological, or chemotaxonomic characters. In the present case, the deviating morphology of ascospore ends appears more significant than the difference in size. The holotype collection has paler pigments in KOH than the material from Northern Ireland, but the ascospores are identical, and it features similar very small gregarious stromata. The cultures are similar to those described for *H. fuscum* by Petrini & Müller (1986) and Ju & Rogers (1996) but have rather stout conidiogenous cells and

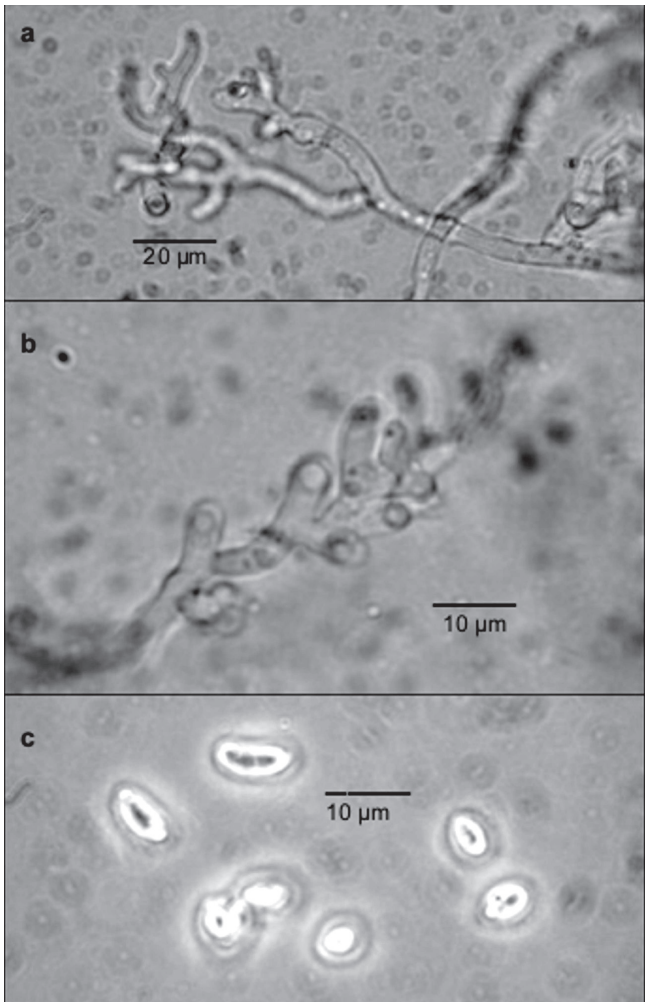

Fig. 4. *Hypoxylon fuscoides*, ex-type strain, from OA culture. a. conidiophores, showing dichotomously branched *Virgariella*-like conidiogenous structures. b. Close-up of conidiophore apex, showing repetitive branching, resulting in stout conidiogenous cells. c. Conidia. Scale is indicated by bars.

slightly larger conidia. In particular, the successive production of numerous small conidiogenous cells from the tip of the same conidiophore is only exceptionally observed in other cultures of *Hypoxylon*, and those of the most frequent morphochemotype of *H. fuscum* from *Corylus* normally produce conidiogenous cells up to 40 µm long. The stromatal HPLC profile also deviates strongly from that observed in numerous collections of *H. fuscum* collected

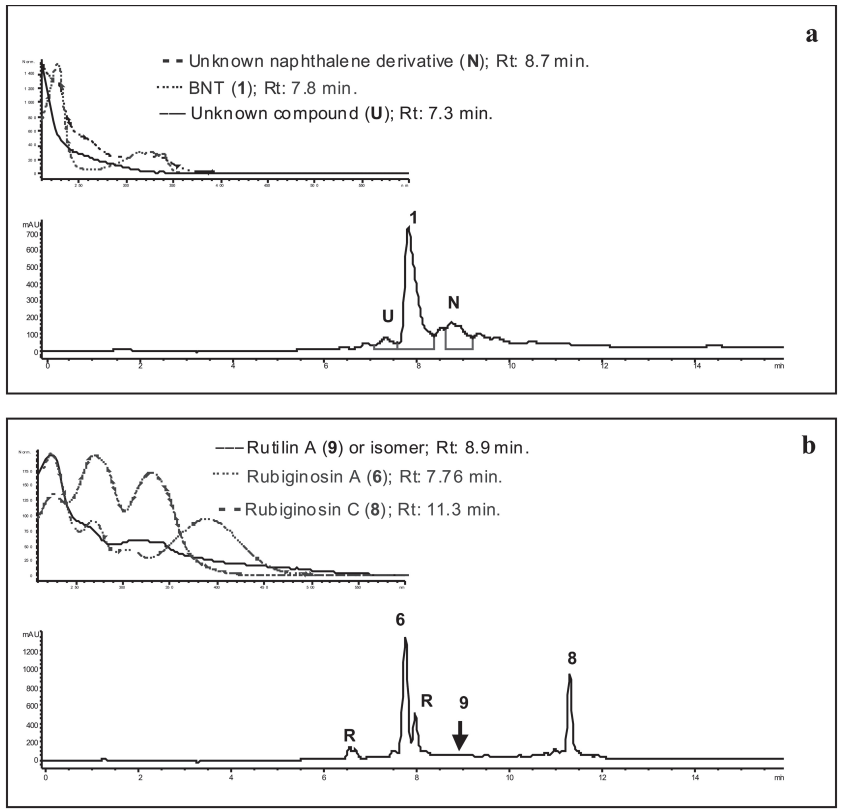

Fig. 5. Stromatal HPLC-UV profiles (210 nm) of holotype specimens of *Hypoxylon fuscoides* (a) and *Hypoxylon lusitanicum* (b). and DAD spectra of major metabolites. In Fig 5a, **U** indicates an unknown major component lacking a characteristic DAD spectrum, and **N** indicates an unknown naphthalene with a DAD spectrum similar to BNT (**1**). Daldinal A and daldinins C and E (**10** - **12**), the characteristic pigments of *H. fuscum*, were not detected. In Fig. 5b, **R** indicates further major components of the rubiginosin type, whose spectra are not depicted). For chemical structures of known compounds see Fig. 1.

from *Alnus* and *Salicaceae*, which apparently also lacks daldinins but contains different pigments that also result in olivaceous colors in KOH in our recent study (Stadler et al. 2008).

Rogers et al. (2008) described *H. rosieri* J.D. Rogers & Lar.N. Vassiljeva, another segregate of *H. fuscum* from the USA (Texas), based on a similar purple KOH reaction. Their species differs from *H. fuscoides* in having markedly longer, more slender ascospores (13.5–15 × 5–6 µm). Furthermore, sigmoid ascospore germ slits were not mentioned in their description.

Hypoxylon lusitanicum J. Fourn., M. Stadler & Priou, **sp. nov.** Figs 6–7

MycoBank MB 516749

A Hypoxylo perforato *differt granulis rufobrunneis in KOH dissolutis et ascis longistipitatis, 158–170 µm longitudine tota, partibus sporiferis 76–91× 7–8 µm, stipitibus 70–80 µm.*

Type: PORTUGAL: Ribatejo Prov., Achete, Ribeirhina, 39° 19' 13" N, 08° 42' 55" W, alt 55 m., on dead blackened wood of *Rhamnus alaternus* (*Rhamnaceae*) in Mediterranean evergreen vegetation, 5.V.2009, J.P. Priou, JF 09125 (**Holotype** – LIP; ex-type culture in MUCL52424).

Etymology: For Portugal (Lusitania in Latin).

