

A STUDY OF *CYATHICULA STROBILINA* AND ITS
CHALARA ANAMORPH IN VITRO

WALTER GAMS

*Baarn**

SUSANNE PHILIPPI

*Karlsruhe***

The connection of *Cyathicula strobilina* (Fr.: Fr.) Korf & Dixon with the *Chalara strobilina* Sacc. anamorph is confirmed by means of ascospore isolations. The in vitro morphology of the latter is described in detail and the heterogeneity of the genus *Chalara* is pointed out.

Abundant apothecia of *Cyathicula strobilina* (Fr.: Fr.) Korf & Dixon in Dixon (1974) can be found mainly in the autumn and winter months at (300–)500–900 m altitude, on moist cones of *Picea* in a rather progressed stage of decomposition. The regular association of the ascomata with a 'subiculum floccoso-furfuraceum,' possibly indicative of an association with a hyphomycete, was already observed by Fries (1822), the genetic association with a *Chalara* as an anamorph was first suggested by Saccardo (1876). Rehm (1894: 722) carefully described the fungus as *Phialea strobilina* as distinct from *Ombrophila strobilina* (Alb. & Schw.) Rehm; on page 1262 (1895) he referred to Brefeld (1891) who had tried to culture the latter, obtaining cylindrical conidia; on page 323, Brefeld (l.c.), however wrote: 'Nicht erfolgreicher war die Untersuchung von Formen der Gattung *Phialea*. ... *Ph. strobilina* (Fr.) [lieferte] dunkelgefärbtes, reichliches Luftmycel ohne Fructification.' He thus obviously worked with Albertini & Schweinitz's fungus and not with Fries's. The confusion between the two taxa was sorted out by Dixon (1974), and the new combination *Ciboria bulgarioides* (Rabenh.) Baral & Krieglst. (1985) was published for the former. Under *Cyathicula strobilina*, Carpenter (1981: 256) discussed its taxonomic position.

Philippi (1984) gave a thorough description of the apothecia with an exhaustive literature review, and confirmed the connection with the anamorph *Chalara strobilina* Sacc. (1876) by means of careful direct observation of the occasional origin of conidiophores from excipular hyphae. This study was quoted by Baral & Krieglsteiner (1985: 47) who endorsed Carpenter's (1981) remark that the fungus was close to *Chloroscypha* and might be placed in *Antinoo*.

The *Ch. strobilina* anamorph was briefly mentioned with the original short diagnosis by Nag Raj & Kendrick (1976) in their monograph of *Chalara*, among the dubious species because of the absence of type material; however, the teleomorph, *Helotium strobilinum*, is

* Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, The Netherlands.

** Ludwig Marum-Straße 29, 7500 Karlsruhe, Germany.

known to be represented in Saccardo's herbarium in Padova (PAD, Gola, 1930: 121) and in many other herbaria (Dixon, 1974), but apparently not the type material of the anamorph, which had been collected in Cansiglio in Oct. 1875. But this cannot be a reason to reject the name of a fungus which is so clearly characterized by its substratum and teleomorph connection. There is also no type or other material of *Peziza strobilina* from Fries preserved at UPS (O. Constantinescu, pers. comm.) and none of *Helotium strobilinum* from Fuckel at G (O.H.E. Monthoux, pers. comm.). The specimens preserved in Saccardo's herbarium under *Helotium strobilinum*, *Phialea strobilina*, and *Ombrophila strobilina* could be examined for this study.

Chalara is so far mainly known to be connected with pyrenomycetous teleomorphs. Its connection with discomycetes is unusual and deserves a thorough vindication. The present study was undertaken to confirm previous conclusions on the anamorph-teleomorph connection by means of ascospore isolations, and to study the variation of the fungus in vitro.

MATERIALS AND METHODS

Cultures isolated by W.G.—CBS 803.84, single-ascospore isolate from suspended squashed ascomata on *Picea abies* cone, 270–300 m alt., Katzenbach-Hintere Diebswiesen near Ettlingen, Nordschwarzwald, Germany, 4 Nov. 1984, *S. Philippi* (Herb. CBS 3644, cultures Herb. CBS 3715). — CBS 804.84, conidial isolate from same substratum and origin. — CBS 805.84, conidial isolate from same substratum, Heckengäu bei Holzbronn, Kreis Calw, Germany, 14 Oct. 1984, *S. Philippi* (Herb. CBS 3643). — CBS 643.85, conidial isolate from same substratum, near Kringsjå, north of Oslo, Norway, Aug. 1985, *W. Gams* (Herb. CBS 3740).

