CERATOCYSTIS VERSUS OPHIOSTOMA: A REAPPRAISAL

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ABSTRACT

In the Ceratocystis complex the genus Ophiostoma is maintained for species which have conidial anamorphs other than Chalaras, have rhamnose in their cell walls, and are resistant to cycloheximide. Fourteen new combinations are proposed in Ophiostoma.

Key Words: Ascomycetes, Ceratocystis, Ophiostoma.

The subdivision of the genus Ceratocystis Ell. & Halst. sensu lato has long been a source of taxonomic controversy. Münch (1907) distinguished the genus Endoconidiophora Münch, with E. coerulescens Münch (the teleomorph of Chalaras quercina Sacc.) as the only species, from the other blue-staining Ascomycetes for which the name Ceratostomella Sacc. was in current use. The use of anamorph characters as the primary taxonomic parameters was a major point of discussion. These characters have since been used frequently for the segregation of infrageneric groups (e.g., Nannfeldt in Melin and Nannfeldt, 1934; Hunt, 1956; Mathiesen-Käärik, 1960). Goidánich (1936) even applied such characteristics at the generic level by introducing the genus Grosmannia Goid. for species with Verticicladiella Hughes anamorphs. Cain (1972) suggested that species with Verticicladiella anamorphs might be more closely related to hyphal yeast genera with hat-shaped ascospores, such as Cephaloascus Hanawa, than to species with phialidic, Chalaras-like anamorphs. De Hoog (1974) therefore classified the major part of the genus, mostly blue-staining species, in Ophiostoma H. & P. Syd. (1919). The few species with Chalaras anamorphs were retained in Ceratocystis sensu stricto. Some of these species are tropical fruit-rotting fungi. A major drawback of such a division is that the teleomorph characters are insufficiently concordant with those of the anamorph.

Upadhyay and Kendrick (1975) proposed another division of Ceratocystis. Species with falcate ascospores were classified in the newly erected genus Ceratocystiopsis Upadhyay & Kendrick. The remaining species were classified in Ceratocystis, regardless of their anamorphs; Upadhyay (1981) distinguished three sections on the basis of ascospore shape. Ceratocystiopsis is morphologically rather well delimited in that hardly any shapes are known intermediate between falcate and crescent-shaped ascospores. Based on the ecology and anamorphs of Ceratocystiopsis species, the majority may be regarded as belonging to the complex of Ophiostoma-like, blue-staining fungi. However, C. falcata (Wright & Cain) Upadhyay has short perithecial necks and two-celled ascospores, and thus is rather similar to Pyxidiophora Bref. & Tav. (Lundqvist, 1980).
Another genus related to *Ophiostoma* is *Europhium* Parker, in which the non-ostiolate blue-staining *Ceratocystis* species are accommodated. The presence or absence of an ostiolum is variable and dependent on environmental conditions (von Arx, 1973), and therefore the genus was not accepted, e.g., by de Hoog (1974) and Upadhyay (1981). However, it was maintained for practical reasons by von Arx (1974).

In 1981, Upadhyay put *Sphaeronaemella* Karst. into synonymy with *Ceratocystis*. This was a rather unfortunate decision. *Sphaeronaemella* has oblate ascospores with narrow germ slits, fleshy, pink to reddish white ascomata, and a *Gabarnaudia*-like anamorph (Samson, 1974). Those species recently accommodated in the closely related genus *Viennotidea* Negru & Verona ex Cannon & Hawksworth (1982) also have *Gabarnaudia* anamorphs. The connection of *Chalara* with *Sphaeronaemella* mentioned by Carmichael et al. (1980) was interpreted from the unnamed anamorph of *Sphaeronaemella helvellae* (Karst.) Karst. This was studied by Malloch (1974) and is actually a *Gabarnaudia* species. The characters mentioned above suggest that *Sphaeronaemella* is related to *Hypocrea* Fr. rather than to *Ceratocystis*. *Sphaeronaemella* and *Ceratocystis sensu lato* were classified in the Hypocreaceae (von Arx, 1974) or Nectriaceae (including Pyxidiophoraceae; von Arx, 1981) and the Ophiostomataceae, respectively.

In recent years the biochemistry of many species of *Ceratocystis sensu lato* has been studied. Marked differences were found, more or less correlated with anamorph distribution. Species with *Chalara* anamorphs lacked cellulose in their cell walls (Rosinski and Campana, 1964; Smith et al., 1967; Jewell, 1974), whereas it was present in the remaining species. In addition, all analyzed strains of the latter group contained rhamnose (Spencer and Gorin, 1971; Weijman and de Hoog, 1975), while none of the species with *Chalara* anamorphs did so. Subsequently, Harrington (1981) found that the species of *Ceratocystis sensu stricto* were unable to grow on media with cycloheximide, whereas species of the *Ophiostoma* complex were mostly resistant to this compound.