Stromata (Fig. 6) effused, ellipsoid to elongated, 8–22 mm long × 2.5–8 mm broad × 0.6–0.8 mm thick, at times coalescent, at times with steep, indented black margins; surface pruinose, slightly uneven, Brown Vinaceous (84), with perithecial contours hardly exposed, with a thick layer of olivaceous yellow waxy granules beneath the surface and around the upper half of perithecia, yielding Sienna (8) pigments in 10% KOH; the tissue beneath the perithecia 50–150 µm thick, dull brown, soft-textured, delimited by a black line spreading over the underlying wood. Perithecia subglobose to obovoid, 0.5–0.6 mm high × 0.3–0.45 mm diam. Ostioles umbilicate, often in a shallow depression, fringed with a disc of white material 70–80 µm diam. Asci (Fig. 7) cylindrical, long-stipitate, 8-spored, 158–170 µm total length, spore bearing-parts 76–91 µm long × 7–8 µm broad, the stipes 70–80 µm long, with a discoid apical ring 0.8–1 µm high × 2.5–3 µm broad, bluing in Melzer's reagent. Paraphyses not seen. Ascospores (Fig. 7) 11–13.5 × 5–7 µm (M = 11.8 × 5.5 µm, n = 30) ellipsoid-inequilateral with narrowly rounded to acute ends, brown, smooth, with a spore- length straight germ slit; perispore readily dehiscent in 10% KOH, faintly striate. Epispore smooth.

Cultures and anamorph: Colonies on YMG and Difco OA covering Petri dish in 2 weeks, at first whitish, becoming umber (9), velvety to felty, azonate, with diffuse margins, with honey (64) pigments diffused beyond colonies; reverse slightly melanizing with age. No conidiogenous structures observed.

Secondary metabolites: In accordance with the orange pigments in KOH, the stromatal HPLC profile of *H. lusitanicum* (Fig. 5b) revealed the presence of azaphilones, with rubiginosins A (**6**) and C (**8**) being major detectable components. A minor metabolite at Rt 8.9 min was also observed, which probably corresponds with rutilin A (**9**) or another yet unknown dimeric azaphilone of the rutilin type. Neither mitorubrins nor hypomiltin (**2** – **5**) were detected. However, the cultures produced 5-methylmellein (**14**) as major component in YMG medium, indicating a relationship to the *H. fuscum* and *H. rubiginosum* species complexes (cf. Bitzer et al. 2008).

Further specimen examined: **PORTUGAL**: **Ribatejo** Prov., **Achete**, Ribeirinha, *Rhamnus alaternus*, 6.V.2009, mixed with old pulvinate stromata of *Hypoxylon perforatum*, J.P. Priou, JPP 29083.

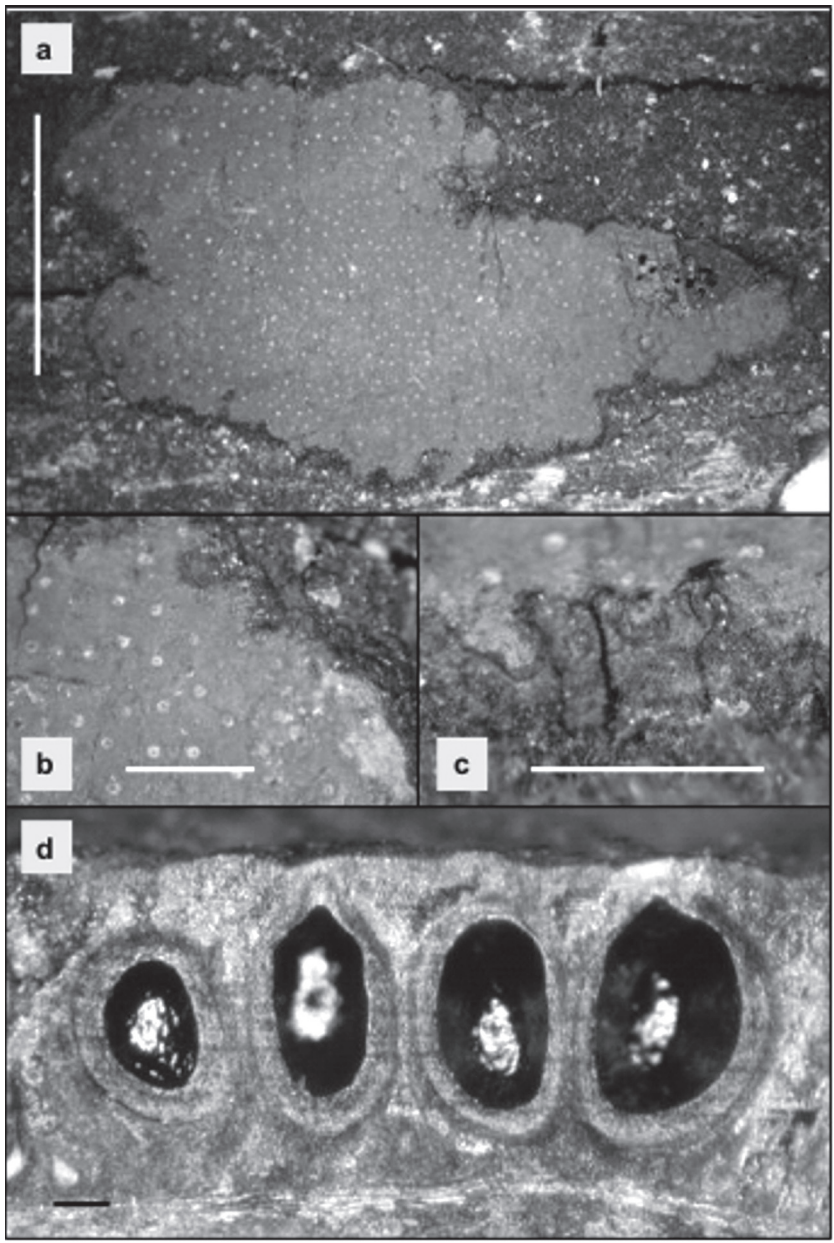

Fig. 6. Stromata of *Hypoxylon lusitanicum*, from holotype (LIP). a. Stromatal habit on the natural substrate. b, e. Close-up of stromatal surface, showing ostioles. c. Close-up of blackened stromatal margin. d. Section through stroma, showing perithecia. Scale bars: a: 5 mm, b, c: 1mm d: 0.1 mm.

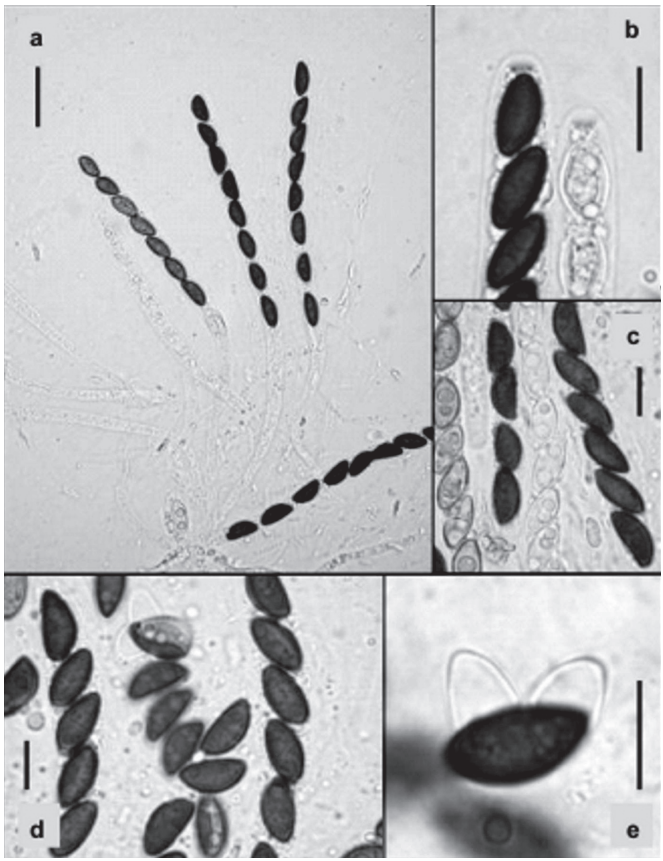

Fig. 7. Microscopic characteristics of *Hypoxylon lusitanicum*, from holotype (LIP). a, Asci in water. b Ascal tip in Melzer's reagent, showing amyloid apical apparatus. c-d Ascospores in water. e Ascospore in KOH, showing dehiscent perispore. Scale bars: a: 20 µm, b, c, d, e: 10 µm.

