HYPOXYLON

in Britain and Ireland

1. Changing perspectives in Hypoxylon

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The taxonomy and understanding of fungi in the Xylariaceae and in particular within the genus *Hypoxylon* has seen a considerable number of changes in recent times, but these appear to have scarcely filtered through to the field mycologist. There are few sources in the popular literature which have adequately explained or illustrated these changes and none which encompasses them all. One of the most interesting developments has been a multiplication of species in the genus, even in apparently well studied areas such as Europe. *Hypoxylon rubiginosum* would have been described twenty years ago as a variable or perhaps polymorphic species, but is now considered to comprise a number of segregate species, some of them former 'varieties' of *H. rubiginosum*, now known to be distinct both chemically and structurally. In a second article I shall demonstrate that at least four species in this category are present in Britain and Ireland. A final article will discuss the remaining British species retained in *Hypoxylon* and in its recent segregate *Annulohypoxylon*. Recorders will be better equipped to recognise these species in the field and so contribute to our understanding of their distribution and habits through records sent in to FRDBI. This first article

Fig. 1. *Annulohypoxylon multiforme* on birch: has papillate ostioles and dull brown KOH-extractable pigments. Photograph © R. Anderson.

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sets the scene by exploring recent changes in nomenclature in relation to literature sources commonly used by British field mycologists. This is followed by a key separating the modern concept of *Hypoxylon* from that of closely related genera formerly included therein.

Historical changes in the scope of *Hypoxylon*

Hypoxylon belongs to the Family Xylariaceae. This was called Sphaeriaceae by Dennis (1981) but it is now generally accepted that Xylariaceae has precedence. The Xylariaceae belong to the Order Xylariales which are characterised by forming perithecia (rather than apothecia, pseudothecia etc.). Perithecia are subglobose and usually ostiolate, opening via beaks or necks. The Xylariaceae include many species with well developed black, carbonised stromata within which multiple perithecia are embedded. Other characteristics include possession of asci, usually with apical rings bluing in iodine, and possession of dark, one-celled or sometimes unequally two-celled, ascospores with a germination pore or slit. It includes genera such as *Xylaria* (including the type genus), *Podosordaria*, *Kretzschmaria* (= *Ustulina*), *Rosellinia, Biscogniauxia, Daldinia* and *Anthostomella*.

Whilst some Xylariaceae are parasitic on plants many are saprotrophic. *Hypoxylon* includes pathogenic species but are mainly found fruiting on dead wood which they metabolise and rot. They are a familiar sight on fallen timber in our woods virtually all the year round. The term 'woodwart' has been coined for them and seems appropriate in view of their spreading or hemispherical blackened growths on bark and wood. However, the historical concept of *Hypoxylon* has been ammended somewhat in recent decades and a number of fungi formerly included in *Hypoxylon* are now placed in separate genera.

Most people interested in ascomycetes will possess or have access to one of the editions of Dennis (1981) and/or Ellis & Ellis (1985, enlarged version 1997). The concept of *Hypoxylon* used by these authors provides a good starting point for discussion. Dennis (1981) included eleven species in *Hypoxylon*. Of these, three are now placed in separate genera leaving eight remaining. Dennis' list is given below with the modern usage where appropriate. It largely follows a useful key by Whalley (1977) that is no longer widely available.

Fig. 2. *Hypoxylon fragiforme* on beech: has umbilicate ostioles and orange pigments. Photograph © R. Anderson.

Dennis (1981)	**Current usage**
Hypoxylon fragiforme	Unchanged.
H. rutilum	Unchanged.
H. howeanum	Unchanged.
H. cohaerens	Transferred to *Annulohypoxylon* as *A. cohaerens.*
H. rubiginosum	Split into a number of segregates of which at least three (plus *H. rubiginosum*) are in Britain and Ireland.
H. multiforme	Transferred to *Annulohypoxylon* as *A. multiforme*.
H. serpens	Transferred to *Nemania* as *N. serpens.*
H. fraxinophilum	Name change to *H. intermedium.*
H. fuscum	Unchanged.
H. semi-immersum	Transferred to *Nemania* as *N. confluens.*
H. nummularium	Transferred to *Biscogniauxia* as *B. nummularia*.

