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NOTES ON THE CYANOPHILY OF SPORES, WITH A DISCUSSION OF THE GENUS LEUCOGYROPHANA (CORTICIACEAE)

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The genus *Leucogyrophana* Pouz. has been placed in the Coniophoraceae by some authors on account of the cyanophilous spores, by which character the Coniophoraceae are said to differ from the Corticiaceae. Contrary to this opinion it is shown that not all the species of the Coniophoraceae exhibit cyanophily. Moreover the same spore reaction has been observed in a rather large number of species of the Corticiaceae. This means that 'cyanophily' is not a conclusive key character for a distinction between the two families mentioned. It is proposed to maintain *Leucogyrophana* in the Corticiaceae. A key to the accepted species of this genus is presented.

In 1958, Pouzar created a monotypic genus for *Merulius molluscus* Fr., viz. *Leucogyrophana*. This genus was said to be closely related to *Serpula* (Pers.) S. F. Gray, a genus of the Coniophoraceae. "Both genera have in common before all the brown colouration of the spore wall in Melzer's reagent and a very intensive blue colouration of endospore in cresyl blue". (p. 31). The name strongly suggests a *Serpula* (syn.: *Gyrophana* Pat.), but "...the difference between these two genera is in colour of the spores which is brown in *Serpula* and hyaline in *Leucogyrophana*". (p. 36). By now this genus comprises six species, in at least one of which the spore wall shows no affinity to cotton blue. After having studied some species of *Leucogyrophana* and of the Coniophoraceae I have my doubts about the value of the character "spore-wall stained in cotton blue", regarding the systematics at family level.

The first to introduce the use of cotton blue in the microscopy of the hymenomycetes were Nannfeldt & Eriksson (1953) who while studying *Coniophora ochroleuca* Bres. were "...surprised to find that the spores ... stained an intense blue colour". This prompted them to examine further species of the genera *Jaapia*, *Coniophora*, and *Serpula*, after which they were satisfied that "... the expected reaction was obtained". After discussing the possible relationships of the genus *Jaapia* Bres. the authors declared that "... all these facts show that *Jaapia* is a true member of the Coniophoraceae, and the structure of the spore-wall (esp. its stain reactions) not only serves as a most useful diagnostic character but affords, in our opinion, convincing proof that the family is a most natural one".

It is necessary here to mention that the authors not only noticed a staining of the spore-wall but were able to demonstrate "... that the stain had been absorbed by the inner layer of the spore-wall, while the thinner outer layer had remained per-

fectly hyaline". This phenomenon mostly in connection with additional characters has been adopted by other mycologists to define the Coniophoraceae. Thus one reads in Donk (1964: 255): "Spore-wall rather thick, presumably always double. . ., both layers smooth, the inner strongly absorbing Cotton Blue (cyanophilous)".

So far the application of this character offers no problems. Difficulties arise when an author ascribes to or removes from the Coniophoraceae a species or genus solely on account of the spores becoming stained in cotton blue or not. This was the very reason why Pouzar (1958) and Donk (1964) attributed *Leucogyrophana* to the Coniophoraceae, which was not accepted by Parmasto (1968) who placed the genus in the Corticiaceae (Athelioideae) as did Eriksson (1958) and Christiansen (1960) with the type species of *Leucogyrophana*, *Merulius molluscus* Fr.

The purpose of my observations recorded below was to (re)study the behaviour in cotton blue of the spores of a number of species of the Coniophoraceae and the Corticiaceae and thus to gain a better insight in the taxonomic value of this behaviour.

Methods and terminology

I used a solution of cotton blue (Baumwollblau - Chroma/Germany) in lactophenol. It is often necessary to boil the material for several seconds in order to accelerate the absorption of the dye. If the cyanophily of the spore-wall is not strong, empty spores have to be examined.