Comments: *Hypoxylon lusitanicum* appears highly similar to *H. perforatum* with regard to its stromatal morphology (conspicuous white discs around umbilicate ostioles and presence of yellowish granules beneath the stromatal surface. It can be distinguished by its red brown pigments in KOH, larger perithecia, long-stipitate asci, and significantly larger ascospores with more narrowly rounded ends. The red brown pigments are due to the presence of rubiginosins as in *H. rubiginosum* and *H. petriniae*, whereas *H. perforatum* produces hypomiltin instead (cf. Stadler et al. 2004). Both, *H. rubiginosum* and *H. petriniae* have often been confused with *H. perforatum;* hence they might be easily confounded with *H. lusitanicum.* Two recently described taxa from the Canary Islands, *H. canariense* and *H. urriesii* (Stadler et al. 2008), might also

Table 1. Diagnostic characters of six species of the *Hypoxylon rubiginosum* complex.

	H. canariense	*H. lusitanicum*	*H. perforatum*	*H. petriniae*	*H. rubiginosum*	*H. urriesii*
Surface color	Fulvous(43), Dark Brick (60), or Brown Vinaceous (84)	Brown Vinac. (84)	Dark Brick (60) to Brown Vinac. (84)	Vinaceous Grey (116) to Brown Vinac. (84)	Rust (39) to Dark Brick (60)	Dark Brick (60)
White- fringed ostioles	frequent	present	present	frequent	occasional	absent
Stromal thickness	0.5–0.6 mm	0.6–0.8 mm	0.5–1–(2.5) mm	0.3–0.8 mm	1–1.3(–2) mm	0.3 mm
Perithecial diam.	0.3–0.4 mm	0.3–0.45 mm	0.25–0.4 mm	0.25–0.4 mm	0.3–0.65 mm	0.15–0.2 mm
KOH pigments	Orange(7) to ienna(8)	Sienna (8)	Amber (47)	Orange (7)	Orange (7)	Orange (7)
Secondary metabolites	mitorubrins, rubiginosins	rubiginosins, rutilin A	hypomiltin	rubiginosins, BNT	mitorubrins, rubiginosins	mitorubrins,r ubiginosins
Ascus stipe length	27–40 µm	70–80 µµ	24–50 µm	37–64 µm	60–98 µm	18–30 µm
Av. ascospore size	10.4 × 4.8 µm	11.8 × 5.5 µm	10.9 × 4.9 µm	10.7 × 5.1 µm	10.1 × 4.4 µm	12.3 × 5.4 µm
Germ slit	straight	straight	straight	straight	straight	sigmoid
Perispore (LM)	smooth	faintly striate	smooth	smooth	smooth	smooth
Reference	Stadler et al. 2008	This study	Fournier & Magni 2004	Fournier & Magni 2004	Fournier & Magni 2004	Stadler et al. 2008

be confused in the field with *H. lusitanicum* because of their effused stromata having similar surface colors. *Hypoxylon canariense* mainly differs from *H. lusitanicum* in having short-stipitate asci and smaller ascospores averaging 10.4 × 4.8 µm with a smooth perispore, while *H. urriesii* differs from the new taxon in having much smaller perithecia, short-stipitate asci and slightly larger ascospores averaging 12.3 × 5.4 µm with a sigmoid germ slit and a smooth perispore. Some important diagnostic characters to discriminate these six species are summarized in Table 1.

Hypoxylon gibriacense J. Fourn., M. Stadler & Gardiennet, **sp. nov.**

MycoBank MB 516750 Figs 8–9

A Hypoxylon shearii *et* Hypoxylon fraxinophili *differt discis annulatis conspicuis, peritheciis ambientibus. A* Hypoxylon addis *differt in ascosporae parviorae, perisporium conspicuiter striatum praeditae.*

Type: FRANCE, Côte d'Or, Gevrey-Chambertin, Combe de Lavaux, on moss-covered bark of a fallen branch of *Acer platanoides* (*Aceraceae*), 3.XII.2009, A. Gardiennet, AG 09033 (**Holotype** – LIP; culture in MUCL 52698).

Etymology: from *Gibriacum*, the Latin name of Gevrey, the locality of the type collection .

Stromata (Fig. 8) corticolous, erumpent through the periderm, glomerate with a narrowly restricted base, containing 12–20 perithecia, scattered to often coalescent, 2–3 mm diam × 1.5–2.5 mm thick, soft-textured; surface Greyish Sepia (106) with a faint olivaceous tone, pruinose, with perithecial contours exposed to strongly exposed; dull yellow granules forming a thin crust beneath the surface and sometimes extending between the perithecia, yielding fugacious Amber (47) then Sienna (8) pigments in 10% KOH (Livid Red (56) under the microscope); subperithecial tissue purplish black, brownish grey at base 1–1.3 mm thick. Perithecia ellipsoid to subglobose, 0.5–0.6 mm high × 0.4–0.5 mm diam. Ostioles umbilicate, opening at the centre of a paler discoid area ca. 0.2 mm diam delimited by a low rim. Asci (Fig. 8) unitunicate, cylindrical, 130–140 µm total length, the spore-bearing parts 85–95 µm long × 9–9.5 µm broad, the stipes 40–45 µm long, apex without apical ring, not bluing in Melzer's reagent. Paraphyses filiform, copious. Ascospores (Fig. 9) 11.5–13 × 6–6.8 µm (M = 12.4 × 6.5 µm, n = 30), ellipsoid-inequilateral with narrowly rounded ends, one side flattened to sometimes slightly concave, brown to dark brown, smooth, with spore-length straight germ slit (arrows). Perispore dehiscent in KOH, with fairly conspicuous striae somewhat anastomosing. Epispore smooth.

Cultures on YMG and OA media covering a 9 cm Petri dish in 2–3 weeks, white, felty to floccose, azonate, with diffuse margins; reverse becoming Honey (64). No conidiophores or other anamorphic structures observed after up to 6 weeks of incubation.