Herbarium specimens obtained from P A D.—1. *Helotium strobilinum* (Fr.) Fuckel — *Sporoschisma strobilinum*, in Saccardo's handwriting (Fig. 2), without any indication of the origin. — 2. *Helotium strobilinum* Fr. — Lb. — 3. *Helotium strobilinum* (Fr.) Fuckel et *Chalara strobilina*, in Belgio in strobilis, *Mouton*. — 4. Phill. 40. *Peziza strobilina* Fr., Derbyshire, *James Renny* Esq. — 5. *Phialea (Ciboria) strobilina*, C. Roumeguère, *Fungi Gall. exs. Cent XIII, 1254, Malmédy, Rel. Libert. No. supp. S 80*. — 6. *Ombrophila strobilina* (Fr.) P. Karst., *Rehm, Ascom. 703, auf faulenden Zapfen von Abies excelsa, Pulsnitz (Sachsen), 1882, Staritz*.

RESULTS

Cyathicula strobilina (Fr.: Fr.) Korf & Dixon *apud* Dixon in *Mycotaxon* 1: 92. 1974.

Peziza strobilina Fr.: Fr., *Syst. mycol.* 2: 125. 1822. — *Helotium strobilinum* (Fr.: Fr.) Fuckel, *Symb. mycol.*: 313. 1870. — *Ombrophila strobilina* (Fr.: Fr.) P. Karst. in *Mycol. fenn.* 1: 92. 1871 [non *O. strobilina* (Alb. & Schw.) *Rehm* 1896]. — *Hymenoscypha strobilina* (Fr.: Fr.) Phill., *Brit. Discom.*: 133. 1887. — *Phialea strobilina* (Fr.: Fr.) Sacc. in *Syll. Fung.* 8: 256. 1889. Description under this name also by Dennis in *Mycol. Pap.* 62: 29. 1956. — *Chlorociboria strobilina* (Fr.: Fr.) Seaver in *Mycologia* 28: 393. 1936. — *Antinoea strobilina* (Fr.: Fr.) Vel., *Monogr. Discom. Bohemiae* 1: 215. 1934. — *Phialea eustrobilina* Korf *apud* Ramamurthi, Korf & Batra in *Mycologia* 49: 861. 1958.

A n a m o r p h.—*Chalara strobilina* Sacc. in *N.G. bot. ital.* 8: 185. 1876; *Michelia* 1: 80. 1877; *Fungi ital. delin.*: 30. 1877.

All herbarium specimens cited above (except No. 4) contained ascomata of the present fungus, although ascospores could not be detected in those mounted. The anamorph was present with very few conidiophores and conidia in specimens 1, 3, 4 and 5. Two more spec-

