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The epitypification of *Ophiostoma minutum*, now *Ceratocystiopsis minuta*

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Abstract — Siemaszko's (1939) illustrations and figure legends for *Ophiostoma minutum* are designated herein as the lectotype for *Ceratocystiopsis minuta*, and a strain UAMH 11218 [= WIN(M) 1532, = R. Jankowiak 705] isolated from perithecia in galleries of *Ips typographus* in stems of *Picea abies*, from Biebrzanski National Park (Polish: Biebrzański Park Narodowy), Werklye Protection Range, grown and dried on wood chips, is then designated as the epitype and deposited in UAMH. This specimen will serve as a reference in future studies on species of *Ceratocystiopsis* that use modern morphological, chemotaxonomic, and molecular approaches. Morphological details are also presented for the epitype material.

Key words — nomenclature, ophiostomatoid fungi, species delimitation

Introduction

The genus *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr., based on *Ceratocystiopsis minuta* (≡ *Ophiostoma minutum*), was erected to accommodate ophiostomatoid fungi that have short perithecial necks and falcate ascospores (Upadhyay & Kendrick 1975). Siemaszko (1939) did not mention a conidial state for his fungus, and none of his material is extant. However Upadhyay (1981) indicated the anamorph of this species is a *Hyalorhinocladiella* H.P. Upadhyay & W.B. Kendr. Additional significant papers in the history of the erection of *Ceratocystiopsis* are those of Davidson (1942) and Mathiesen (1951).

Davidson (1942) took up Siemaszko's name as "*Ceratostomella (Ophiostoma) minutum* Siem." for several isolates he obtained from stained sapwood and grubs of *Monochamus titillator* (Fabr.) infesting a single dead pine near the District of Columbia, U.S.A. And while fertile perithecia were produced by his isolates, cultures he started from single ascospores were sterile; no perithecia were formed. He also recorded that this fungus produced a "cephalosporium-

like" conidial state, but that the perithecia it produced were smaller than those measured by Siemaszko.

Next Mathiesen (1951) provided an amended description for *O. minutum*, based on a number of the collections from both spruce and pine trees that had beetle galleries in them of one of four different bark beetles; perithecia were found regularly amongst the frass in the galleries. She noted some differences in that several of her measurements fell between those recorded by Siemaszko and Davidson. She also provided a detailed description of what she, too, called a "cephalosporium-like" conidial state, but her figures illustrate more complex fruiting structures than do those of Davidson.

Upadhyay's (1981) treatment of the fungus, by then known as *Ceratocystiopsis minuta,* was based entirely on North American material, for he did not record a single off-shore specimen as having been examined. Yet while Davidson reported the perithecial necks were 45–90 µm in length, Upadhyay stated they were 45–150 µm long. He also assigned the conidial state to the genus *Hyalorhinocladiella*, even though *Hyalorhinocladiella minutibicolor*, the type species of that anamorphic genus, does not resemble the conidial state of *Ceratocystiopsis minuta* as figured by Mathiesen (1951). [The original spelling of the specific epithet "*minuta-bicolor*" has been corrected throughout in accordance with Articles 60.8 and 60.9 of the ICBN (McNeill et al. 2006).]

Historically, the genus *Ceratocystiopsis* was not accepted by Wingfield et al. (1988), Hausner et al. (1993), or Van Wyk & Wingfield (1993); indeed Hausner et al. (1993) formally reduced it to synonymy with *Ophiostoma*. Subsequently, Zipfel et al. (2006), who discussed the taxonomic placement of the falcate-ascospored, short-necked, ophiostomatoid fungi, re-instated the genus name *Ceratocystiopsis*. They placed eleven species in the genus, but addressed only briefly the taxonomic and phylogenetic inconsistencies that existed amongst the species, and it now appears likely that the specimen they selected to represent *Ceratocystiopsis minuta* in their study was an unfortunate choice; it was not explained. They also listed, with brief notes, eleven other species that might be linked in some way to accepted members of this genus.

Recently Plattner et al. (2009) reviewed the taxonomic and phylogenetic inconsistencies that surround strains and isolates of this species studied previously by various authors, and attempted to resolve them using a molecular-based approach. They made much progress, but the final result did not allow convincing conclusions to be drawn, although the extent of genetic diversity they uncovered within the complex showed clearly that several phylogenetic species have been combined under the name *Ceratocystiopsis minuta*. Unfortunately however, because neither herbarium material nor a viable culture exists that can be linked to Siemaszko's (1939) original description of the basionym *Ophiostoma minutum*, they could make no further progress. Thus there is still no true nomenclatural type to serve as a reference for current workers.