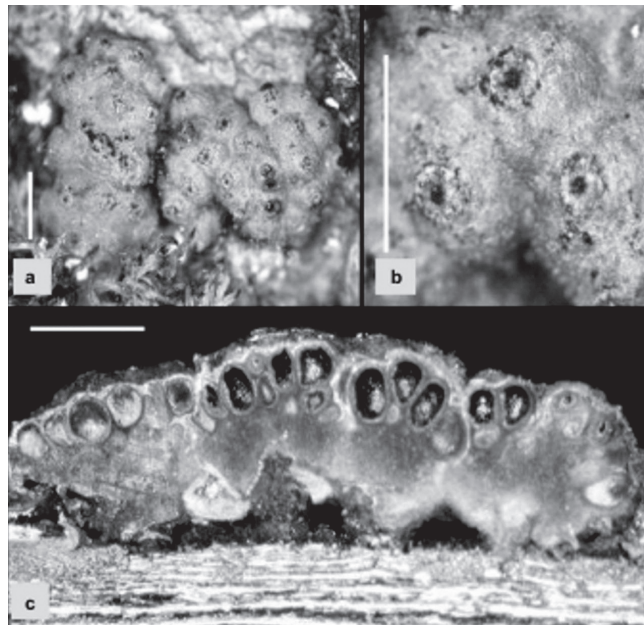

Fig. 8. Stromata of *Hypoxylon gibriacense*, from holotype (LIP). a. Stromatal habit on the natural substrate. b. Close-up of stromatal surface, showing perithecial mounds and ostiolar disks. c. Section through stroma, showing perithecia. Scale bars: a, b, c: 1 mm.

Secondary metabolites: HPLC of the stromatal MeOH extract of the holotype specimen (Fig. 13) revealed rubiginosin C (**6**) and another major peak that was revealed to be a mixture of BNT (**1**) and hypomiltin (**5**) only by HPLC-MS because the chromatographic method used to separate the components in the crude extract by HPLC-DAD appeared insufficient to discriminate these compounds. The DAD spectrum therefore at first appeared unique because it was actually caused by two components showing different absorption maxima in the UV-visual detection range. The mass spectra derived from this peak, labeled ("**1** + **5**") containing both compounds are included in Fig. 13 for comparison. All these compounds also occur in various other species of the *H. rubiginosum* complex (cf. Stadler et al. 2008). The cultures (Fig. 15) produced mellein (**13**) and several other metabolites, most of which were not yet identified, but 5-methylmellein (**14**) was not observed.

Comments: *Hypoxylon gibriacense* is distinctive in featuring ostiolar discs and in having asci lacking an apical apparatus. Despite the clearly differentiated discs around the ostioles, it is considered best placed in *Hypoxylon* rather than *Annulohypoxylon* based on the soft-textured stromata and ascospores with transversally dehiscent and striate perispores that lack a dorsal thickening

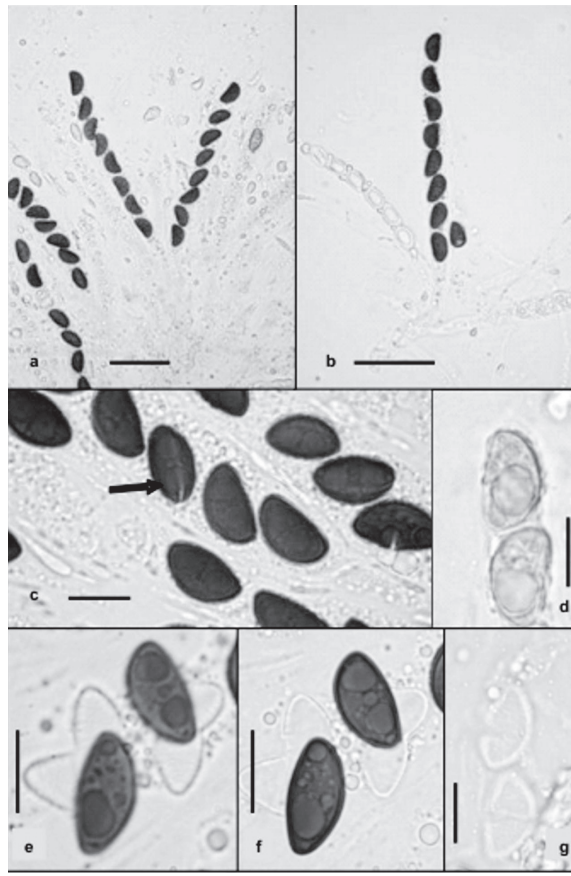

Fig. 9. Microscopic characteristics of *Hypoxylon gibriacense*, from holotype (LIP). a, Asci in chlorazol black. b. ascus in water. c Ascospores in water. d. Ascus tip in Melzer's reagent e, f. Ascospores in KOH at different focuses, showing dehiscent ornamented perispores. g. Free perispores. Scale bars: a b:. 30 μm, c- g: 10 μm

(Fig. 9). The inconspicuous glomerate stromata with conspicuous perithecial elevations and thick subperithecial tissue, the presence of yellow granules beneath the surface yielding red brown pigments in KOH, the lack of ascal apical rings, and the conspicuously striate perispores make a combination of characters not known from any temperate or tropical taxon of *Hypoxylon*.

Of the known European (and Northern temperate) taxa, *H. fraxinophilum* (Pouzar 1972) appears most similar. The stromatal, ascal, and ascospore morphology of this fungus are reminiscent of *H. gibriacense*. As previously shown by Stadler et al. (2004, as "*H. intermedium*"), *H. fraxinophilum* also contains hypomiltin (**5**), but neither rubiginosin C (**6**) nor BNT (**1**) were

detected in its stromata. Furthermore, it differs in its stromata lacking ostiolar disks, and in its host specificity for *Fraxinus*, rather than *Acer*. The new species is, however, remarkably similar to *H. addis* (see below) in having ostiolar discs, similar pigment colors in KOH, and its asci lacking an amyloid apical apparatus. *Hypoxylon gibriacense* and *H. addis* differ in their ascospore dimensions and in the merely faintly striate perispores in *H. addis;* moreover, the stromatal secondary metabolite profiles of the two species differ completely.

Hypoxylon addis J. Fourn., M. Stadler & U. Lindem., **sp. nov.**

MycoBank MB 516751 Figs 10–11

A Hypoxylon shearii *et* Hypoxylon fraxinophili *differt discis annulatis conspicuis, peritheciis ambientibus. A* Hypoxylon gibriacense *differt in ascosporae maiorae.*

Type: ETHIOPIA: Giyon/Wolisso, Negash Lodge, 2000m, +8° 32' 1.73", +37° 58' 52.65", on a corticated dry twig of *Croton sylvaticus* (*Euphorbiaceae*). 3.X.2009, U. Lindemann, JF-09302 (**Holotype**– LIP).

Etymology: Ethiopian "Addis", meaning "new".

Stromata (Fig. 10) corticolous, scattered, glomerate-pulvinate, erumpent through the periderm, 1–3 mm diam × 1–1.2 mm thick, soft-textured; surface Vinaceous Buff (86) to dark Brick (60), pruinose, with perithecial contours exposed to strongly exposed, at times rosellinioid; dull yellow granules beneath the surface and between the perithecia yielding Luteous (12) to Orange (7) pigments in 10% KOH; subperithecial tissue brownish with black streaks, 0.3–0.5 mm thick. Perithecia subglobose, 0.5–0.55 mm diam. Ostioles umbilicate, most often opening at the centre of a raised disc ca. 0.35 mm diam. Asci (Fig. 11) unitunicate, cylindrical, 170–190 µm total length × 9.5–10.5 µm broad, the spore-bearing parts 85–100 µm long, the stipes 70–90 µm long, easily broken, apex without apical ring, not bluing in Melzer's reagent. Paraphyses filiform, copious. Ascospores (Fig. 11) 13–16.5 × 6–7.7 µm (M = 14.6 × 7 µm, n = 20), ellipsoid-inequilateral with narrowly rounded ends, one side flattened to often slightly concave, dark to blackish brown, smooth, with spore-length straight germ slit. Perispore dehiscent in KOH, striate, with striae visible in brightfield microscopy but inconspicuous, epispore smooth.

No cultures obtained. Anamorph not seen.

