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## **The "red Hypoxylons" of the temperate and subtropical Northern hemisphere**

**Marc Stadler<sup>1,2</sup>, Jacques Fournier<sup>3</sup>, Alfred Granmo<sup>4</sup> and  
Esperanza Beltrán-Tejera<sup>5</sup>**

<sup>1</sup>University of Bayreuth, Dept. Mycology, Universitätstraße 30, D-95447 Bayreuth, Germany; <sup>2</sup>InterMed Discovery GmbH, Otto-Hahn-Strasse 15, D-44227 Dortmund, Germany; <sup>3</sup>Las Muros, F-09420, Rimont, France;  
<sup>4</sup>Department of Natural Science, Tromsø University Museum, University of Tromsø, NO-9037 Tromsø, Norway;  
and <sup>5</sup>Departamento de Biología Vegetal (Botánica), Universidad de La Laguna,  
38071 La Laguna, Tenerife, Canary Islands, Spain.

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Corresponding author: Marc Stadler; marc.stadler@t-online.de. Accepted for publication December 11, 2007.  
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**Abstract:** Selected taxa of *Hypoxylon* from the Northern hemisphere were compared with numerous type and authentic specimens of *H. fuscum*, *H. rubiginosum*, and presumably related taxa. Besides morphological analyses, we used secondary metabolite profiles based on high performance liquid chromatography, coupled with diode array detection and mass spectrometry (HPLC-DAD/MS). Chemotaxonomic studies on the Nitschke and Persoon types of the above names and further ancient type specimens turned out to be rather conclusive. Along with the information provided in the world monograph of *Hypoxylon* by Ju and Rogers, the results of our HPLC profiling studies are regarded as key asset to provide a better overview on the diversity and biogeography of these species complexes. The utility of this approach is demonstrated by a study on *Hypoxylon* in the Canary Islands. From a

comparison of morphological and chemical traits with the above mentioned material, we recognize two new species (*H. canariense* and *H. urriesii*). The predominantly tropical *H. anthochroum* and *H. subrutulum* were also found from this archipelago, but representatives of these taxa from different parts of the world showed heterogeneous HPLC profiles. The number of accepted species in *Hypoxylon* might increase substantially, once an inventory of their tropical representatives, based on holomorphic morphology and considering the importance of their stromatal extrolites, has been completed.

**Key words:** Biogeography, chemotaxonomy, evolution, fungal pigments, Xylariaceae, Xylariales.

**Introduction:** The monograph of the late Julian H. Miller on *Hypoxylon* (Miller 1961) was instrumental, because his taxonomic concepts provided the basis for modern taxonomy of the hypoxylid Xylariaceae. From several thousands of specimens, Miller (1961) evaluated the morphology of the teleomorph, emphasizing features such as stromatal morphology and color, ostioles, and the size of asci and ascospores. Continuous studies of this fungal group revealed other characters such as shape and dehiscence of perispores, morphology of germ slits, spore surface structures (as assessed by light microscopy and scanning electron microscopy), and stromatal pigments to be useful for species discrimination. Several of his broad species concepts were meanwhile further resolved, and the genus rearranged (Hsieh et al. 2005; Ju et al. 1998, 2002; Læssøe et al. 1989; Pouzar 1979, 1985a, 1985b; Rogers and Ju 1998). Pioneering studies on anamorphs of *Hypoxylon sensu lato* were performed by, e.g., Greenhalgh and Chesters (1968), Jong and Rogers (1972), Petrini and Müller (1986), and van der Gucht (1995). Ju and Rogers (1996) provided for the first time a comprehensive revision of this genus, based on holomorphic morphology. They traced and studied numerous type specimens, and ultimately provided a "List of Names" that is most helpful to everybody who wishes to study xylariaceous taxa in future. For the first time they studied stromatal pigments of a representative number of specimens including all available type material. In 10% potassium hydroxide (KOH) soluble stromatal pigments are now a standard

diagnostic tool to identify *Hypoxylon* species. Numerous new species were recognized from this approach, and subsequent additions. Ju et al. (2004) provided an update on the taxonomy of the genus, listing all additional taxa that were published since 1996.

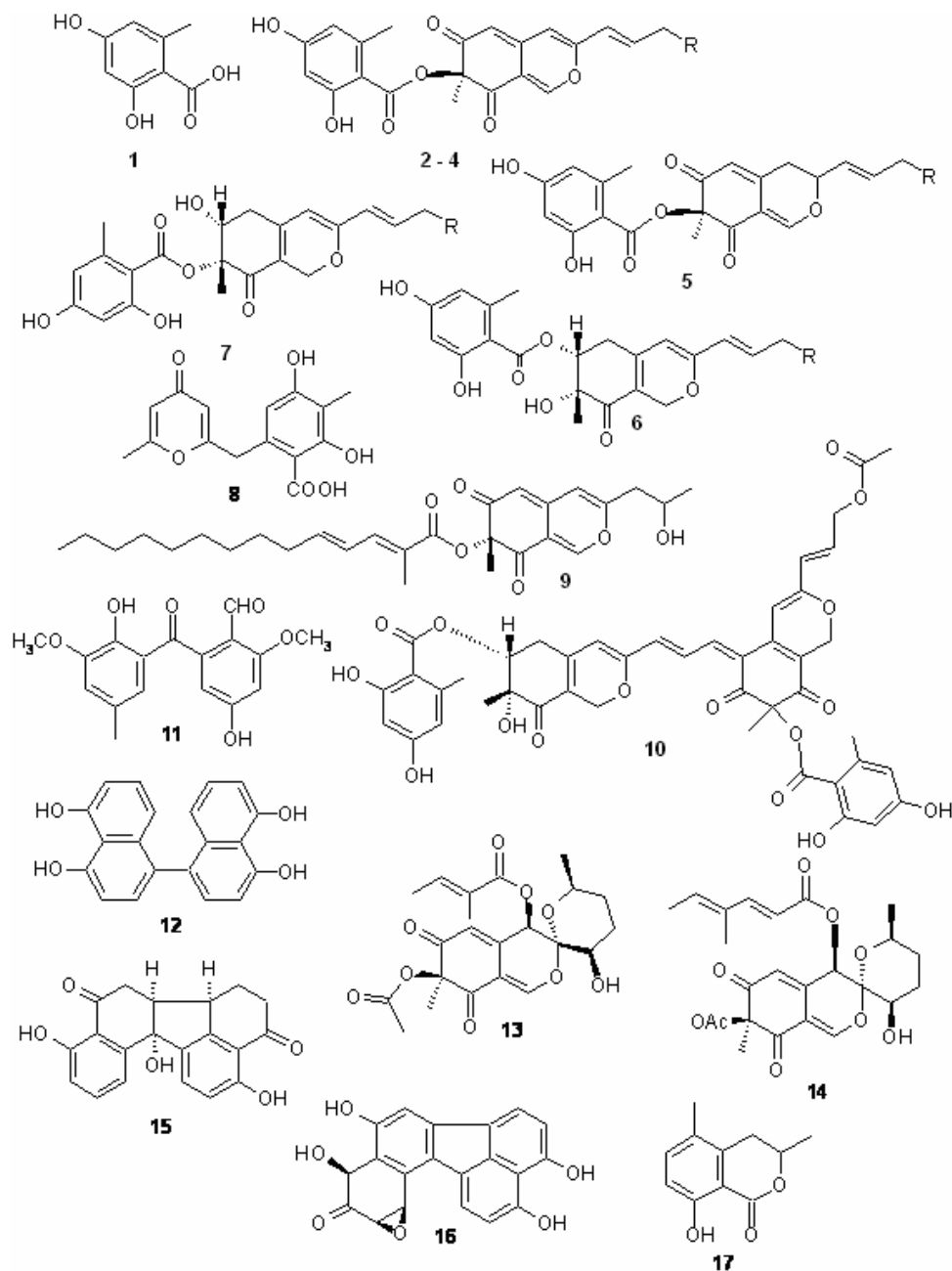
In addition, high performance liquid chromatography (**HPLC**) and other methods of analytical chemistry were carried out in conjunction with the methods deemed diagnostically helpful by Ju and Rogers (1996). This resulted in the identification of various stromatal pigments that are common in *Hypoxylon* and allies, or even specific for certain groups as seen for taxa in the ***H. rubiginosum* complex** (Stadler et al. 2004a, Hellwig et al. 2005). Several of these metabolites were isolated to purity and identified by spectral methods. Standards of the pure compounds now facilitate their detection in crude extracts of other specimens. The distribution of stromatal pigment classes (Quang et al. 2005a, Stadler and Fournier 2006b) in *Annulohypoxylon* (Hsieh et al. 2005; Ju and Rogers 1996 as *Hypoxylon* sect. *Annulata*, resp.) agreed also well with morphological data. Even in an ancient specimen, such as the type specimen of *Sphaeria cohaerens* Pers. (Quang et al. 2005a), stromata of *Daldinia* Ces. & De Not. (Stadler et al. 2001), and several other genera of Xylariaceae (*Entonaema* A. Möller, *Phylacia* Lev., *Rhopalostroma* D. L. Hawksw., *Sarcoxylon* Cooke, and *Thamnomycetes* Ehrenb.; see Stadler et al. 2004c) chemotypes as revealed by HPLC

profiling proved to be consistent in recently collected material and old herbarium specimens, even if the latter had been collected over 200 years previously. If deviations in the chemotypes were observed, those were frequently also accompanied by deviating morphological characters. As an exception, some species may show a successive production of different metabolite family during stromatal ontogeny (Stadler et al. 2006a). However, even such metabolite profiles appear to be quite species-consistent and only dependent on the stage of stromatal development in a given species, rather than on external, environmental factors, and extremely consistent in fully mature material (i.e., the type specimens!). The above results demonstrated the utility of chemotaxonomic methods to revise herbarium specimens, not only in Xylariaceae.

Nonetheless, in the holomorphic species concept applied by Ju and Rogers (1996), it is important to study also material that can be cultured and examined for anamorphic traits. In fact, Ju and Rogers (1996) described the majority of the cultured specimens from fresh material collected in Mexico, Taiwan, and the USA. However, the types of many names in *Hypoxylon* were frequently collected from other geographic regions than the cultured material studied in the latter monograph. Except for some of the most common species, a few cultures of *Hypoxylon* that were identified according to the modern taxonomy are available in public collections. Corresponding herbarium material of strains deposited earlier on in these collections is often missing. Moreover, most herbarium specimens of *Hypoxylon* have never been revised using the modern taxonomic concept. Examples are *H. anthochroum* Berk. & Broome and *H. fusco-purpureum* (Schwein. : Fr.) Fr., which were treated as synonyms of *H. rubiginosum* by Miller (1961). Ju and Rogers (1996) showed that they have close affinities to *H. fuscum* (Pers. : Fr.) Fr. HPLC profiling studies including numerous type and authentic specimens of species with red or

purple pigments, many of them former synonyms of *H. rubiginosum* or *H. fuscum* (Miller 1961), from the temperate and subtropical Northern hemisphere, aimed to establish further correlations between morphological and chemotaxonomic traits. An overview is provided here.

The above mentioned method also proved useful to study *Hypoxylon* in the Canary Islands (IC – Islas Canarias). The biota of these islands were not affected by the Quaternary ice ages. They have been isolated from the large continents since the opening of the Atlantic ocean between Africa and America in the mid-Tertiary. Besides the contributions to the Ascomycetes in the IC by the mycological department of the University of La Laguna (Beltrán-Tejera and Wildpret 1975; Bañares Baudet et al. 1986; Beltrán-Tejera et al. 1987; Bañares Baudet et al. 1987; Beltrán-Tejera et al. 1989; Bañares Baudet et al. 1991; Beltrán-Tejera et al. 2003; Beltrán Tejera et al. 2004; Beltrán-Tejera et al. 2008, in press), the most exhaustive studies on macaronesic discomycetes have been made by Richard P. Korf and his collaborators (Korf 1978; 1981 a,b,c; 1992; Korf et al. 1978; Ouelette and Korf 1979; Greenleaf and Korf 1980; Dissing and Korf 1980; Korf and Zhuang 1991 a, b, c, d, e, f, g; Iturriaga 1995; Iturriaga and Korf 1997, 1998; Lizon et al. 1998; etc.). These publications included several taxonomic revisions and even a large number of taxa new to Science. More recently, new species have been described for *Hyphodiscus* (Galán and Raitviir 2004), and *Orbilina* (Baral and Marson in Karasch et al. 2005), and some records of new species of anamorphic fungi (Castañeda Ruiz et al. 1996, 1997). In fact, IC and other Macaronesian islands (Azores, Madeira, Cabo Verde) are well-known to harbor a large number of endemic plants (Francisco-Ortega et al. 2000). At the present time there are around 320 catalogued species of Ascomycota for the IC (Beltrán-Tejera 2004). This number has about the same dimension as the number of species we estimate to be extant world-wide in *Hypoxylon* alone, suggesting that Xylariaceae and many of



**Fig. 1.** Chemical structures of characteristic pigments and other metabolites of the *H. rubiginosum* complex and the *H. fuscum* complex **1**: orsellinic acid; **2**: mitorubrin (R = H); **3**: mitorubrinol (R = OH); **4**: mitorubrinol acetate (R = OCCH<sub>3</sub>); **5**: hypomiltin (R = OCCH<sub>3</sub>); **6**: rubiginosin A (R = OCCH<sub>3</sub>); **7**: rubiginosin B (R = OCCH<sub>3</sub>); **8**: Macrocarpon A; **9**: rubiginosin C; **10**: rutilin A; **11**: Daldinal A; **12**: Binaphthalene tetrol (BNT); **13**: daldinin C; **14**: daldinin E; **15**: Daldinone A; **16**: daldinone B; **17**: 5-methylmellein. All metabolites but compound **17** have so far only been found in stromata.

the important groups of plant-associated pyrenomycetes remain to be studied in-depth. *Daldinia* is the only Xylariaceae genus that has been treated using the taxonomic concepts as understood in this study (Stadler et al. 2004b).

The last published accounts of *Hypoxylon* in the IC date back to the time when Miller's monograph had been the standard identification work (Urries 1955, Bañares Baudet et al. 1987). With respect to floristic work on Xylariaceae,

even very recent papers are still based on literature that has been outdated several decades ago.

### Materials and Methods:

HPLC profiling and morphological studies were carried out in a similar manner as described by Stadler et al. (2004a, 2006a). The prominent metabolites were characterized using their retention times (Rt) in standardized HPLC systems in conjunction with their UV-visible spectra, recorded by diode array detection (DAD) and electrospray mass spectrometric (MS) detection. Preparative HPLC was carried out as described previously (Bitzer et al. 2008, Hellwig et al. 2005). Color codes follow Rayner (1970) and thus, also Ju and Rogers (1996). Anamorph types are used as defined by Ju and Rogers (1996), and if not indicated otherwise we use the names of *Hypoxylon* spp. as described in this monograph. SEM was conducted as described by Stadler et al. (2002). Specimens from the personal herbaria of Jack D. Rogers, J. Fournier, and M. Stadler are abbreviated thus: JDR, JF, and STMA. Duplicates of some of the STMA and JF specimens are also kept at the Fuhlrott-Museum, Wuppertal; all other herbaria are listed by Holmgren and Holmgren (1998) on the Internet.

## Results

### 1. Notes on the biogeography and secondary metabolite profiles of various species of the *H. rubiginosum* and *H. fuscum* complexes sensu Ju and Rogers

As mentioned in the Introduction, the species complex that was once lumped in *H. rubiginosum* sensu Miller (1961) and the *H. fuscum* group were not clearly distinguished from one another by Miller (1961), as he did not evaluate stromatal pigments in KOH, but used mainly spore size for discrimination. While *H. fuscum* and its allies have greenish, olivaceous, or isabelline pigment colors, due to the presence of

daldinins (*H. fuscum* chemotype), those of *H. rubiginosum* and allies are typically orange, orange-brown, or yellowish green, due to the presence of mitorubrin (*H. fragiforme* chemotype), rubiginosin (*H. rubiginosum* chemotype), or hypomiltin (*H. hypomiltum/perforatum* chemotype) pigments (cf. Stadler et al. 2004a; Hellwig et al. 2005 and comprehensive overviews by Stadler and Hellwig 2005a and Stadler and Fournier 2006b). *Hypoxylon macrocarpum* has olivaceous pigments but lacks the characteristic pigments of both the above groups. Instead it contains macrocarpones (Mühlbauer et al. 2002). These results were already based on over 200 specimens, refined here by the inclusion of additional material, including several type specimens that were not studied before, and various further species. Previously studied specimens are not listed here, but if not indicated otherwise, the previously obtained data are in concordance with those of the current study. Macrocarpones, hypomiltin, and rubiginosin derivatives have been reported from *Pyrenomyxa* (Stadler et al. 2005b), a cleistocarpous genus showing close affinities to *Hypoxylon*, as recently confirmed by molecular data (Bitzer et al. 2008). The latter study also included HPLC profiles of various *Hypoxylon* spp. in culture. Correlations reported by Whalley and Edwards (1995) regarding the production of dihydroisocoumarins and other secondary metabolites in the hypoxyloid Xylariaceae were thus verified for more than 150 cultures, using several different fermentation conditions.

Here, we also include new results on the species erected by Granmo (1999a, 2001), part of which have been treated on the Internet (Fournier and Magni 2004). Scientific names are used fide MycoBank ([www.mycobank.org](http://www.mycobank.org)), except that we followed the recommendations of the Botanical Code (Greuter et al. 2000) by including the sanctioning authors. Some predominantly tropical species of the *H. rubiginosum* complex are not treated here, but will be included in a separate study with emphasis on the neotropics.

Hsieh et al. (2005), Quang et al. (2006) and Bitzer et al. (2008) have recently shown that *H. carneum* Petch is also a member of the *H. rubiginosum* complex. For *H. fragiforme* (Pers. : Fr.) J. Kickx fil. and *H. howeanum* Peck, we refer to comprehensive treatments by Petrini and Müller (1986) and Ju and Rogers (1996). Stadler et al. (2006a, 2007) have studied the succession of their secondary metabolite production during stromatal ontogeny. The latter work revealed that a comparison of HPLC profiles should regard the state of development of individual specimens, which was also taken into account during interpretation of the HPLC data recorded here. With regard to the synonyms established by Ju and Rogers (1996) we have listed here only those cases where type or authentic material was studied, hence the lists are not complete as in an ordinary monograph. In case the synonyms were even confirmed by matching HPLC profiles in comparison to the type material, we have listed those names without any further comments. Question marks are posted before the names in case we obtained inconclusive results by HPLC profiling, or if secondary metabolite profiling suggested that polythetic studies based on fresh material will eventually reveal the presence of additional taxa.

***Hypoxylon anthochroum* Berk. & Broome [MB209615]**

**Confirmed synonyms:** *H. albstigmatosum* Speg. [MB213085]; *H. fuscopurpureum* (Schwein. : Fr.) M. A. Curtis f. *corticola* Starbäck [MB443846]; *H. guarapiense* Speg. [MB155263]. This species was first described from Sri Lanka, and said to have a wide distribution in warmer climates (Ju and Rogers 1996). It was also reported from America, Africa, and New Zealand in the latter monograph, but has so far not been encountered in the geographic region that politically belongs to Europe. However, two specimens in TFC from IC keyed out as *H. anthochroum* and even showed similar HPLC profiles as the lectotype specimen. Miller (1961) considered *H. anthochroum* as conspecific with

*H. rubiginosum*, because of its ascospore size and gross stromatal morphology. HPLC profiling confirmed the view of Ju and Rogers (1996), who accepted *H. anthochroum* as a species, suggesting that it is more closely related to *H. fuscum*. In accordance with the lack of orange stromatal pigments in all specimens listed below, all of them were found devoid of rubiginosins, mitorubins and orsellinic acid. Daldinins and BNT were encountered in the type specimen from Sri Lanka, and in all specimens from America, New Zealand, and IC. The ascospore size range of the type specimen was ca. (10)-11-14 x 6-6.5 µm, i.e., within the upper limit of that reported by Ju and Rogers (1996). Some specimens showing deviating HPLC profiles had smaller spores in average or a narrower size range. The heterogeneity of morphological and chemical traits among the teleomorphs we studied suggests that this taxon is a complex species, as in the apparently related *H. fuscum* (see further below). The concept adopted by Ju and Rogers (1996) appears workable, since most specimens examined keyed out easily. We would rather like to point out some peculiarities that may prove more significant later on nevertheless: In the Mexican specimens San Martín 1055 and 1224B, we observed differences in the HPLC profiles, with daldinins E and F being absent and daldinin C being overlaid by unknown compounds. These two specimens also slightly deviated from typical material (including the type of *H. anthochroum*, all South American specimens listed below, and San Martín 1024a), in having larger perithecia (up to 400 µm diam vs. 280-325 µm diam in the type), and in having KOH pigments Amber (47) to Isabelline (65).vs. Sepia (63). Their ascospores were 10-11 x 5-5.5 µm, i.e., also smaller than in the type. Interestingly, both San Martín 1224a and 1055 were cultured by Ju and Rogers (1996) and reported to have the same *Nodulisporium*-like anamorph (in contrast to *H. fuscum* and *H. fuscopurpureum*, which have similar pigment profiles but show a *Virgariella*-like branching pattern). Notably, cultures from countries other than Mexico (and none from stromata showing

the deviating chemotypes described below) have so far not been obtained.

Material from Africa, classified as *H. anthochroum* by Ju and Rogers (1996) or as *H. hypomiltum* Mont. by Dennis (1963), respectively, also showed deviating HPLC profiles, apparently lacking daldinins. Specimen BR-Myc 035874,81 had Grey Olivaceous (107) pigments and contained BNT and macrocarpones, indicating that it constitutes an undescribed taxon with chemotaxonomic affinities to *H. macrocarpum*. The depauperate specimens BR-Myc 033583,21/GAM 12859 (from South Africa; det. as *H. anthochroum* by Ju and Rogers 1996), however, showed similar HPLC profiles to that of a specimen of *H. duranii* from the JDR herbarium reported on previously (Hellwig et al. 2005). All other specimens studied, including the second specimen from South Africa (GAM 12859) contained daldinins and BNT. Specimen BPI 592373 from *Fraxinus* in USA also keyed out as *H. anthochroum*, but had smaller ascospores (10-11 x 5-6 µm, with narrowly to broadly rounded ends) than the type material and other tropical collections. It might therefore still correspond to an undescribed taxon. The species complex comprising, among other taxa, *H. anthochroum*, *H. duranii*, and *H. hypomiltum* appears to be rather complicated; further results will be reported separately, based on a comparison of fresh materials from the tropics that is pending. As discussed below (*H. fuscopurpureum*), the affinities of *H. anthochroum* are with *H. fuscopurpureum*, rather than with *H. fuscum*, with regard to the similarity of their  $\alpha$ -actin and  $\beta$ -tubulin sequences (see Hsieh et al. 2005).

#### Specimens examined:

**Brazil:** Matto Grosso: Rosario, 15 May 1894, A. M. Lindman (S-F 44614, S-F44615; 2 packets - **holotype** of *H. fuscopurpureum* f. *corticola*. - **Democratic Republic of Congo** ("Zaire"): Leopoldville, Yindu, Oct. 1908, H. Vandereyst det. Dennis (1963) as *H. hypomiltum*, rev. Ju and

Rogers 1996 as *H. anthochroum* (BR-Myc 033583,21); Leopoldville, vicinity of Kisantu, 1907, H. Vandereyst, det. Dennis (1963) as *H. hypomiltum* (BR-Myc 035874,81). - **Mexico:** Quintana Roo state, San Felipe Bacalar, San Martín 1224A and 1224B (JDR); Oaxaca state, Temazcal, 7 Oct. 1988, San Martín 1055 (JDR). - **New Zealand:** Taupo: Kaimanawa Forest Park, Clements Rd., wood of *Nothofagus*, 27 Mar. 1992, P. R. Johnston G211 (PDD 60129); Buller: *Nothofagus*, 7 May 1994, P. D. Johnston (PDD70130). - **Paraguay:** Guarapi, Oct. 1878, B. Balansa 2781 (LPS- **lectotype**; BPI 738471; NY-**isoelectotypes**, selected by Shear 1945, of *H. albstigmatosum*); Guarapi, 29 July 1881, B. Balansa 2764; Fungi Guar. 199 (LPS- **holotype**; NY- **isotype** of *H. guarapiense*). - **South Africa:** Pietermaritzburg, Town Bush Valley, 1934, W. G. Rump 188, 28569 (GAM 12862); Boschfontein, near Wolhuter's Kop, wood of *Mimusops zeyheri*, 5 May 1938, E. M. Doidge & A. M. Bottomley 31068 (GAM 12859). - **Spain:** Canarias: La Palma, Puntallana, Barranco del Cubo de La Galga, laurisilva, *Ocotea foetens*, 28 Apr. 1989, E. Beltrán, J.L. Rodríguez-Armas & J. Leal (TFC Mic. 3636). - **Sri Lanka:** corticated wood, Nov. 1867, G. H. K. Thwaites 160, as *Sphaeria anthochroa* Berk. & Broome, ined. (K(M) 121849-**lectotype** selected by Miller 1961, of *H. anthochroum*). - **USA:** Pennsylvania, Bethlehem and North Carolina, Salem, Syn. 1209, Collins Coll. 38, as *Sphaeria fuscopurpurea* (PH); Louisiana, A. B. Langlois, "with" 2147, corticated wood, together with "*H. parasiticum*" ined., as *H. fuscopurpureum* (NY).