Plattner et al. (2009) considered designating a neotype (new nomenclatural type) for *O. minutum* but were unable to do so because none of the cultures they considered to be appropriate candidate strains-these were from Poland-produced fully mature perithecia. However, using a modified culture technique and one of Plattner et al.'s designated candidate strains, R.J. 705 (UM 1532), we have obtained a very substantial number of mature perithecia on both wood chips and agar surfaces, but neotypification is not the appropriate course.

Along with his formal Latin description, Siemaszko provided photographs of two separate perithecia (possibly separate photographs of the same perithecium at different magnifications), a photograph of a bark sample with beetle galleries filled throughout with frass in which perithecia can be seen, and a line drawing of 22 ascospores showing their shape as enclosed within their mucilaginous sheaths. All these illustrations are referenced with his description and are certainly part of the material upon which the Latin description validating the name was based. Thus lectotypification based on these elements, followed by designation of an epitype, will serve to define this name.

Materials and methods

Air-dried (20°C) wood chips were obtained from the face of the outer sapwood next to the inner bark of laboratory air-dried discs that had been cut from the stem of a healthy specimen of both *Picea glauca* (Moench) Voss and *Pinus sylvestris* L.; the chips, which ranged from 3.5–5 mm long, 1.5–2 mm wide, and up to 1 mm thick, were placed in a clean 600 ml beaker and flooded with enough of a solution containing 20 g malt extract, 1 g of yeast extract, and 0.02 g of thiamine hydrochloride per L distilled water to ensure the chips would be still fully covered when they became saturated. Next the beaker was placed in a sealed Nalgene Vacuum desiccator (Fisher Scientific, Fair Lawn, NJ), and the latter was then evacuated and allowed to stand overnight. The next day, after adding nutrient solution to ensure the chips were covered, the beaker was sealed with aluminum foil and autoclaved for one hour at 121°C. The stimulatory effect of thiamine on perithecial production has long been known (Barnett & Lilly 1947; Hawker 1957), and it has been used recently for this purpose with other ophiostomatoid fungi (van Wyk et al. 2004, 2006).

When cooled, in a sterile chamber the chips were aseptically placed flat on the medium surface of sterile Petri dishes; in the latter the medium had the same composition as the above nutrient solution, but with solidifying agar added at 20 g/L. Two separate inoculation series were undertaken; one used pine chips, the other spruce. Depending on the size of the chips, two to four were placed in each plate. Plates were then inoculated using 1 mm square blocks of mycelium cut aseptically from colony margins of stock plates of isolate RJ705. One block, mycelium face down, was placed immediately adjacent to each wood chip in a plate, and the plates were then incubated in the dark at 20°C for up to 60 days. In total, forty plates were inoculated: 18 with spruce chips (total 54) and 22 with pine chips (total 65).

Although *Ceratocystiopsis minuta* has been reported to occur on *Abies, Larix*, *Picea*, and *Pinus* spp. in association with a variety of bark beetle species, we used pine and spruce chips in our trials because spruce was the host recorded by Siemaszko (1939). Mathiesen (1951: 205), who recorded this species from both spruce and pine, observed that it occurred most commonly on pine, "meist auf Kiefer, weniger oft auf Fichte".

Six agar plates containing only the amended malt extract agar plus thiamine hydrochloride were also inoculated centrally with single blocks of mycelium to serve as controls. Morphological structures were mounted in 85% lactic acid (Fisher Scientific, Fair Lawn, N.J.), and processed for observation according to Hausner et al. (2003). For photography we used Melzer's Reagent (Kohn & Korf 1975) to contrast the spore bodies with their surrounding sheaths.

Results

As our purpose was to obtain appropriate material to serve as an epitype for *Ceratocystiopsis minuta*, only incidental cultural characteristics were recorded. Mycelium growth was slow, as Davidson (1942) and Mathiesen (1951) both noted with their isolates.

By day 10, the mycelium had grown onto the pine-wood chip surfaces, but less abundantly than onto the adjacent agar. On the agar surface it was white initially, but later became pale grey around the inoculation blocks; on wood, the mycelium remained white, and by this time small, pale grey, spherical bodies were present on both the wood and agar. Over the next 50 days many more spherical bodies formed in irregular patches on the mycelium on both the wood and agar surfaces, and a surprisingly large number of them matured into fertile perithecia.