Secondary metabolites: Surprisingly, the HPLC profile of the stromata of *H. addis* did not reveal any known metabolites of *Hypoxylon* or other *Xylariaceae* that we have characterized or observed in the past. As shown in Fig. 14, the stromatal extract contained a predominant peak with a rather characteristic chromophore. Another, presumably related, minor component showing a highly similar DAD spectrum was observed at a lower Rt. A search in the HPLC library used for dereplication of natural products in crude extracts that represents several thousands of pure compounds (Bitzer et al 2007) revealed

that the prevailing stromatal metabolite of *H. addis* corresponds to lecanoric acid (**16**). The DAD and MS spectra and the Rt of lecanoric acid (**16**) are depicted in Fig. 14.

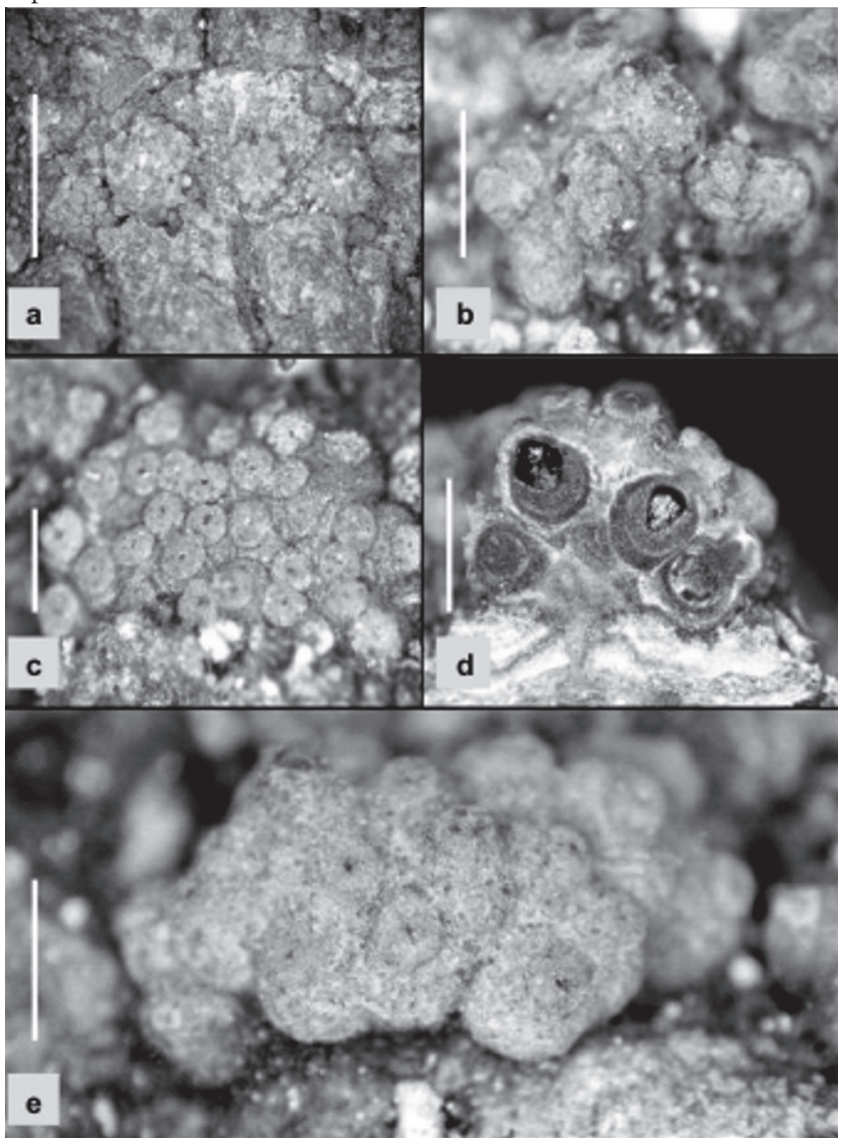

Fig. 10. Stromata of *Hypoxylon addis*, from holotype (LIP). a. Stromatal habit on the natural substrate. b, e. Close-up of stromatal surface, showing rosellinioid perithecial mounds. c. Surface of a glomerate stroma, showing characteristic ostiolar disks. d. Section through stroma, showing perithecia. Scale bars: a. 5 mm, b: 0.5 mm, c, d, e: 1 mm.

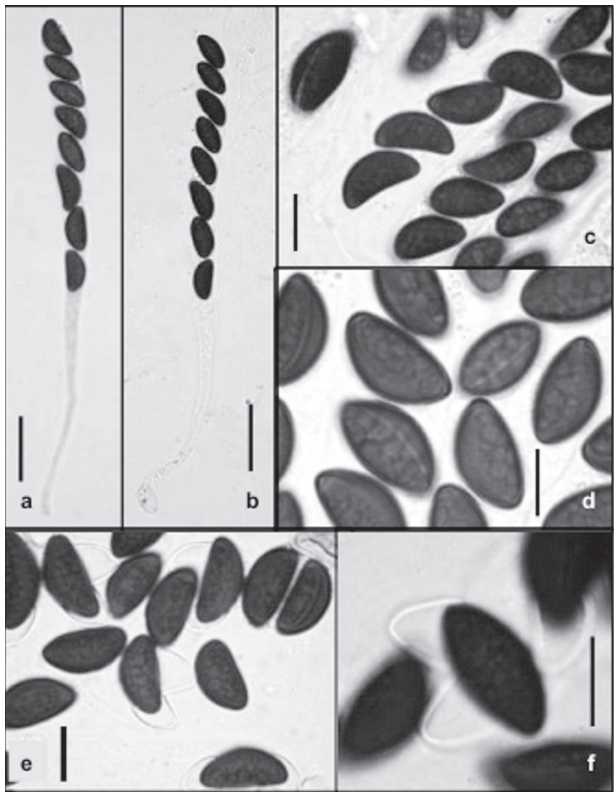

Fig. 11. Microscopic characteristics of *Hypoxylon addis* from holotype (LIP). a, b Asci in chlorazol black. c Ascospores in water. d-f Ascospores in KOH, showing germ slit (d) and dehiscent perispore. Scale bars: a, b: 20 µm, c, d, e, f: 10 µm.

Material studied for comparison (Fig. 12): **USA**: **Louisiana**, **East Baton Rouge Parish**, corticated wood of *Quercus*, IV.1980, J.D. Rogers & J.P. Jones (WSP 69637 – holotype of *H. shearii*).

Comments: *Hypoxylon addis* is distinctive in its small glomerate stromata with large discoid ostioles and microscopically in its asci lacking an apical ring and rather large, dark-colored ascospores with a faintly striate perispore. The collector stated that he also found this species on a dry twig of *Cordia africana*, but that specimen was moldy and needed to be discarded. From a comparison of teleomorphic characters, *H. shearii* Y.M. Ju & J.D. Rogers (Ju & Rogers 1996) appears most similar with respect to its stromatal and ascospore morphology and the color of its stromatal pigments. The type specimen of *H. shearii* was studied for comparison (Fig. 12) and as previously reported (Stadler et al. 2008), its HPLC profile revealed mitorubrins as well as