#### *Hypoxylon californicum* Ellis & Everh.

**emend. Y.-M. Ju & J. D. Rogers**

[MB210167]

The type specimen revealed the *H. fragiforme* chemotype, with orsellinic acid and mitorubins as major components, in agreement with its orange pigments in KOH. The most distinctive feature is the conspicuously striated ascospore epispore (Ju and Rogers 1996). Its stromata resemble those of *H. rubiginosum*, hence it can

be easily overlooked by a superficial investigation.

**Specimen examined:**

**USA:** California: 5 Aug. 1894, corticated wood of *Adenostoma fasciculatum*, A. J. McClatchie 755 (NY-**holotype**).

***Hypoxylon cercidicola* (Berk. & M. A. Curtis ex Peck) Y. M. Ju & J. D. Rogers [MB414952]**

**Synonym:** (?) *Hypoxylon moravicum* Pouzar [MB315731].

This fungus, amply described by Pouzar (1972), is characterized by its apparent host specificity for ash and its discoid stromata featuring a crown of ruptured, involute host periderm, which causes the appearance of irregular stromatal margins. It grows on dying branches before they fall down to the ground. In Europe, it occurs in shadowy and damp places and usually stops growing when branches are in contact with the soil. Its anamorph was described as *Hadrotrichum pyrenaicum* (Petrini and Candoussau 1983), based on material from France. Several specimens, mostly from *Fraxinus* in North America, showed the characteristic morphology of *H. cercidicola*, matching the type in NYS (Stadler et al. 2004a). The type of its European counterpart, *H. moravicum* from PRM, and other specimens from Europe (Stadler et al. 2004a, 2007) were checked by HPLC for comparison. European and American material could neither be segregated by their stromatal pigment colors nor by morphological characters. However, we were surprised to see that they have no common major metabolites. Orsellinic acid and azaphilones prevailed in European specimens (i.e., *H. moravicum*), while American materials (i. e., *H. cercidicola*) contained a different class of yet unknown pigments. Previous HPLC studies on the types in NYS (Stadler et al. 2004a) had been inconclusive, but duplicates in BPI gave better results, fairly agreeing with the remainder of American specimens studied. Orsellinic acid or a similar compound was detected just in traces in

those by the highly sensitive HPLC-MS technique. Instead, they contained pigments with characteristic chromophores (cf. Fig. 2), which were apparently absent in European materials. Possibly, the different chemotypes are indicative of phenomena relating to gene drift, involving geographic isolation that occurred in the co-evolution of these highly host-specific populations. Only European material has so far been cultured. We propose that material from ash in Eastern North America should be collected fresh, cultured and studied by various means of investigation, to establish further differences between these "chemical races".

**Specimens examined:**

**Canada:** Ontario: Aurora, York Co., *Fraxinus nigra*, 2 May 1936, G. D. Darker 5636 as *H. rubiginosum* (BPI 592429 ex Mo. Bot. Gard. Herb. 158313); London, bark of dead *F. nigra*, Mar. 1892, J. Dearness as *H. rubiginosum* (BPI 592427). - **Czech Republic:** Moravia: virgin forest „Cahnov“ near Lanžhot, on dead branch of *F. angustifolia* (still attached to the tree), 14 Oct. 1971, Z. Pouzar (PRM 712591- **holotype** of *Hypoxylon moravicum*); Virgin forest Ranšpurk near Lanžhot, on fallen branches of *F. angustifolia* ssp. *danubialis*, 16 Oct. 1985, Z. Pouzar as *H. moravicum* (PRM 870679). - **Germany:** Badenia-Württemberg: Schwäbisch-Gmünd, *F. excelsior*, 31 Jan. 2004, L. Krieglsteiner (KR-0011260). - **Slovakia:** vic. of Partizánske, forest „Chynoranský luh“, fallen branch of *F. angustifolia*, 21 Oct. 1985, Z. Pouzar as *H. moravicum* (PRM 870676); Province Tisovec: valley on foot of Mount Cigánka near Muráň, branch of *F. excelsior*, 11 Oct. 1989, Z. Pouzar as *H. moravicum* (PRM 871673). - **Ukraine:** Kharkiv district, Gomolshansky National Nature Park, *F. excelsior*, 11 July 2005, CWU (Myc) AS 1376. - **USA:** New York: Buffalo, G. W. Clinton (BPI 578055, 578056, 797326, all ex herb. Clinton, representing portions of the **type** of *Diatrype cercidicola* Peck; Canandaigua, *Fraxinus*, Oct. 1888, O. F. Cook as *H. rubiginosum* (BPI 592353) - Michigan: Ann



Arbor, *F. nigra*, May 1911, C. H. Kauffman 4837 as *H. rubiginosum* (BPI 592430) - New Hampshire: Keene, *F. nigra*, P. Spaulding as *H. rubiginosum* (BPI 592432) - Vermont, Middlebury *F. nigra*, 26 Nov. 1896, A. B. Langlois & E. A. Burt as *H. rubiginosum* (BPI 592426) - Wisconsin, St. Croix Falls, *Fraxinus* sp., Nov 1897, C. F. Baker 557, det. J. H. Miller as *H. rubiginosum* (BPI 592361).

***Hypoxylon commutatum* Nitschke  
[MB213885]**

The lectotype of this monotypic species contained essentially the same metabolites as *H. fragiforme* and *H. howeanum* (see Stadler et al. 2001, 2004a), with mitorubins and orsellinic acid prevailing. Differences to the former species (see Ju and Rogers 1996) are therefore only evident from the ascus and ascospore morphology of the lectotype specimen. Different parts of the stromata in B did not really yield different HPLC profiles, although it clearly constitutes a mixed collection as already stated by Ju and Rogers (1996). We have been searching for this fungus, said to be associated with *Carpinus*, in various European herbaria and in the field, taking special care to examine hornbeam wood. So far we only found *H. fragiforme* and *H. howeanum* (which are not treated in detail here), among the *Hypoxylon* spp. with orange pigments that produced stromata on this substrate. It may be difficult to find this fungus again, but Nitschke's description and the results confirmed by other mycologists on the absence of amyloid apical rings indeed suggest it is different from the above mentioned species.

**Specimen examined:**

**Germany:** sylvia Hostrichiensi, Fuckel, Fung. Rhen. 1056, corticated wood of *Carpinus* (?), as *H. coccineum* (B, **lectotype**, selected by Ju and Rogers 1996).

***Hypoxylon crocopeplum* Berk. & M.A. Curtis [MB444458]**

This species had been regarded as cosmopolitan

in warmer climates by Ju and Rogers (1996). Hsieh et al. (2005), however, recently segregated specimens from regions outside Eastern North America (mostly from the tropics), and accommodated those in *H. polyporoideum* Berk. ex Cooke. Interestingly, *H. crocopeplum sensu stricto* (Hsieh et al. 2005) was recently found in Europe for the first time. Specimens from Belgium and France and a culture obtained from the French material showed the typical morphological characteristics described by Ju and Rogers (1996). As judged from the collection data of the above specimens and the fact that this species was not found among the old herbarium specimens we examined, it has possibly made it to Western Europe only recently. On the other hand, the records from the core zone of the laurisilva of La Palma Island (IC) suggest that this fungus may eventually have been widely distributed throughout the Northern hemisphere.

**Specimens examined** (immature specimens were identified by comparison of their HPLC profiles and gross stromatal morphology, which allows for a reliable distinction from all other species treated here, and sometimes verified by the presence of a *Virgariella*-like anamorph on stromata):

**Belgium:** Namur: Denderleeuw, angiosperm wood, 19 Feb. 1989, H. de Meulder 3953 as *H. rubiginosum* (BR-Myc 012267,45). - **France:** Haute Garonne: Martres-Tolosane, Le Moulin, 13 Dec. 2003, rotten trunk of *Ulmus* sp. (immature), JF-03233. - Loiret: Saint Georges du Cher, Camping, *F. excelsior*, 16 Nov. 2003, leg. P. Leroy (immature). - Pyrénées Atlantiques: Auterive, île du Gave d'Oloron, rotten trunk of *Ulmus*, 6 Nov. 2003, JF-03223 (mostly immature); loc. cit., on bark of *F. excelsior* (mostly immature), same date, JF-03224; loc. cit., on bark of *F. excelsior*, 30 June 2004, J.F. and M. S, JF-04101, culture CBS 119004). - **Spain:** Canarias: La Palma, Los Sauces, Los Tiles, 12 Dec. 1987, E. Beltrán, J.L.Rodríguez-Armas & J.Leal as cf. *Hypoxylon* (TFC Mic. 3598); loc. cit., Fayal-Brezal, *Ocotea foetens*, 2 Dec. 1989, E. Beltrán, Á. Bañares,

J. Leal, M.C. León & F. Cabrera as cf. *Hypoxylon* (TFC Mic. 5213, immature); loc. cit., same host, 29 Nov. 1991, E. Beltrán, J.L. Rodríguez-Armas, Á. Bañares & J. Leal as cf. *Hypoxylon* (TFC Mic 7059, immature). - **USA**: Iowa: Decorah, *Prunus* ("on plum tree"), July 1883, E. W. Holway 347 (NY- part of the type specimen of *H. commutatum* ssp. *holwayanum*., which does not correspond to the protologue (see Ju and Rogers 1996). South Carolina: (corticated wood of oak?) H. W. Ravenel, Car. Inf. 1906, as *Sphaeria croceopepla* ined. [K(M) 120978- **holotype**].

***Hypoxylon dearnessii* Y.M. Ju & J.D. Rogers [MB414956]**

This species was also included in the broad concept of *H. rubiginosum sensu* Miller (1961). It appears to be associated with *Acer*, and rather shows affinities to *H. howeanum*, from which it mainly differs by its ascospore morphology. Accordingly, its stromata are devoid of rubiginosin derivatives and contain mitorubrin type azaphilones only (see HPLC profile in Fig. 2 below). In contrast to *H. howeanum*, mitorubrin was not detected in the stromata of this species. Two prominent apparently specific metabolites also occurred. One of those (HD1 in Fig. 2) was hitherto not observed in other *Hypoxylon* spp., while the other (HD2) is also present in *H. fragiforme* and *H. howeanum*. It showed a rutilin-like DAD spectrum, and corresponding MS data point toward its identity with another dimeric azaphilone molecule.

In addition to further records from North America, a culture of a specimen in MUCL, originally determined by G. Hennebert as *H. rubiginosum sensu* Miller (1961) also has a similar secondary metabolism as *H. howeanum* and *H. fragiforme* (Bitzer et al. 2008). We have not yet seen conidiogenous structures of this culture because it did not sporulate. Despite extensive field work and studies on herbarium material, this fungus still remains to be found on *Acer* in Europe. Even the related *H. howeanum* appears to be rare on that host.

**Specimens examined:**

**Canada**: Ontario: London, bark of *Acer*, Oct. 1903, J. Dearness, Fung. Columb. 3028 as *H. perforatum* [NY- **holotype**, K(M) 123176 – **isotype**]; same locality and host, 12 Mar. 1890, J. Dearness 1283 (B 70 0010853). – Québec: Gatineau Park, Fortune Lake, Sep. 1961, G. Hennebert as *H. rubiginosum*; rev. J. F. & M. S. (designation of stromata and corresponding culture MUCL 2709, see Bitzer et al. 2008). - **USA**: Colorado: Minnehaha, bark of *Acer glabrum*, 4 July 1906, F. E. & E. S. Clements, Crypt. Form. Colorado 212, det. J. H. Miller as *H. rubiginosum* (NY - **paratype**); Maine: Piscataqua Co., near Milo, bark of red maple, 2-6 Sep 1905, W. A. Murrill 2102 as *H. rubiginosum* (NY - **paratype**).

***Hypoxylon fendleri* Berk. ex Cooke [MB206066]**

This fungus was studied for comparison with the new species reported in the taxonomic part. The specimens we so far examined all showed affinities to *H. fragiforme*, since mitorubrin, rather than rubiginosins, constituted its orange stromatal pigments. See also discussion below in Notes on *H. canariense*. Another specimen of *H. fendleri* was recently cited by Bitzer et al. (2008) from the Netherlands, collected from a tropical greenhouse.

**Specimen examined:**

**Guadeloupe** (French West Indies): Saint Louis de Marie Galante, Les Sources, 2 Dec. 2005, on dead decorticated wood, C. Lechat, CLL 5503, det J.F.; for HPLC studies on type and conspecific material see Hellwig et al. (2005).

***Hypoxylon ferrugineum* G.H. Otth [MB166031]**

This fungus was considered a variety of *H. rubiginosum* by Miller (1961) and treated as a species by Petrini and Müller (1986) and Ju and Rogers (1996). Stadler et al. (2004a) found that European material differs from *H. rubiginosum sensu stricto* in being devoid of rubiginosins.

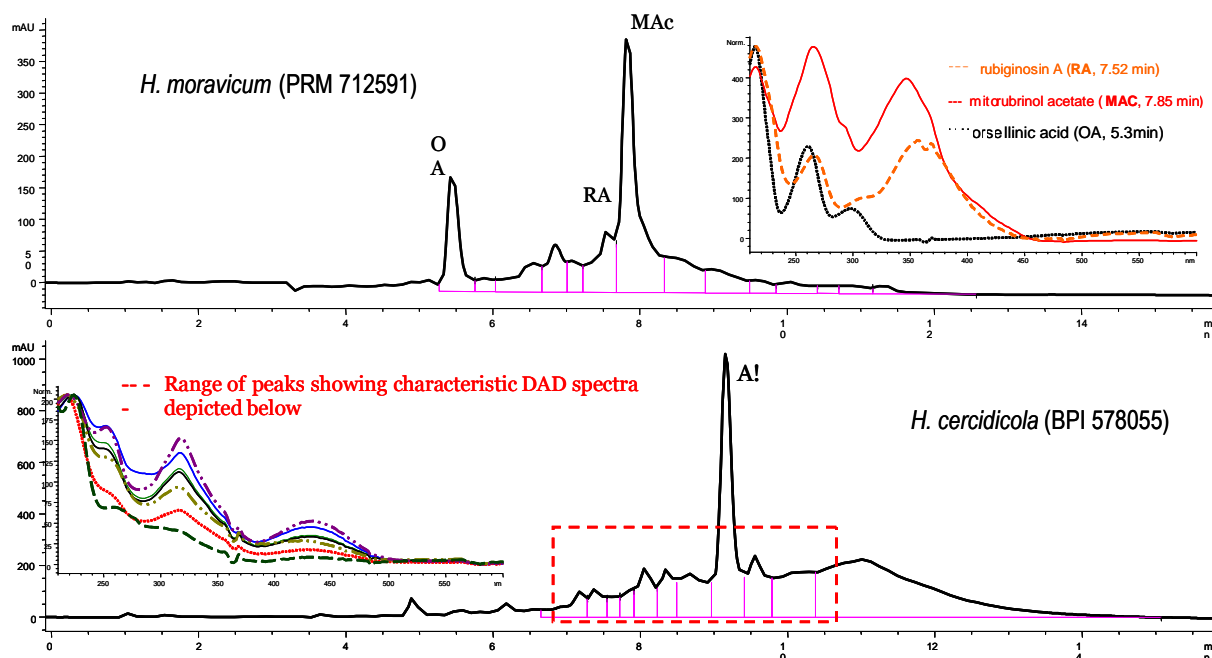


Fig. 2. Comparison of HPLC-UV chromatograms (210 nm) of stromatal methanol extracts of the type specimens of *Hypoxylon moravicum* (PRM, above), *H. cercidicola* (BPI, center), and *H. dearnessii* (below) and DAD spectra of their characteristic metabolites. For *H. moravicum* the characteristic components of the *H. rubiginosum* chemotype (Stadler and Fournier 2006b) are indicated. In *H. cercidicola*, no known metabolites of other *Hypoxylon* spp. were detected as major components, and a series of peaks showing a different DAD spectra were observed. Those appeared in the range of retention times indicated by a square. An artifact is present as previously described by Stadler et al. 2004b), for *Daldinia bakeri*, indicated by an (A!).

The taxon is certainly not common, but a meticulous search of pyrenomycetes on *Tilia* revealed it from Austria (Scheuer et al. 2001). We studied two additional specimens from the Czech Republic and Canada, confirming unpublished results on the specimen label by T. Læssøe on its first record from America. However, the Canadian specimen, while being in full accordance with regard to its morphological characters, showed a HPLC profile similar to *H. rutilum*. It contained rubiginosin A and rutilins, besides the mitorubrins found in the European material (Fig. 4). The Canadian material might be an undescribed taxon, but this remains to be assessed by culturing both forms, for no anamorph has ever been reported even from the European *H. ferrugineum*.

#### Specimens examined:

**Canada:** Ontario: London, decorticated wood of cf. *Populus*, 9 Sep. 1937, E. H. Moss, det. T.

Læssøe, K(M) 16944. - **Slovakia:** Muráňska Plateau Natural Park, reserve Poludnica below Bodolová, on fallen twigs of *Tilia cordata*, 12 Oct. 1988, Z. Pouzar & P. Turis, det. Z. Pouzar (PRM 870224).

#### *Hypoxylon fraxinophilum* Pouzar [MB315708]

**Synonyms:** *H. intermedium* (Schwein.: Fr.) Y.M. Ju & J.D. Rogers 1996 (nom. illeg. ICBN 2000 art. 53); non Spegazzini 1884 [MB416089]. *H. argillaceum* (Pers.) Nitschke 1867 (nom. illeg. ICBN 2000 art. 53); non (Fr. : Fr.) J. Kickx f. 1835.

We previously reported that this species (as *H. intermedium*) resembles *H. perforatum* in containing hypomiltin (Hellwig et al. 2005). This was now confirmed by studies of the type material of *H. fraxinophilum*. We only examined superficially the type of *S. argillacea* Pers. (another invalid synonym) in L and did not

record its HPLC data for comparison. Little material is left, but previous morphological studies undertaken by various mycologists leave no doubt about its identity. When comparing specimens of this species, all associated with ash, collected in various countries we found no differences in either the morphological features or the HPLC profile.

### Specimens examined:

**Czech Republic:** Moravia: Mikulčice near Hodonín, forest „Skarina”, on fallen branch of *Fraxinus angustifolia*, 28 Aug. 1984, leg M. Tortić, det. Z. Pouzar as *H. fraxinophilum* (PRM 712595- **holotype** of *H. fraxinophilum*); vic. of Lanžhot, near Breclav, virgin forest „Ranšpurk” on dead branch of *F. angustifolia* ssp. *danubialis*, 18 Sep. 1984, Z. Pouzar as *H. fraxinophilum* (PRM 870427) - **Germany:** Rheinland-Pfalz: Landkreis Mayen-Koblenz, vic. of Rhens, *F. excelsior*, 23 May 1938, J. Sponheimer 6706, det. Kirschstein as *H. rubiginosum* (B 70 0010838). - **Slovakia:** Skalica near Holič, forest „Holičský státny les”, on fallen branch of *F. angustifolia*, 13 Oct. 1971, Z. Pouzar as *H. fraxinophilum* (PRM 712600). USA: Pennsylvania, corticated rotten wood of *Fraxinus*, Mühlenberg 58 (L 910, 270-516, **type** of *Sphaeria argillacea*; not studied by HPLC).

### *H. fuscopurpureum* (Schwein. : Fr.) M.A. Curtis [MB120511]

**Confirmed synonyms:** *H. ianthinum* Cooke [MB158513]; *H. vogesiacum* (Currey) Sacc. var. *microsporum* J. H. Miller [MB349609]. This fungus may be confused with both *H. fuscum* and *H. rubiginosum* and is thus easily overlooked. We agree with Ju and Rogers (1996) that *H. ianthinum* and *H. vogesiacum* var. *microsporum* are synonyms of *H. fusco-purpureum*. We also confirm their view that Miller (1961) has repeatedly identified specimens of typical *H. fuscopurpureum* as *H. rubiginosum* (see BPI specimens below). The types of all of the above names have similar HPLC profiles, containing essentially the same metabolites as

collections of the most frequent chemotype of *H. fuscum* from *Corylus*, as represented by the type in L. (see below). BNT and daldinins C, E, and F were detected in all of the above species and in the other specimens listed below from countries other than Slovakia.

The Slovakian specimens, annotated by Z. Pouzar as "*H. lacrymosporum* ined." but then revised by himself (presumably after the monograph by Ju and Rogers had been published), have slightly larger ascospores (15-17 x 5.5-7.5 µm vs. 12-15 x 5.5-7 µm in the types and other specimens from herbaria other than PRM), and contained different major components. The absence of daldinal A and daldinins E and F strongly points toward the status of the Slovakian specimens as an undescribed taxon. KOH extracts were Isabelline (65) or Honey (47), as in "regular" *H. fuscopurpureum*. However, as can be seen in Fig. 3 (comparison of HPLC profiles), this is partly due to the presence of daldinone B in the "*H. lacrymosporum*" specimens. We have never previously observed daldinone B in any member of the *H. rubiginosum* and *H. fuscum* complexes. The "*H. lacrymosporum*" specimens also only contained traces of BNT. This compound was detected only by the more sensitive HPLC-MS, because a large peak representing daldinin C overlaid signals of other metabolites occurring at the same retention time upon DAD analysis. In all other specimens of *H. fuscopurpureum*, BNT and daldinins E and F prevailed, and daldinin C was only detected as a minor component, regardless of the stage of stromata development. Curiously, the only other European records of *H. fuscopurpureum*, reported by Mühlbauer et al. (2002) showed the same "typical" HPLC profile as the American type specimens.

The highly similar morphology of *H. fusco-purpureum* (aside from the ascospores) to *H. fuscum*, and the quasi identical HPLC profiles of both species, would suggest that they are phylogenetically closely related. Nevertheless, Hsieh et al. (2005) found that material of *H.*

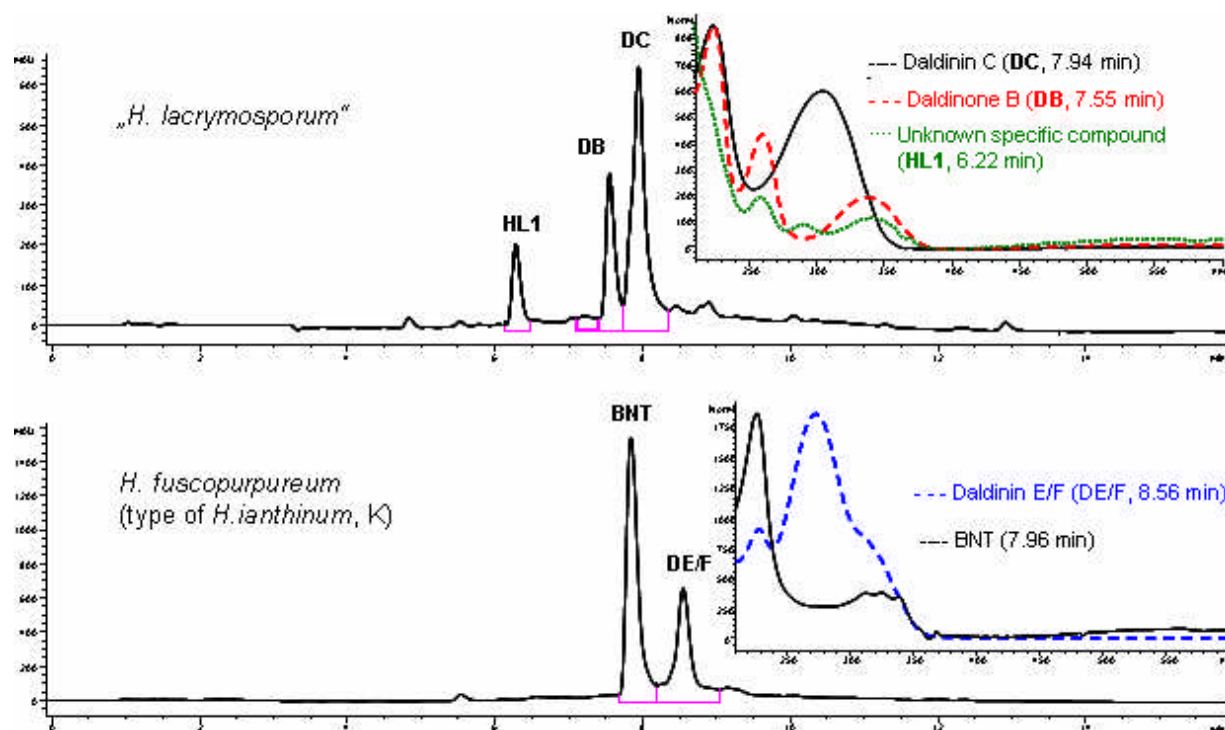
*fuscopurpureum* from Taiwan appeared far apart from *H. fuscum* in their phylogenetic tree based on  $\alpha$ -actin and  $\beta$ -tubulin sequences. There, it appeared more closely related to *H. anthochroum*. Taken together, and if eventually corroborated by studies on a broad range of specimens, these results would suggest that either daldinin biosynthesis along with the *H. fuscum*-like stromatal habit have evolved twice in *Hypoxylon*, or that a daldinin-containing lineage must have separated early in the evolution of the genus. Triebel et al. (2005) retrieved only rDNA sequences of what is probably a non-xylariaceous contaminant of the *H. fuscopurpureum* stromata, in two different specimens collected from Austria and Germany. Unfortunately we did not obtain cultures or DNA sequences suitable for phylogenetic alignments from the PRM specimens.

Immature specimens with effused stromata on bark (cited below), were only tentatively identified here as *H. fuscopurpureum*. They could as well correspond with *H. anthochroum*, revealed as closest relative by Hsieh et al. (2005) inferred from molecular data.