Although initially the developing perithecial necks were darker than the perithecial bases, this slight "bicolored" condition disappeared as the perithecia continued to mature and was never as pronounced as that seen in perithecia of *Ceratocystiopsis minutibicolor* (R.W. Davidson) H.P. Upadhyay & W.B. Kendr. (Upadhyay & Kendrick 1975).

By day 20 there were abundant maturing/matured perithecia in localized patches on the agar and numerous more dispersed perithecia on the wood-chip surfaces. And by then the majority of the perithecia had become uniformly dark colored, and spore tendrils/droplets were seen at many neck apices (Fig. 1 A – E).

Over the next 40 days increasing numbers of mature perithecia formed, particularly on the wood chips, and at day 60 the plates were dried at 20 C in a drying oven, and stored for further study.

Although both Scots pine and white spruce chips were used in parallel inoculation trials, mature fertile perithecia formed only in the culture plates containing pine chips.

Nomenclature

Ceratocystiopsis minuta (Siemaszko) H.P. Upadhyay & W.B. Kendr., Mycologia 67(4): 800. 1975 FIGS 1A–E, 2

≡ *Ophiostoma minutum* Siemaszko, Plant Polonica 7: 23. 1939

≡ *Ceratocystis minuta* (Siemaszko) J. Hunt, Lloydia 19: 49. 1956

≡ *Ceratostomella minuta* (Siemaszko) R.W. Davidson, Mycologia 34: 655. 1942

Lectotypification

The status of the genus *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. is in doubt because no holotype specimen exists for the species upon which it was based, i.e. *Ceratocystiopsis minuta* (≡ *Ophiostoma minutum*); indeed none of Siemaszko's herbarium material is now extant. However, in accord with Article 9.2 of the ICBN (McNeill et al. 2006), Siemaszko's (1939) illustrations typify his description.

LECTOTYPUS (designated here): Siemaszko's Planta Polonica 7: plate III: 10, 11, 12; fig. 1B. 1939.

Siemaszko (1939) did not refer to any specific collection but merely the tree host species and beetle with which the fungus is associated in a specific geographical area. Plate III: 10 and 12 are photographs of two perithecia, 11 perithecia in beetle galleries, and Fig. 1B shows line drawings of ascospores.

Epitypification

From the foregoing, the concept of *Ceratocystiopsis minuta* is now technically fixed, but Siemaszko's illustrations and description cannot possibly accommodate the needs of mycologists employing more modern morphological techniques or of those currently using chemotaxonomic and molecular methods to unravel relationships amongst various widespread populations of fungi such as *Ceratocystiopsis minuta*. Therefore we have chosen to designate an epitype.

EPITYPUS (designated here): UAMH 11218 (= a dried culture of WIN(M) 1532 = R. Jankowiak 705). ISOEPITYPE BPI 880579 (= R.Jankowiak(R.J. 705)/1532).

The strain was isolated from perithecia in galleries of *Ips typographus* (L.) in stems of *Picea abies* (L.) H. Karst., Biebrzanski National Park (Polish: Biebrzański Park Narodowy), Werklye Protection Range, Northeastern Poland, R. Jankowiak, date not given, and grown and dried by us on wood chips on agar.

Description of the epitype

Perithecia on wood and agar form initially as pale grey, spherical bodies with a lighter coloured central area from which the neck develops. Young necks initially darker than the perithecial base, bicolored phase gradually disappears with maturity; the upper portion of the base darkens first, the