In this connection some remarks on the terminology are necessary. The dyes cotton blue and cresyl blue have been used by agaricologists since Kühner introduced the latter in his study of *Marasmius* (1933) without creating a new name for the reaction. Later on the term "metachromatic" has been adopted for a spore-wall staining red in the blue solution of cresyl blue whereas — more rarely — "orthochromatic" is spoken of when the spore-wall becomes blue-coloured, but the term metachromatic is also used to indicate a blue colouration of gloeocystidia by several blue dyes. Not seldom authors speak simply of a "cresyl blue reaction". A new term has been introduced by Kotlaba & Pouzar (1964: 131): "We propose the term 'cyanophily' for the staining of the spore-wall, or its ornamentation, and the hyphal wall in Cotton Blue and 'acyanophily' when they do not stain, as a precise term is desirable for describing this reaction".

Mention should be made of a note by Eriksson & Ryvarden (1973) that the spores of *Amphinema byssoides* (Fr.) J. Erikss. stained in cotton blue are ". . . ± red in phase contrast". This, however, is nearly always the case with spores that have taken up a blue stain as well as in all other stained parts of the basidiocarp such as hyphae, basidia, cystidia which have been broken in small fragments or which are strongly curved (thus the red colour is well visible in sterigmata e.g.). This means that the colour change from blue to red visible in phase contrast in blue-stained objects is of no taxonomic importance.

In my opinion the expression 'cyanophilous reaction' is not a very felicitous one, but I am using it for want of better.

SPECIAL PART

I first studied some species of the Coniophoraceae to become more acquainted with a well-established cyanophilous reaction, and obtained the following results.

The spores of *Coniophora arida* (Fr.) Karst. are distinctly cyanophilous. Normally the inner wall is strongly stained, suffused with a slight purple tint, whereas the outer wall becomes only slightly bluish. The capacity to become stained seems to be independent of time (collected between 1915 and 1965) and state of preservation.

MATERIAL STUDIED (all collected in the Netherlands)[1].—1915 (L 959.313–269), 1952 (L 952.287–905), 1957 (L 956.312–066), 1958 (L 958.284–013), 1965 (L 966.116 –036).

Coniophorella olivacea (Pers.) Karst. has also cyanophilous spores; but here particularly the outer layer of the spore-wall stains, whereas the inner layer shows a less intense colour.

MATERIAL STUDIED.—Mycotheca Estonica 15 (*Parmasto* 1956; L); Kryptogamae exsiccatae, Wien, 1601 (*v. Höhnel*; L).

Jaapia ochroleuca (Bres. apud Brinkm.) Nannf. & J. Erikss. shows also cyanophilous spore-walls; a distinction between the two layers is not always easy to detect.

MATERIAL STUDIED.—Type, *Brinkmann* 1897 (S); Brinkmann, Westf. Pilze 28 (*Brinkmann* 1899; L).

Serpula himantioides (Fr.) Boud.: The spores of all the specimens I studied were not cyanophilous; only the young spores with almost hyaline walls showed a reaction to cotton blue, whereas the older spores with brown walls remained unstained. After several weeks and once more boiling of the spore-suspension in cotton blue solution, some spores acquired a greenish-olivaceous colour, owing to the addition of a blue stain to the yellowish-brown colour already present in the spores. No strong affinity to cotton blue could be demonstrated in this species. Nevertheless it may be possible — by repeated heating or treating with chemicals — to force the spores to take up a greater amount of the cotton blue stain, but this was not the aim of the present study. In any case it is clear that after the 'normal' process, that is treating the spores with a cotton blue solution and boiling for several seconds, this species is not cyanophilous.

MATERIAL STUDIED.—Netherlands, 1958 (L 958.319–091), 1959 (L 955.283–158), 1960 (L 960.262–506), 1965 (L 966.116–035), 1968 (L 968.101–006); England, 1954 (L 943.250–136); Parmasto, Mycotheca Estonica 16, 1956 (L).

From these results it is clear that the character "the inner layer of the spore-wall strongly absorbing cotton blue" does not apply to all typical species of the Coniophoraceae. First of all, after the normal treatment the colour reaction cannot be

[1] In order to save space, most collections are referred to by their collecting date and admission number.

obtained in all species; secondly, it is not always especially the inner layer that stains: sometimes it is not possible (with a light microscope) to distinguish between the two layers, in other cases the outer layer seems to have a stronger affinity to cotton blue than the inner layer.