### Specimens examined:

**Canada:** Ontario: London, decorticated wood of *Fraxinus*, July-Aug. 1904, J. Dearness, Fung. Columb. 2034 as *H. fuscopurpureum*, det. J. H. Miller as *H. rubiginosum*, original identification conf. by Ju and Rogers 1996 (NY). - **Slovakia:** Muranska Plareau Natural Park, reserve Poludnica, Bodolová near Muráň, fallen branch of *F. excelsior*, 11 Oct. 1989, leg. P. Turis, det. & rev. Z. Pouzar, as "*Hypoxylon lacrymosporum* (ined.)"; later rev. as *H. ianthinum* Cooke, subsequently rev. as *H. fuscopurpureum* (PRM 871847); near Muráň, on hill Ciganka, fallen branch of *F. excelsior*, 2 Aug. 1989, Z. Pouzar, determinations see previous specimen (PRM 870454). - **USA:** Locality unknown: ex herb. Schweinitz 1209 (BPI 738623 - **isolectotype** of *Sphaeria fuscopurpurea* (selected by Ju and

Rogers 1996). - California: Inverness, Marin Co., *Quercus agrifolia*, 19 Feb. 1931, H. E. Parks 3536, det. T. Laessøe & A. J. S. Whalley as *H. vogesiaceum* var. *microsporum* (BPI 641633); Mt. Shasta, Siskiyou Co. (alt. 5700 ft.), Wagon Camp, *Ceanothus velutinus*, 8 Aug. 1967, W. B. & V. G. Cooke 38638 as *H. vogesiaceum* var. *microsporum* (BPI 594092). - Colorado: Denver, decorticated wood, E. Bethel 254, as *H. rubiginosum* var. *purpurea* ined. (NY, cited by Ju and Rogers 1996 as from „Canada“). - Louisiana: near St. Martinsville, on dead pieces of oak (*Quercus*), 30 Aug. 1889, A. B. Langlois 1993 ex herb. Ellis as *H. fuscopurpureum*, det. J. H. Miller as *H. rubiginosum*, original det. confirmed by us. - Maine: Orono, F. L. Harvey as *H. rubiginosum* var. *purpurea* ined., det. J. H. Miller as *H. rubiginosum*, rev. Ju and Rogers (1996) as *H. fuscopurpureum*, confirmed here (NY); Piscataquis Co., Medford, Camp Sunday (No. 5), corticated wood, 28 Aug. 1905. W. A. Murrill, det. J. H. Miller as *H. rubiginosum* (NY); loc. cit., near Milo, Duck Point Camp, corticated wood of *Acer rubrum*, 2-6 Sep. 1905, W. A. Murrill, det. J. H. Miller as *H. rubiginosum* (the latter two specimens are annotated by Ju "immature" and were det. by us as *H. cf. fuscopurpureum* from characteristic stromatal habit, effused even on bark, and by characteristic chemotype). - New York: Adirondacks, 24 Aug. 1934, R. F. Cain (BPI 594091 - **type** of *H. vogesiaceum* var. *microspora*); Potsdam, on wood, M.B. Ellis ex herb. M.C. Cooke 1885, K(M) 121851- **type** of *H. ianthinum*); loc. cit., on *Ulmus* sp., 1879, M. B. Ellis, det. T. Laessøe as *H. vogesiaceum* var. *microsporum*, K(M) 121848; Syracuse, *Fraxinus sambucifolia*, 27 Oct. 1916, L. H. Pennington as *H. fuscopurpureum*, rev. J. H. Miller as *H. rubiginosum* (BPI 716801 ex Lloyd herb. No. 11424); Potsdam, *Fraxinus* sp., Jan 1865, J. B. Ellis as *H. rubiginosum* (BPI 592352). exact locality unknown: "on dead wood and bark from various localities", Ellis & Everhart: Fungi Columbiani No. 1324, as *H. rubiginosum* (PH-1031231).



**Fig. 3.** Comparison of HPLC-UV chromatograms (210 nm) of stromatal methanol extracts of "*Hypoxylon lacrymosporum*" (PRM 870454, above) and of the type specimen of *H. ianthinum* (K, below) and DAD spectra of their characteristic metabolites. Notably, the major components of one species were not detected as prominent peaks in the other despite they both key out as *H. fuscopurpureum*. For correspondence of known metabolites see Fig. 1.

***Hypoxylon fuscum* (Pers. : Fr.) Fr.  
[MB163954]**

**Confirmed synonyms:** *H. lianincola* Rehm [MB167365], *H. purpureum* Nitschke [MB211415].

Like *H. rubiginosum*, this species belongs to the most frequently reported Xylariaceae. It preferentially occurs on betulaceous hosts but is not restricted to those plants. Ju and Rogers (1996) delineated differences to morphologically similar species (e.g., *H. fuscopurpureum* and *H. chathamense* Y.-M. Ju & J. D. Rogers), but still included various synonyms from temperate as well as tropical regions. Their concept was based on morphological characters of the teleomorph and the anamorph, including an extraordinary large ascospore size range (8-20 x 4-8 µm). On the other hand, their cultures, obtained from stromata growing on various hosts (Betulaceae,

*Fagus*, *Sorbus*) were rather similar. Attempts to segregate this taxon based on ascospore size (Petrini et al. 1987) revealed certain correlations to its apparent host-specificity, which were confirmed by our own work. Nevertheless, it is still regarded as a complex species. To address this problem from a chemotaxonomic standpoint, the major stromatal pigments of *H. fuscum* from *Corylus* were isolated and characterized as daldinins C, E, and F (Quang et al. 2004b). We have recently described two different general chemotypes, (or "chemical races") occurring on *Corylus* and *Carpinus*, and on *Alnus* and the Salicaceae, respectively (Stadler and Fournier 2006b, Stadler et al. 2007), as inferred from HPLC profiling. The chemotype of type material in L was determined to provide chemotaxonomic data that may help to resolve this species complex in future.

The HPLC profiles of subsurface granules, carefully prepared from two type materials in L., both yielded daldinal A, daldinins C, E, and F, besides BNT. One of those was derived from decorticated wood with the stroma apparently still intact, while the other contained depauperate stromata on corticated wood reminiscent of *Corylus avellana*, as judged from bark structure. Even though the woody substrate was not studied carefully, evidence provided by HPLC profiling, and previous examination of both specimens (Petrini and Müller 1986, Ju and Rogers 1996), leaves no doubt about the identity of this species. The type material represents the typical morphochemotype of *H. fuscum* constantly associated with hazel in the Northern hemisphere. Interestingly, a number of specimens from *Alnus* in USA and Taiwan, and the type specimen of *H. lianincola* from a liana in the Philippines showed the same chemotype as those from *Carpinus* and *Corylus* in Europe. Merely daldinal A was not detected in them. Specimens from other host plants other than Salicaceae (including, e.g., *Acer* and *Lonicera*, see Stadler et al. 2007) collected in Europe mostly corresponded to the *Corylus/Carpinus* chemotype, which is therefore apparently less host-specific, but the number of specimens from such plants is not sufficient to draw further conclusions at this time.

The other variety that frequently occurs on *Alnus* and the Salicaceae in Europe is characterized by containing particular large amounts of cytochalasin-like metabolites that are lacking in the mature stroma. Those contain some unknown compounds, reminiscent of naphthalene derivatives (Stadler et al. 2007, here classified as "chemotype A/S" in contrast to the "typical" above described chemotype (named "C/C" for *Carpinus/Corylus*). This deviating chemotype contains no or little of the daldinin pigments. Further work on the identification of the pigments of the latter chemical race might allow for a comparison of biosynthetic features. A significant number of fresh specimens should

be studied also by molecular methods, to evaluate whether cryptic species are involved.

**Specimens examined** (if not stated otherwise, the original determination was *H. fuscum*; if substrate unknown or other than *Alnus/Salix* and *Carpinus/Corylus*, respectively, or in case of deviations from the above discussed host specific HPLC profiles, also including the chemotype as A/S or C/C):

**Austria:** Burgenland, Bez. Oberwart, in forest W of Jormannsdorf, on dead standing trunk of *Carpinus betulus*, 2 Mar. 1980, J. Poelt (S-F44473); Frohsdorf. Rosaliengebirge, *Alnus glutinosa*, 21 Sep. 1935, H. Huber, det. W. Kirschstein, annotation: "also in Hartberg near Mönchkirchen" (B 70 0010728). - Lower Austria: Niederdonau, vic. of Seebenstein, Fuchsriegel, *Alnus glutinosa*?, leg. H. Huber, det. Kirschstein (B 70 0010731; largely immature, containing cytochalasins, bark structure of host like *Alnus*); loc. cit., Bezirk Neunkirchen, near Pitten. Leiding, *A. glutinosa*, leg. Huber, det. Kirschstein (B 70 0010732); loc. cit., Zihlsgraben near Gleißfeld, *Betula*, H. Huber (B 70 0010758; chemotype A/S); Tulln, Donau-Auen, bark of *Salix*, May 1958, F. Petrak, Reliqu. Petrak. (Graz) No 2038 (B 70 0010702; S-F44479). - Steiermark: Windische Bühel, 12 km SE of Leibnitz, ca. 4 km S from Straß, "kleines Waldstück unmittelbar NW von Obegg", *Carpinus betulus*, 3 Oct. 1999, C. Scheuer (S-F21576); Grazer Bergland, Schöcklgebiet. Falschgraben near Stattegg, *Ca. betulus*, 30 Sep. 1995, leg. C. Scheuer, det. L. E. Petrini (S-F44470). - **Belgium:** Antwerpen: Geel, de Zegge, 15 May 1976, L. Imler (BR-Myc 042603,20, chemotype A/S); Herselt, de Langdonken, 14 Jan 1995, H. de Meulder 11005 (BR-Myc 046483,20; chemotype A/S); Oelegem: Vrieselhof, 26 Oct 1988 H. de Meulder 2591 (BR Myc 009571,65; host det. as *Alnus*) - Luxembourg: Villers-sur-Semois, Bois de Brossar, 29 Apr 1993, A. Fraiture 1797 (BR-Myc 018227,88; host det. as *Corylus*) - Namur: Dourbes, Montagne aux Buis, „dead *Juglans*



*regia*", 15 June 1970, P. Pierart, det. K. van der Gucht (BR-Myc 060839,20; chemotype C/C; substrate rather looks like *Corylus* in our mind); Malonne, 14 Apr. 1983, G. Thiry, det. K. van der Gucht & K. Laureys as *H. rubiginosum* (BR-Myc 060786,64; chemotype C/C except for granules bright orange); Heure-en-Famenne, Jalna, *Corylus*, 2 Apr. 1994, H. de Meulder 9657 (BR-Myc 025952,53); Nismes, *Corylus*, 14 Mar. 1993, H. de Meulder 8407 (BR-Myc 019175,66). - Oost-Vlaanderen: Bazel, Natuurpark Scheideland, „op dode elzetakken“ 18 Jan. 1969, J. Moens 687, det. K. van der Gucht & K. Laureys (BR-Myc 060793,71; chemotype A/S). - West-Vlaanderen, Slijpe, *Acer campestre*, 29 Nov. 2000, H. Ruysseveldt as *H. rubiginosum* (BR-Myc 131878,55; chemotype C/C). - **Canada:** Ontario: London, Aug. 1904, corticated wood of *Ostrya virginiana*, J. Dearness, Fung. Columb. 2122 as *H. fusco-purpureum* (NY), duplicate in WSP rev. as *H. fuscum* by Ju and Rogers 1996; n.v.); Quebec: Cap-Rouge, *Corylus americana*, leg. Abbé Provencher 1876-1877 (de Thümen, Mycoth. univers. Nr. 871; 2 packets; B 70 0010743; B 70 0010744). - **Czech Republic:** Bohemia: Teplitz-Schönau, "*Fagus*", Autumn 1872, (de Thümen: Fungi austriaci Nr. 664; B 70 0010706; host is probably not *Fagus*, chemotype A/S, with cytochalasins in young stromata); vic. of Böhmisches Zinnwald, Oct. 1873, ex herb. de Thümen (B 70 0010724; Chemotype A/S). - **Germany:** Badenia-Württemberg: Constance, "on dead *Fagus* branches", G. Bauer & Leiner 1858 (Jack, Leiner & Stizenberger, Kryptog. Badens, Nr. 151; B 70 0010705; host was identified as *Corylus*; chemotype C/C); vic. of Rastatt. 2 Dec. 1874, Schroeter (B 70 0010723; chemotype A/S); Landkreis Heidenheim, Dischingen, Krembs ex herb. Rieber (B 70 0010752; host identified as *Corylus*); exact locality unknown, Württemberg. leg. Krembs ex herb. Rieber (B 70 0010753, host identified as *Corylus*); Landkreis Reutlingen, cascade "Uracher Wasserfall", "*Fagus*", 1891, Rieber (B 70 0010755, host det. as *Corylus*); Schörzingen,

*Corylus* (B 70 0010767); Häfnerhaslach, vic. of Sommerburg, 01.05.1995, A. Gminder as *H. rubiginosum* (STU 10779, host det. as *Corylus*); Schwenningen, "Schwenninger Moos, 13 Jan 1971, M. Mark (STU 10506; host det. as *Corylus*); Stuttgart-Weilimdorf, *Carpinus*, 5 Mar. 1975, Baral (STU 10292); Weisweil, Hechtsgraben, 13 Apr. 1987, Maser (STU 10602); Warthausen, 1874, König as *H. multifforme*, rev. H. Baral (STU 10536; host det. as *Carpinus*); same locality and collection data, *Carpinus* (STU 1041). - Bavaria: Landkreis Erlangen, Höchststadt, Heroldsberg, "*Fagus silvatica*", Zahn (B 70 0010713; host det. as *Corylus*, chemotype C/C). - Berlin: Spandau, Stadtforst, *A. glutinosa*, W. Kirschstein (B 70 0010751); loc. cit., *C. avellana*, W. Kirschstein (B 70 0010739); Jungfernheide, Zopf, B 70 0010741; chemotype A/S; host det. as *Alnus*); Bezirk Zehlendorf, Grunewald, Krumme Lanke, dead trunks of *A. glutinosa*, 11 Feb. 1906, Laubert (B 70 0010710); loc. cit., 24 Apr. 1909, Laubert (B 70 0010711); Grunewald, near Paulsborn, *A. glutinosa*, Laubert (B 70 0010714). - Brandenburg: Landkreis Teltow-Fläming, vic. of Luckenwalde, Felgentreuer Busch, *Alnus*, 24 Oct. 1999, D. Benkert (B70 00909371); Landkreis Märkisch Oderland, near Strausberg, Apr. 1896, leg. E. Pritzel, det. P. Hennings (B 70 0010757; host identified as *Corylus*); Landkreis Havelland, vic. of Kleinbehnitz, Hasellake, *A. glutinosa*, W. Kirschstein (B 70 0010734); loc. cit., same substrate and collector (B 70 0010735; B 70 0010736); loc. cit, same collector, *Corylus* (B 70 0010747); loc. cit., same collection data (B 70 0010748); loc. cit., Brieselang. *C. avellana*, W. Kirschstein (B 70 0010737); loc. cit., *A. glutinosa*, same collector (B 70 0010738); Nauen, vic. of Bredow, Bredower Forst, *Carpinus betulus*, W. Kirschstein (B 70 0010750); "Stieurtzen", *C. avellana*, W. Kirschstein (B 70 0010749); Landkreis Havelland, Brieselang, *C. avellana*, Kirschstein (B 70 0010782) - Hessen, Landkreis Alsfeld, Kestrich. Sumpf beim Park, *Populus nigra*, H. Hupke (B 70 0010759; chemotype A/S);



Dillkreis, vic. of Langenaubach, *C. avellana*, A. Ludwig (B 70 0010818); same collection data, *A. glutinosa*, A. Ludwig (B 70 0010822); loc. cit., vic. of Haiger, *C. avellana*, A. Ludwig (B 70 0010823). - Mecklenburg-Vorpommern, vic. of Schwerin, ca. 1860, leg. Fiedler 30, ex herb. Nitschke (B 70 0010785; chemotype C/C); same collection data, Fiedler, ex herb. Nitschke B 70 0010794; chemotype A/S); Landkreis Parchim, near Parchim, Kannenberg. *Corylus*, Dahnke, det. A. Ludwig (B 70 0010812). - North Rhine Westphalia: Münsterland, Münster-Wolbeck, 1863, T. Nitschke (B70 00909252, **type** of *H. purpureum*; chemotype C/C); loc. cit., Feb 1863, Nitschke 11 (B 70 0010786; host det. as *Corylus*); loc. cit., May 1865, Nitschke (B 70 0010795, host det. as *Corylus*); loc. cit., Dec. 1864 (B 70 0010793; host identified as *Corylus*) loc. cit., March 1864, Nitschke (B 70 0010791; host det. as *Corylus*); loc. cit., vic. of Münster, Nienburg, Nitschke (B 70 0010788; host det. as *Corylus*); loc. cit., Münster-Nienberge, Oct. 1866, Nitschke (annotated as „Conidienform“), immature (B 70 0010790, showing only a few mature perithecia and chemotype C/C; several further specimens from the Nitschke herbarium, all det. as *H. fuscum* by him, have also been examined but are not listed here for economy of space); Landkreis Siegen, between Rödgen and Wilnsdorf, *A. incana*, A. Ludwig (B 70 0010816). - Rheinland-Pfalz: Sankt Goarshausen, above Loreley, *Corylus*, leg. J. Sponheimer, det. Kirschstein (B 70 0010777); Landkreis Mainz-Bingen, vic. of Nieder-Heimbach, Soonecker Grund, *Corylus*, leg. J. Sponheimer, det. Kirschstein (B 70 0010778); Rhein-Hunsrück-Kreis, Mühlthal near Boppard-Bad Salzig, angiosperm forest, Schlanigbach creek, *C. avellana*, J. Sponheimer 6681, det Kirschstein (B 70 0010779). - Saxonia: Leipzig, *A. glutinosa*, R. Staritz (B 70 0010760 chemotype A/S). - Saxonia-Anhalt: Kreis Mansfelder Land, near Eisleben, Schloß Mansfeld, dry branch of *C. avellana*, J. Kunze (B 70 0010721); same locality, Geistholz, *C. avellana*, J. Kunze (B 70 0010722); Kreis Mansfelder Land, near

Eisleben, Zopf (B 70 0010733). - Thuringia: Kyffhäuserkreis, Bad Frankenhausen. near Mt. Kyffhäuser, *C. avellana*, May 1901, Aderholt (B 70 0010712). - Exact locality unknown, "*Rheinland, ad ramos varios putridos, frequens*", Fuckel, Fungi rhen. Nr. 1054. (B 70 0010716; BR Myc 097062,62; host det. as *Corylus*). - **Hungary**: Szekszárd, *Carpinus betulus*, 10 June 1928, L. Hollos (S-F44478). - **Ireland**: Enniskerry Co., Wicklow, *Corylus*, 18 Apr. 1951 P. O'Connor (MA 22717 ex DBN) - **Italy**: Piemont: Provincia di Alessandria, presso Serravalle Scrivia, *A. glutinosa* („su pali secchi di Ontàno“), autumn 1858, Ferrari (Herb. Crittogam. Ital. Nr. 644, B 70 0010725) - Veneto: Prov. di Treviso, Selva di Cadore, *A. glutinosa*, D. Saccardo 850 (Mycoth. Ital. Nr. 79; B 70 0010707) - **Letonia**: Vidzeme: vic. of Vetiena, *A. incana*, 26 Dec. 1936, K. Starcs 5260 (B 70 0010817); same collection data, 25 Mar. 1932, K. Starcs 102 (B 70 0010708); loc. cit., "Baltezus", *A. incana*, A. Kirulis 93, det. W. Kirschstein (B 70 0010729) - Distr. Madona, Vestiena, *A. incana*, 26 Aug. 1931, K. Starcs 19, det. Sydow (B 70 0010773); loc. cit., *C. avellana*, 10 Aug. 1934, K. Starcs, det. Ludwig (B 70 0010774); loc. cit., Rigas Rajons, Ropazi, *A. incana*, K. Starcs 6027, det. Ludwig (B 70 0010776). - **Norway**: Hordaland: Voss, Bulken, *C. avellana*, leg. A. Lundberg, comm. A. Granmo (B 70 0010709); Troms: Bardu, Setermoen, Moegga, *Alnus*, 10 June 1999, A. Granmo 38/99 (TROM 39789; chemotype C/C despite *Alnus* is the host). - **Philippines**: Laguna: Mt. Maquiling, near Los Baños, dead stems of liana, 24 May 1913, M. B. Raimundo 1186 (S-F10684-**holotype** of *H. lianincola*). - **Poland** (then Germany): Bezirk Landsberg, near Tamsel, *Carpinus betulus*, 22 Feb. 1906, P. Vogel (B 70 0010756); Neumark, Landkreis Krossen (Budachów), W. Kirschstein (B 70 0010781, chemotype C/C). - **Romania**: Muntenia, Distr. Dâmbovita, Nucet., *A. incana*, 18 Mar. 1926, Savulescu & Sandu 18 (B 70 0010720 ex Herbar. Mycol. Romanicum; chemotype A/S) - **Spain**: Cangas: Barra, *A. glutinosa*, 1 Feb. 1992, M. I.

López-Prada, det. M. Aneiros (MA 33955). - Girona: *C. avellana*, 30 Oct. 1974, R. Bertault (MA 38527); same locality and collection data (MA 38528). - Huelva: Rivera de Múrtiga, *A. glutinosa*, 25 Mar. 1998, L. Romero de la Osa (MA 40383). - Huesca: Bielsa, P. N. de Ordesa, Ermita de Nuestra Señora de Pineta, twigs of *C. avellana*, 17 Oct. 1989, J. Checa (MA 31861 ex Herb. Univ. Alcalá 14072); P. N. de Ordesa, Cotatuero, bark of *C. avellana*, 16 Oct 1973, J. Checa (MA 31862 ex Herb. Univ. Alcalá 14055). - Lleida: exact locality not stated, *Populus* sp., 9 Nov. 1973, L. Corbins, det. G. Malençon (MA 45801 ex MUB 13565; chemotype A/S) - Navarra: Orilla del Arga, *A. glutinosa*, 15 May 1941, Urries [Pireniales 3] as *H. cohaerens* (MA 17533). - Oviedo: Reserva Biológica de Muniellos (wood det. as *Corylus*), 7 May 1984, M. T. Tellería et al. (MA 099997); same collection data (MA 12023); same locality, 8 May 1984 (MA 12017, chemotype A/S); Monasterio de Hermo, in beech forest, 16 June 1983, M. T. Tellería et al. (MA-12020, chemotype A/S); Picos de Europa, Fuente Dé, Parador Nacional, 21 Nov. 1984, M. Dueñas et al. (MA-12021; chemotype A/S). - **Sweden**: Hälsingland: Ängersjö, Vännberget, on *Sorbus*, 12 May 1900, leg. M. Östman, det. T. Laessøe (S-F44475; chemotype C/C); Ängersjö, *Betula*, 19 May 1902, leg. M. Östman, det. A.J.S. Whalley & T. Laessøe (S-F44476; chemotype C/C). - Stockholm, South Djurgården, *Acer platanoides*, 27 Dec. 1920, leg. R. Vestergren, det. F. Petrak (S-F44471, chemotype C/C). - **Switzerland**: St. Jacob, 20 Apr. 1815, Nees v. Esenbeck (B 70 0010810; host determined as *Corylus*). - **Taiwan**: I-lan Co., Ta-tung, Chi-lan-shan, 14 Sep. 1991, Y.-M. Ju 80091402 (JDR, chemotype C/C). - **UK**: England, London, Hampstead, Cooke, Fungi Britann. Exs. 246.; (host det. as *Corylus*; 2 packets, B 70 0010699; B 70 0010700); loc. cit., Highgate, Cooke, Fungi Britann. Exs., Edit. Sec., 467 (B 70 0010701; chemotype C/C); Salop, Wyre forest, "oak" twigs, Nov. 1927, P. G. Rhodes (S-F44480; chemotype C/C, host is probably *Alnus*). - Exact locality unknown, 1873, Plowright, Sphaeriaceae.

Brit. Cent. 1., No. 20 (B 70 0010703; chemotype C/C). - North Wales, Apr 1974, A. J. S. Whalley 175 (BR-Myc 097057,57, host det. as *Corylus*). - **USA**: California: Humboldt Co., Trinidad, *A. incana*, 24 Mar. 1931, H. E. Parks (Univers. of California: California Fungi 320 (B 70 0010726; BR Myc 074154,46, the latter in bad condition, chemotype C/C, even though *Alnus* is given as host); Sonoma Co., Mt. Jackson Resort, dead twigs of *Alnus*, 8 July 1945, V. M. Miller (WSP 31550). - Iowa: Decorah, on dead limbs of *Corylus* (Ellis. North American Fungi No. 678a; PH-1031233); loc. cit. (?), *Alnus*, N. J. Newfield (Ellis. North American Fungi No. 678, PH-1031234; chemotype C/C). - New Jersey: Newfield, *A. serrulata*, 1874, J. B. Ellis (de Thümen; Mycotheca univers. Nr. 367; B 70 0010745); loc. cit., April 1952, H. E. Parks 7542, as *H. fuscopurpureum*, rev. Ju and Rogers (1996) as *H. fuscum* [K(M) 121853]; - Hawaii: Oahu, Forest Reserve. C. L. Shear 187, det. J. H. Miller as *H. rubiginosum* (B 70 0010854 ex USDA 71670; chemotype C/C); - Maine: Loring, 6 Mar. 1991, J. D. Rogers (JDR). - Maryland: Beltsville, *Carpinus*, 1 July 1950, F. Petrak in Reliqu. Petrakianae 2884 ex herb Graz as *H. rubiginosum* (B 70 0010857; chemotype C/C). - South Carolina: Pee Dee, *Alnus*, C. L. Shear 9802, det. J. H. Miller as *H. rubiginosum* (USDA 71665, B 70 0010855; chemotype A/S). - Virginia: Smith Island, *Osmanthus americana*, C. L. Shear, det. J. H. Miller (USDA No. 71662, B 70 0010763; chemotype C/C). - Washington D.C.: on fallen limb of "oak", geological park, 29 Dec 1906 (S-F44482; chemotype C/C, bark structure of host reminiscent of *Corylus*). - **Locality unknown** (Europe): corticated rotten wood of cf. *Corylus* (L 910, 267-664, **holotype** of *Sphaeria fusca* Pers.); decorticated wood, as *Sphaeria rubiginosa* (L 910, 263-1191; chemotype C/C).