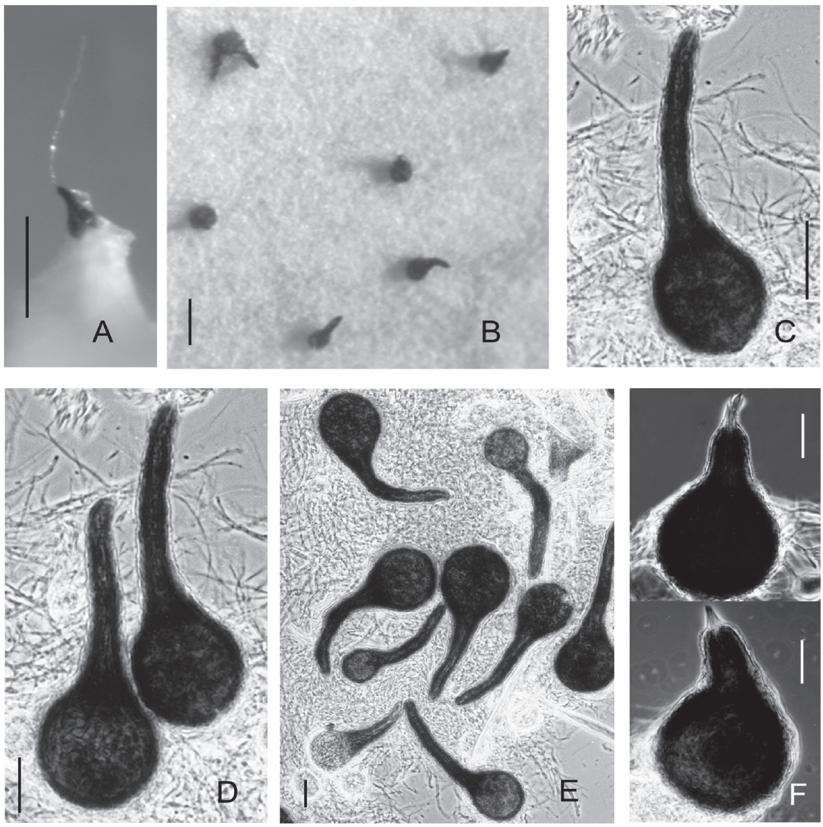

Fig. 1: A–E. *Ceratocystiopsis minuta* epitype UAMH 11218 [WIN(M) 1532]. A. A single perithecium, with an extruded spore tendril, on hyphal elements. B. Mature perithecia on the mycelium surface. C–E. Maturing and mature perithecia. Note the color variations in the young perithecia in E, and the extruded ascospores in D. F. *Ceratocystiopsis minuta* CBS 116795 [WIN(M) 1511]. Perithecia produced by this isolate in culture on wood; show the significant morphological differences apparent between the appearance of these perithecia and those produced by UAMH 11218. Scale bars: A and B = 250 µm; C–F = 30 µm.

darkening spreading downwards over time. Mature perithecial bases globose to obpyriform, surface smooth to slightly irregular, very rarely with short brown hairs, dark-brown to black in color; base width 37.5–87.5 (sd = 59.41 ± 13.17) µm, base height 37.5–97.5 (sd = 58.7 ± 14.58) µm. Perithecial necks with smooth to irregular surfaces, 20–45 (sd = 28.89 ± 6.8) µm wide at the base, tapering to 7.5–17.5 (sd = 13.8 ± 4.98) µm and narrowest at the apex; 70–175 (sd = 113.4 ± 17.98) µm long, ostiolar hyphae not included. Ostiolar hyphae up to 12 µm long); 1.5 µm wide at the base and tapering to a slightly blunted point; hyaline