Even if the cyanophilous reaction cannot always be obtained within the Coniophoraceae it would nevertheless be a useful character if it were restricted to this family. It is, however, also a main character of the Gomphaceae. Fortunately, this creates no great problems since in this family the spores show a distinct cyanophilous ornamentation and, in addition, never have such thickened walls as is the case in typical species of the Coniophoraceae. Also in the polypores and in the Agaricales there are some genera with cyanophilous spores, but as these can be easily distinguished by means of other characters, no difficulties arise here either. Furthermore it was found that in some unnamed specimens of *Tomentella* the young spores are distinctly cyanophilous. The main problem, however, remains how to distinguish the Corticiaceae from the Coniophoraceae. Nannfeldt & Eriksson (1953: 178) stated that ". . . . several other aphyllophorous species with ± thick-walled spores were tested in the same way but reacted negatively". Since they did not state which species they examined, the uncertainty remains whether among the material investigated there were any Corticiaceae. Pouzar (1958) mentioned one corticiaceous species with cyanophilous spores, viz. *Merulius molluscus* Fr., which he placed in the separate genus *Leucogyrophana* that according to him belonged to the Coniophoraceae. Kotlaba & Pouzar (1964: 137) said that ". . . no species of the Corticiaceae are known to have cyanophilous spores, except for some species of the genus *Hypochnicium*". In a footnote, (1964: 134) they mentioned *Peniophora mollis* (Fr.) Bourd. & Galz. as having amyloid as well as cyanophilous spores. Maas Geesteranus (1971), who warns with reason of overestimating this colour reaction, found ". . . a very marked cyanophily . . ." in *Cristella fastidiosa* (Pers. per Fr.) Brinkm. My own studies show that cyanophily is present in some other genera of Corticiaceae, too.

In *Piloderma* Jülich, a genus segregated from *Athelia*, the two common species *P. bicolor* (Peck) Jülich and *P. byssinum* (Karst.) Jülich have been studied. In both species the spore-wall reveals a cyanophilous reaction. Since the spores are slightly yellowish one may philosophize about a possible relationship with the Coniophoraceae. Against this view speak the much smaller spores and the lack of clamps.

MATERIAL STUDIED.—*Piloderma bicolor* (spores of all specimens studied are cyanophilous), Austria, 1968 (Herb. Jülich 1299b); Denmark, 1964 (L 964.281–460); France, 1952 (Herb. Donk 11173).

Piloderma byssinum (cyanophily sometimes rather weak), Sweden, 1932 (Herb. Donk nos. 3577, 3580), 1962 (Herb. J. Eriksson 10454); France, 1909 (Herb. Donk 1172); Austria, 1968 (Herb. Jülich 1247).

In the genus *Byssocorticium* Bond. & Sing. the spores of most species are slightly thick-walled and of an olivaceous to grey-bluish colour. This means that cyanophily

is not always easily detectable. Nevertheless the spores of *B. atrovirens* (Fr.) Bond. & Sing. are distinctly cyanophilous while those of *B. pulchrum* (Lund.) Christ. are only slightly so.

MATERIAL STUDIED.—*B. atrovirens*, France, *L. Maire* (L 931.71–112), *Bourdot & Galzin* (L 931.71–97); England, 1961 (L 961.204–061).
B. pulchrum, Germany, Jaap, Fungi selecti exsiccati 281 (L).

Amphinema byssoides (Pers. ex Fr.) J. Erikss. has weakly cyanophilous spores.

MATERIAL STUDIED.—Netherlands, 1928 (Herb. Donk 975); Germany, 1968 (Herb. Jülich 955, 993, 1016, 1203, 2083, 2101).

Botrybasidium laeve (J. Erikss.) Parm. is a species with thin-walled spores which are weakly to distinctly cyanophilous. This reaction is naturally not as distinct as e.g. in *Piloderma* since the spore-wall is much thinner, but cyanophily (best seen in empty spores!) is definitely present. The basidia and especially the hyphae exhibit a remarkably strong cyanophily.