**Deviating forms and dubious synonyms of *H. fuscum* from America and Spain**  
(?) *H. subchlorinum* Ellis & Calkins [MB443926]; (?) *H. commutatum* Nitschke

subsp. *holwayanum* Sacc. & Ellis) [MB137507]. We wish to summarize our results on deviating specimens of *H. fuscum*, which might eventually be recognized as new taxa, but the data available as now (and especially the lack of cultures and fresh material) is insufficient to make new taxonomic proposals at this time.

A specimen that is apparently distributed to many herbaria as exsiccatum [USA: California: Marin Co., vic. of Inverness, *Quercus agrifolia*, H. E. Parks as *H. fuscum* (Univers. of California: California Fungi Nr. 321; B 70 0010727; BR Myc 074155,47; S-F F44481) deviated from *H. fuscum* as well as from *H. porphyreum* and may constitute another undescribed taxon associated with *Quercus*. The HPLC profile deviated from typical *H. fuscum* by little extractable pigment. Daldinins C and E were only detected tentatively besides daldinone A, and no BNT was observed. KOH-extractable pigments were dull green. Ascospores measure 15-18 x 7-9 µm, i. e. larger than in typical *H. fuscum*, but no asci were seen. Another duplicate that we have not seen (BPI 641633) was studied by Ju and Rogers (1996).

The type of *H. subchlorinum* [USA: Florida, Jacksonville, "1836", W. W. Calkins, N. Amer. Fung. 2115, (NY, 3 packs, co-types of *H. subchlorinum*, isotype in K(M) 123179, deviating in collection data „Winter 1886“; probably the correct collection date) had rather small ascospores (8-10 x 4-5 µm), but showed a similar chemotype as material from *Corylus* (yielding daldinins and BNT but no daldinin A). Another fungus listed *sub H. fuscum* by Ju and Rogers (1996) was also collected from *Quercus* in the USA [Iowa: Decorah, corticated wood of *Quercus*, July 1883, E. W. Holway 3679 (NY-**holotype** of *H. commutatum* subsp. *holwayanum*). When we received the specimen, it had already been separated by Yu-Ming Ju from an element that corresponds with *H. crocophellum* (see above) because it did not fit the protologue of this variety. We agree with Ju and Rogers (1996) that the remaining stromata

have the same general appearance of *H. fuscum* from corticated wood and agree well with the protologue. Their KOH-extractable pigments are hazel (88 in Rayner 1970). Ascospores are 10-13(-15) x 4.5-5.5 µm, basically in agreement with *H. fuscum* but mostly lacking sigmoid germ slits. However, HPLC revealed macrocarpones A and C and a series of unknown compounds besides BNT, and daldinins were not found. Characteristic pigments of *H. porphyreum* were not detected, either. This "chemical race" may represent another host-specific variety or even a different species of the *H. fuscum* complex, with chemotaxonomic affinities to *H. macrocarpum*. It is not surprising to see so many aberrant forms on *Quercus* in the USA, where the Fagaceae shows a considerably higher diversity than in Europe, which could have influenced their associated mycobiota.

Material from **Belgium** [Liege: Malmedy, *Fagus* ("sur l'ecorce du chene et du hetre"), C. Roumeguere (ex Reliq. Libertianae 701) Fungi Gallici exsiccati no1384 det. K. van der Gucht & K. Laureys (BR-Myc 060832,13)] resembled *H. fuscum* in its morphology as well as in its chemotype, however, the ascospores measured 13-18 x (5.-5-)6-7 µm, and the HPLC profile was quite inconclusive; daldinin C and BNT were only detectable by HPLC-MS in the old depauperate specimen.

Material from the Spanish Pyrenées (**Spain**: Huesca: Bielsa, Boda de Campolino, P. N. de Ordesa, "*Buxus sempervirens*," 17 Oct. 1989, J. Checa, as *H. rubiginosum* (MA 31863 ex Herb Univ. Alcalá 14096) actually resembled *H. fuscum* as well. The stromata were reminiscent of the effused form of this species that is easily confused with *H. rubiginosum*. However, they were small (max. 9 mm diam) and somewhat erumpent. The HPLC profile was in accordance with mature *H. fuscum* from *Alnus* and *Salicaceae*, however, ascospores averaged larger than usual [14.5-15.5 (-17) x 6-6.5(-7) µm]. Ascus dimensions were recorded as follows: p. sp. 120-130 µm, stipes 45-80, total up to 205 mm long,

with apical ring 1-5-1.75 x 3.5-3.75 µm. The host was definitely not *Buxus*, but more likely *Corylus*, considering the wood structure and the thin, silvery periderm. The layers beneath the stromatal surface were atypical as they showed a reddish brown color, yielding dark olivaceous to dark brick pigments in 10% KOH. We conclude that those and similar specimens may have prompted Ju and Rogers (1996) to adopt a rather broad concept of *H. fuscum*. We have included here more detailed data to allow others to further pursue this problem, based on fresh material from the above localities and hosts. A significant number of specimens from hosts on which this fungus has not frequently been reported should be made available from all regions of the world for further polythetic studies.

***Hypoxylon julianii* L.E. Petrini  
[MB103465]**

This species resembles *H. rutilum* in its HPLC profile of stromata (Stadler et al. 2004a) and cultures (Bitzer et al. 2008), but is easily segregated by its ascospore morphology and stromatal habit. Rutilins (see *H. rutilum* below) are responsible for the scarlet colors of the granules located beneath the stromatal surface. The culture produces 5-methylmellein, like most other species of *Hypoxylon* treated in this paper.

**Specimen examined:**

**Switzerland:** Kt. Graubünden: Solas near Filisur, 3.Sep. 1982, L. E. Petrini, decorticated wood of *Alnus incana* (ZT- holotype; culture ATCC 58967).

***Hypoxylon laschii* Nitschke [MB153239]**

Examination of the holotype confirmed previous results on material from France in that mitorubins prevailed in its stromata (Stadler et al. 2004a), suggesting differences from *H. rubiginosum sensu stricto*. These are also reflected in morphological features and the apparent host specificity of this species for *Populus*. The specimen in NY did not deviate in

its HPLC profile from the above materials.

**Specimens examined:**

**Poland:** (previously Germany, Pommern), Neumark, Landkreis Friedeberg, near Driesen (Drezdenko) corticated rotten wood of *Populus*, leg. Lasch ex herb. Nitschke (B70 00909231-**holotype**). - **USA:** Maine: Lexington Co., Brackett Hill, corticated branch of *P. tremuloides*, 14 Aug. 1971, H. E. & M. E. Bigelow as *H. rubiginosum*, rev. Y.-M. Ju (NY).

***Hypoxylon liviae* Granmo [MB375052]**

This species is associated with *Sorbus* and was so far only found in Norway (Granmo 2001). It can be separated rather easily from its relatives through the combination of sepia effused-pulvinate stromata, small perithecia with umbilicate, black ostioles, a well-developed brown tissue between and beneath perithecia with Luteous (12) to Sienna (8) KOH-extractable pigments. The ascospores are dark brown, nearly equilateral, 9.5-11.5 x 4.5-5.5 µm, with a straight, very conspicuous germ slit spore-length and perispores dehiscent in 10 % KOH. In the specimen studied we frequently found anomalous ascospores in 4-spored asci, up to 15 x 7.5 µm. Several asci were even 1-spored and contained a single giant ascospore (40-56 x 5.5-7.5 µm), cylindrical with narrowly to broadly rounded ends, with a spore-length germ slit. It remains unclear whether this anomalous development is characteristic of this species but it does not occur too frequently in related taxa. *Hypoxylon liviae* can also be distinguished from *H. rubiginosum sensu stricto* by its *Virgariella*-like anamorph in culture. The cultures of *H. liviae* that we obtained grew extremely slowly and soon ceased growth at 23 °C.

The stromatal HPLC profile revealed mitorubins and orsellinic acid. In addition, a series of further, unknown azaphilones were detected, which were reminiscent of the unidentified pigments of *Pyrenomyxa morgani* M. Stadler, Laessøe & Lar.N. Vassiljeva (see

Stadler et al. 2005 as PM1). One of them was also found in *H. saliciola* (see further below and Fig. 4) and is therefore named here "HS1". The other two peaks were only found in *H. liviae* and are named "HL1-2" in Fig. 4. Possibly those compounds account for the slight deviations in pigment colors as compared to the species of the *H. fragiforme* chemotype, which are normally Orange (7 in Rayner 1970). In any case, the HPLC profile of this species appears quite specific, even though this remains to be confirmed by studying additional specimens. Neither BNT nor rubiginosins were detected, which discriminates *H. liviae* from various other members of the *H. rubiginosum* complex.

#### Specimens examined:

**Norway:** Nordland: Evenes, Forra, Hoggvik, 0.6 km NE of Forrahaugen, *Sorbus aucuparia*, 6 Aug. 2001, A. Granmo AG 32/01 (TROM 39600); Troms: Bardu, Sørødal, 1.5 km N of Sørømo, *S. aucuparia*, 11 June 1999, A. Granmo 66/99 (TROM 39588); loc. cit., Storffjord, Skibotn, Aksugaikuvvarri, *S. aucuparia*, 20 June 1997, leg. L. Mølster & E. Arneberg, ex. herb. A. Granmo 1/97 (TROM 39598); loc. cit. Målselv, Rostavatn, on living *S. aucuparia*, in an area with the bark flaking off, 25 July 2000, A. Granmo 13/2000 (TROM 39586).

#### *Hypoxylon macrocarpum* Pouzar [MB315729]

We have confirmed by chemotaxonomic studies on type material as well as by inclusion of various further specimens that this species is present all over the Northern hemisphere, where its stromata may appear on various hosts. Even immature specimens can be easily recognized by HPLC, due to the presence of the apparently specific macrocarpones (see Mühlbauer et al. 2002 and Introduction). See also in the section "deviating forms of *H. fuscum*" for another, possibly undescribed species of *Hypoxylon* that also contains macrocarpones, in combination with different co-metabolites than those observed in *H. macrocarpum*.

#### Specimens examined:

**Belgium:** Antwerpen: Hemiksem, Kallebeek, *Salix*, 7 Aug. 1994, H. de Meulder 10154 as *H. rubiginosum* (BR-Myc 029253,56). - **Czech Republic:** Bohemia: forest „Veltruský park“, Kralupy, Egyptský pavilon, on fallen branches of *Acer campestre*, 02 Sept. 1998, Z. Pouzar (PRM 882575); loc. cit., protected area "Voškov" near Karlstejn, fallen branch of *Carpinus betulus*, 4 Sep. 2002, Z. Pouzar (PRM 896583). - **Germany:** Brandenburg: Landkreis Märkisch Oderland, near Buckow (Märk. Schweiz), *Carpinus*, 25 Mar 1997, D. Benkert (B70 00909366). - North Rhine Westphalia: Haan-Gruiten, *A. pseudoplatanus* H. Wollweber (Ww 3243); loc. cit., *F. excelsior*, 16 May 2005 (STMA 05121; duplicate in PDD, culture: CBS 118188) - **Slovakia:** forest „Panónsky hój“, Dárniky, near Čierna voda (h. p. Bratislava), fallen branch of *F. angustifolia*, 25 Oct. 1971, Z. Pouzar (PRM 807840 – **holotype** of *H. macrocarpum*). - **Ukraine:** Kharkiv district, Gomolshansky National Nature Park, *Populus tremula*, 21 July 2005, CWU (Myc) AS 1363 (mature); Kyiv district, "Rastochie" Natural Reserve, *Fagus sylvatica*, 20-21 Aug. 2002, CWU (Myc) AS 999 and -1421. - Kyiv, Goloseevo forest, *Carpinus betulus*, 2 June 2005, CWU (Myc) AS 1365 and -1366 (all the above treated in Stadler et al. 2007); **USA:** Colorado: 1910, F. J. Seaver & E. Bethel, Fungi of Colorado, det. J. H. Miller as *H. rubiginosum* (NY).

#### *Hypoxylon macrosporum* P. Karst. [MB161373]

This species is characterized by its host specificity for *Salix* and its large ascospores. It has a boreal-montane distribution. We have not yet studied the lectotype from Finland but included a specimen that was regarded synonymous by Ju and Rogers (1996) and represented the type of Miller's *H. vogesiacum* var. *macrosporum*. All specimens studied contained BNT and daldinins, which accounts for the greenish pigments of this species and revealed affinities to *H. fuscum* (cf. Fig. 5).

**Specimens examined:**

**France:** Ariège: Mérens les Vals, chemin de l'étang de Bésines, Bois Long (1950 m), *Salix bicolor*, 3 Sept. 2000, JF-00183. - **Norway:** Nordland: Grane, Øvre Fiplinvatnet, S of Jensnes, on dead trunk of *Salix nigricans* ssp. *nigricans*, 1 July 1996, G. Mathiassen GM 3335 (TROM 2723); loc. cit., Trofors, *Salix nigricans* var. *nigricans*, 27 July 1989, G. Mathiassen GM 7781 (TROM 2779); loc. cit., Rana, Stokkalia, *Salix lapponum*, 21 July 1989, G. Mathiassen GM 7534 (TROM 2767). - Troms: Kåfjord, Kåfjorddalen, SE of Sabitjåhka, *Salix glauca* ssp. *glauca*, 20 Aug. 1983, G. Mathiassen GM 2494 (TROM 1950); loc. cit., Dalen, *Salix myrsinifolia* ssp. *borealis*, 18 July 1981, G. Mathiassen GM 772 (TROM 30286); Målselv, Dividalen, Dødesvann, *Salix lanata* ssp. *lanata*, 21 Aug. 1983, G. Mathiassen GM 2502 (TROM 1951); Tromsø, Kaldsletta, *Salix*, 2 Aug. 1995, A. Granmo 2/95 (TROM 30240); loc. cit., Bardu, Sørvalen, 1.5 km N of Sørmo, *Salix*, A. Granmo & G. Mathiassen AG 66/99 (TROM 39570). - **USA:** Wyoming: Medicine Bow Mts., wood of *Salix*, 1922, C. H. Kauffman and L. E. Wehmeyer (BPI 594093- **isotype** of *H. vogesiacum* var. *macrosporum*).

***Hypoxylon notatum* Berk. & M.A. Curtis [MB156703]**

This species is said to be a relative of *H. perforatum* that differs in stromatal morphology, as well as by its reduced ascus apical ring and in having larger ascospores (Ju and Rogers 1996). We have not yet studied many specimens of this taxon, whose type is from Eastern continental USA, aside from the type material in K and a specimen from Taiwan, in which daldinin C had been detected tentatively (Hellwig et al 2005). As additional material became available, we re-interpreted the HPLC data reported before by comparison of the spectra data in our HPLC library. We ultimately found that type material of *H. mbaiense*, from the neotropics, showed similar HPLC profiles as the type of *H. notatum*. Their prevailing

pigments are mitorubrin-like azaphilones (see Fig. 6 for material from the Ravenel herbarium in PH which was still suited well for HPLC analyses). The reason for this discrepancy remains unclear. The latter pigments were only detected by HPLC-MS by comparing their molecular peaks, which were overlaid by a mixture of signals that could not be interpreted further. Hence, the specimen may have been subjected to excess heat, and only daldinin C may have remained stable enough to be detected by a superficial HPLC analysis. In any case, the three specimens from USA gave more or less dilute Umber (9) to Luteous (12) pigments in KOH; Pure Yellow (14) or Greenish Yellow (16) pigments reported by Ju and Rogers (1996) for this species were only confirmed for the Taiwanese specimen. The absence of hypomiltin derivatives is in accordance with these findings, however, material from USA also contained some unknown azaphilones that may cause yellowish pigment colors in KOH if they are present in higher concentrations than the normal mitorubrins and rubiginosins. It will hardly be possible to verify these findings without having fresh material at hand for comparison of these complicated HPLC profiles.

The related *H. ulmophilum* (see below) has some distinctive morphological features and appears to be more host specific. It diverged phylogenetically from material of *H. notatum* in the molecular study by Hsieh et al. (2005), albeit both otherwise clustered in a group of *Hypoxylon* which are known to have mitorubrin like stromatal pigments. Considering our results on other taxa of this group, it remains unclear whether the material from which the culture used in the latter study really corresponds with the material from USA. The specimen cultured and studied for molecular data by Hsieh et al. (2005) was from Hawaii, whereas the cultures described earlier on by Ju and Rogers (1996) were made from Taiwanese specimens and not used for the molecular study. One of the specimens from Taiwan was kindly provided to

us by J. D. Rogers and showed a different HPLC profile to those from Brazil and USA. The corresponding stromata were scanty and did not allow us to conduct a complete morphological survey. Possibly, this species (or the members of this species complex) have the ability to produce daldinins and mitorubrin type azaphilones in concert. If so, the prevalence of these metabolites may depend on either the state of development or may differ among local and/or host-specific populations. Quang et al. (2004a) have reported the occurrence of small quantities of daldinin C in *H. rubiginosum* too.

#### **Specimens examined:**

**Brazil:** Mbay, decorticated wood of *Quebrachia lorentzii*, 17 Feb. 1882, B. Balansa 3419 (LPS-**holotype**; K(M) 123173; NY-**isotypes** of *Hypoxylon mbaiense*). - **Taiwan:** Ping-tung Co., Heng-chun, Ken-ting, 26 Nov. 1987, Y.M. Ju 76112604 (JDR, see Ju and Rogers 1996, Hellwig et al. 2005, with deviating chemotype). - **USA:** Florida: Thaxter 7183 ex FH (JDR); South Carolina: on twigs of *Celtis* sp., ex herb. Berkeley No. 1910, **syntype** (of *Sphaeria notata*), depauperate, **lectotype** of *H. notatum* selected by Miller (1961); K(M) 123174; loc. cit. corticated wood of *Quercus*, 1855, H. W. Ravenel, Fung. Carol. Exs. Fasc. IV, no. 36, as "*H. notatum* Berk. et Curt., sp. nov." (PH-1031228; duplicate in BPI studied by Ju and Rogers 1996).

#### ***Hypoxylon novemexicanum* J.H. Mill. [MB332468]**

We have studied type material by HPLC and found that this species contains mitorubrins and orsellinic acid, which agrees well with its stromatal pigments in KOH, but it differs from other members of the *H. rubiginosum* complex by the absence of rubiginosins. *H. novemexicanum* appears morphologically similar to *H. crocopenum*, from which it differs in its ascospore morphology. Cultures and (*Virgariella*-like) anamorphs, however, are rather similar in those two species (cf. Ju and Rogers 1996).

#### **Specimens examined:**

**USA:** New Mexico: Clonderoft, 1 Sep. 1904, T. H. Macbride 3247 (GAM 5896 – **holotype**; BPI 590962 – **isotype**).

#### ***Hypoxylon perforatum* (Schwein.: Fr.) Fr. [MB187004]**

**Confirmed synonyms:** *H. plumbeum* Speg. [MB188957]; *H. rubiginosum* (Pers. Fr.) Fr. var. *microcarpum* Speg. [MB137474].

Despite its morphological similarity to *H. rubiginosum sensu stricto*, this species can be easily segregated because its stromata contain hypomiltin and lack other, chemically related pigments of the mitorubrin and rubiginosin types (Hellwig et al. 2005). Therefore, its stromatal pigments in KOH are citrine or greenish yellow, rather than orange. We have studied a number of further specimens from the Northern hemisphere and found the results remarkably consistent. Traces of hypomiltin were still detected in a very small piece of the type material (BPI 738766) by HPLC-MS. This species is frequent on *Fraxinus* in Southern Europe and even in the USA, but colonizes other hosts in various parts of the world. It is one of the few taxa in this species complex that can really be regarded as cosmopolitan and was recorded from the IC, in contrast to *H. rubiginosum* as understood here. The specimen in question had been identified by Rogers as *H. rubiginosum sensu* Miller (1961).

**Specimens examined** (all obtained as *H. rubiginosum* if not stated otherwise):

**Brazil:** Sao Leopoldo, on twigs, 1907, Rick, Fungi Austro-americani No. 357 as *H. mbaiense* (2 packets, one ex FH), rev. Ju and Rogers (1996) as *H. perforatum* (NY). - **Belgium:** Luxembourg: Villers-sur-Semois, Bois de la Fosse, Pulmonario-Carpinetum, dead wood of *Fraxinus*, 30 Apr. 1993, A. Fraiture 1800 (BR-Myc 018230,91). - **Czech Republic:** Moravia: "Mährisch-Weißkirchen" (Hranice), Podhorn, *Ulmus*, July 1931, F. Petrak as *H. rubiginosum* [Rel. Petrakianae (Graz) Nr. 1456,

B 70 0010825]. - **Germany:** Badenia-Württemberg: Sulzbach, Feb. 1979, G. Krieglsteiner (STU K219/79, host det. as *Fraxinus*); Nordwürttemberg, Schwäbisch Hall, vic. of Gelbingen am Kocher, 26 Mar. 1986, L. Krieglsteiner (STU K132/86). - Rheinland-Pfalz: Landkreis Mayen-Koblenz, vic. of Rhens, *Fraxinus*, 10 June 1938, J. Sponheimer 6758, det. W. Kirschstein (B 70 0010837). - **Italy:** Veneto: Prov. di Treviso, near Selva di Cadore, in ramis emortuis, D. Saccardo (Mycotheca italica Nr. 81 (B 70 0010827)). - **New Zealand:** Coromandel: 31 Aug 1958, J. M. Dingley, det. Ju and Rogers 1996 (PDD 62068). - **Paraguay:** San Pedro, Misiones, corticated wood of *Ilex paraguayensis*, Feb. 1907 (LPS 2017- **holotype** of *H. rubiginosum* var. *microcarpum*); Guarapi, corticated hard wood, 2 Aug. 1881, B. Balansa 2760 (LPS 1949- **holotype** of *H. plumbeum*). - **Spain:** Barcelona: Coll de Olzinelles, *Arbutus unedo*, 31 Oct. 1974, R. Bertault 13728 (MA 38529). Canarias: Tenerife, Los Silos, Monte del Agua, wood, 27 Oct. 1986, det. J. D. Rogers as *H. rubiginosum* (TFC Mic. 2750). - **USA:** Alabama: Tuskegee, Macon Co., *F. nigra*, 28 Nov. 1935 (BPI 592425). - Florida: Big Tree, *Fraxinus* sp., 7 Feb. 1946, C. L. Shear (BPI 592357). - Georgia: Athens, *F. lanceolata*, 20 Jan. 1925, det J. H. Miller as *H. rubiginosum*, Lloyd herb. No. 10714 (BPI 716798). - Maryland: Cabin John, *Fraxinus* sp., 12 Dec. 1918, C. H. Kauffman (BPI 592356). - New York: Aquetuck, *Fraxinus* sp., 1 Sep. 1925, C. L. Shear (BPI 592359). - Virginia: Vienna, *Fraxinus* sp., 19 Jan. 1908, C. L. Shear (BPI 592355); Prices Fork, *Fraxinus* sp., 17 Oct. 1935, C. L. Shear (BPI 592358). - **Exact locality unknown** (but probably North America): on dry, dead oak limbs (*Quercus*); Ellis: North American fungi, No. 165 (2 packets; PH-1031227, PH-1031232). - Locality and substrate unknown, ex herb. Schweinitz (BPI 738766 - **holotype** of *Sphaeria perforata*).

***Hypoxylon petriniae* M. Stadler & J. Fourn. [MB488928]**

This species occurs mostly, but not exclusively

on *Fraxinus* and was reported by Stadler et al. (2004a) from France after Ju and Rogers (1996) had mentioned it *sub H. rubiginosum* (Notes, based on material from UK and Mexico) and Petrini and Müller (1986) had already described it earlier as *H. rubiginosum* var. *cercidicola*. We have meanwhile confirmed that this fungus occurs in temperate North America (including the first record from USA) and further European countries. Interestingly, Plowright has apparently already recognized that this fungus is something special in the 19<sup>th</sup> century, since he recorded a "*f. fraxini-excelsioris*", but did apparently not publish this validly. We also confirmed that *H. cercidicola sensu* Granmo (1999a) is identical to *H. petriniae*, based on specimens included in his monograph. The Norwegian material contained relatively small amounts of BNT, overlaid by larger amounts of azaphilones, with rubiginosin C as prevailing compounds but otherwise was rather typical of this species.