Ellis & Ellis (1985) largely used Dennis' system but with the following additional species:

Ellis & Ellis (1997)	**Current usage**
Hypoxylon chestersii	Transferred to *Nemania* as *N. chestersii.*
H. mammatum	Transferred to *Entoleuca* as *E. mammata.*
H. mediterraneum	Transferred to *Biscogniauxia* as *B. mediterranea.*
H. serpens var. *effusum*	Transferred to *Nemania* as *N. effusa.*
H. udum	Transferred to *Euepixylon* as *E. udum.*

There are still other names in the British literature. Cannon *et al*. (1985) in the *British Ascomycotina* checklist give the following:

Cannon *et al*. (1985)	**Current usage**
Hypoxylon gwyneddi	Transferred to *Nemania* as *N. gwyneddi*; described from Wales.
H. cohaerens var. *microsporum*	Transferred to *Annulohypoxylon*, elevated to species status and hence requiring a different specific epithet; now *A. minutellum.*
H. serpens var. *diffusum*	Transferred to *Nemania* as *N. diffusa.*
H. stygium	Transferred to *Annulohypoxylon* as *A. stygium*; old greenhouse record from Chatsworth, probably on imported wood.

Finally, there are some other names in the Fungal Records Database of Britain and Ireland .

FRDBI	**Current usage**
Hypoxylon annulatum	Transferred to *Annulohypoxylon* as *A. stygium* var.*annulatum*; a single record in FRDBI - more precisely identifying the Chatsworth collection listed above as *H. stygium*.
H. cercidicola	A name of very uncertain application; two records in the database; these probably refer to *Hypoxylon petriniae*, a segregate of *H. rubiginosum.*
H. investiens	Unchanged; included in FRDBI without records; native range in N. & S. America.

Hypoxylon macrocarpum	Unchanged; segregate of *H. rubiginosum*.
H. michelianum	Transferred to *Annulohypoxylon* as *A. michelianum*; single record in FRDBI is an error of mine for a *Nemania*; southern in Europe.
H. perforatum	Included in FRDBI without records; probably present in Britain; segregate of *H. rubiginosum*.
H. petriniae	Unchanged; segregate of *H. rubiginosum*.
H. subticinense	Unchanged; segregate of *H. rubiginosum*.

The segregate genera

Four segregate genera, namely *Entoleuca* (1922), *Euepixylon* (1867), *Biscogniauxia* (1891) and *Nemania* (1821), are included in a key by Rogers & Ju (1998). This key is adequate, though not very user-friendly, for separating them. As with Dennis (1981), Rogers & Ju (1998) give eight species of *Hypoxylon* for Britain and Ireland. Changes taking place since include the recognition of additional species in the *H. rubiginosum* complex and the separation of the annulate *Hypoxylon* (- those with papillate ostioles i.e. *H. cohaerens*, *H. cohaerens* var. *microsporum* and *H. multiforme*) into a new genus *Annulohypoxylon* (Hsieh *et al*. 2005). These changes will be reviewed in more detail in articles 2 and 3 of the series but are summarised in the species list below. A non-British mycota published recently also has keys to the extra-*Hypoxylon* genera. This is Hansen & Knudsen (2000) Volume 1 (Ascomycetes) of *Nordic Macromycetes*. Here also, a key to the genera of Xylariaceae is a little awkward and technical - see p.236. It should be noted that in this volume the authors have opted for *Ustulina* in place of *Kretzschmaria*. It also appears that the species they refer to as *Hypoxylon rutilum* (on *Salix caprea*) is not in fact the rare beech species referred to in the present article. Hansen & Knudsen's *H. rutilum* is likely to refer to yet another segregate of *H. rubiginosum*: *H. salicicola* Granmo, probably confined to willow.

Clearly the number of changes to names and dispositions will be confusing for anyone dependent upon the out of date mainstream British literature. Many of these developments are the result of DNA analysis or research into the secondary metabolism of the Xylariaceae. The latter has demonstrated that pigments secreted by some genera, notably *Hypoxylon*, are characteristic of specific taxa. Despite this, early attempts to construct a taxonomy based on pigments in *Hypoxylon* foundered. But species recognition nowadays, after all the dust has settled, is actually quite simple. It often requires no more than a simple chemical test and a knowledge of which tree species the fungus is growing on. A piece of the fruiting body placed in 10% potassium hydroxide (KOH) will release any pigments present in granules beneath the surface of the stromata. If a colour develops at all it is reasonably safe to assume that one is dealing with *Hypoxylon* / *Annulohypoxylon*. The other four genera formerly in *Hypoxylon*, i.e. *Biscogniauxia*, *Euepixylon*, *Entoleuca* and *Nemania*, do not possess KOH-extractable pigments so their reaction is nil.