MATERIAL STUDIED (both specimens determined by J. Eriksson).—Germany, 1933 (Herb. Donk 8070); Netherlands, 1928 (Herb. Donk 1290).

Botryohypochnus isabellinus (Fr.) J. Erikss.: The spore-walls, spines, and basal hyphae are distinctly cyanophilous.

MATERIAL STUDIED.—England, 1959 (L 961.3–925); Germany, 1968 (Herb. Jülich 1143); France, *Bourdot* (L 931.82–28).

In the genus *Hypochnicium* two species with more or less cyanophilous spores have been studied, viz. *H. bombycinum* (Sommerf.) J. Erikss. (distinctly cyanophilous) and *H. punctulatum* (Cooke) J. Erikss. (weakly cyanophilous).

MATERIAL STUDIED.—*H. bombycinum*, Germany, Brinkmann, Westf. Pilze 11 (L); *H. punctulatum*, Netherlands, 1952 (L 951.328–008); England, 1960 (L 961.3–936); Germany, 1967 (Herb. Jülich 927).

Two specimens of *Cristella sulphurea* (Pers. ex Fr.) Donk have been studied, in both of which the spore (incl. warts) are cyanophilous. The same has been said for *Cristella fastidiosa* by Maas Geesteranus (1971). In other unnamed specimens of *Cristella* no cyanophily could be detected. According to Eriksson (1954: 191) the spores of *Cristella* "... do not stain with Cotton Blue".; unfortunately he did not mention which species he studied.

MATERIAL STUDIED.—Germany, 1967 and 1968 (Herb. Jülich 768 and 1499).

Since it was now clear that cyanophily is a character also present in genera of the Corticiaceae, I examined only three more species of *Corticium*, viz. *C. confluens* (Fr.) Fr., *C. evolvens* (Fr.) Fr., and *C. coprophilum* Wakef. The two former species have more

or less thin-walled, hyaline spores, and show a weak cyanophily. The last named species has thick-walled and strongly cyanophilous spores.

MATERIAL STUDIED.—*C. confluens*, Germany, Brinkmann, Westf. Pilze 13 (L); *C. evolvens*, Germany, 1968 (Herb. Jülich 1014); *C. coprophilum*, Netherlands, 1973 (L).

Furthermore the observation of Eriksson (1954) has to be mentioned that in *Peniophora abietis* both the outer layer of the spore wall and the aculei stain in cotton blue.

From the observations above it seems clear that cyanophily is by no means such a remarkable and conclusive feature as has been thought by some authors. At specific level it is often valuable, but for the delimitation of genera or even families it seems less suited. The transfer of *Leucogyrophana mollusca* from the Corticiaceae to the Coniophoraceae, merely because its spores are cyanophilous, is ill-founded, as several Corticiaceae have cyanophilous spores and many more species of this family probably will show the same reaction. Hence *Leucogyrophana mollusca* should remain in the Corticiaceae. This view is further supported by the observation that not all of the species of *Leucogyrophana* have cyanophilous spores:

Leucogyrophana mollusca (Fr.) Pouzar. All specimens seen have cyanophilous non amyloid spores.

MATERIAL STUDIED.—USSR, Parmasto, Corticiaceae URSS 140 (UPS); Sweden, Lundell & Nannfeldt, Fungi exs. suecici 86 (UPS); Netherlands, 1933 (Herb. Donk 5135).

Leucogyrophana mollis (Bres.) Parm. According to Kotlaba & Pouzar (1964) "... only the young spores have cyanophilous walls". I myself have not seen such young spores and observed only absolutely acyanophilous spores. Instead, I noticed that the spores are distinctly amyloid. Since within the genus *Leucogyrophana* the microscopical features do not show great diversity, this is an important distinctive character.

MATERIAL STUDIED.—Czechoslovakia, *Pouzar* 1963 (L 963.168–662); Netherlands, 1931 and 1932 (Herb. Donk 2319 and 4059); Sweden, 1932 (Herb. Donk 3731).