Thus several groups of independent characters have now proven to have a more or less concordant distribution. However, Harrington (1981) noted that this concordance does not coincide exactly with the distinction between endogenous and exogenous conidiogenesis, as tabulated by Weijman and de Hoog (1975). In the present paper the variability and pleomorphism of these fungi is again discussed, in an attempt to settle upon optimal anamorph criteria to distinguish the *Ophiostoma* complex on the one hand, and *Ceratocystis sensu stricto* on the other.

**ANAMORPH CHARACTERS**

Early in this century, Hedgcock (1906) indicated that cultural and anamorph characters could be important contributors to more balanced taxonomic concepts. Indeed, the anamorphs present a rich source of diversity, with numerous potential taxonomic criteria. However, these are more variable and subject to change during maintenance than are the characters of the teleomorph. The pleomorphism of the *Ceratocystis* complex concerned has been carefully studied by earlier workers, but has received insufficient attention in recent literature.

As an example, the anamorphs of the type strain of *C. araucariae* Butin, CBS 114.68, are listed here. The species was originally described (Butin, 1968) as having a sporodochial state, which was referred to by Upadhyay (1981) as *Hyalopesotum*, with “holoblastic sympodial” conidiogenesis. The latter author recognized correctly the occurrence of two major conidial states. The type culture, CBS 114.68, exhibits five types of propagation. A first type is sporodochial, the hyaline but
Ceratocystis araucariae. Three-week-old culture on CMA (CBS 114.68), various types of anamorph reproduction.

Fig. 1. Ceratocystis araucariae. Three-week-old culture on CMA (CBS 114.68), various types of anamorph reproduction.

thick-walled conidiophores arising from narrow, hyaline hyphae and being strongly branched in a penicillate manner (Fig. 1a). The conidia are formed holoblastically from sympodially proliferating conidiogenous loci and leave indistinct scars. They finally aggregate in large, slimy masses which are recognizable macroscopically as sessile, whitish drops. In a second type, expanding, wide, brown hyphae are formed, on which thin-walled, hyaline, short, remote branches arise at narrow isthmi (Fig. 1d). Only a few conidia are produced. A third type involves disarticulation of the hyphae. A fourth type occurs in the submerged mycelium of the sporodochial state as a more loosely branched anamorph resembling the Hyalopesotum anamorph. However, the conidia are formed in small, slimy heads from indistinct phialide-like conidiogenous cells (Fig. 1b). Finally, a fifth type is late-stage budding of conidia (van Oorschot and de Hoog, 1981; Fig. 1c).

The five types of propagation are clearly distinguishable from each other, either in morphology or conidiogenesis. Formally they may thus be referred to as synanamorphs. Two groups of synanamorphs (sympodial-1/phialidic/budding, and sympodial-2/arthric) can easily be obtained as separate cultures. Of particular taxonomic interest is the occurrence of both holoblastic and phialidic conidiogenous cells in segregants which seem to form a biological entity.

Many strains of other species of Ceratocystis exhibit similar morphological plasticity. From the foregoing example, in which phialides and sympodial cells
are found in the same segregant of a single strain, it is clear that rigid application of conidiogenesis alone will not show the natural relationships of these fungi. The differentiation of species with endogenous or exogenous conidiogenesis (Weijman and de Hoog, 1975) is too schematic. In CBS 114.68 the phialidic anamorph has much more in common with the sympodial morph of this strain than with the Chalara anamorphs of Ceratocystis. Similarly, the conidiogenous cells of Phialographium Upadhyay & Kendrick, though referred to as phialides, are more similar to the annellides of Leptographium Lagerberg & Melin than to the phialides of Chalara. For example, the anamorphs of C. europhioides Wright & Cain, CBS 275.65, and C. vesca Davids., CBS 800.73, treated by Upadhyay (1981) as Leptographium and Phialographium, respectively, have conidiogenous cells very similar to one another (Fig. 2). In both, the first conidium breaks through an outer wall and leaves a collarette, as in Phialographium. Later conidia are often formed by percurrent elongation, leading to irregularly annellated zones reminiscent of those known in Leptographium. A criterion by which to distinguish between proliferating phialides and annellides may be their formation of several or one conidium, respectively, after each proliferation. However, this criterion seems to be unworkable. It is therefore doubtful whether the genus Phialographium should be maintained at all.