**Specimens examined** (if not stated otherwise, all were obtained as *H. rubiginosum*):

**Austria:** Burgenland: Frohsdorf (Rosaliengebirge), *Fraxinus excelsior*, H. Huber, det. W. Kirschstein (B 70 0010843). - **Belgium:** Liege: Esneux, on dead branch A. Fraiture 2892 (ex Troupin), 25 Apr 2002 (BR-Myc 150505,58, host det. as *Fraxinus*). - **Luxembourg:** Villers-sur-Semois, Bois de Rastad, Pulmonario—Carpinetum, dead wood of *Fraxinus*, 26 Nov. 1994, A. Fraiture 2360 (BR-Myc 029566,78). - **Oost-Vlaanderen:** Ostbroek, 20 May 1991, H. de Meulder 6595 (BR-Myc 015453,30, host det. as *Fraxinus*). - **Germany:** Brandenburg: Landkreis Oberhavel, near Oranienburg, Am Malzer Kanal, *Fraxinus*, 5 Oct. 1997, D. Benkert as *H. rubiginosum* (B70 00909367); Landkreis Teltow-Fläming, vic. of Woltersdorf, Bürgersbusch, *Fraxinus*, 9 May 1999, D. Benkert as *H. rubiginosum* (B70 00909368). - **Netherlands:** Zuid-Limburg: Elsloo, Bunderbos, dead wood of *Fraxinus*, 8 Sep. 1995, A. Fraiture 2418 (BR-Myc



034936,16). - **Norway:** Hordaland: Granvin, Eide, *F. excelsior*, 24 July 1994, A. Granmo 45/94 (TROM 3814); Rogaland, Suldal, Hylsfjord, Hysten, *F. excelsior*, 25 July 1994, A. Granmo 58/94 as *H. cercidicola* (TROM 3812). - **UK:** Devon: Slapton wood, *Fraxinus*, June 1974, A. J. S. Whalley 183 (BR-Myc 097110,13). - Norfolk: King's Lynn, *Fraxinus excelsior*, "in ligno putrido, raro", annotated as "*H. rubiginosum* f. *fraxini-excelsioris*", Plowright 1876 in de Thümen, Mycoth. univers. Nr. 1071. (B 70 0010851, BR Myc 097108,11); loc. cit., *Fraxinus*, Jan. 1882, C. Roumeguere ex herb. C. B. Plowright, Fungi Gallici exsiccati no 2185 (BR-Myc 097107,10). - England, exact locality unknown, Plowright, ca. 1873 in Plowright; Sphaeriac. Britann., Cent. 1, No. 21. (B 70 0010826). - **USA:** New Hampshire, Thornton, *F. americana*, 8 Oct. 1933, H. J. Eno (BPI 592411).

***Hypoxylon porphyreum* Granmo [MB464669]**

**Synonym:** *H. bicolor* Ellis & Everh. [MB213023], nom. illeg. (ICBN2000 Art. 53), non Berk. & M.A. Curtis 1875  
The "type" of the illegitimate name *H. bicolor* Ellis & Everh. and authentic specimens of *H. porphyreum* Granmo agree very well with one another with respect to their morphological features and their highly specific HPLC profiles. *H. bicolor* was synonymized with *H. fuscum* by Ju and Rogers (1996). This name, however, is a later homonym of *H. bicolor* Berk. & M. A. Curtis 1875, which happens to be a *Diatrype*. *H. porphyreum* is thus reported for the first time from America. This fungus has never been found on hosts other than Fagaceae, in contrast to *H. fuscum*, which, according to our knowledge, has never been recorded with certainty from *Quercus* or other fagaceaeous hosts. Morphological differences, however, are rather slight and can be best seen when material of genuine *H. fuscum* is compared. The granules surrounding the perithecia of *H. porphyreum* are citrine yellow, while being orange brown in *H. fuscum*, and the stromatal pigments in KOH

are pale brown and generally less intense than in *H. fuscum*. Ascospores are slightly paler, and smaller than in most collections of *H. fuscum*, but within the size range accepted for that species by Ju and Rogers (1996) and Petrini et al. (1987). Their frequently sigmoid germ slit is noteworthy as it is also a distinctive feature of *H. fuscum*.

Besides Norway (see Granmo 1999a), this species was now encountered at rather high elevation in the French Pyrenées. We have checked concurrently various collections of *H. fuscum*-like stromata but were so far unable to find further records of *H. porphyreum* from *Quercus*. Nevertheless, some specimens collected from *Fagus* in B and BR from Belgium, the Czech Republic, and Germany were reminiscent of *H. porphyreum*. They were rather depauperate, and contained only a few mature ascospores. However their HPLC profiles were also devoid of daldinins, and they contained similar specific compounds besides BNT that were otherwise only found in *H. porphyreum*. The immature parts of the specimen in BR contained cytochalasin-like compounds as young stromata of *H. fuscum* from *Alnus* (cf. Stadler et al. 2007, and *H. fuscum* above). Albeit *Fagus* belongs to the same family as the typical host, their correspondence should be confirmed based on fresh material. The HPLC profile of *H. porphyreum* (cf. Fig. 5) is quite different from all data on *H. fuscum* that we have so far recorded, including over 200 specimens from various non-fagaceous host species and unidentified substrates (see below for a selection of such specimens). Daldinin type azaphilones as well as the putative binaphthalene derivatives known from specimens associated with *Alnus* and the Salicaceae (Stadler et al. 2007) lacked in *H. porphyreum*. We attempted to isolate the apparently specific pigments from specimen JF-03167, but failed to identify them because they proved highly unstable. Even fractions from preparative HPLC generated in a similar manner

as described by Stadler et al. (2006a) were all found devoid of daldinins. These results confirmed the preliminary HPLC data presented by Granmo (1999a), who also reported *H. porphyreum* to be different from *H. fuscum* with respect to its rDNA sequence (Granmo et al. 2001), and by HPLC profiling in the absence of standard metabolites.

The close relationship of *H. porphyreum* to *H. fuscum* is also expressed by their similar anamorphs. A culture of the specimen JF-03167 showed a *Virgariella*-like branching pattern as defined by Ju and Rogers (1996), similar to that of *H. fuscum* as described for the latter species by the latter authors as well as by Petrini and Müller (1986). It mainly differed in having slightly smaller conidiogenous cells (15 – 30 x 1–2 µm vs. 20–42 x 1.5–2 µm in *H. fuscum*) and conidia 4–5.5 x 2–2.5 vs. 4–6 x 2–2.7 µm in *H. fuscum*). Our culture is highly similar to that described by Granmo (1999a, cf. Fig. 23) for *H. porphyreum*.

#### Specimens examined:

**Belgium** ("Borussia, Malmedy", meaning the town of Malmedy which formerly belonged to Prussia) *Fagus sylvatica*, no date, de Thümen (ex M.A. Libert), Mycotheca universalis, as "*H. fuscum* f. *fagi sylvaticae*" (Reliquiae Libertianae Nr. 1861; B 70 0010742; BR Myc 097076,76). -

**Czech Republic:** Mährisch Weisskirchen, Teplitz, on bark of *Fagus sylvatica*, March 1889, in herb. F. Petrak, ex Dr. G. Eichler (Fungi Eichleriani No 224 - BR Myc 097065,65). -

**France:** Ariège: Orlu, Les Forges d'Orlu, Côte des Bouychels, 950 m., 16 May 2003, JF-03062, on *Quercus petraea*. - Same location and substrate, 19 Sept. 2003, JF-03167 (culture CBS 119022). - **Norway:** Aust-Agder: Grimstad, Einarfjell, *Quercus*, 16 July 1995, A. Granmo AG 133/95 (TROM 38214); same collection data (TROM 38212); same collection data (TROM 38213) - **Germany:** North Rhine Westphalia: vic. of Münster, near the "Coburg", Apr. 1864 Nitschke as *H. fuscum* (B 70 0010799; substrate

identified as *Fagus*). - **USA:** Louisiana: Pointe'a la Hache, dead limbs of *Quercus virens*, 22 Mar. 1886, A. B. Langlois 344 (NY- **holotype** of *H. bicolor* Ellis & Everh., nom. illeg.).

#### *Hypoxylon retpela* van der Gucht & van der Veken [MB358217]

Van der Gucht and van der Veken (1992) and Ju and Rogers (1996) described this species based on a specimen with the same details but only cited holotype material from GENT. We have studied the isotype in K for comparison with one of our new species from the IC (see taxonomic part). HPLC revealed orsellinic acid, mitorubins and rubiginosins. As judged from our results with respect to the species-specific azaphilone pigment patterns in *Hypoxylon* with orange stromatal pigments, this feature is deemed important not only for comparison with the related *H. fendleri*, which contains no rubiginosins but mitorubins (cf. Hellwig et al. 2005) but may even be significant for segregation of other related species, especially in the tropics.

#### Specimen examined:

**Papua New Guinea:** Madang Prov.: Balek Wildlife Sanctuary, on dead wood, 11 Nov. 1989, K. van der Gucht & L. de Meester, K(M) 22344 – **isotype**.

#### *Hypoxylon rubiginosum* (Pers.: Fr.) Fr. [MB213808] *sensu stricto*

**Confirmed synonyms:** *H. botrys* Nitschke [MB202754]; *Sphaeria granulosa* Pers. [MB443853]

As with *Sphaeria fusca* we have studied the type materials cited by Miller (1961) and Ju and Rogers (1996) in L and found that their HPLC profiles clearly corresponded with the "plurivorous" form of *H. rubiginosum*. This was described in detail by Petrini and Müller (1986, as *H. rubiginosum* var. *rubiginosum*), as well as Ju and Rogers (1996), Granmo (1999a) and Stadler et al. (2004a). Besides the anamorphic and teleomorphic characters described there,

this species is characterized by the lack of apparent host-specificity with respect to the occurrence of its stromata.

We had previously reported that it lacks BNT and contains rubiginosin A and other compounds that were identified by Quang et al. (2004a) as major metabolites. Out of many data recorded from ancient type specimens, those of the type in L is depicted in Fig. 6., to demonstrate the stability of the chemotaxonomic metabolites even after over 200 years of storage. Such a metabolite profile is not specific for *H. rubiginosum*, however, certain morphologically similar taxa differ from this pattern, containing other types of azaphilones, or other compounds in addition (cf. *H. salicicola*, *H. liviae* elsewhere herein).

Our studies on *Hypoxylon* have never revealed this species as understood here to occur in any tropical country. We did not yet verify all previous reports on the occurrence of this species *sensu* Miller (1961) but note that Ju and Rogers (1996) with whose morphological concept we agree, have reported it from Florida. Suwannasai et al. (2005) reported *H. rubiginosum* to occur in Thailand, even including ITS nrDNA data. However, the latter authors did not use any sequences from public databases of verified European material for comparison. They did not report details on anamorphic features, let alone HPLC profiles, hence it remains to be seen whether their "*H. rubiginosum*" actually corresponds to the taxon as understood here. The 5.8S/ITS nrDNA sequences published by Suwannasai et al. (2005) strongly deviate from our own sequences based on European material (D. Peršoh, H. V. Tichý & M. S., unpubl., but see for comparison the data from the paper by Bitzer et al. 2008 published in GenBank). It is noteworthy that we were even unable to find *H. rubiginosum sensu stricto* among the specimens collected from the Canary Islands, which are geographically much closer to Europe than Thailand. The specimens listed

below, however, were all in agreement with the typical form, and their HPLC profiles corresponded to the type in L.

**Specimens examined** (if not indicated otherwise, all were originally determined as *H. rubiginosum*):

**Austria:** „*ad lignum putridum*“, K. W. Fuckel, Fungi rhenani No 1051 (BR-Myc 097109,12; differing from the specimen so labeled in B!). - **Belgium:** Antwerpen: Hemiksem, Kallebeekbos, *Salix*, 5 July 1994, H. de Meulder 9874 (BR-Myc 028151,21). - Namur: Nismes, Fondri des Chiens, 24 July 1993, H. de Meulder 8668 (BR-Myc 019853,65). - Oost-Vlaanderen: Bazel Natuurpark, Scheldeland, 24 Jan. 1970, J. Moens 1116, det. K. van der Gucht and K. Laureys (BR-Myc 060794,72); Denderleeuw, *Populus X canadensis*, 29 Sep. 1992, H. Ruysseveldt (BR-Myc 048373,67); Moerzeke, Kastel, *Salix*, 12 Sep. 1992, H. de Meulder 7395 (BR-Myc 016246,47); Wellemeersen, 27 Aug. 1989, H. de Meulder 4093 (BR-Myc 012842,38). - **Canada:** Ontario: High Park, Toronto, *Fraxinus* sp., 31 Oct. 1912, J. H. Faull 12043 (BPI 592351). - **Czech Republic:** Moravia: „Sudetenland, near Sternberg, in truncis putridis“, Sep. 1925, F. Petrak (B 70 0010833). - **Germany:** Bavaria: Oberbayern, Landkreis Bad Tölz, Wolfratshausen, near Bad Heilbrunn, *Fagus*, W. Kirschstein (B 70 0010844). - Brandenburg: Landkreis Märkisch Oderland, Altfriedland, Am Stöbberow, Lapnover Mühle, 13. Sep. 1997, D. Benkert (B70 00909369). - Hessen: Rheingau-Taunus-Kreis, Oestrich-Winkel [Hostrichiam]. "*In sylva Hostrichiensi, ad lignum putridum, raro*", Fuckel, Fungi rhen. Nr. 1051 (B 70 0010824). - Badenia-Württemberg: Nordwürttemberg, Schwäbisch Hall-Breitenstein, *Salix*, 17 Oct. 1985, L. Krieglsteiner (STU K936/85). - North Rhine Westphalia: Westfalen, Nienberge near Münster, corticated wood of *Fagus*, Oct. 1865 (B - **holotype** of *H. botrys*); Münsterland, vic. of Münster-Nienberge, *Acer campestre*, Oct. 1865, Nitschke (B 70 0010845); loc. cit., Oct. 1866,

Füisting ex herb. Nitschke (B 70 0010850); loc. cit., "bei Erdmanns", Gasselstiege, June 1864 (B 70 0010846); loc. cit., same collection data (B 70 0010847); Münsterland, Warborgshügel, *Fagus*, Sep. 1865, Nitschke (B 70 0010848); Landkreis Unna, Thiergarten zu Cappenberg, „*Cerasus*“ (*Prunus cerasi*), Aug. 1866, Nitschke (B 70 0010849); Landkreis Euskirchen, vic. of Bad Münstereifel, Bodenbachtal, am Hirschmieser Bach, *Carpinus*, leg. Sponheimer, det. W. Kirschstein (B 70 0010842). - Rheinland-Pfalz: Landkreis Mainz-Bingen, vicinity of Nieder-Heimbach, Soonecker Grund, dry branch of *Fraxinus* lying on the ground, 8. Mar 1939, J. Sponheimer 8171, det. W. Kirschstein (B 70 0010834); Kamper Wald am Hermannsberg, *Populus tremula*, leg. Sponheimer, det. W. Kirschstein (B 70 0010835); Lkr. Mayen-Koblenz, vic. of Kapellen-Stolzenfels. Am Lauxbach, *Salix*, Sponheimer 6506, 25 Mar. 1938 (B 70 0010836); loc. cit., Rhens, Oberbergbach, *Salix caprea*, 13 July 1937, Sponheimer 6151 (B 70 0010839); Sankt Goarshausen, Loreley, *Salix caprea*, J. Sponheimer 6258, det. W. Kirschstein (B 70 0010841); „Rheinland“, Gebüsch am Bickelbach am Buchholz“, *Acer campestre*, 9 Apr. 1938, J. Sponheimer 6580, det. W. Kirschstein (B 70 0010840). - **Letonia**: Latgale: Vidsmueza, *Betula verrucosa*, 30 Oct 1934, K. Starcs 2499 (2 packets; B 70 0010829; B 70 0010830). - **Portugal**: Minho: Parque Nacional de Peneda-Gerês, Carvalhal da Ermita, *Laurus nobilis*, J. Checa, 28 Apr. 1989 (MA 31895 ex herb. Univ. Alcalá 15852). - **Spain**: Baleares: Menorca: Binifamis, Alayor (substrate given as *Pinus halepensis* but looks like angiosperm wood), 15 Nov. 1990, J. Checa (MA 31776 ex herb. Univ. Alcalá 14600); loc. cit., Sta. Eulalietel, Mercadal, *Arbutus unedo*, 16 Nov. 1990, J. Checa (MA 31775 ex herb. Univ. Alcalá 14601); loc. cit., twigs of *Phyllirea angustifolia*, 16 Nov. 1990, J. Checa (MA 31777 ex herb. Univ. Alcalá 14602). - **USA**: Delaware: Faulkland, *Populus* ("on decaying poplar boards"), Oct. 1897, A. Commons (Ellis & Everhart: North American Fungi 2nd series. No.

1949; PH-1031225). - Georgia: Athens, *F. lanceolata*, 21 Jan. 1924, J. H. Miller, Lloyd herb. No. 10628 (BPI 716799). - Louisiana: near St. Martinsville, on dead pieces of oak (*Quercus*), 31 Aug. 1889, A. B. Langlois 2148 ex herb. Ellis, det. J. H. Miller as *H. fusco-purpureum*; same collection data, 2 Sep. 1889, A. B. Langlois 2147 ex herb. Ellis, det. J. H. Miller as *H. rubiginosum* (BPI). - New York: Alcove, *F. nigra*, Oct. 1892, C. L. Shear (BPI 592433); same collection data (BPI 592431); Berne, *Fraxinus* sp. 31 Aug. 1925, C. L. Shear, det. J. H. Miller (BPI 592360); Newcomb, *F. nigra*, 28 Sep. 1928, H. G. Eno (BPI 592428). - Pennsylvania: Philipsburg, *F. nigra*, 1922, L. W. Nuttall (BPI 592434). - Vermont: Bethel, *F. americana*, 12 May 1927, P. Spaulding 16100, det. J. H. Miller (BPI 592410). - Virginia: Annandale, *Acer*, 9 July 1927, C.L. Shear, det. J. H. Miller (B 70 0010852 ex USDA 71664). - **Locality unknown** (Europe): corticated wood (L 910, 263-1194, **lectotype**, selected by Miller 1961, of *Sphaeria rubiginosa*; decorticated wood (L 910, 267-362, **holotype** of *Sphaeria granulosa*).

#### An undescribed taxon similar to *H. rubiginosum* from USA

Even in the United States, there are probably still numerous undescribed members of this species complex to be found. For instance, a specimen in B [USA: Ohio: Cincinnati, Mt. Airy forest, 5 Apr. 1936, *Celtis occidentalis*, leg. Hansbrough, det. J. H. Miller as *H. rubiginosum* (USDA 91670, B 70 0010856)] showed a stromatal morphology reminiscent of *H. rubiginosum sensu stricto*, but differed in its HPLC profile in containing BNT and a series of unknown components. Its ascospores were (15-)16-18 x 7.5-8.5 µm, ellipsoid-inequilateral with narrowly rounded ends and perispore dehiscent in KOH, and the KOH-extractable pigments Umber (9) to Hazel (88). No mitorubins or other known azaphilones were detected.

This species does not key out in Ju and Rogers

(1996), and we found no indications as to its affinities to other *Hypoxylon* spp. even from its pigment profiles. A case could be made to describe this fungus here as new, but we rather wish to encourage others to search for fresh material and study it to provide a holomorphic description.

***Hypoxylon rutilum* L.R. Tul. & C. Tul.  
[MB213459]**

It is widely reported that this species differs from related taxa by its apparent host specificity for *Fagus*. However, according to intensive field studies in Southwestern France, this fungus is rather more frequent on *Prunus avium* and *Corylus*. Among other morphological features described by Ju and Rogers (1996), it can be segregated from related species by the presence of scarlet granules immediately below the stromatal surface. The pigments concentrated in these granules were detected first by Stadler et al. (2004a, see HPLC profile in Fig. 2) and recently identified as the dimeric azaphilones, rutilins A (see Fig. 1) and the chemically similar rutilin B (Quang et al. 2005b), i.e., metabolites that arise from a condensation of the mitorubrin and rubiginosin azaphilones. Their biogenesis is apparently mediated by specific enzymatic reactions, which are not overexpressed in other members of the *H. rubiginosum* complex. We were meanwhile able to confirm the correspondence of our recently collected specimens with type material and another specimen in K that ended up in the Cooke herbarium from PC and was labeled "*H. rutilans*", which is not an accepted name in *Hypoxylon* (Ju and Rogers 1996). However, it also contained rutilins.

**Specimens examined:**

**U.K.** (exact locality unknown): corticated twigs of *Fagus*, Aug. 1858, Valvins (PC – **holotype**); "British Isles", sine loc., on bark of *Fagus sylvatica*, 1879, ex herb Paris (ex herb Cooke 1885). K(M) 124722 as "*H. rutilans* Tul. (ined.)"

***Hypoxylon salicicola* Granmo  
[MB464670]**

This species was segregated by Granmo (1999a) from the *H. rubiginosum* complex by its host specificity for *Salix*. Its ascospores are smaller than in *H. rubiginosum* and ellipsoid-equilateral. They resemble those of *H. subticinense* but have dehiscent perispores. Perithecia are smaller than in *H. rubiginosum sensu stricto*. We found additional differences by HPLC profiling. All specimens examined corresponded with the *H. fragiforme* chemotype with mitorubrinol acetate as major component. A specimen in BR keyed out as *H. salicicola*. However, it had numerous anomalous ascospores, and asci were often 4-spored. Another specimen from Belgium was collected fresh from the typical host plant and had normal ascospores and a highly similar HPLC profile as the Norwegian material.

Interestingly, an unknown compound with HPLC characteristics suggests that it constitutes another azaphilone (HS1). Traces of daldinone B were also detected in its stromatal extract after careful re-analysis of the HPLC data. Neither of these compounds were detected in *H. rubiginosum sensu stricto*. It remains to be confirmed by additional collections from the area whether *H. salicicola* jumps to other hosts when occurring further south in Europe, and whether the above metabolites are chemotaxonomic markers for this species.

**Specimens examined:**

**Belgium:** Luxembourg: Villers sur Semois, Bois de Rastad, on dead wood of *Fraxinus* (acc. to label), 5 May 1994, A. Fraiture 2100 (BR Myc 026548,67); Orval, Ruisseau de Williers, *Salix*, 29 Sep. 2006, JF-06243. - **Norway:** Nordland: Rana, Skugghei at Holmen, *Salix*, 23 Oct. 1995, leg. F. Skugghei, det. A. Granmo (TROM 38683); Evenes, Forra Hoggvik, *Salix*, 18 Sep. 1995, A. Granmo AG 236/95 (TROM 38682). - Troms: Bardu, Sjørdalen, 0.5 km S of Sørmo, on decaying branch of *Salix* sp (wood and cortex),

11 June 1999, A. Granmo AG 50/99 (TROM); loc. cit., Bjørnsund, Kjosén, *Salix caprea*, 12 Sep. 1998, A. Granmo AG 77/98; loc. cit., Setermoen, Moegga, *Salix*, 29 July 2000, A. Granmo AG 46/2000 (TROM 39619); loc. cit., Storfjord, Skibotn, Lullesletta v. Iselva, *Salix*, 25 Aug. 1996, A. Granmo and I. Mølster AG 27/96 (TROM 38684). - **Sweden:** Åsele Lappmark: Vilhelmina, near Lillån, *Salix myrsinifolia* ssp. *myrsinifolia*, leg. G. Mathiasen GM 6311 (TROM 3821).

***Hypoxylon shearii* Y.M. Ju & J.D. Rogers [MB414978]**

This species is only known from two collections thus far, which were both from *Quercus* in the Southeastern USA. It resembles *H. notatum* in having a reduced ascus apical ring as well as by its gross stromatal morphology but deviates in its ascospore morphology (Ju and Rogers 1996). We have studied the type in WSP and found that it contains mitorubins as well as rubiginosins, and therefore differs from *H. notatum sensu stricto* with respect to its HPLC profile.

**Specimen examined: USA:** Louisiana, East Baton Rouge Parish, corticated wood of *Quercus*, Apr. 1980, J. D. Rogers & J. P. Jones (WSP 69637 - **holotype**).