Leucogyrophana subtessulata (Parm.) Jülich has cyanophilous spores. Judging by the specimens seen, *Leucogyrophana cremeo-isabellina* (Litsch.) Parm. is conspecific. Both have inamyloid but cyanophilous spores of the same size, while hyphae, basidia, and rhizomorphs (which are not always present) seem to be identical.

MATERIAL STUDIED.—a) *Leucogyrophana subtessulata*, URSS, Parmasto, Corticiaceae URSS 126 (paratype, UPS). — b) *Leucogyrophana cremeo-isabellina*, Sweden: "An altem Zaunholz. Sdl. Neglinge-Skogsö, unweit Saltsjöbaden, 14.11.1906, *L. Romell*" (type; W no. 18266); Sweden: Upland, Knifsta, 13.12.1908, L. *Romell 1908* (W); URSS: Parmasto, Corticiaceae URSS 111 and 112 (UPS).

Leucogyrophana subillaqueata (Litsch.) Jülich, *comb. nov.* — Basionym: *Corticium subillaqueatum* Litsch. *in* Annls mycol. **39**: 128–129. 1941.

This species has an interesting history. Litschauer based his description on two collections made by Romell in Sweden. According to this description, the hyphae are sometimes ampulliform at the septa, and microscopical details too are similar to those of *Corticium illaqueatum* Bourd. & Galz. [now: *Ceraceomyces tessulatus* (Cooke) Jülich] except that the rhizomorphs are lacking and the spores are somewhat smaller. Oberwinkler (1965), who restudied the two specimens, found the basidia to be cylindrical-pedunculate, and consequently placed this species in his "ad interim" genus *Athelopsis* Oberw.; according to him the spores are hyaline, thin-walled and inamyloid. Parmasto (1968), validly publishing and emendating the genus *Athelopsis*, did not include this species but left it in his list of species of doubtful position. On studying the two specimens myself, I found the spores slightly yellowish, slightly thick-walled, amyloid becoming greyish, and cyanophilous. The morphology of hyphae, basidia, and spores leaves no doubt that the place of this species is in the genus *Leucogyrophana*. The key characters are: hymenial surface even, spores amyloid and cyanophilous, thus differing from all other species of the genus *Leucogyrophana*.

MATERIAL STUDIED.—Sweden, "Stockholm, Lidingö, 29.5.1910, *L. Romell 2068* (type; M); Upl., Lohärad sn, Erken, "Bibacken", 3.11.1918, *L. Romell 4095* (UPS).

I had no occasion to study two other species described by Parmasto, viz. *Leucogyrophana pouzarii* Parm., and *L. pseudomollusca* (Parm.) Parm. According to his descriptions the spores are not amyloid but dextrinoid; he mentions no cyanophilous reaction.

KEY TO THE SPECIES OF LEUCOGYROPHANA

1a. Hymenial surface in dried specimens even, in fresh condition sometimes slightly merulioid. 2
  2a. Spores amyloid.
    3a. Spores not cyanophilous, (4.5–)5.5–6.5(–7.5) × 2.5–3(–4) µm. *L. mollis* (Bres.) Parm.
    3b. Spores cyanophilous, 4–5 × 3–4 µm. . . . . *L. subillaqueata* (Litsch.) Jülich
  2b. Spores inamyloid, cyanophilous, 4–7 × 2.7–4 µm. *L. cremeo-isabellina* (Litsch.) Parm.
    Syn.: *L. subtessulata* (Parm.) Jülich.
1b. Hymenial surface in fresh and dried condition distinctly merulioid.
  4a. Spores cyanophilous but not amyloid and not dextrinoid, 4.5–5.5(–6) × 3.3–4.3 (–4.5) µm. . . . . *L. mollusca* (Fr.) Pouzar
  4b. Spores dextrinoid, not cyanophilous (?), about 6–7 × 4–5 µm.
    5a. Cystidia present, 20–30 × 7–8 µm. . . . . *L. pouzarii* Parm.
    5b. Cystidia lacking. . . . . *L. pseudomollusca* (Parm.) Parm.

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