The phialides of, for example, C. adiposa (Butler) Moreau are clearly different from those of Phialographium. All conidia are formed endogenously from a meristematic zone deep within the collarette. Coherent chains of conidia are extruded through tubular phialide openings (Cole and Samson, 1979). This is in contrast to the phialides of fungi such as C. araucariae and species of Phialographium, which produce globose heads of conidia. Minter et al. (1982) suggested that this
Chalara  Phialographium etc.  non-phialidic anamorphs  Gabarnaudia

Weijman and de Hoog 1975

Upadhyay and Kendrick 1975

Upadhyay 1981

de Hoog and Scheffer 1983*

Ceratocystis  Ophiostoma  Ceratocystiopsis  Sphaeronaemella

borderline species:

Ceratocystis
*Generic key features:
perithecia black
ascospores hyaline,
variously shaped but not falcate, without germ-slits
cellulose –
rhamnose –
cycloheximide growth –

Ceratocystiopsis
*Generic key features:
perithecia black or whitish
ascospores hyaline, falcate, without germ-slits
cycloheximide growth +

Ophiostoma
*Generic key features:
perithecia black or whitish
ascospores hyaline, variously shaped but not falcate, without germ-slits
rhamnose +
cycloheximide growth +

Sphaeronaemella
*Generic key features:
perithecia pinkish
ascospores brown, oblate, with germ-slits

cellulose +

Fig. 3. Diagram of current classifications of the Ceratocystis complex, showing distribution of anamorphs.
difference provides a better criterion for the distinction meant by Weijman and de Hoog (1975).

As might be expected, the distinction between species with Chalara-like and those with other anamorphs is not unequivocal either. In the CBS culture collection, there are several Chalara strains which fit the description of the anamorph of Ceratocystis autographa Bakshi given by Nag Raj and Kendrick (1975) and Gams and Holobová-Jechová (1976). These strains are resistant to cycloheximide, like the majority of Chalara species tested, but quite in contrast to the Chalara anamorphs of Ceratocystis sensu stricto. A further exception is Ceratocystiopsis falcata, which was recently found to have a Chalara anamorph (Rayner and Hudson, 1977), thus confirming its relationship to Pyxidiophora.

Despite these exceptions, we feel that on the whole, distinction of Ceratocystis sensu stricto from Ophiostoma (including Europhium) and Ceratocystiopsis is warranted (Fig. 3). The accepted species of Ceratocystis s. str. were listed by de Hoog (1974), those of Ceratocystiopsis by Upadhyay (1981). The remaining species without Chalara anamorphs listed in Upadhyay’s book should thus be regarded as Ophiostoma species. The necessary new combinations proposed in Ophiostoma for those which are maintained in the CBS culture collection follow.

Ophiostoma araucariae (Butin) de Hoog & Scheffer, comb. nov.
= Ceratocystis araucariae Butin, Canad. J. Bot. 46: 61. 1968. (Basionym)

Ophiostoma bacillisporum (Butin & Zimmermann) de Hoog & Scheffer, comb. nov.
= Ceratocystis bacillispora Butin & Zimmermann, Phytopathol. Z. 74: 281. 1972. (Basionym)

Ophiostoma brevicolla (Davids.) de Hoog & Scheffer, comb. nov.
= Ceratocystis brevicolli Davids., Mycologia 50: 667. 1958. (Basionym)

Ophiostoma distortum (Davids.) de Hoog & Scheffer, comb. nov.
= Ceratocystis distorta Davids., Mycologia 63: 10. 1971. (Basionym)

Ophiostoma dryocoetidis (Kendrick & Molinar) de Hoog & Scheffer, comb. nov.

Ophiostoma francke-grosmanniae (Davids.) de Hoog & Scheffer, comb. nov.

Ophiostoma huntii (Robins.-Jeff.) de Hoog & Scheffer, comb. nov.

Ophiostoma megalobrunneum (Davids. & Toole) de Hoog & Scheffer, comb. nov.
= Ceratocystis megalobrunnea Davids. & Toole, Mycologia 56: 796. 1964. (Basionym)

Ophiostoma nigrum (Davids.) de Hoog & Scheffer, comb. nov.
= Ceratocystis nigra Davids., Mycologia 50: 662. 1958. (Basionym)

Ophiostoma populinum (Hinds & Davids.) de Hoog & Scheffer, comb. nov.
= Ceratocystis populina Hinds & Davids., Mycologia 59: 1102. 1967. (Basionym)

Ophiostoma rostrocoronatum (Davids. & Eslyn) de Hoog & Scheffer, comb. nov.

Ophiostoma seticolle (Davids.) de Hoog & Scheffer, comb. nov.

Ophiostoma sparsum (Davids.) de Hoog & Scheffer, comb. nov.
Ophiostoma tremulo-aureum (Davids. & Hinds) de Hoog & Scheffer, comb. nov.
= Ceratocystis tremulo-aurea Davids. & Hinds, Mycologia 56: 794. 1964. (Basionym)

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