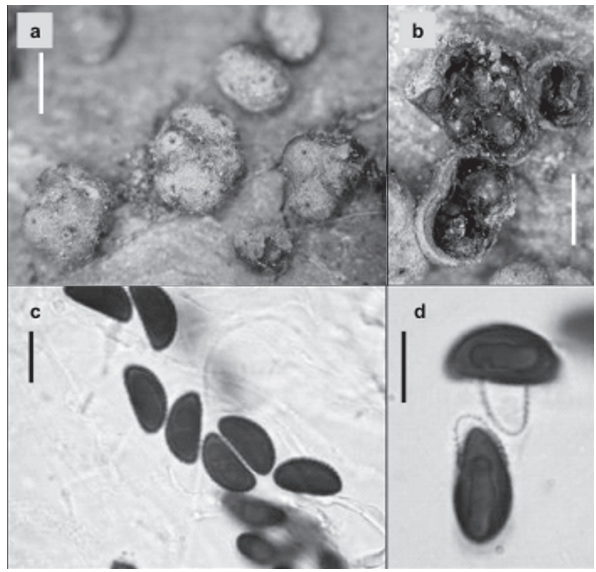

Fig. 12. Morphological characteristics of *Hypoxylon shearii*, from holotype (WSP). a. Stromata. b. Sectioned stromata showing yellow granules and globose perithecia. c. Ascospores in water. d. Ascospores in KOH, showing dehiscent perispore. Scale bars: a,b: 1 mm, c, d: 10 µm.

rubiginosins, all of which are absent in *H. addis*. The new species also differs in having conspicuous raised discs around the ostioles and microscopically in having larger ascospores (13–16.5 × 6–7.7 µm vs. 12–14 × 5.5–6.5 µm in *H. shearii*) with much less conspicuous ornamentation on the perispore. We have not yet studied authentic material of *H. shearii* var. *minor* F. San Martín et al. (1999), which differs from the typical variety by having smaller ascospores, 7–8 × 3.5–4 µm. Interestingly, both varieties of *H. shearii* have been collected thus far exclusively from *Quercus*.

One of the most intriguing features encountered in *H. addis* is the stromatal pigment profile, almost exclusively revealing lecanoric acid. This molecule is widely distributed in lichenized ascomycetes (Huneck 2001 and references cited therein) but has so far not often been encountered in non-lichenized fungi. According to our knowledge, the present study reveals lecanoric acid from a member of the *Xylariaceae* for the first time. Our retrospective analysis of the previously recorded HPLC profiling data in our *Xylariaceae* metabolite library confirms that lecanoric acid has indeed not been detected as major component of any of the previously studied 3500 specimens of *Hypoxylon*, including the majority of currently accepted taxa and their type specimens.

Lecanoric acid is formally derived from condensation of two molecules of orsellinic acid (**7**), which is widespread in the *H. rubiginosum* complex

as well as in the *H. fragiforme* group (Stadler et al. 2008). All the ubiquitous molecules of the mitorubrin, rubiginosin, and hypomiltin azaphilones contain an orsellinic acid moiety, attached to the azaphilone core molecules by an ester bond. Accordingly, the free orsellinic acid was found in many of the corresponding stromatal extracts of the respective *Hypoxylon* spp. as major component. The azaphilone core moieties were not detected in *H. addis*, although its stromata showed similar pigment colors in KOH as many species of the *H. rubiginosum* complex. Therefore, *H. addis* might represent a rather derived member of *Hypoxylon*, which has early abandoned or never attained azaphilone biosynthesis and developed the specific pathway for lecanoric acid instead, in convergence to the *Lecanorales* and other lichenized taxa of *Ascomycota*. It should be interesting to compare this species using molecular phylogenetic data in order to assess its closest relatives. However, we have so far been unable to obtain viable cultures from the stromata.

Although lichenized ascomycetes have been studied intensively for secondary metabolites for over a century, with many taxa of *Hypoxylon* and allied *Xylariaceae* studied intensively for such compounds in the past decades, there are not many examples for the parallel occurrence of the same compound

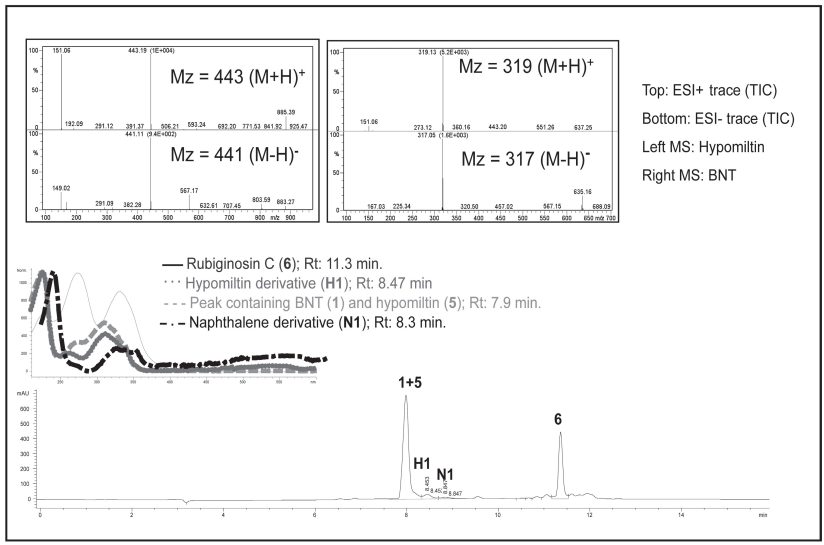

Fig. 13. Stromatal HPLC-UV profile (210 nm) of the stromatal methanol extract derived from the holotype specimen of *Hypoxylon gibriacense*, including DAD and ESI-MS spectra of some major metabolites. Rubiginosin C (**6**), a peak containing hypomiltin (**5**) overlaid by BNT (**1**), and other yet unidentified derivatives of hypomiltin (**H1**) and BNT (**N1**) were the major detectable components.

classes in both groups. However, the major stromatal constituents of *H. aeruginosum* J. H. Mill. and other *Xylariaceae* featuring blue or green stromatal surfaces have been recently identified as derivatives of the lichen constituent, lepraric acid (Læssøe et al. 2010).

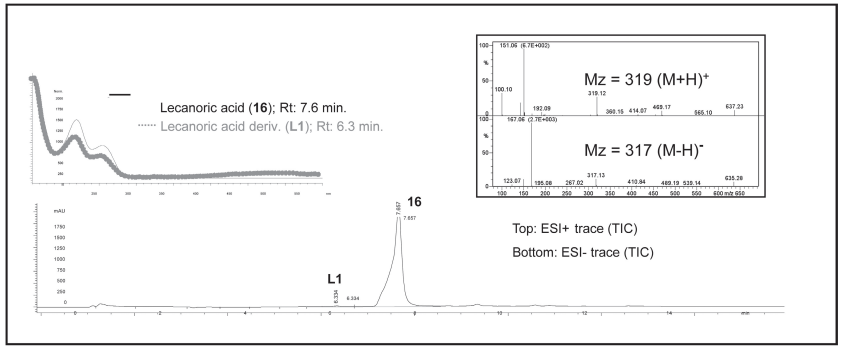

Fig. 14. HPLC-UV profile (210 nm) of the stromatal methanol extract derived from the holotype specimen of *Hypoxylon addis*, including DAD and ESI-MS spectra of some major metabolites. Lecanoric acid (**16**) was clearly the major detectable component, accompanied by a derivative (**L1**), but no known metabolites of *Hypoxylon* were detected