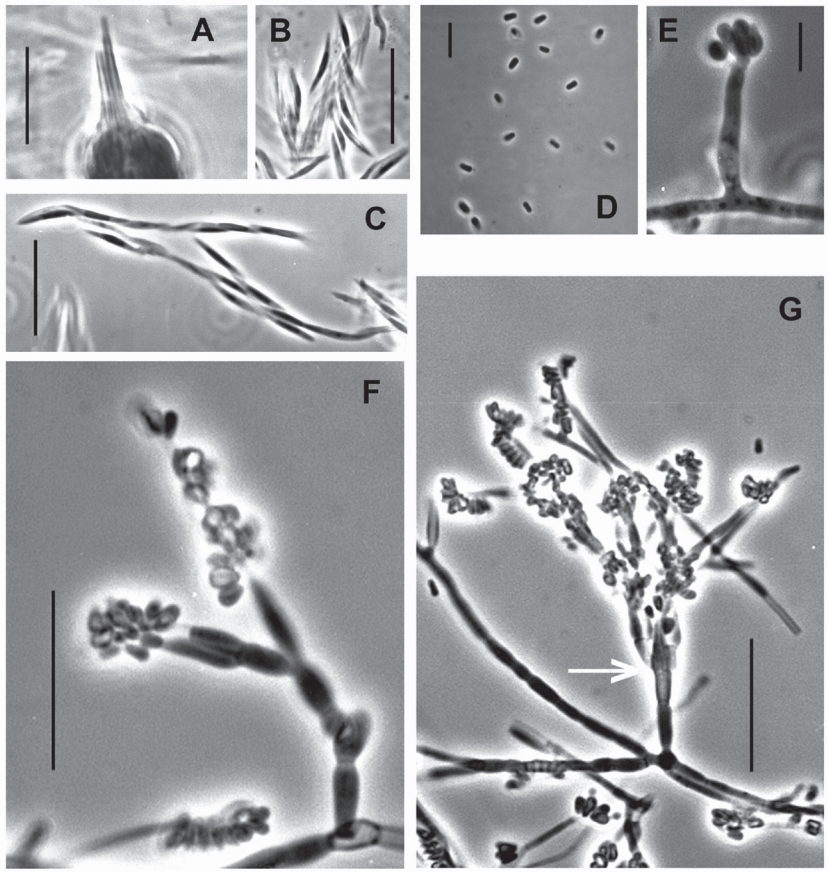

Fig. 2: *Ceratocystis minuta* epitype UAMH 11218. A. Neck apex of a mature perithecium with ostiolar hyphae. B and C. Ascospores stained with Melzer's reagent. D. Conidia. E. Simple conidiophore with adhering conidia. F. Branched conidiophore. G. Complex (macronematous) branching conidiophore; whorled branches origin is denoted by arrow. Scale bars: A, B and C = 20 μm; D and E = 10 μm; F and G = 30 μm.

and convergent. Asci deliquesce rapidly and are seen rarely in mounting media; the free ascospores are usually extruded in long tendrils from the ostiole, but sometimes they collapse into mucilaginous droplets). Ascospores with sheath appear falcate, 10–13 (sd = 11.4 ± 1.18) μm long and widest, 1–2 (sd = 1.38 ± 0.48) μm, at their mid-point, tapering sharply to their tips. Ascospores without the sheath rarely seen.

Mycelium on agar white initially, becoming pale grey centrally and in infrequent patches with maturity. Aerial mycelium sparse, producing two types

of conidiophores. First type short, simple, randomly produced, with droplets containing numerous conidia at apices. Second type arise as short hyphal elements that continue to grow producing verticillate to irregular lateral and secondary branches. Conidiogenous cells appearing holoblastic, terminal, and percurrent. Conidia produced in slime drops, one-celled; oblong to slightly tapered with rounded ends, oval; small, 2–4 (sd = 3.34 ± 0.59) µm long and 1–2 (sd = 1.38 ± 0.48) µm wide hyaline and smooth.

We do not accept the synonymization of *Ceratocystis dolominuta* H.D. Griffin (Griffin 1968) with *Ceratocystiopsis minuta* (Upadhyay 1981). We concur with Griffin and Olchowecki & Reid (1974) that its consistently shorter ascospores separate it from *Ceratocystiopsis minuta*.

Discussion

Ideally a designated epitype would be based on an isolate from the Białowieski Park Narodowy (Białowieski National Park) area of eastern Poland, as this is where Siemaszko (1939) made his collections. Therefore, Plattner et al. (2009) sequenced three strains isolated from hosts in that area by T. Kirisits in June, 2002; specifically CBS 116795, 116796 and 116963. Of these, only strain 116795, closely allied to 116963 in their tree, fruited in culture, but it did not fit well with either Siemaszko's protologue for *Ophiostoma minutum*, or the latter as amended by Mathiesen (1951); 116796 did not fruit either and grouped with four other Polish strains and one Japanese strain in a different clade of Plattner et al.'s tree (2009). None of these four latter Polish isolates were from Białowieża, but three were from northeastern Poland, between 150 and 210 miles north northeast of that locality. Plattner et al. (2009: 884) noted that one of these "... , R.J. 705, which produced what appeared to be mature perithecia but no ascospores, showed an imperfect state almost identical to that described by Mathiesen (1951) and Davidson (1942)" would be a candidate for neotypification if it had produced ascospores. It is this strain we used for epitypification.

In Europe, *Ceratocystiopsis minuta* is associated commonly with a wide range of bark beetles on more than one host tree species (Kirisits 2004). In some cases there is a common association between the bark beetle and this fungus, e.g. *Ips typographus* and *Ceratocystiopsis minuta* on *P. abies* in certain areas of France (Viri & Lieutier 2004), but in other geographical areas the association is rare. For example, during a study of the fungi associated with *Tomicus piniperda* (L.) attacking *P. sylvestris* at eight locations in Poland, Jankowiak (2006) found it at only one of the locations sampled, and then only at very low levels. Clearly, *Ceratocystiopsis minuta* is beetle-vectored, although with more than one species (Kirisits 2004).