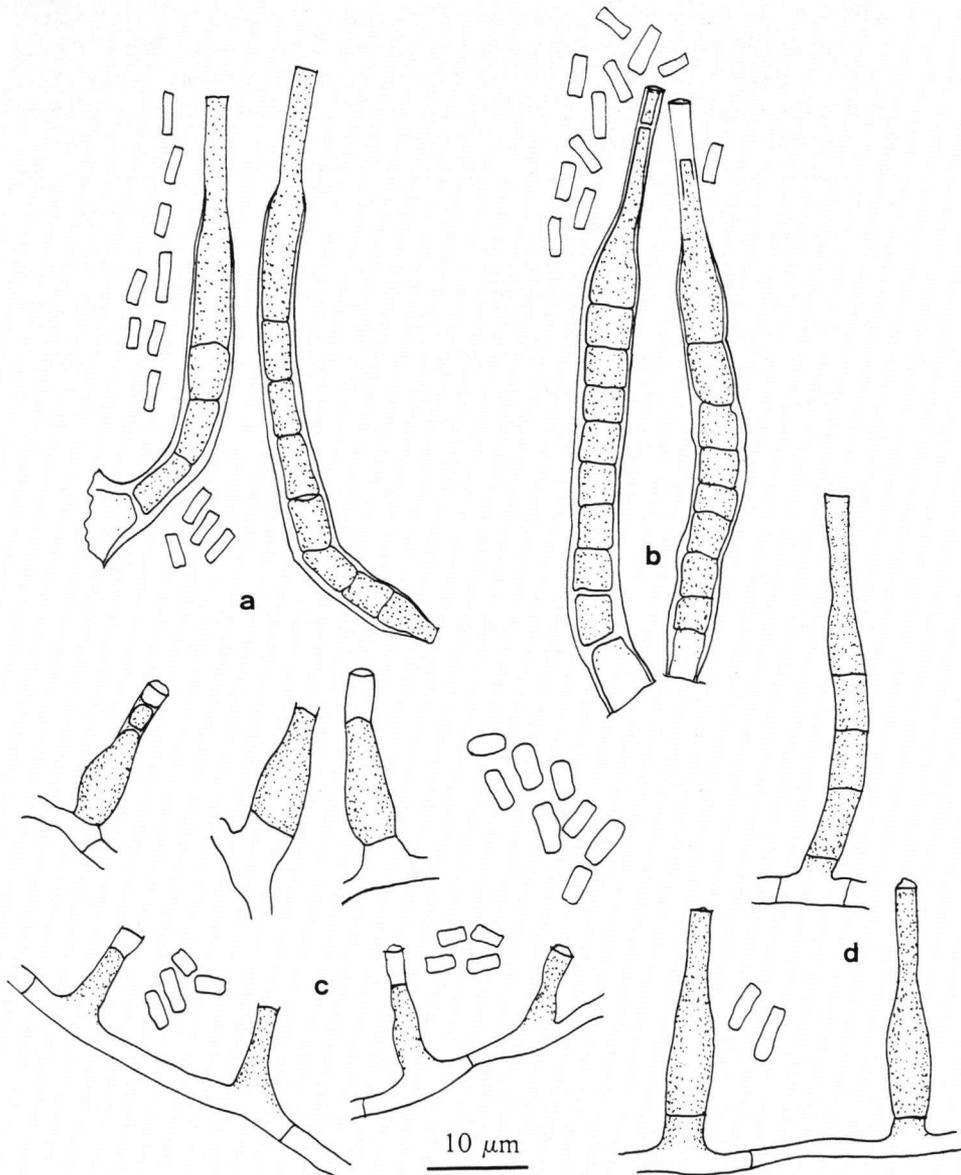


Fig. 1. *Cyathicula strobilina*.— a, b. Anamorph developing on the natural substratum (a. herb. CBS 3644, b. herb. CBS 3643). — c, d. From 8-day-old cultures (c. CBS 804.84 on PCA, d. CBS 803.84 on MEA).

imens preserved under *Ombrophila strobilina*, Rehm Ascomyceten No. 1005, and Sydow, Mycoth. germ. No. 332, contain the larger ascومات of *Ciboria bulgarioides*. It is likely that specimen No. 1 (Fig. 2) is in fact the holotype of *Chalara strobilina*, for which Saccardo may have thought of placing it in *Sporoschisma*. The measurements of phialide and conidia on the label agree with those of the protologue.

As this material is rather scanty and no longer very representative of the anamorph, representative material is deposited as herb. CBS 3644, the material from which isolates CBS 803.84 and 804.84 were made.

For more details on nomenclature and delimitation against *Ciboria strobilina* (Alb. & Schw.) Sacc. [*Ciboria rufofusca* (Weberb.) Sacc. or *Ciboria bulgarioides* (Rabenh.) Baral & Krieglst.], see Dixon (1974).

Ascospores were found to germinate easily on 2% malt extract agar. Numerous single-ascospore isolates invariably produced cultures identical with conidial isolates. Though the possibility cannot be ruled out that some conidia might have been present in the ascospore suspension used during isolation, the massive appearance of identical colonies proves the origin of at least most cultures from ascospores and the genetic connection with the anamorph.

Morphology in pure culture (Fig. 1c, d). — Colonies slow-growing, hardly reaching 10 mm diam. in 14 days on 2% malt extract agar (MEA) or potato-carrot agar (PCA) at 20°C. Mycelium olivaceous brown, with few aerial hyphae; sporulation beginning after about 6 days. Vegetative hyphae generally slightly pigmented, often roughened due to encrustation, 1.5–3.5(–4.5) µm wide. Globose roughened pigment concretions, 4–5 µm diam., formed in the medium. Chlamydozoospores absent. Conidiophores arising from submerged hyphae, erect, either simple phialides separated from the paler subtending hypha by a basal septum, often also lacking the basal septum, or more rarely compound, up to more than 50 µm tall, with several septa, about 5 µm distant, with an integrated terminal phialide. Conidiophores and phialides rather uniformly medium brown. Phialides flask-shaped, tapering in the middle part from a cylindrical to ellipsoid venter rather sharply into a cylindrical collarette; total length 11–24 µm; greatest width 2.5–4.5 µm. Conidia forming long chains (sometimes slimy heads), cylindrical, with both ends sharply truncate or slightly rounded, hyaline, 2.5–5 × 1.5–2.2 µm. A structure comparable to the 'macroconidia' described by Philippi (1985) could not be observed in vitro.