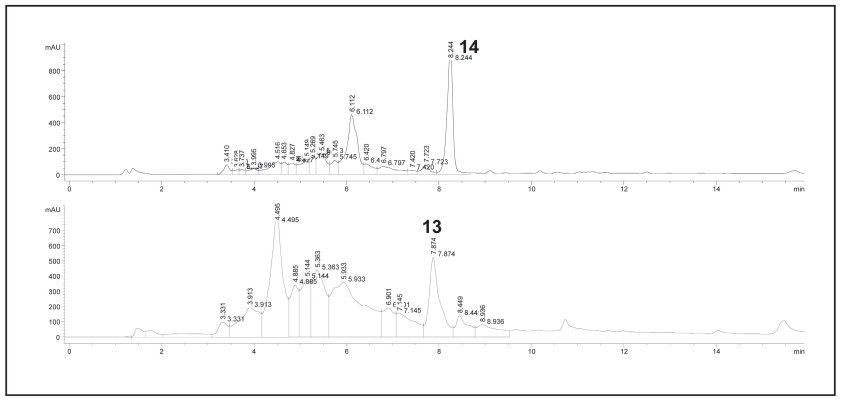

Fig. 15. HPLC-UV profile (210 nm) of the ethyl acetate extracts prepared from YMG cultures of the ex-holotype strains of *Hypoxylon fuscoides* and *H. gibriacense* after 8 days of cultivation, according to Bitzer et al. (2008). The HPLC profile of *H. fuscoides* (above) revealed .5-methylmellein (**14**) as major component, while *H. gibriacense* (below) produced mellein (**13**) and a series of other, mostly unknown compounds. The HPLC profile of *H. lusitanicum* (data not shown) closely resembled that of *H. fuscoides* and, therefore, most other members of the *H. fuscum* /*H. rubiginosum* complexes so far studied.

An updated key to European species of *Hypoxylon*

We found it practical to update our key to the species of *Hypoxylon* that have so far been encountered from regions that politically or geographically belong to Europe. This key is based on the one published by Stadler et al. (2004) but taking new results on the chorology of the species into account. In addition, the key incorporates recently published species (Stadler et al. 2008) as well as those newly described in the present study.

Species of *Annulohypoxylon* (formerly regarded as *Hypoxylon* sect. *Annulata* sensu Ju & Rogers 1996), however, have been expelled from the key published in 2004; for morphological characters and differences to *Hypoxylon,* see Hsieh et al. (2005). To safely identify hypoxyloid specimens with papillate ostioles [*A. cohaerens* (Pers.) Y.M. Ju et al. = *H. cohaerens* (Pers.) Fr.; *A. minutellum* (Sydow & P. Sydow) Y.M. Ju et al. = *H. cohaerens* var. *microsporum* J.D. Rogers & Cand.; *A. multiforme* (Fr.) Y.M. Ju et al. = *H. multiforme*(Fr.) Fr.] and with ostioles encircled by a disk [*A. michelianum* (Ces. & De Not.) Y.M. Ju et al. = *H. michelianum* Ces. & De Not.; *A. stygium* var. *annulatum* (Rehm) Y.M. Ju et al. = *H. stygium* var. *annulatum* (Rehm) Y.M. Ju & J.D. Rogers], a comparison with certain *Annulohypoxylon* species keyed by Stadler et al. (2004) as *Hypoxylon* therefore remains indispensable.

1 Mature stromata carbonaceous, black, without KOH-extractable pigments [but immature stromata orange, with Dark Purple (80) to Dark Vinaceous (82) pigments]. Ascospores 9.5–11.5 × 4–5.5 µm. (USA, France) ..... ***H. submonticulosum*** Y.M. Ju & J.D. Rogers

1 Mature stromata waxy to woody, not carbonaceous, colored other than black and with KOH-extractable pigments **2**

2(1) Stromata hemispherical to almost spherical **3**

2 Stromata effused to pulvinate **6**

3(2) Stromatal surface Vinaceous Grey (116), Sepia (63) or Grayish Sepia (106), KOH-extractable pigments Pure Yellow (14), Greenish Yellow (16) or Citrine (13), ascospores 17–22 × 9–11 µm; on *Fraxinus* ..... ***H. fraxinophilum*** Pouzar

3 Stromatal surface Rust (39), Bay (6), or Dark Brick (60), KOH-extractable pigments orange (7), ascospores averaging less than 15 µm long **4**

4(3) Ascal apical ring present, amyloid; widespread **5**

4 Ascal apical ring absent; monotypic, not recorded since 1867, ascospores 9–11 × 4.5–5.5 µm ..... ***H. commutatum*** Nitschke

5(4) Mainly on *Fagus*; ascospores 11–13.5 × 5–6.5 µm ..... ***H. fragiforme*** (Pers.: Fr.) J. Kickx f.

5 On other hosts, rarely on *Fagus*; ascospores 7–9 × 3.5–5 µm ..... ***H. howeanum*** Peck

6(2) Stromatal surface with Purple (35) or Vinaceous (57) colors. **7**

6 Stromatal surface with Orange (7), Rust (39), Brick (60), or brown colours . . . **17**

6 Stromatal surface with a greenish tone, Isabelline (65), without visible colored granules beneath surface but with Fawn (87) to dilute Umber (9) KOH-extractable pigments; ascospores 11–12.5(–13.5) × 6–6.5 µm, ellipsoid-equilateral with straight germ slit, perispore indehiscent in 10% KOH (M.S. & J.F.; unpublished data on specimen in K collected in Poland; identified by Z. Pouzar). . . . . .***H. papillatum*** Ellis & Everh.

7(6) Ascospores averaging more than 20 µm long. **8**

7 Ascospores averaging less than 15 µm long **9**

8(7) KOH-extractable pigments Pale Vinaceous Grey (115) to Vinaceous Grey (116) in fresh specimens or absent in aged specimens; ascospores 18.5–23 × 8–10 µm with germ slit spore-length . . . . .***H. vogesiacum*** (Pers.) Sacc.

8 Boreal distribution, frequently on *Salix*, KOH-extractable pigments dense, Greenish Olivaceous (90); ascospores 22–31 × 8.5–11µm with faint germ slit less than spore-length . . . . . ***H. macrosporum*** P. Karst.

9(7) KOH-extractable pigments Livid Purple (81) or absent **10**

9 KOH-extractable pigments pigments Orange (7) to Sienna (8). **11**

9 KOH-extractable pigments Amber (47), Isabelline (65), Olivaceous (48), Gray Olivaceous (107), Greenish Olivaceous (90), Citrine (13) or otherwise with yellow, green or brown tones. **13**

10(9) Stromata effused, 0.4–0.5 mm thick, with KOH-extractable pigments dilute, Livid Purple (81) or absent; ascospores 8–11.5 × 4.5–5 µm with a straight germ slit. Distribution world-wide; major stromatal metabolites: carneic acids (Quang et al. 2006) . . . . . .***H. carneum*** Petch

10 Stromata pulvinate, 0.8–1.4 mm thick, with KOH-extractable pigments Vinaceous Purple (101); ascospores 9.5–12.5 × 5–6 µm with a sigmoid germ slit (France, UK, present study) . . . . . .***H. fuscoides***

11(9) Ascospores 11–13.5 × 5–7 µm, with perispore faintly striate by LM; known from Portugal (present study) . . . . . ***H. lusitanicum***

11 Ascospores 9.5–11.5 × 4.5–6 µm, with perispore smooth by LM **12**

12(11) Stromata widely effused with jagged black margins; ascospores 9–11.5 × 5–6 µm; host preference for *Fraxinus*; Temperate Europe and USA (Stadler et al. 2008) . . . . . ***H. petriniae*** M. Stadler & J. Fourn.

12 Stromata less widely effused to effused-pulvinate, with concolorous margins; ascospores 9.5–11.5 × 4.5–5 µm; known from the Canary Islands (Stadler et al. 2008) . . . . . .***H. canariense*** J. Fourn. et al.