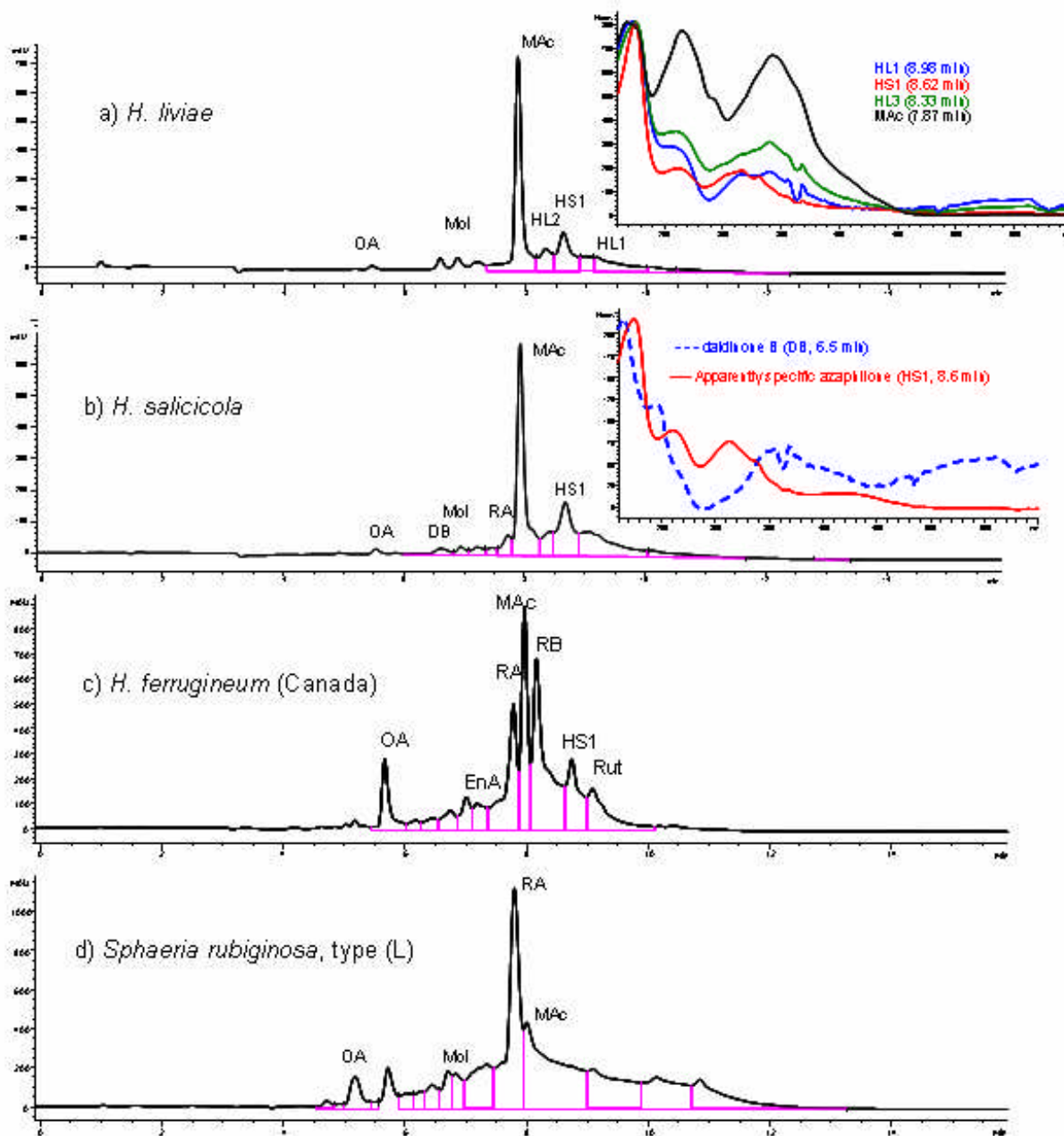
***Hypoxylon subrutilum* Starbäck [MB215471]**

**Synonyms** fide Ju and Rogers 1996 (provided with a question mark in case we doubt the conspecificity for results presented below): *Hypoxylina umbilicata* Starbäck [LEG; MB208147]; (?) *H. rubrostromaticum* J. H. Miller [MB332474]; (?) *H. haematites* Lév. var. *macrosporum* Theiss. [MB155375]; (?) *H. haematites* Lév. ex Theiss. f. *microspora* Theiss. [MB443860] ≡ *H. haematites* Lév. ex Theiss. var. *microsporum* (Theiss.) Rick [MB444455]; (?) *H. indicum* Syd. & P. Syd. [MB158467]; (?) *H. congoanum* Torrend ex Beeli [MB438100]. Some specimens from TFC keyed out as *H. subrutilum*, and this fungus is therefore

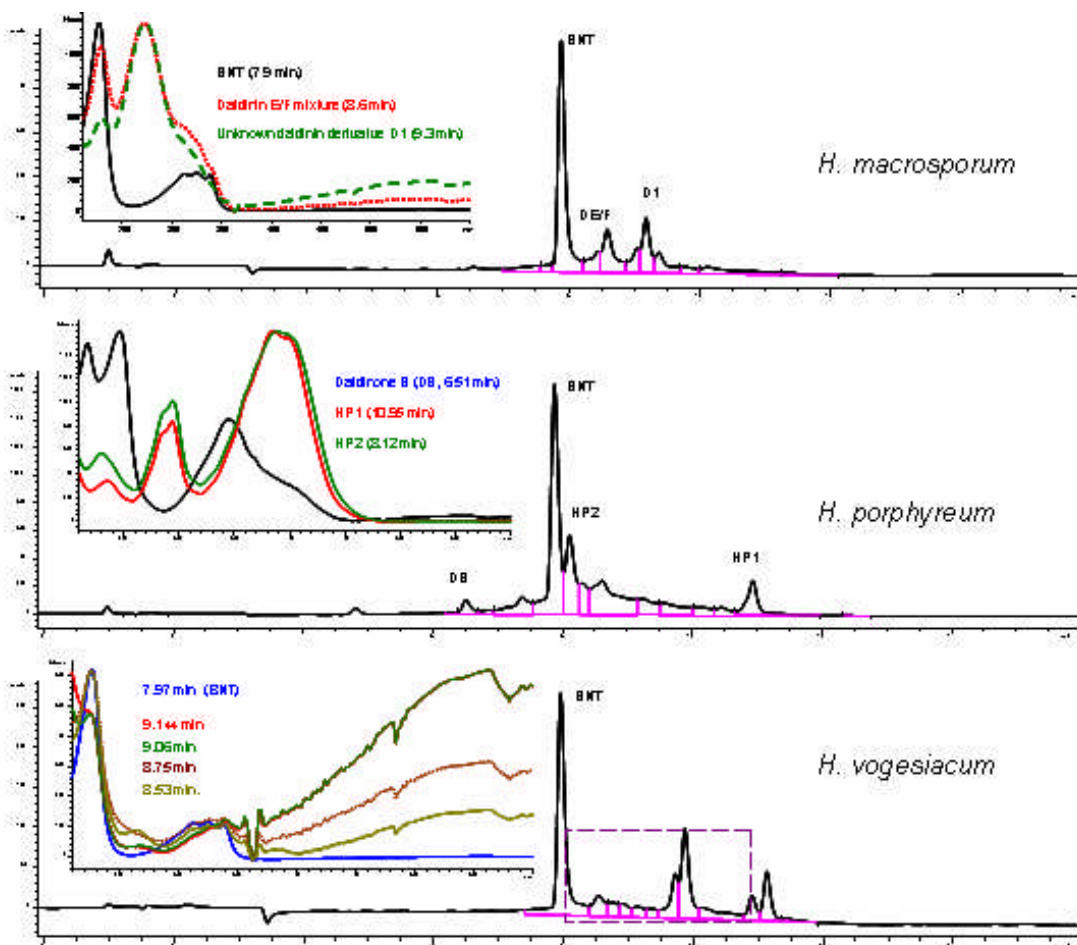
reported here for the first time from IC. However, we have to point out some incongruities between our own results and those reported by Ju and Rogers (1996), with regard to the characteristics of this species. The type specimen of *H. subrutilum* is from Brazil, but Ju and Rogers (1996) reported it from Asia and Africa as well. The culture on which their description of anamorphic features is based was obtained from a specimen collected in Hawaii, which we have not yet seen. They reported greenish olivaceous pigments for this species and emphasized affinities to *H. anthochroum*, based in their observations on anamorphic morphology, discussing the spore size range of the two above mentioned species to be a continuum.

On the other hand, they also stated that *H. anthochroum* was in their mind rather closely related to *H. placentiforme* [= *Daldinia placentiformis* (Berk. ex Curt.) Theissen in the current sense], while segregating some collections resembling *H. subrutilum* from New Zealand with similar glomerulate stromata and different ascospore morphology, as *H. subrutiloides*. Material of the latter species was studied by HPLC and found to contain mitorubins by Hellwig et al. (2005), and we meanwhile confirmed by studies on further specimens that these azaphilones are its major stromatal pigments (J. F. & M. S., unpubl.). The only chemical data hitherto available on *H. subrutilum* were recorded by Mühlbauer et al. (2002), who studied a specimen in CABI referred to by Martin (1969) as *H. rubrostromaticum* (i.e., a synonym of *H. subrutilum*). We have meanwhile studied the holotype of *H. subrutilum* and some other type and authentic specimens, which are listed further below. Except for the Deighton specimen from Sierra Leone, those were all listed by Ju and Rogers (1996) sub *H. subrutilum*. Since we have also found material from IC to correspond with this taxon *sensu* Ju and Rogers, we wish to present our results on the heterogeneous

chemotypes.



**Fig. 4.** HPLC-UV chromatograms (210 nm) of the stromatal methanol extracts of *Hyxpoxyton* spp. **a)** *H. liviae* TROM 39600, showing mitorubrinol acetate (MAc) and three unknown compounds with azaphilone-like DAD spectra. **b)** *H. salicicola* TROM 38683, showing MAc, rubiginosin A (RA), another unknown azaphilone (HS1) and traces of daldinone B (DB). **c)** *H. ferrugineum* (from Canada, K), showing Entonaemin A (EnA, the chemically related rubiginosins A and B (RA, RB) and rutilin besides HS1 and other known compounds. If detected, orsellinic acid (OA) and mitorubrinol (Mol) are indicated in the chromatograms but their DAD spectra are not shown. **d)** *H. rubiginosum* (type of *S. rubiginosa*, L), revealing RA as major component.



**Fig. 5.** HPLC-UV chromatograms (210 nm) of stromatal methanol extracts of *Hypoxylon* spp. including DAD spectra of some characteristic metabolites. **Above:** *H. macrosporum* TROM 2723, revealing the binaphthyl BNT, daldinins E/F (DE/F; mixture of isomers inseparable by reversed phase HPLC), and another apparently specific unidentified daldinin derivative (D1). Further unidentified peaks are similar to those detected in *H. vogesiaceum*), probably constituting unidentified binaphthalenes. **Center:** *H. porphyreum* TROM 38214, showing the binaphthyl BNT, its derivative daldinone B, and two hitherto unknown specific pigments. Notably, the HPLC profiles of *H. macrosporum* are much more similar to that of *H. fuscum*, whereas *H. porphyreum* has no common metabolite with the former species aside from BNT. **Below:** *H. vogesiaceum* TROM 30234, including DAD spectra of BNT and unknown derivatives thereof. No other known metabolite classes of the Xylariaceae were detected.

a) In the type specimen in S, mitorubrinol acetate, rubiginosin A and orsellinic acid were clearly revealed by HPLC (Fig. 6d). In contrast to Ju and Rogers (1996), who had characterized this species to have Olivaceous (48), Isabelline (65), or Honey (64) pigments, we found them to be faint Luteous (12), i.e., an orange color. In our experience, ancient specimens sometimes yield little stromatal pigments. It is thus difficult to

decide whether the dilute KOH extract has a yellowish, olivaceous, or rather a orange color. In addition, the stromatal HPLC profile showed large amounts of an artefact (A!) that we have previously also seen in various other ancient herbarium specimens, including the types of *D. bakeri* Lloyd (Stadler et al. 2004b) and *H. cercidicola* (Fig. 2). We have previously discussed that this compound might be a preservative



added to the specimens to prevent insect infestations (Stadler et al. 2004b), which evidently falsified the determination of stromatal pigments. Disregarding these artefacts, the results of HPLC profiling suggest that the type specimen of *H. subrutilum* would have yielded orange pigments in KOH in fresh state. A similar pigment color and essentially the same HPLC profile and morphological characters as in the type of *H. subrutilum* was noted also in the type of *Hypoxylina umbilicata*, the only name we can confirm to be a synonym of *H. subrutilum*. However, this specimen contained rubiginosin A besides mitorubrinol and orsellinic acid. Since the concentrations of azaphilones can in some instances vary in a given species, we do not regard the above differences as taxonomically significant. The ascospore size range in both the above specimens was identical, ranging from (13-)15-18 x 6.5-9 µm [vs. 13-23 (-24) x (6-) 6.5-10 (-10.5) µm as stated by Ju and Rogers (1996)].

#### Specimens examined:

**Bolivia:** Gran Chaco, Tatarenda, twigs of Mimosa, 9 Apr. 1902, R. E. Fries 387 [S-F10638, **lectotype**, selected by Laessøe (1989) of *Hypoxylina umbilicata*]. - **Brazil:** Rio Grande do Sul: Porto Alegre, 29 Sep. 1892, Malme (S-F 40195, **holotype** of *H. subrutilum*).

b) Only BNT was observed in traces in the type specimen of *H. indicum* [**India:** Pusa, 18 Apr. 1906, E. J. Butler 1103 (S-F10675-**holotype** of *H. indicum*), which yielded no KOH-extractable stromatal pigments at all when in our hands and had ascospores ranging to up to 22 µm long.

c) The following specimens resembled the above mentioned *H. rubrostromaticum sensu* Martin (1969) in CABI, studied by Mühlbauer et al. (2002) with respect to their stromatal secondary metabolites, containing BNT and some yet unknown prominent metabolites. The material from IC and the type material of the varieties of *H. haematites* examined also belongs here,

suggesting that this fungus is present in the neotropics as well as in the African palaeotropics. The ascospore size range varies among the specimens belonging to this chemotype but was in general wider than that of the typical group a), ranging from 13-22 x 8-12 µm. Even old specimens generally showed rather dense olivaceous to isabelline stromatal pigments. In the African specimens, daldinin C was tentatively detected besides the unknown metabolites, which indicates affinities to the *H. fuscum* complex as postulated by Ju and Rogers (1996). Pursuing Miller's concept and maintaining *H. rubrostromaticum* as a valid species name in the genus might be practical to accommodate at least the specimens listed below. However, the situation may in reality be even more complicated even in this chemotype group which has previously been segregated into varieties according to ascospore size range by other authors.

Neotypification of *H. subrutilum* using a specimen from the neotropics that will provide an unambiguous HPLC profile and can be cultured should be accomplished first. Group c) matched best the description of *H. subrutilum* by Ju and Rogers (1996) but drastically differs from chemotype a) which includes the holotype specimen. Group a), on the other hand, shows chemotaxonomic affinities to *H. subrutiloides*. It should eventually be attempted to further segregate this conglomerate of *Hypoxylon* spp., but fresh material of the various chemotypes will be needed for culturing.

We refrain from taking any taxonomic consequences, but the situation that arose from the confusion caused by Miller (1961) upon erection of *H. rubrostromaticum* may in truth be even more complicated than already discussed by Ju and Rogers (1996) in their "Notes" to *H. subrutilum* and *H. diatrypeoides* Rehm. At least two additional species may eventually have to be erected to resolve this species complex.

**Specimens examined:**

**Brazil:** São Leopoldo, corticated wood, 1905, J. Rick, Fung. Austro-Amer. 88 ex herb G. Bresadola as *Hypoxylon latissimum* Speg.; loc. cit., corticated wood, J. Rick, Fung. Austro-Amer. 88, as *H. latissimum* var. *purpurea* Rick, ined. (NY, **type** of *H. haematites* var. *macrospora* fide Ju and Rogers 1996); loc. cit., corticated limbs, 1908, F. Theissen (GAM 2738 - **type** of *H. haematites*); J. Rick 505 as *H. glomerulatum* var. *macrospora* J. H. Miller, ined. (GAM 12808). - **Democratic Republic of Congo** ("Zaire", Leopoldville): Kinshasa, on corticated wood, Oct. 1908, H. Vanderyst (BR-Myc 033167,90-**holotype** of *H. congoanum* Torrend ex Beeli, a *nomen nudum* fide Dennis 1963). - **Sierra Leone:** bark of *Codiaeum variegatum* (Euphorbiaceae), 15 Nov. 1926, F. C. Deighton 55. as *H. rubrostromaticum* [K(M) 124724]. - **Spain:** Canarias: La Palma, Breña Alta, La Pavona, in Fayal-Brezal, *Chamaecytisus proliferus*, 13 Dec. 1987, E. Beltrán, J.L.Rodríguez-Armas & J.Leal as *Hypoxylon* sp. (TFC Mic. 3581); loc. cit., Los Sauces, Los Tiles, in laurisilva, on corticated wood of *Erica arborea*, 3 Dec. 1989, E. Beltrán, Á.Bañares, J.Leal, M.C.León & F.Cabrera as cf. *Hypoxylon* (TFC Mic. 5178).

***Hypoxylon subticinense* Y.M. Ju & J.D. Rogers 1996 [MB414983]**

This species has about the same stromatal morphology and general appearance as *H. ticinense* but differs in its ascospore and anamorph morphology (Ju and Rogers 1996). Interestingly, it contains rubiginosins, while *H. ticinense* only contains mitorubins. We found it among the specimens from Belgium in BR, aside from previous reports on its occurrence in France and America (Ju and Rogers 1996, Stadler et al. 2004a). Its host range includes *Hedera helix* and Asteraceae (*Baccharis*).

**Specimens examined:**

**Belgium:** Antwerpen: angiosperm wood, 3 Apr 1988 H. de Meulder 2066 (BR-Myc 006202,91). -

West Vlaanderen: Waulsort, 4 Apr. 1987, H. de Meulder 3912 (BR-Myc 012200,75); Knokke, 3 Nov. 1995, H. Ruysseveldt (BR Myc 049019,34). - **USA:** Louisiana: Baton Rouge, 1 July 1914, C. J. Humphrey & C. W. Edgerton, det. J. H. Miller as *H. sclerophaeum* (BPI 716660).

***Hypoxylon ticinense* L.E. Petrini [MB103466]**

This species, first recognized by Petrini and Müller (1986) was recently shown to have chemotaxonomic affinities to *H. fragiforme*, because it contains mitorubins but lacks rubiginosins (Stadler et al. 2004a) and produces major metabolites other than 5-methylmellein in culture (Bitzer et al. 2008). Even molecular data suggest close relationships to the type species of *Hypoxylon*, rather than to *H. rubiginosum* (Hsieh et al. 2005). This species is still only known from Europe and shows no apparent host preference. An immature specimen from La Gomera resembled *H. ticinense* in its stromatal morphology and HPLC profile, suggesting that this species might soon be found on the IC in mature state.

**Specimen examined:**

**Germany:** Badenia-Württemberg: Weisweil, Zollgrund, *Crataegus oxyacantha*, May 2006, E. Strittmatter et al. (M, culture MUCL 47714). - **Spain:** Canarias: La Gomera, Parque Nacional de Garajonay, path from Las Mimbreras (Arroyo del Cedro) to Garajonay (1090 m), Fayal-brezal arbóreo-arbustivo, *Persea indica*, 28 Apr. 2001, E. Beltrán, Á.Bañares & J.L.Rodríguez-Armas (TFC Mic. 10145).

***Hypoxylon ulmophilum* Lar. N. Vassiljeva [MB450691]**

An authentic specimen was studied by HPLC, which revealed orsellinic acid and mitorubrin derivatives (i.e., similar profiles as in the type of *H. notatum*, which contained daldinin C in addition, see above). The ascospores of *H. ulmophilum* are dark brown, ellipsoid-inequilateral (not strongly curved as in *H.*

*notatum*), with narrowly to broadly rounded ends, (13-) 15-18 x (6.5-)7.5-9 µm, with straight to slightly sigmoid germ slit spore-length (i.e., larger than in the type of *H. notatum*, which has them only 13-15 µm long). Their morphology is different from that of *H. notatum*, and they rather resemble those of *H. ferrugineum* out of the *Hypoxylon* spp. with orange stromatal pigments. The status of *H. ulmophilum* as separate taxon appears justified, as corroborated by the molecular phylogeny provided by Hsieh et al. (2005) The latter authors did not report anamorphic features of *H. ulmophilum*, and we failed to culture the VLA specimen, hence the asexual stage of this fungus is still unknown.

#### **Specimen examined:**

**Russia:** Primorsky Territory: Khanka Lake Reserve, *Ulmus japonica*, 21 June 2003, L. Vasilyeva (VLA).

#### ***Hypoxylon vogesiacum* (Currey) Sacc. [MB170307]**

**Synonym:** *H. rubiginosum* (Pers. : Fr. ) Fr. var. *insigne* Rehm [MB137459].

This species is included for comparison, as one of the varieties erected by Miller has in the past been confused with members of the *H. rubiginosum* complex. As discussed by Granmo (1999a), the name sanctioned by Saccardo (1882) goes back to Currey (1858), rather than to Persoon. *Hypoxylon vogesiacum* lacks KOH-extractable pigments, and its ascospores have a spore-length germ slit at the centre of a dotted band (Ju and Rogers 1996). The type of this species is from the Vosges region, which nowadays belongs to France. We did not yet get hold of fresh material from the type locality but studied a number of specimens from southwestern France, Germany, and Norway, and the type of *H. rubiginosum* var. *insigne* from Austria in comparison with material also treated by Ju and Rogers (1996). Specimens that we studied fresh, share with *H. vogesiacum* sensu Ju and Rogers (1996) a similar purplish and pruinose stromatal surface, and ascospores

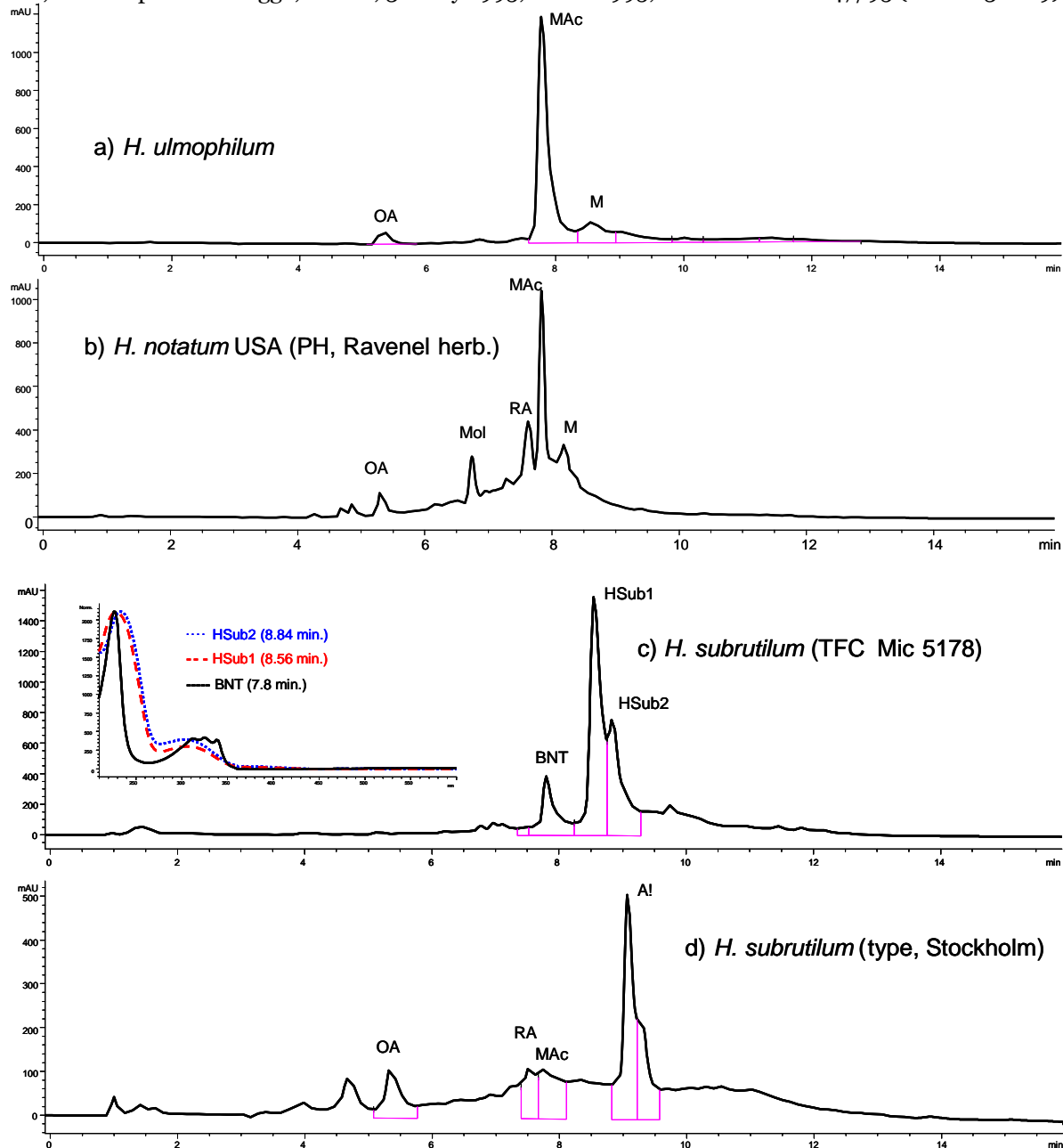
averaging more than 20 µm long, with a spore-length germ slit. They differ from the typical *H. vogesiacum* in producing livid violet to grey pigments in KOH and in having slightly larger ascospores lacking a dotted or darker band along the germ slit. Only the Austrian specimen had shorter ascospores and its perispore did not easily dehisce, as already stated in the above monograph. Even in immature parts of the recently collected stromata, we were so far unable to detect compounds other than the ubiquitous BNT. Carneic acids (the characteristic metabolites of *H. carneum*, which also has purple stromatal pigments in KOH) or daldinins (as in *H. fuscopurpureum* and *H. macrosporum*) were not detected. Also, no differences were seen in the HPLC profiles of specimens with dehiscent and indehiscent perispores, respectively, albeit we have so far been unable to include material of the latter type in fresh state. If this fungus has affinities to the other taxa treated here, its stromatal pigments might have been reduced in the course of its evolution.

#### **Specimens examined:**

**Austria:** Buchenbey, 3 Dec. 1906, P. Mrouses (S-F10748- **holotype** of *H. rubiginosum* var. *insigne*). - **Czech Republic:** Moravia: near Wsetin West Berkiden, Bery Cab, on branches, F. Petrak as *H. vogesiacum*, Mycotheca carpatica No. 238, K(M) 121846. - **France:** Ariège: Orlu, Rés. Nat. d'Orlu, Jasse de Justiniac (1280 m), banks of Oriège, *Ulmus montana*, 29 July 2001, JF-01152,; same location and host, 28 Sep. 2001, JF-01212. - Haut Rhin: Vosges, (*in ligno indurato*) *A. pseudoplatanus*, Dec. 1823, Mougeot et Nestler Stirp, Vogosorum exs. No. 765 [K(M) 121844 - **isolectotype** of *Sphaeria vogesiaca*. selected by Miller (1961), replaced as type by Granmo (1999a) by another specimen not included in this study] - Hautes Pyrénées: Saint Lary, Loudenvielle, Vallée d'Aure, *Ulmus*, 28 Apr. 2002, leg. P. Valet, comm. F. Candoussau (JF-02077). - **Germany:** Badenia-Württemberg: Schwäbische Alb, vic. of Schelklingen, Bannwald "Tiefental", Aceri-Fagetum on calcarious soil,

*Acer pseudoplatanus*, 11 Sep. 1990, G. Krieglstainer (STU K1050) - **Norway**: Sogn og Fjordane: Vik, Arnafjord, *Ulmus glabra*, 30 July 1995, A. Granmo 205/95 (TROM 30234); loc. cit., west slope of Krokegga, *Ulmus*, 30 July 1995,

A. Granmo 204/95 (TROM 30232); Sogndal, Norum, hillside NE of church, *Fraxinus*, 29 July 1995, A. Granmo 198/95 (TROM 30235). Vest-Agder: Mandal, Nornevatnet, *Ulmus*, 18 July 1995, A. Granmo AG 147/95 (TROM 30229).