Collections suspected of being *Hypoxylon* should have a small piece placed in a few drops of 10% potassium hydroxide on a glass slide placed over white paper. The colours will develop in sequence and should be noted (there is usually one dominant colour but more than one colour may be observed). This may take a few minutes. Taken with the sample's physical appearance and tree host (very important!) identification is usually then possible. It should be noted that the extracted colours are not necessarily the same shade as the coloured pigment granules observed beneath the surface of fruiting bodies, nor indeed as the colours on stromatal surfaces. The KOH colours can be

accurately identified by comparison with a colour chart although, in the majority of cases, this is probably unnecessary. If in doubt Fournier & Magni's excellent website (2003) should be consulted, where most of the European *Hypoxylon* species are described with colour photos of the stromata and of KOH-extractable pigments.

One other genus in the Xylariaceae contains species secreting KOH-extractable pigments. This is *Daldinia* (King Alfred's cakes). Curiously, *Daldinia* is predicted to belong within *Hypoxylon sensu stricto* in several DNA studies published recently. As yet no one has plucked up the courage to actually synonymise *Daldinia* with *Hypoxylon*, but watch this space! Nor is the close relationship between *Daldinia* and *Hypoxylon* a new idea. The Tulasne brothers (1863) included *Daldinia* within *Hypoxylon* a century and a half ago. Small *Daldinia* could conceivably be confused in the field with some of the more spherical *Hypoxylon* but may be separated by slicing the fruitbody vertically and examining carefully. *Daldinia* species, even when old, show a series of concentric layers. These may have partly disintegrated in small species possessing a gelatinous rather than a solid context (e.g. *Daldinia fissa* on *Ulex*), but are always observable with care. *Hypoxylon* may show what appears to be a limited zonation near the surface i.e. a layer of perithecia then a subiculum, but not the series of zones seen in *Daldinia*.

Key to *Hypoxylon* and related genera in Britain and Ireland

1 Stromata mainly or entirely each with a single perithecium (perithecia are usually separate, not fused; if a stroma appears to form by fusion of several, this is small and appears as small spots or stripes on wood) **2**
- Stromata multiperitheciate, and relatively large; perithecial outlines usually evident in the stroma **3**

2 Stromata immersed in very decayed wood; spores each with a tiny, oval germ pore . . . . . ***Euepixylon***
- Stromata superficial on wood or bark; spores with long (2/3 to full length) germ slits . . . . . ***Rosellinia***

3 Immersion of stromata in 10% KOH leads to rapid release of coloured pigments **4**
- Immersion of stromata in KOH produces no coloured pigments **6**

4 Fruiting body always with a series of concentric zones in cross section, globular and often large, but may be small, rarely down to 1 cm in diameter; . . . . . ***Daldinia***
- Fruiting body never with a series of concentric zones in cross section, variable in shape, flattened, pustular or globular; if globular then usually less than 1 cm in diameter **5**

5 Perithecia with papillate ostioles – opening higher than the stromatal surface - ostioles may also be encircled by a flattened disc [Fig. 1] . . . . . ***Annulohypoxylon***
- Perithecia with umbilicate ostioles – opening lower than the stromatal surface – no encircling disc present [Fig. 2] . . . . . ***Hypoxylon***

6. When young covered with a white then grey fleshy tissue (entostroma); when old becoming black and very brittle . . . . . ***Kretzschmaria*** (*Ustulina*)
- When young may be covered with a pale brown or black soft tissue; more often black but not becoming brittle when old **7**

7 Superficially similar to *Diatrype* stromata and erupting from wood or bark as thin, flat, black stromata in which greyish ostioles are scattered; or, as flat stromata distorted by depressed centres and raised edges (cupulate); spores brown, either unicellular (common) or unequally divided into two cells (one species only) [Fig. 3] . . . . . ***Biscogniauxia***
- Rounded perithecial outlines evident on stromata; stromata raised, pulvinate not flattened **8**