13(9) Perithecia obovoid to frequently tubular, up to 1 mm high; stromatal surface with a metallic shine when mature. Recorded from Central and Western Europe and North America (various hosts). Ascospores 9.5–11.5 × 4–4.8 µm . . . . . .***H. macrocarpum*** Pouzar

13 Perithecia spherical to obovoid, not tubular; stromatal surface lacking a metallic shine when mature **14**

14(13) Ascospores with straight germ slit. **15**
14 Ascospores with slightly sigmoid germ slit. **16**

15(14) Ascospores ellipsoid-inequilateral in lateral view, 9–12 × 4–6 µm, perispore dehiscent in 10% KOH. KOH-extractable pigments Amber (47), Greenish Yellow (16) or Citrine (13) . . . . . ***H. perforatum*** (Schwein.) Fr.
15 Ascospores ellipsoid, nearly equilateral in lateral view, often pyriform, 12–15 × 5.5–7 µm, perispore indehiscent in 10% KOH. KOH-extractable pigments Olivaceous (48), Greenish Olivaceous (90), Gray olivaceous (127), or Olivaceous Gray (121). So far known from Austria, Germany, Slovakia, and North America. . . . . ***H. fuscopurpureum*** (Schwein.) M.A. Curtis

16(14) Stromata with pure yellow (14) to luteous (12) granules and greenish olivaceous (90) KOH-extractable pigments; apparently restricted to *Quercus*, with a boreal distribution; ascospores 10–13.5 × 4–5 µm. So far known from France (Stadler et al. 2004), Scandinavia (Granmo 1999) and USA (Stadler et al. 2008) . . . . . ***H. porphyreum*** Granmo
16 Stromata with sienna (8) or otherwise orange brown granules and KOH-extractable pigments amber (47), isabelline (65), olivaceous (48), gray olivaceous (107), or greenish olivaceous (90); widespread, preferably on *Betulaceae* and other hosts, but not yet safely recorded from *Quercus*; ascospores 11–16 × 5–8 µm . . . . . ***H. fuscum*** (Pers.) Fr.

17(6) Young stromata with a bright yellow to orange fimbriate margin; perithecia small, 0.1–0.3 mm diam, seated on a well developed black basal tissue **18**
17 Young stromata lacking a bright yellow to orange fimbriate margin. **19**

18(17) Ascospores 5–7 × 2.5–3.5 µm, ellipsoid-inequilateral in lateral view. So far recorded from Austria, Croatia, Germany (Bitzer et al. 2008). France, Italy, Slovakia (Ripková & Hagara 2003) and Switzerland . . . . . ***H. ticinense*** L.E. Petrini
18 Ascospores 8–11 × 4–5 µm, ellipsoid-equilateral in lateral view. So far recorded from France and USA. . . . . ***H. subticinense*** Y.M. Ju & J.D. Rogers

19(17) KOH-extractable pigments yellow or orange. **20**
19 KOH-extractable pigments with shades of olivaceous brown **33**

20(19) Ascal apical ring highly reduced or lacking, inamyloid **21**
20 Ascal apical ring present, amyloid. **23**

21(20) Stromata glomerate with ostioles encircled by a ring; ascospores 11.5–13 × 6–6.8 µm with perispore conspicuously striate by LM (France, present study) . . . . . ***H. gibriacense***
21 Stromata applanate to pulvinate, with ostioles lacking a ring; ascospores with perispore smooth by LM **22**

22(21) Stromata discoid, encircled with a swollen stellate margin, on bark of *Fraxinus*; ascospores 9.5–12 × 5–6 µm. Europe and North America . . . . . ***H. cercidicola*** (Berk. & M.A. Curtis ex Peck) Y.M. Ju & J.D. Rogers
22 Stromata pulvinate to hemispherical, reported from *Carpinus*, ascospores 9–11 × 4.5–5.5 µm (see also **4**). . . . . ***H. commutatum*** Nitschke

23(20) Ascospores averaging more than 14 µm long **24**
23 Ascospores averaging less than 12 µm long **25**

24(23) Stromata pulvinate restricted at base, with rust brown to ochraceous brown granules beneath surface; apparently rare, known only from *Tilia* and *Sorbus*; ascospores 14–17 × 6.5–8 µm, Recorded from Switzerland (Petrini & Müller 1986), Slovakia and Canada (Stadler et al. 2008) . . . . . ***H. ferrugineum*** G. H. Otth
24 Stromata effused to pulvinate, with blood red granules beneath surface, recorded from various hosts; ascospores 15–18 × 6–7.5 µm . . . . . ***H. julianii*** L.E. Petrini

25(23) KOH-extractable pigments Amber (47), Greenish Yellow (16) or Citrine (13); ascospores 9.7–11.5 × 4.7–5.3 µm (see also **15**) . . . . . ***H. perforatum*** (Schwein.) Fr.
25 KOH-extractable pigments Orange (7), Sienna (8), Rust (39) or Scarlet (5) . . . **26**

26(25) Ascospores almost equilateral in lateral view. **27**
26 Ascospores inequilateral in lateral view **28**

27(26) Ascospores 7–10 × 3–4.5 µm; stromatal surface dark rust (39) to sepia (63), with dark orange granules beneath surface and KOH-extractable pigments fulvous (43) to rust (39); recorded from *Salix* in Northern Europe (Granmo 1999), Belgium (J.F. & M.S., unpublished data), and USA (Ju & Rogers 1996, as unnamed segregate in "Notes to *H. rubiginosum*") . . . . . ***H. salicicola*** Granmo
27 Ascospores 9.5–12.5 × 4.8–6 µm; stromatal surface vinaceous buff (86), greyish sepia (106) to brown vinaceous (84) with bright yellow granules beneath surface and between perithecia; KOH-extractable pigments hazel (88), sienna (8) to umber (9). Apparently restricted to *Sorbus*, with a boreal distribution (Granmo 2001) . . . . . ***H. liviae*** Granmo

28(26) Ascospores averaging less than 11 µm long **29**
28 Ascospores averaging more than 11 µm long. **31**

29(28) Stromata with papillate ostioles. **30**
29 Stromata with umbilicate ostioles . . . . . ***H. rubiginosum*** (Pers.) Fr.

30(29) Stromata erumpent, pulvinate, small, with orange granules beneath surface; known from Europe and USA, restricted to *Populus*; ascospores 8–10 × 3.5–4.5µm . . . . . ***H. laschii*** Nitschke
30 Stromata superficial, effused to pulvinate, with blood red granules beneath surface; distribution apparently world-wide, without apparent host specificity ascospores 7.5–10 × 4–4.8 µm . . . . . ***H. rutilum*** Tul. & C. Tul.

31(28) Perithecia up to 0.2 mm diam; ascospores 11–14.5 × 5–6 µm with slightly sigmoid germ slit; Canary Islands . . . . . ***H. urriesii*** J. Fourn. & M. Stadler
31 Perithecia 0.3–0.45 mm diam; ascospores with straight germ slit **32**

32(31) Ascospores 9.5–11.5 × 4.5–5 µm; known from the Canary Islands (see also **12**) . . . . . ***H. canariense*** J. Fourn. et al.
32 Ascospores 11–13.5 × 5–7 µm; known from Portugal (see also **11**) . . . . . ***H. lusitanicum***

33(18) KOH-extractable pigments Hazel (88), Sienna (8) to Umber (9); perithecia obovoid, up to 0.4 mm high; ascospores dark brown, ellipsoid, nearly equilateral, 9.5–12.5 × 4.8–6 µm; apparently restricted to *Sorbus* (see also **27**) . . . . . ***H. liviae*** Granmo

33 KOH-extractable pigments isabelline (65), umber (9), or grayish sepia (106); perithecia frequently tubular, up to 1 mm high; ascospores inequilateral and narrower, 9.5–11.5 × 4–4.8 µm (see also **13**). . . . . ***H. macrocarpum*** Pouzar

Acknowledgements

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