**Fig. 6.** HPLC-UV chromatograms (210 nm) of stromatal methanol extracts of *Hypoxylon* spp. including DAD spectra of some characteristic metabolites. **a)** *H. ulmophilum* (VLA), containing orsellinic acid (OA), mitorubrin (M) and mitorubrinol acetate (MAc). **b)** *H. notatum* (PH, containing the above compounds, mitorubrinol (Mol), and rubiginosin A (RA). **c)** *H. subrutilum* (TFC, chemotype C), containing BNT and unknown metabolites (HSub1, HSub2). **d)** *H. subrutilum*, holotype specimen (S), containing MAC, RA, OA, and large amounts of artefacts (A!) that falsified its stromatal pigment colors in KOH.

Table 1. Chemotypes (see Stadler et al. 2004a, Hellwig et al. 2005, except for "vogesiicum", which is characterized by containing only binaphthalenes and was not defined elsewhere) of the species examined in this study. E, NA: so far only known from Europe, or North America, respectively. AHS: apparent host specificity. IC: Canary Islands.

	<b>Chemotype</b>	<b>Remarks (on HPLC profile)</b>	<b>Other peculiarities</b>
<i>Hypoxylon</i>	fuscum	deviating profiles from fuscum in some specimens, e.g. from Africa	IC; pantropical (?)
<i>anthochroum</i>			
<i>H. californicum</i>	fragiforme		striate epispore (NA)
<b><i>H. canariense</i></b>	<b>rubiginosum</b>		<b>IC</b>
<i>H. carneum</i>	specific	contains carneic acids (Quang et al. 2006)	Cosmopolitan in warmer climates
<i>H. cercidicola</i> (Europe)	rubiginosum		AHS for <i>Fraxinus</i> , erumpent stromata
<i>H. cercidicola</i> (America)	specific	no fresh material examined yet, no known metabolites observed	AHS for <i>Fraxinus</i> , erumpent stromata
<i>H. commutatum</i>	fragiforme	no fresh material examined yet	AHS for <i>Carpinus</i> (?)
<i>H. croceopileum</i>	rubiginosum		warmer climates, subtropical regions of E, NA
<i>H. dearnessii</i>	fragiforme	mitorubrin lacking, some specific compounds detected	AHS for <i>Acer</i>
<i>H. ferrugineum</i>	fragiforme	deviating chemotype and host range in NA	AHS for <i>Tilia</i>
<i>H. fragiforme</i>	fragiforme	young stromata produce cytochalasins	AHS for <i>Fagus</i> , also in North Asia
<i>H. fraxinophilum</i>	perforatum		AHS for <i>Fraxinus</i>
<i>H. fuscopurpureum</i>	fuscum	Deviation morphochemotype in specimens from Slovakia in PRM	AHS for <i>Fraxinus</i> (?)
<i>H. fuscum</i>	fuscum	Different subtypes	Cosmopolitan species complex
<i>H. howeanum</i>	fragiforme	young stromata produce cytochalasins	AHS for Betulaceae, also in Southern hemisphere
<i>H. julianii</i>	rubiginosum	Dimeric rutilins present	
<i>H. laschii</i>	fragiforme		AHS for <i>Populus</i> , erumpent stromata
<i>H. liviae</i>	rubiginosum		AHS for <i>Sorbus</i> (Norway); E
<i>H. macrocarpum</i>	macrocarpum		
<i>H. macrosporum</i>	fuscum		AHS for <i>Salix</i> (boreal distribution)
<i>H. notatum</i> (type)	rubiginosum	Deviating chemotypes and inconclusive HPLC profiles observed	heterogeneous
<i>H. novemexicanicum</i>	fragiforme		NA
<i>H. perforatum</i>	perforatum		IC, cosmopolitan?
<i>H. petriniae</i>	rubiginosum	BNT present	AHS for <i>Fraxinus</i>
<i>H. porphyreum</i>	specific		AHS for <i>Quercus</i>
<i>H. retpela</i> (T)	rubiginosum	Quite specific with respect to the pattern of its rubiginosin derivatives	tropical
<i>H. rubiginosum</i>	rubiginosum		no AHS!
<i>H. rutilum</i>	rubiginosum	Dimeric rutilins present	
<i>H. shearii</i>	rubiginosum		NA
<i>H. subticinense</i>	rubiginosum		
<i>H. salicicola</i>	rubiginosum		AHS for <i>Salix</i> (Norway), E
<i>H. subrutilum</i>	rubiginosum	type specimen from South America shows diverging HPLC profile from material collected in IC; three strongly divergent chemotypes observed	IC (" <i>H. rubrostromaticum</i> " chemotype only)
<i>H. ticinense</i>	fragiforme		E
<b><i>H. urriesii</i></b>	<b>rubiginosum</b>		<b>IC</b>
<i>H. vogesiicum</i>	vogesiicum	mainly binaphthyls	AHS for <i>Ulmus</i> (E, NA); species complex ?

## 2. Taxonomic part

As outlined above by comparison of various *Hypoxylon* taxa that occur in temperate and subtropical regions of the world, as well as by a comparison with our unpublished data and the work by Ju and Rogers (1996) and Ju et al. (2004), we have not found *H. rubiginosum sensu stricto* among the *Hypoxylon* spp. we obtained from the IC. Instead we have found two undescribed species that key out as *H. rubiginosum* but still have distinctive features, which are constantly represented by a relatively large number of specimens. Therefore, these taxa are described as new.

### *Hypoxylon urriesii* J. Fournier & M.

Stadler, sp. nov.

MB 511026

Figs. 7, 8, 11, , 12a, 13a

**Typus:** Spain: Canarias: Tenerife, Chamorga (Anaga), near Cabeza del Tejo, on bark of an unidentified recently felled lauraceous tree, 7 Dec. 2005, STMA 05329 (TFC Mic. 19667 - **holotype**, culture MUCL 47223).

**Etymology:** Named for J. M. de Urries y Azara, who explored the Xylariaceae of the Canary islands before us.

*Stromata in ligno corticato, irregulariter effusa vel elongata, applanata, 6-28 mm longa x 3-6 mm lata x 0.2-0.3 mm crassa, margi stromatibus fusi vel nigri et recti. Externe rufobrunnea, demum denigrata, superficie leviter rugosa, sine tumulis perithecorium, sub superficie granulis luteo-aurantiacis conspersis, granulis aurantiacis in KOH dissolutis; textura inter perithecia brunnea medullosa, textura sub peritheciis tenua, exigua. Perithecia sphaerica, 0.15-0.2 mm diametro. Ostiola umbilicata, exigua. Asci 95-110 µm longitudine tota, ad 8-8.5 µm lati; partibus sporiferis 72-85 µm longi, stipitibus 18-30 µm longi. Annuli apicali discoidi, 1.5-1.8 µm alto x 2.8-3.4 µm lato, in liquore iodato Melzeri cyanescentes. Ascosporae*

*brunneolae, unicellulares, ellipsoideo-inequilaterales, saepe subtortae, apicibus angustatis, 11-14.5 x 5-6 µm, rima germinativa recta vel leviter sigmoidi, sporilonga in latere convexo praeditae, perisporium in KOH dehiscens, leve, episporium leve.*

**Stromata** effused, 6-28 mm long x 3-6 mm broad x 0.3 mm thick, Dark Brick (60) with a concolorous to blackish effused margin; surface pruinose, dull, with perithecial contours about ½ exposed; yellow and dull orange granules abundant under the surface, yielding Orange (7) pigments in 10% KOH; subperithecial tissue inconspicuous. **Perithecia** spherical, 0.15-0.2 mm diam. **Ostioles** umbilicate, inconspicuous. **Asci** 8-spored, 95-110 µm total length, p sp 72-85 µm long x 8-8.5 µm broad, stipes 18-30 µm long, with apical apparatus discoid, amyloid, 1.5-1.8 µm high x 2.8-3.4 µm broad. **Ascospores** brown, ellipsoid-inequilateral to navicular, with narrowly rounded to acute ends, often slightly twisted, 11-14.5 x 5-6 µm (M = 12.3 x 5.4 µm, n = 30), with straight to slightly sigmoid, spore-length germ slit; perispore dehiscent in 10% KOH, smooth; episore smooth. Conspicuous transverse striations observed by SEM (10.000x; Fig. 12a).

**Cultures** covering a 9 cm Difco OA plate in 2 wk, at first white, velvety to felty, azonate, with diffuse margins; becoming hazel (88), (Fig. 13b), reverse uncolored. So far no sporulating regions observed after prolonged incubation times (>6 wk) in either OA, or YMG medium.

**Notes:** This species keys out as *H. rubiginosum* in Ju and Rogers (1996) because of the dull yellow brown stromatal granules and ellipsoid-inequilateral ascospores with dehiscent perispores, but it differs in its perithecial dimensions (0.1-0.2 mm vs. 0.4-0.5 mm) and ascospore morphology (11-14.5 x 5-6 µm with narrowly rounded to acute ends vs. 9-12 x 4.5-5 µm with broadly to narrowly rounded ends in *H. rubiginosum sensu stricto*). Macroscopic features

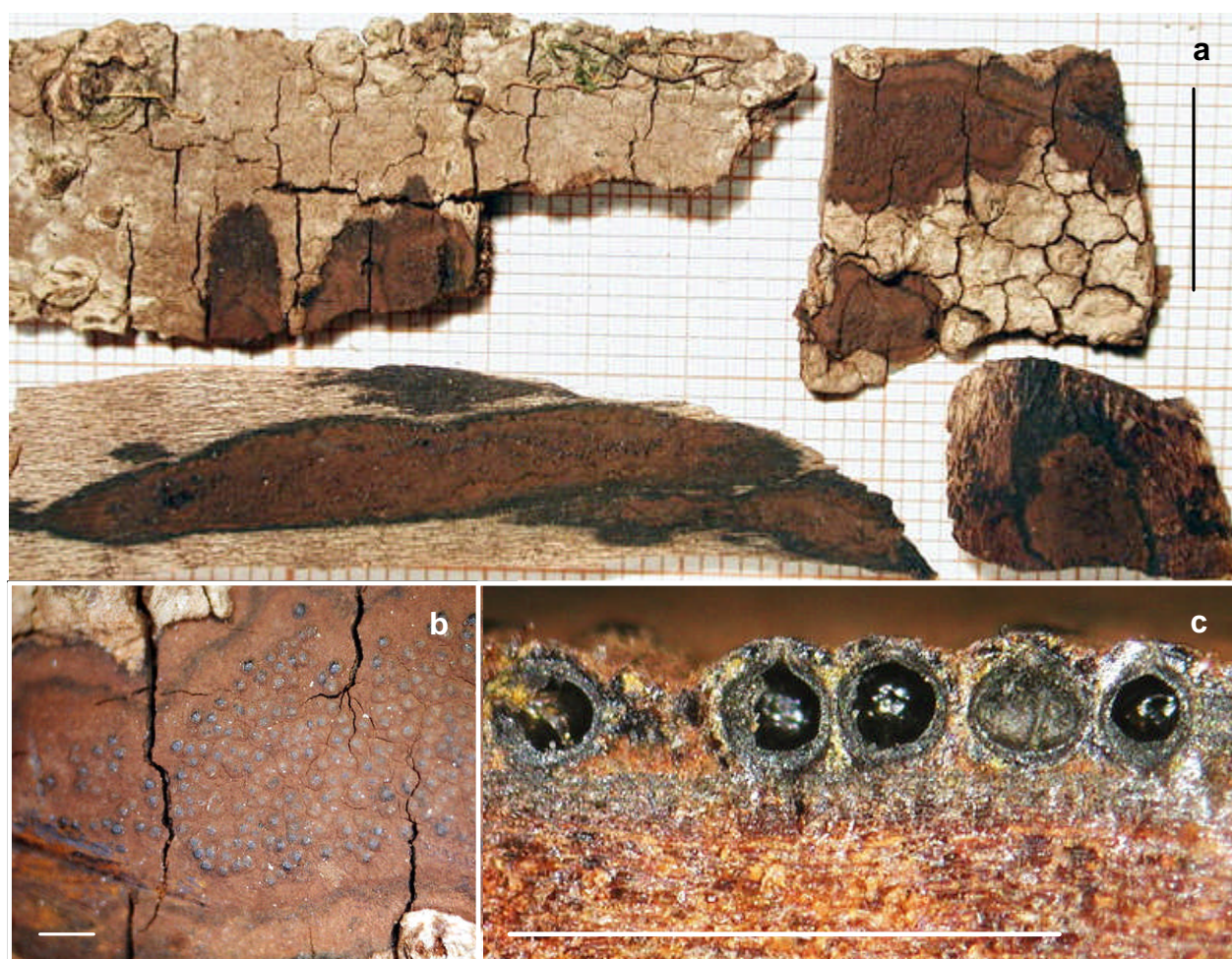


of the stromata including surface color and very small spherical perithecia of the new species recall those of *H. salicicola*, which, however, has smaller ( $7\text{--}10 \times 3\text{--}4.5 \mu\text{m}$ ), nearly equilateral ascospores and quite a different geographic distribution and HPLC profile.

Similar navicular, somewhat twisted ascospores as in *H. urriesii* are encountered in *H. fendleri*, but in the latter ascospores are smaller ( $9\text{--}12 \times 4.5\text{--}5 \mu\text{m}$ ), darker, and feature a more strongly sigmoid germ slit. Moreover *H. fendleri* has a more vinaceous stromatal surface and more

brightly pigmented granules surrounding larger perithecia.

Main morphological distinctive characters for the new species are therefore the combination of thin stroma with minute perithecia and ascospore shape and dimensions. *H. salicicola* was segregated from *H. rubiginosum* on similar grounds, in addition to the presence of a deviating anamorph.



**Fig. 7.** *Hypoxylon urriesii*, from holotype specimen TFC Mic. 16667. a) Stromatal habit. b) Stromatal surface. c) Section through perithecial region showing perithecia and granules embedded in waxy layers; a, bar = 1 cm; b, c, bar = 1 mm

***Hypoxylon canariense* J. Fournier, M. Stadler, Beltrán-Tej. & Granmo, sp. nov.**  
MB 511027 Figs. 9 – 11, 12b, 13b.

**Typus:** Spain: Canarias: Tenerife, Anaga mountains, near Cruz del Carmen, on bark and decorticated wood of *Erica arborea*, 1 Dec.

2005, M. Stadler, STMA 05301 (TFC Mic. 19666 - **holotype**).

**Etymology:** Named for its frequent occurrence on all IC that still dispose of large indigenous angiosperm (laurisilva) forests.

*A Hypoxylon rubiginoso differunt stromatas parviora, 5-30 (-50) mm longa x 4-11 mm lata x 0.5-0.6 mm alta, superficie vinacea, stipitesque ascorum semper breviores partibus sporiferis, et status anamorphosis generi Virgariellae similis.*

**Stromata** effused to effused-pulvinate, 5-30 (-50) mm long x 4-11 mm broad x 0.5-0.6 mm thick, with abrupt to effused concolorous margin; surface Fulvous (43) when immature, Dark Brick (60) when mature, at times Dark Vinaceous (82), pruinose, matt, plane to slightly undulate, with perithecial contours hardly visible to conspicuous, at times nearly perithecioid; pruinose coating of mixed granular elements, partly turning bluish grey in 3% KOH; yellow and dull orange granules abundant under the surface and around perithecia, forming a thick orange brown crust just beneath the surface, yielding Orange (7) to Sienna (8) pigments in 10% KOH; subperithecial tissue inconspicuous.

**Perithecia** spherical, 0.3-0.4 mm diam.

**Ostioles** umbilicate, inconspicuous, in mature and overmature stromata often surrounded by a ring of white granules 70-90 µm diam.

**Asci** 8-spored, total length 95-120 µm, the spore-bearing parts 66-80 µm long x 6-8 µm broad, the stipes 27-40 µm long, with apical apparatus discoid, 0.5-1 µm high x 2.5-3 µm broad, bluing in Melzer's reagent. **Ascospores** brown, ellipsoid-inequilateral to often slightly crescentic, 9.5-11.5 x 4.5-5 µm (M = 10.4 x 4.8 µm, n = 90) with a faint, straight, spore-length germ slit on the more convex side; perispore dehiscent in 10% KOH, with inconspicuous

transverse striations (barely visible by light microscopy at up to 1000x, but clearly seen by SEM at 10.000x, see Fig. 12b).

**Cultures** covering a 9 cm Difco OA plate in 9-11 d, at first white, velvety to felty, azonate, with diffuse margins; becoming hazel (88), then sometimes reddish brown, due to production of exudates (Fig. 13a), reverse melanizing in age. Sporulating regions scattered over entire surface of colony, grey. **Anamorph** on natural substrate at margins of immature stromata, on old stromata Honey (64) to Fulvous (43), *Virgariella*-like, smooth to finely roughened, with conidia broadly ellipsoid, 5-6.5 x 3.5-4.5 µm (in 3% KOH). developing after 3 wk on Difco OA, Anamorph in culture similar, with slightly smaller conidia (not shown).

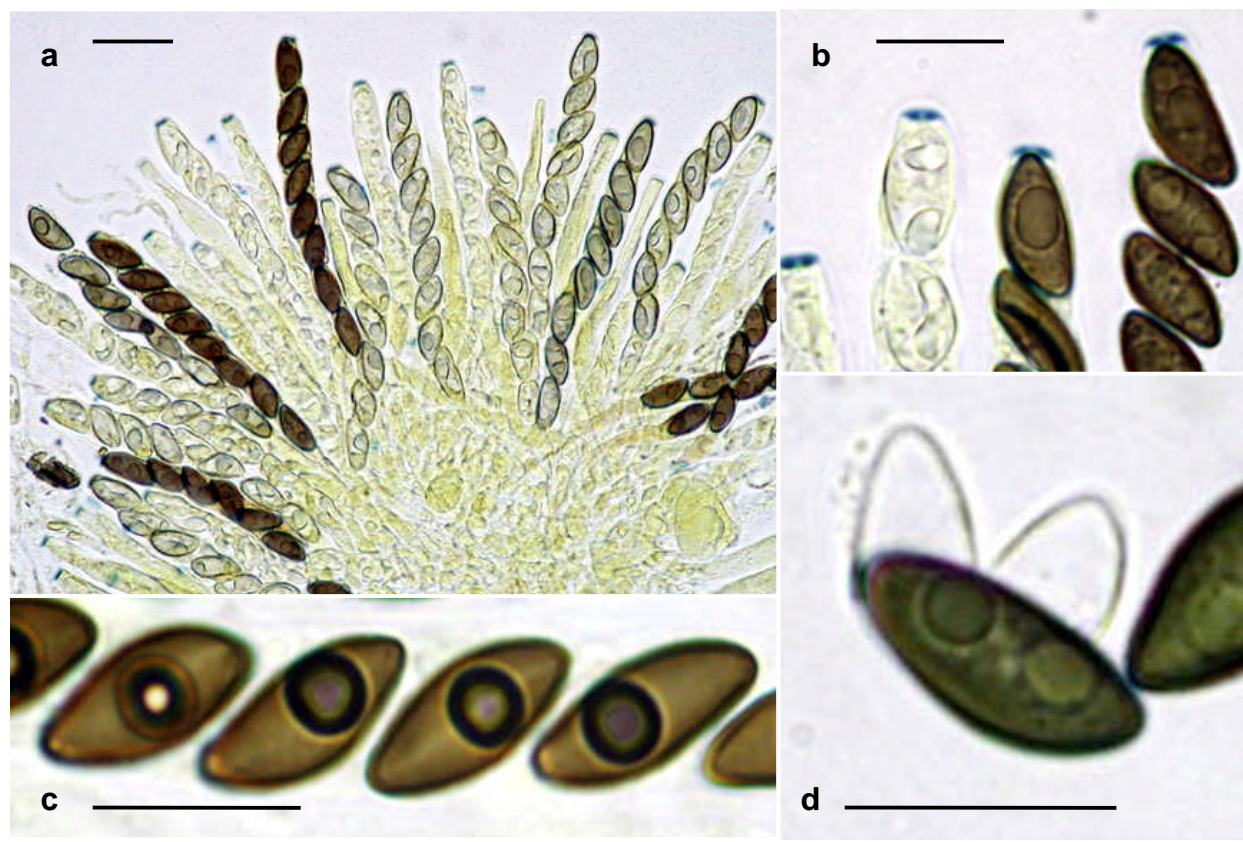
#### Further specimens examined:

**Spain:** Canarias: Gran Canaria, Finca Ossorio, on dead branches, 25 Apr. 1987, E. Beltrán & J.L.Rodríguez-Armas (TFC Mic. 2888); loc. cit., Tilos de Moya, on dead branches in Laurisilva forest, 12 Nov. 1987, E. Beltrán & J. L. Rodríguez-Armas (TFC Mic. 3962); loc.cit. on bark of *Ocotea foetens*, 6 Jan. 2006, A. Granmo & L. Mølster (TROM). - La Gomera, Parque Nacional de Garajonay, sendero de Las Mimbreras a Meriga, Laurisilva, dead branch of *Laurus novocanariensis*, 14 Feb. 2002, E. Beltrán, J. L. Rodríguez-Armas, Á.García & E.Martín (TFC Mic. 14308; TFC Mic. 15681); dto, road to recreational area "Juego de Bolas", Fuensanta, 1050m, Laurisilva, on dead branches of *Persea indica*, 15 Apr. 2000, E. Beltrán et al. (TFC Mic. 14241); loc. cit., 1350m, Laurisilva, *L. novocanariensis*, 13 Nov. 2001, E. González & J. Barrera (TFC Mic. 14357). - El Hierro, El Golfo, on dead wood, 9 Apr. 1987, E. Beltrán (TFC Mic. 16465). - La Palma: Barlovento, Barranco Gallegos, Laurisilva, *L. novocanariensis*, 2 Apr. 1989, E. Beltrán, J. L. Rodríguez-Armas & J. Leal as *Hypoxylon* (TFC Mic. 3500); Puntallana, Cubo de La Galga, *Ocotea foetens*, 16 Dec. 2004, STMA 05021 (TFC Mic. 19665, culture CBS 119025); loc. cit., *Laurus novocanariensis*, no



date, J. M. Castro (TFC Mic. 9700); Los Sauces, Los Tiles, laurisilva, wood, 28 Jan. 1989, E. Beltrán, Á. Bañares, J. L. Rodríguez-Armas & J. Leal as *Hypoxylon* (TFC Mic. 3431); loc. cit., *Laurus novocanariensis*, 28 Apr. 1989, E. Beltrán, Á. Bañares, J. L. Rodríguez-Armas & J. Leal as *Hypoxylon* (TFC Mic. 3553); loc. cit., 2 Feb. 1990, C. Hernández-Padron as cf. *Hypoxylon* (TFC Mic. 5592); loc. cit., *Ocotea foetens*, 29 Nov. 1991, E. Beltrán, J. L. Rodríguez-Armas & J. Leal as *Hypoxylon* (TFC Mic. 7063); loc. cit., 14 Apr. 1994, J. M. Castro

(TFC Mic. 9715). - Tenerife, La Laguna, Anaga, Las Yedras, Laurisilva, dead wood, 13 Apr. 1987, E. Beltrán, J. L. Rodríguez-Armas & A. Losada as cf. *Hypoxylon* (TFC Mic. 18603); Los Silos, on dead wood, 27 Oct. 1986, E. Beltrán et al. (TFC Mic. 3116); loc. cit., on dead branches, 27 May 1985, E. Beltrán et al. (TFC Mic. 3120); loc. cit., on dead wood, 27 Apr. 1987, E. Beltrán et al. (TFC Mic. 3119); Teno Mountains, vic. of Erjos, wood of *Persea indica*, gathered as firewood in recreational area, 9 Dec. 2005, M. Stadler STMA 05341 (largely immature, culture MUCL 47224).



**Fig. 8.** *Hypoxylon urriesii*, from holotype specimen TFC Mic. 16667. Microscopic details. a) Asci in Melzer's reagent. b). Ascus apical regions stained with Melzer's Reagent, showing amyloid apical rings. c) Ascospores in detail in Melzer's reagent. d) Ascospores with perispore dehiscing in 10% KOH. a, bar = 25 µm; b, c, d, bar = 10 µm.

**Notes:** Except for the STMA specimens, all collections were obtained by us as *H. rubiginosum* and indeed keyed out under this name according to Miller (1961) and Ju and Rogers (1996). The present fungus is similar to *H. rubiginosum sensu stricto* in several respects:

effused-pulvinate stromata; surface color with shades of orange brown, similar yellow and orange stromatal granules yielding orange pigments in KOH and a similar ascospore morphology and size range. These close morphological affinities are corroborated by a

similar HPLC profile characterized by the presence of mitorubrin and derivatives and rubiginosin A, representing the "*rubiginosum*" chemotype. However, numerous available collections of this new fungus allowed to establish the wide range of its morphological variations, e.g., the small size of the stromata, their thickness (never over 0.6 mm) and the vinaceous tones occurring on the surface.