Plattner et al. (2009) concluded that the name *Ceratocystiopsis minuta* referred to several phylogenetic species, and that different species misidentified as *Ceratocystiopsis minuta* might be present in different geographical locations. Our results indicate that populations of these putative different phylogenetic species may coexist within fairly restricted geographical areas; based on combined morphological and molecular criteria, this appears to be the case in northeastern Poland.

The molecular analysis in Plattner et al.'s tree (2009; Fig. 1) suggests that two distinct populations of *Ceratocystiopsis minuta* exist in eastern Poland. Isolate R.J. 705, which we have designated as epitype, is representative of one of these populations, while CBS 116795 belongs to the other. Their molecular distinctness is confirmed by the morphological differences that we observed between these two isolates (Table 1, Figs. 1 A–F).

Table 1. Comparison of perithecial and ascospore measurements for *Ceratocystiopsis minuta* strains R.J. 705 and CBS 116795.

Isolate	R.J. 705	CBS 116795
Perithecium (μm)		
Base width	37.5–87.5; sd = 59.41 ± 13.7	52.5–100; sd = 78.44 ± 13.03
Base height	37.5–97.5; sd = 58.7 ± 14.58	57.5–100; sd = 77.39 ± 12.58
Neck length	70–175; sd = 113.4 ± 17.98	30.0–75; sd = 49.8 ± 12.65
Neck base width	20–45; sd = 28.89 ± 6.8	20–35(–37.5); sd = 26.81 ±5.0
Neck tip width	7.5–17.5; sd = 13.8 ± 4.98	(15–)17.5–25; sd = 20.56 ± 2.34
Ostiolar hyphae	up to 12. 4 in length	up to 12.5 in length
Ascospores (μm)		
Length	10–13; sd = 11.4 ±1.18	9–15; sd = 10.86 ± 1.34
Width	1–2; sd = 1.19 ± 0.29	1–2; sd = 1.14 ± 0.26
Conidiophore type	macronematous & micronematous	micronematous

Although not precisely the same, another unusual situation is evident in reports of *Ceratocystiopsis minuta* from Japan. Plattner et al. (2009) noted that Japanese strains of this fungus from two different tree species, *Picea jezoensis* (Siebold & Zucc.) Carrière (Yamaoka et al. 1997) and *Larix kaempferi* (Lamb.) Carrière (Yamaoka et al. 1998) shared a common phylogenetic ancestor, although they were placed in two distinct monophyletic groups (clades). These two isolates, JCM 9367 (YCC-139) from *P. jezoensis* and JCM 9816 (YCC-294) from *L. kaempferi*, are similarly morphologically distinct from each other in both their perithecial appearance and the nature of their anamorphs (Yamaoka et al. 1997, Figs. 1–5; Yamaoka et al. 1998, Figs. 2–5), as isolate R.J. 705 is from CBS 116795. Also, JCM 9367 (YCC-139), is placed in the same clade as R.J. 705

in Plattner et al.(2009, Fig.1), and resembles the latter isolate in morphological features, i.e. perithecial neck shape and conidiophore complexity.

Upadhyay & Kendrick (1975) erected the genus *Hyalorhinocladiella*, based on *Hyalorhinocladiella minutibicolor*, to accommodate the anamorph of *Ceratocystiopsis minutibicolor*. While their description and photographs do represent accurately the conidial state of *Ceratocystiopsis minutibicolor* as described by Davidson (1966), their concept of *Hyalorhinocladiella* does not fit the anamorph of *Ceratocystiopsis minuta* as defined herein.

Benade et al. (1996) emended the description of conidiogenesis in *Ceratocystiopsis minutibicolor* to percurrent and sympodial extensions of the conidiogenous cell, but did not indicate whether the conidiophores could be more complex than the simple hyphal elements described and/or figured by Davidson (1966) or Upadhyay & Kendrick (1975).

Our photographs of isolate R.J. 705, plus those of isolate YC-139 (Yamaoka et al. 1997) and the drawings of Mathiesen (1951) of *Ceratocystiopsis minuta* all show that this species, as epitypified herein, most commonly produces quite complex conidiophores; the simple *Hyalorhinocladiella*-like structures are less abundant.

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