Good growth occurred in the range 10–20°C, at 24°C minimal outgrowth was observed.

Morphology on the natural substratum (Fig. 1a, b). — Conidiophores usually much longer, up to 130 µm, and 3–>10-septate; phialides usually with a shorter, 5–12 µm long and (3.5)–4(–4.5) µm wide, venter and even sharper delimitation of the collarette; total length 18–35 µm. Conidia 3–5 × 1.0–1.5 µm.

DISCUSSION

The present species is a very typical member of *Chalara*. *Chalara* is normally known as the anamorph of the pyrenomycetous genera *Ceratocystis* (Ophiostomataceae), *Cryptendoxyla* (Pseudeurotiaceae), *Pyxidiophora* (Pyxidiophoraceae) (Carmichael & al., 1980; Blackwell & Malloch, 1989), *Melanochaeta* (Trichosphaeriaceae) (Müller & Samuels, 1982), *Chaetosphaeria* (Trichosphaeriaceae, Holubová-Jechová, 1984), and *Quasiconcha* (Dothideales,

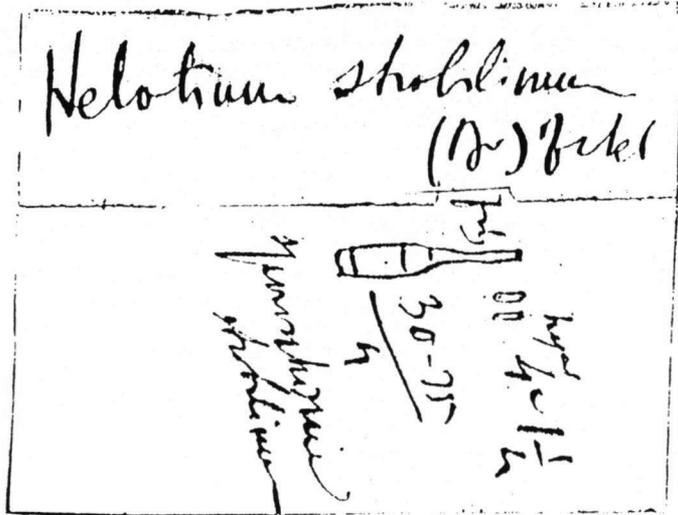


Fig. 2. Label of presumed type specimen of *Chalara strobilina* in Saccardo's handwriting.

Lophiaceae) (Blackwell & Gilbertson, 1985). A connection of *Chalara*-like anamorphs with discomycete teleomorphs has been known for the *Bloxama truncata* Berk. & Br. anamorph of *Bisporella sulfurina* (Quél.) Carpenter (Leotiaceae) and that of *Belonidium albidum* Grelet & Crozals from the studies of Berthet (1964), and the possible connection of *Chaetochalara* anamorphs with *Calycellina carolinensis* Nag Raj & Kendrick (Leotiaceae or Hyaloscyphaceae?) and *Hyaloscypha cladii* Nag Raj & Kendrick (1976: 183) (Hyaloscyphaceae). The latter, with its sometimes setose anamorph *Chalara* (*Chaetochalara*) *cladii* M.B. Ellis, was reclassified as *Phaeoscypha cladii* by Spooner (in Kirk & Spooner, 1984). *Chalara*-like anamorphs were also found in *Hamatocanthoscypha* (Hyaloscyphaceae) by Huhtinen (1990).

Chalara obviously is a heterogeneous genus, but no morphological criteria can be offered so far to correlate groups of anamorph-species with particular ascomycete orders.

The *in vitro* observations show that the criterion of conidiophore septation can be rather unreliable in this genus, unless the conditions of cultivation are carefully standardized.

According to the key in Nag Raj & Kendrick (l.c.), the anamorph might key out as *Chalara parvispora* Nag Raj & Hughes (1974), but this species is reported to have conidia which are rounded above and the phialophores have very large numbers of septa. Thus *Ch. strobilina* is a well-defined, easily collected and cultured species.

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