Microscopically, the stipes of asci are always definitely shorter than the spore-bearing parts, while asci of *H. rubiginosum* are always long-stipitate. Ju and Rogers (1996) gave a wide range of ascal stipe length for *H. rubiginosum*, but Stadler et al. (2004a) showed that they included some characters of the meanwhile distinct *H. petriniae* characterized by short-stipitate asci. Furthermore, the anamorph on the natural substrate and in culture shows a *Virgariella*-like branching pattern, while the typical *H. rubiginosum* has a *Nodulisporium*-like anamorph as defined by Ju and Rogers (1996).

*Hypoxylon perforatum* and *H. petriniae* (see above) also have effused stromata with small spherical perithecia and short-stipitate asci with ascospores similar to those of our new species. Moreover, they both have a *Virgariella*-like anamorph and purplish tones on stromatal surface, (the latter feature occurs occasionally in *H. perforatum* but typically in *H. petriniae*). Aside from minor differences in stromatal morphology, they can be clearly distinguished from *H. canariense* by chemotaxonomic features: *H. perforatum* contains hypomiltin instead of mitorubrins and rubiginosin, and gives yellow pigments in 10% KOH, which allows for an easy distinction from related taxa. On the other hand, *H. petriniae* contains BNT along with the characteristic compounds found in the "*H. rubiginosum*" chemotype. This additional compound does not change the KOH reaction but accounts for the typical vinaceous, lilac or purple stromatal surface of that species.

*Hypoxylon salicicola* was likewise separated by Granmo (1999a) from *H. rubiginosum*, mainly because of smaller perithecia and smaller ascospores (7-10 x 3-4.5 µm), which are nearly equilateral even in lateral view, aside from biogeographic aspects. It therefore can be easily distinguished from the present fungus.

Since the IC are geographically much closer to the tropics than to the European mainland, *H. canariense* needed to be compared to taxa known from warmer climates that have been reported to possess similar morphological characters and pigments, such as *H. retpela* and *H. fendleri* (hence we studied representative specimens for comparison that are listed in Part 1). The former can be distinguished based primarily on its conspicuous ornamentation on perispore, while the latter has ascospores with narrowly rounded ends, with a sigmoid germ slit. Both differ in their HPLC profiles too (Table 1). The HPLC profile of the type specimen of *H. retpela* is compared to that of *H. canariense* in Fig. 11, showing that they differ drastically from one another. Likewise, *H. fendleri* was shown by Hellwig et al. (2005) to contain only mitorubrins; a feature confirmed in meantime by studies on several further specimens. As already stated in the introduction, the tropical members of the "red Hypoxylons" will be treated in detail in a subsequent study, and data on the above tropical species will be included there.

Most specimens examined occur on bark, but some also grew on dead decorticated wood. Judging from bark and wood anatomy, this fungus is not host specific, but rather linked to a specific environment. National parks and regional parks such as Los Tiles de Moya, one of the places where there are still some remnants of Laurisilva on Gran Canaria, as well as similar, more extensive protected areas on the westernmost IC have all been proven to harbor this species. From collection work in the Teno and Anaga Mountains of Tenerife, it can be safely assumed that this fungus is not rare in

those regions where it occurs.

The species listed as hosts on the specimen labels are all endemic to or common in the Laurisilva or the Fayal-Brezal of the Macaronesian Islands (Hohenester and Weiß 1993; Pott et al. 2003). This fungus or similar forms might soon turn up in Madeira, the Capverdian Islands, or even the Azores. On the other hand, considering that the geographic isolation has also lead to various endemic plants that only occur on one or several of the IC, its being endemic to IC can not be excluded.

### Comments on *Hypoxylon resinosum*

#### Urries and conclusive remarks

*Hypoxylon resinosum* was so far the only name in the genus whose type is from the IC, hence we feel compelled to comment upon this species. With kind assistance by the curators of MA, we have received and studied all materials left from the work of Urries in MA on Xylariaceae of the IC, but found no record of the type specimen of this name there or in TFC. Ju and Rogers (1996) also stated that they could not locate the type specimen.

This species, described by Urries (1955), and later characterized in detail by the same author (Urries 1957) was defined as follows (most important characters literally translated here from the protologue): Stromata "sparse, erumpent (developing from below the periderm)", effused-pulvinate, of ca. 5 mm diameter x 600 – 800 µm high, with surface plane or slightly roughened; at first reddish and with "white perforated" ostioles, later blackening. Context of the hyphae with "crystalline conglomerates" and those hyphae excrete large amounts of an "amber- to fuscous colored resin", which finally even covers the stromatal surface (hence the etymology). Perithecia monostichous, immersed (lower than the stromatal surface), densely crowded, spherical to laterally compressed, 400-500 µm diam. Asci octosporous, "cylindrical" (probably

referring to the p . sp.), 70-90 x 8-9 µm. Ascospores brown, 12-14 x 7-8 µm, "ellipsoid-lenticular" (which could refer to ellipsoid-inequilateral shape as understood here), "first enclosed in a hyaline capsule, from which they are later emerging" (possibly referring to an easily dehiscent perispore). Disregarding the resin covering the stromatal surface, this description may in principle be applied to many of the species described above, including *H. fuscum* and some members of the *H. rubiginosum* complex.

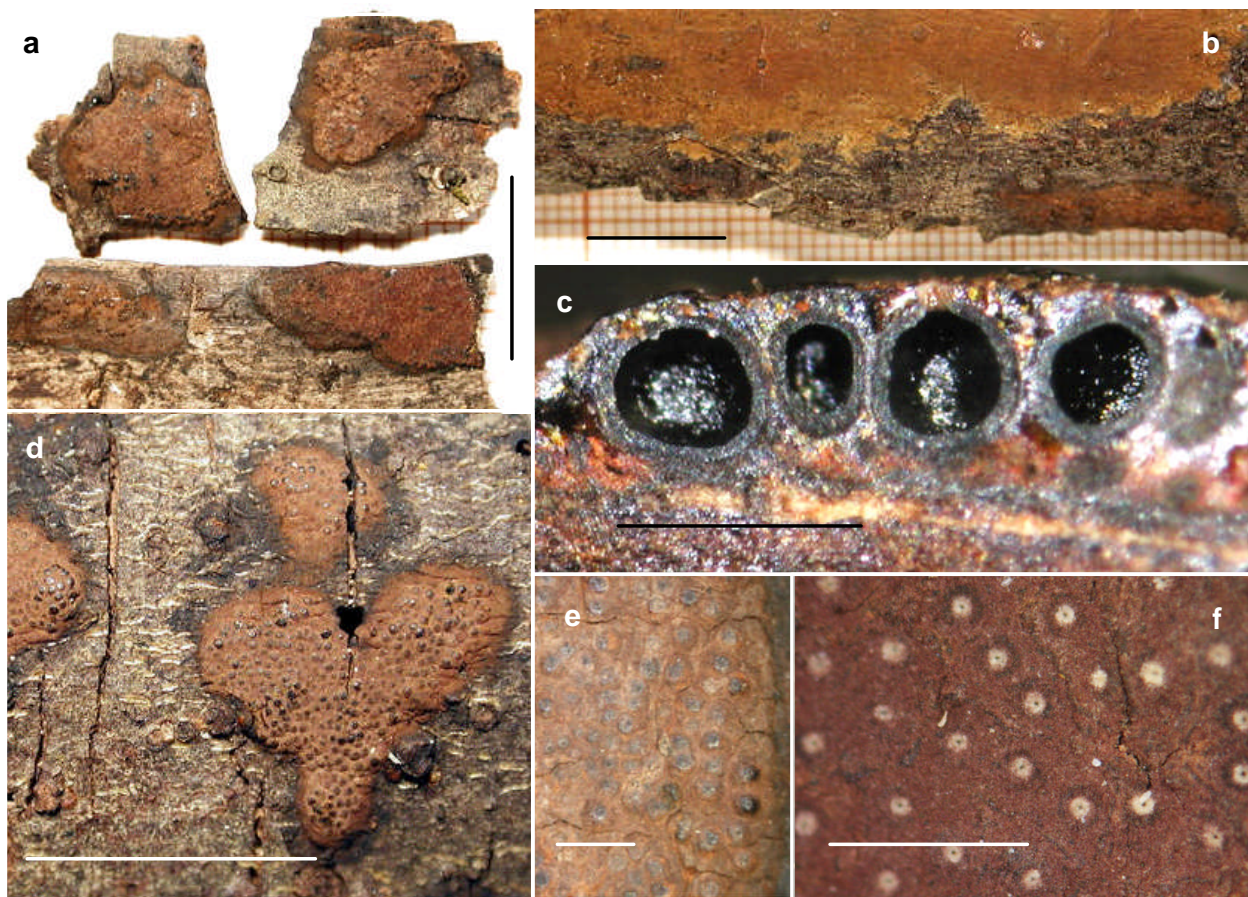
Urries (1957) also described the "anamorph" of the fungus as "initially pruinose", later producing "chlamydospore-like structures of ca. 5 µm diam, which apparently arise from the hyphae" and stated he had not observed any production of "regular" conidia. From our own examinations of *Hypoxylon* and everything that has so far been reported in the literature on the biology of Xylariaceae, we think it is most unlikely that such a fungus exists. The "anamorph" reported could be derived from another fungus colonizing the *Hypoxylon* stromata. On the other hand, it remains possible that the *Hypoxylon* studied by Urries was overmature and had already had part of the pruinose surface worn off, revealing a waxy, "resinous" layer of subsurface granules. This is frequently the case in species like *H. macrocarpum*, whose stromata only develop the characteristic metallic shine of the stromatal surface at this stage of development.

Then again, we should not doubt the existence of a validly and meticulously described species but rather search for the type – or a representative specimen that can be used as neotype. The example of *H. resinosum* shows how little we know about the true diversity of the Xylariaceae (and other fungi) in places that are remote from any geographic region that has already been thoroughly explored. In any case, *H. fuscum* and *H. rubiginosum* themselves still remain to be found in the IC, even though they could well be



present on the endemic willows ("Los Sauces", referring to *Salix*), or on introduced potential host species. From our studies on numerous immature and overmature Xylariaceae, we expect that several further species of *Hypoxylon* and various other pyrenomycetes might soon be discovered there. While the deviating chemotaxonomic and morphological data occasionally reported on species from temperate regions might soon reveal additional taxa to be present in Europe and North America, which can be regarded as relatively well studied. We are

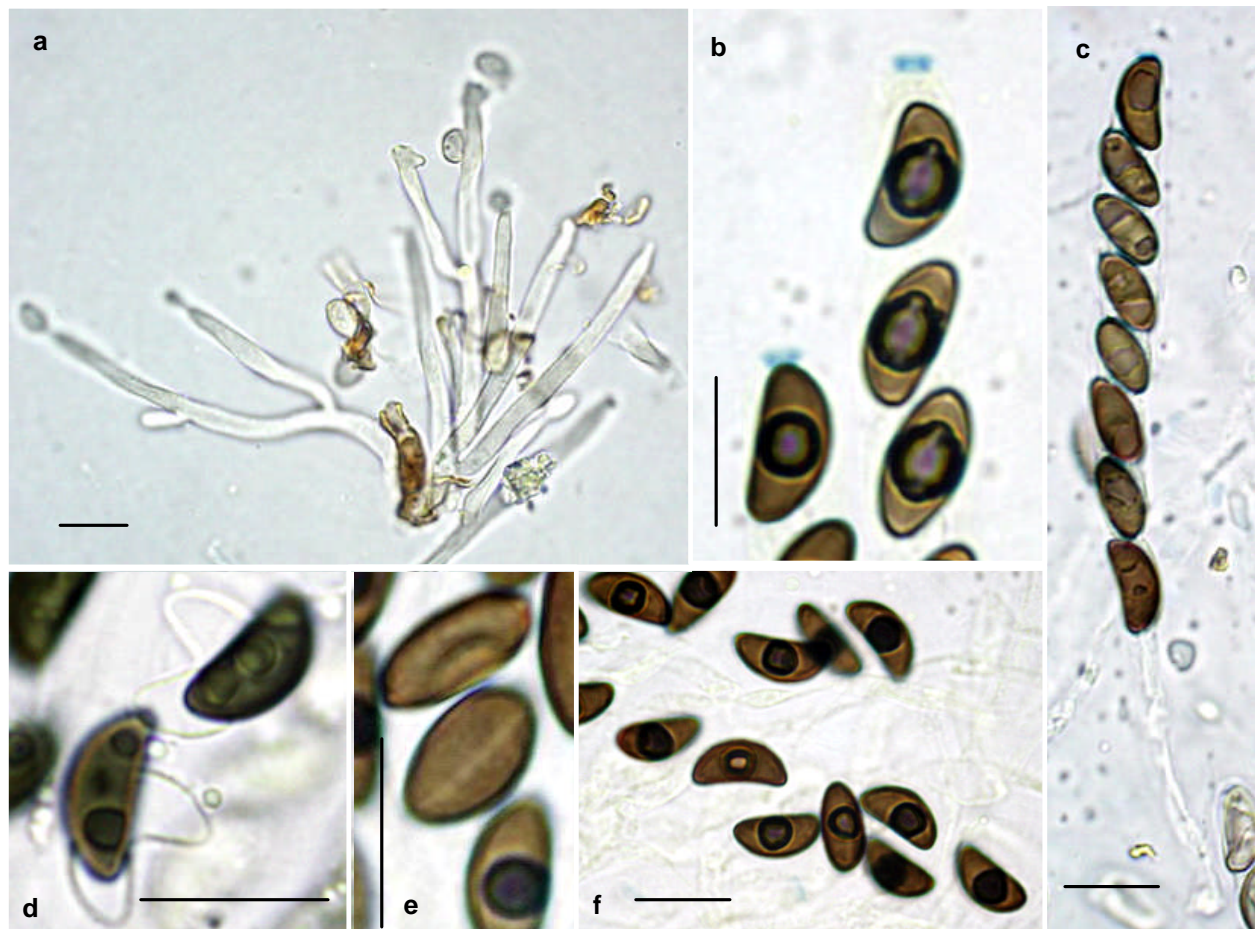
confident that additional undescribed *Hypoxylon* species will turn up if the Northern temperate regions of Asia are explored. Ju and Rogers (1996) already noted the presence of several unknown species of this genus in New Zealand, relying on a relatively small number of recently collected specimens, but their monograph did not include any culturable material from South Africa and Southern South America. Therefore, it is to be expected that even the temperate regions of the world harbor an unprecedented diversity of *Hypoxylon* species.



**Fig. 9.** Stromata of *Hypoxylon canariense*, from various specimens. a) holotype (TFC Mic. 19666), pulvinate stromata on wood. b) TFC Mic. 14308, immature stroma with anamorph. c) holotype, section through stroma, showing perithecia and pigment granules. d,) TFC Mic. 3120 effused stromata, on bark, with black margins. e) TFC Mic. 3120, stromatal surface. f) TFC Mic. 3962, stromatal surface showing vinaceous tones and ostioles fringed with white substance. Bars: a, b, d = 1 cm; c = 500  $\mu$ m; e, f, = 1 mm.

Comparing HPLC profiles and morphological features of the few tropical representatives of the species complexes treated here has also resulted in an unexpected variability of chemical traits, but plurivorous species like *H. perforatum* indeed appear to be almost cosmopolitan. The

results so far obtained therefore suggest that many *Hypoxylon* spp. are not restricted to certain geographic areas or climate zones, while others seem to have diverged from their closest relatives due to their long term geographic isolation or highly host specific adaptations.



**Fig. 10.** *Hypoxylon canariense*, microscopic details, from holotype (TFC Mic. 19666). a) *Virgariella*-like anamorph on stromata in 3% KOH. b) ascospores and ascus apical rings staining blue in Melzer's reagent. c) ascus with ascospores in water. d) ascospores in 10% KOH, revealing dehiscent perispore. e) ascospore in Melzer's reagent, showing dorsal germ slit. f) ascospores in lateral view in Melzer's reagent. Scale is indicated by bars., bar = 10  $\mu$ m.

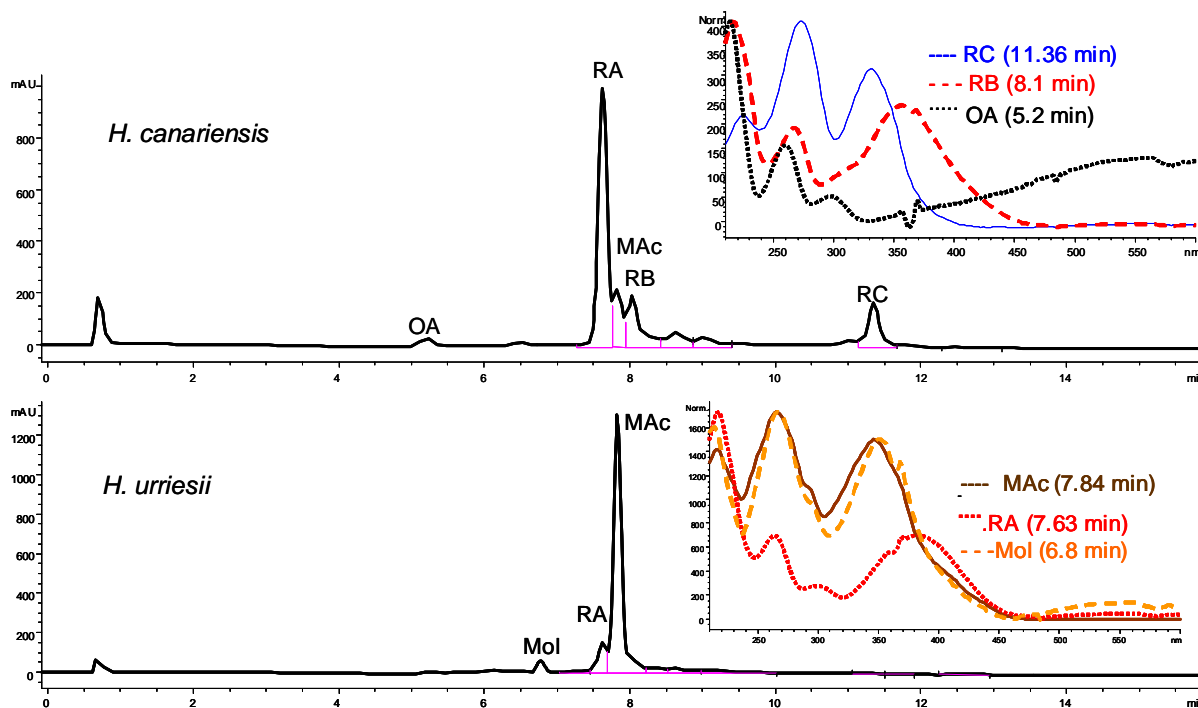
On the other hand, one should not forget that *Hypoxylon* is among the fungal genera that have recently been shown to be represented ubiquitously among the endobionts of plants. The number of "endophytic" records in

databases like GenBank classified as "*Hypoxylon*" from molecular phylogenetic studies is steadily increasing. Most of those isolates (if they were isolated and preserved in public culture collections at all), however, have

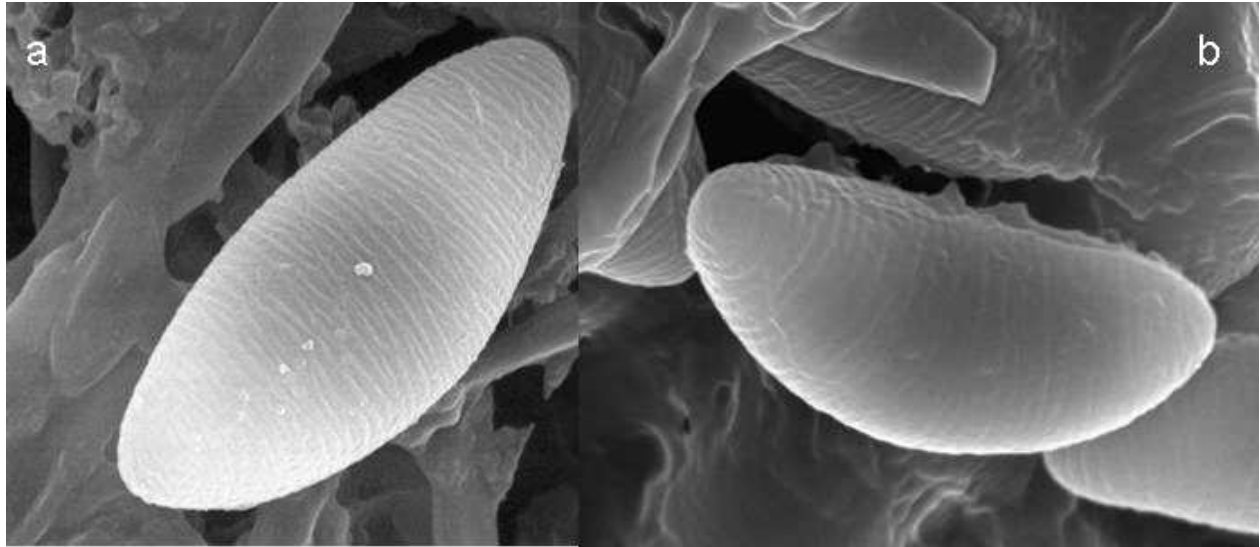


never been proven conclusively to correspond to a certain teleomorph. Still, they are sometimes referred to in even peer-reviewed papers by their full species names, based on comparisons with the most similar DNA sequence published on the Internet. Such procedures are certainly not helpful in the scope of functional biodiversity assessments. A mere inventory of molecular traits that does not rely on phenotype characters also, would rather disguise the true biodiversity of *Hypoxylon* as much as Miller's broad species concepts. The same certainly holds true for other groups of fungi.

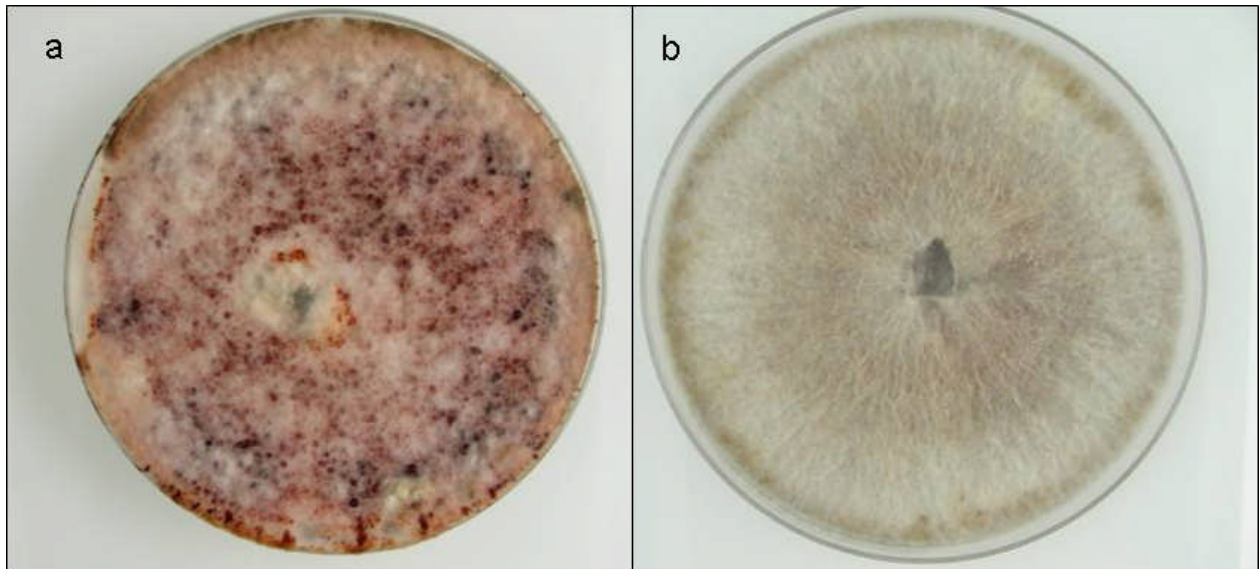
In any case, it is difficult to estimate the true diversity in this genus. However, we believe that the number of accepted species in *Hypoxylon* will soon double, as compared to the monograph by Ju and Rogers (1996), once extensive field work and a polythetic taxonomic approach considering chemical data of the ancient type specimens as well as molecular and anamorphic traits, or even characters that can only be observed in fresh specimens, have been accomplished also in the tropics and the Southern hemisphere.



**Fig. 11.** *Hypoxylon canariense* and *H. urriesii*. - HPLC profiles of stromatal extracts of holotype specimens showing DAD spectra of prominent and characteristic components. RA, RB, RC, represent rubiginosins A-C, respectively; mitorubrinol and its acetate are abbreviated by Mol and MAc, and orsellinic acid by OA). For comparison, the profile of the isotype of the tropical *H. retpela* (which is morphologically similar to *H. canariense*) is also depicted to demonstrate that it contains specific azaphilones (including the prominent major component HRet 1) in addition to a broad variety of known and unknown derivatives of the mitorubrin and rubiginosin family.



**Fig. 12.** *Hypoxylon canariense* and *H. urriesii*, from holotype specimens. Scanning electron micrographs (10.000x) of ascospores. a) *H. urriesii*, ascospore 12.8  $\mu\text{m}$  long. b) *H. canariense*, ascospore 9.4  $\mu\text{m}$  long.



**Fig. 13.** Cultures of *Hypoxylon canariense* and *H. urriesii*. OA plates (9 cm diam) after 3 wk of incubation. a) culture CBS 119025, showing reddish exsudates. b) MUCL 47223.

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