8 Stromata large, multiperitheciate and effused into irregular elongate colonies, only occasionally as spots; about 1 mm thick [Figs 4,5] . . . . . ***Nemania***
- Stromata small appearing as small spots or stripes on wood; few perithecia present; 1 mm, or greater in thickness **9**

9 Stromata forming small spots, semi-circular to circular in shape and 2 mm or greater in thickness; perithecia large (>2 mm) and with prominent ostioles; spores with spore-length germ slit. . . . . ***Entoleuca***
- Stromata forming spots or stripes, 1 mm in thickness; perithecia small (1 mm) and ostioles less prominent; spores with small, oval germ pore [Fig. 6] . . . . . ***Euepixylon***

Provisional list of British species of *Annulohypoxylon* and *Hypoxylon*

Genus ***Annulohypoxylon*** Ju, Rogers & Hsieh
Annulohypoxylon cohaerens (Pers.: Fr.)Ju, Rogers & Hsieh
Annulohypoxylon minutellum (Syd. & P. Syd.)Ju, Rogers & Hsieh
[syn. *H. cohaerens* var. *microsporum* in Rogers & Ju (1998)]
Annulohypoxylon multiforme (Fr.: Fr.)Ju, Rogers & Hsieh

Genus Hypoxylon Bull.: Fr.
Hypoxylon fuscum (Pers.: Fr.)Fr.
Hypoxylon fragiforme (Pers.: Fr.)Kickx.
Hypoxylon howeanum Peck
H. intermedium (Schwein.: Fr.) Ju & Rogers [*H. fraxinophilum* Pouzar in earlier works]
Hypoxylon macrocarpum Pouzar [segregate of *H. rubiginosum*]
Hypoxylon petriniae Stadler & Fournier [segregate of *H. rubiginosum*]
Hypoxylon rubiginosum (Pers.: Fr.)Fr.
Hypoxylon rutilum Tul. & C. Tul.
Hypoxylon subticinense Ju & Rogers [segregate of *H. rubiginosum*]

Fig. 3. *Biscogniauxia anceps* on blackthorn, with a flat, *Diatrype*-like erumpent stroma; no KOH-extractable pigments. Photograph © R. Anderson.

Fig. 4. *Nemania aenea* var. *macrospora* on holly; black with a brassy tint but no KOH-extractable pigments. Photograph © R. Anderson.

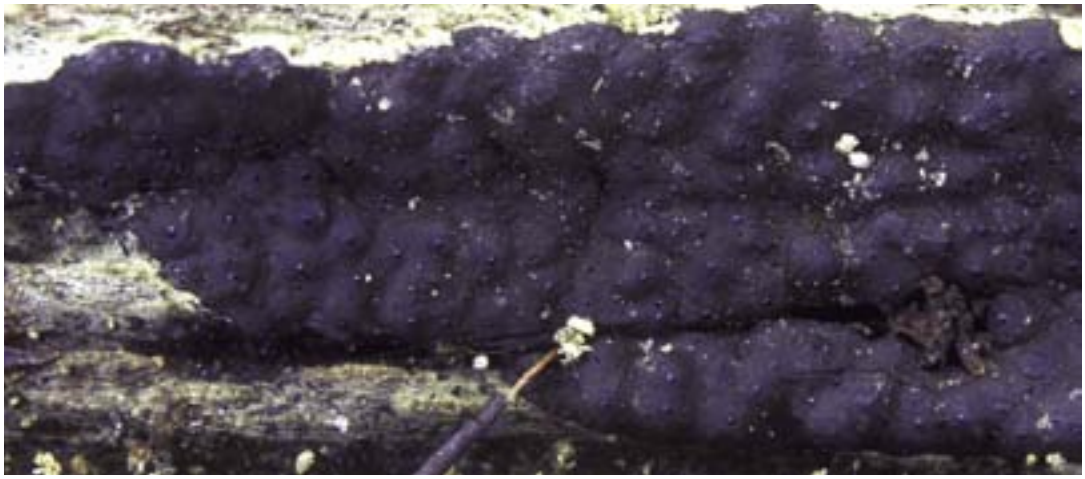

Fig. 5. *Nemania serpens* on beech; black and with no KOH-extractable pigments. Photograph © R. Anderson.

Fig. 6. *Euepixylon udum* on decayed oak heartwood: stromata small, often with a single perithecium. Photograph © R. Anderson.

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