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On the variability of *Ophiostoma piceae*

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Abstract. The morphology and cultural characteristics of *Ophiostoma piceae* were described on the basis of 22 strains from the CBS collection supplemented with 25 fresh isolates from Poland, mostly from necrotic parts of *Quercus robur*. The pleomorphism of anamorphs of the fungus is described in detail. The various cultural types could be reproduced by replicate transferance. The identity of the anamorph strains was confirmed using some strains that produced perithecia. It is concluded that cultures of *O. piceae* may be quite different in appearance, and that several names that are in current use should be regarded as synonyms. An emended description of the taxon is provided.

Introduction

Members of the genus *Ophiostoma* Syd. & P. Syd. are widely known as blue stain fungi on various trees in the northern hemisphere. *Ophiostoma* differs from the mainly tropical genus *Ceratocystis* Ellis and Halsted by characters of the anamorphs, which have mainly sympodial or percurrent conidiogenesis in *Ophiostoma* but a phialidic one in *Ceratocystis* (De Hoog & Scheffer 1984). This segregation of genera is supported by biochemical characteristics (Weijman & De Hoog 1975) and cycloheximide resistance (Harrington 1981, 1987). In summary, *Ophiostoma* species have cellulose and rhamnose in their cell walls and are resistant to cycloheximide, while these characters are not found in *Ceratocystis s. str.* The major part of the species treated in Upadhyay's (1981) monograph as *Ceratocystis,* among which *C. piceae,* should be referred to as *Ophiostoma* species.

The species under study was initially described from sapwood of *Picea* by Münch (1907) under the name *Ceratostomella piceae.* In later studies confusion was caused by the pleomorphism of the species. Its markedly different synnematous and mononematous stages may be found separately from each other and different anamorph-generic names were assigned to them, partly

with own epithets: *Graphium pirinum* Goidànich (= *Pesotum piceae* Crane and Schoknecht) and *Hyalodendron pirinum* Goidànich, respectively. Nomenclatural histories of the species were presented by Crane & Schoknecht (1973) and De Hoog (1974). The first mentioned authors found that in *O. piceae* (Münch) Syd. and P. Syd. the conidia in both the mononematous and synnematous structures are produced by sympodial proliferation. Crane & Schoknecht (1973) segregated the genus *Pesotum,* with *P. piceae* as the type species, from *Graphium* on the basis of sympodial versus percurrent conidiogenesis. De Hoog (1974) described the sympodial, mononematous form as a member of the anamorph-genus *Sporothrix* Hektoen and Perkins.

Strains similar to *O. piceae* have been found to be pathogenic to trees other than *Picea,* viz. *Pyrus, Fagus* and particularly *Quercus.* A main issue therefore is the question whether these represent different taxonomic entities, or are just variants of a single, variable species, *O. piceae.* In the last decennia several closely related taxa have been introduced, viz. *Ceratostomella pilifera* var. *dryina, C. quercus, C. fagi, C. catoniana, Ophiostoma roboris, O. valachicum, O. kubanicum* and *Ceratocystis floccosum.* The aim of the present study was to describe the variability of *O. piceae* in pure culture, in order to establish whether separate taxonomic entities within this complex are justified. A study on cross-inoculations on the various host trees is in progress.

Material and methods

The 22 strains studied from the CBS culture collection and 25 fresh isolates are listed in Table 1. Strains were grown in Petri dishes with oatmeal agar (OA) and incubated at room temperature in daylight. Expansion growth was measured after 10 days and colony characters were recorded with regular intervals from the 10th day onwards. Slides were mounted in lactic acid and Amman's lactophenol.

Fresh isolates came from decaying parts of standing oaks (*Q. robur,* occasionally one of its hybrids) from various localities in Poland. Samples were taken from margins of necrotic spots with a sterile knife after removal of superficial tissues. In the laboratory, two methods of isolation were used:

- Samples were washed with running tap water, superficially sterilized with 70% alcohol and incubated in a wet chamber in daylight. After 10–14 days conidial slime from synnemata was inoculated onto potato dextrose agar (PDA) and malt extract agar (MEA) in petri dishes, which were incubated at room temperature in darkness.
- Small sections were cut from large samples, brought onto the above media and incubated at 25° C in darkness.

Single-spore isolations were made using strongly diluted spore suspensions.

Petri dishes were examined under low magnification and small pieces of agar bearing single conidia were cut out and transferred onto OA and incubated under standard conditions.

Results and discussion

Colonies

On the basis of their cultural characteristics the strains could be assigned to three different groups.

Table 1. Origins of strains studied.

CBS Strains
CBS 108.21*, T of *Ceratostomella piceae*, ex *Picea abies*, FRG, E Münch
CBS 120.31*, ex *Quercus*, FRG, E Lehmann
CBS 236.32*, T of *Ceratostomella fagi*, ex *Fagus sylvatica*, FRG, W Loos
CBS 251.33 and 252.33, mating partners ex CBS 236.32, CJ Buisman
CBS 263.35*, T of *Ceratostomella catoniana*, *Graphium pirinum* and *Hylodendron pirinum*, ex *Pyrus communis*, Italy, G Goidànich
CBS 142.37, 143.37, FRG
CBS 126.39*, K Zobl
CBS 153.54*, ex paper pulp, Norway, L Anker
CBS 180.69, ex *Picea sitchensis*, Northern Ireland, DA Seaby
CBS 799.73, authentic for *Ceratocystis floccosa*, ex wood, Sweden, A Mathiesen-Käärik
CBS 353.83*, culture contaminant
CBS 776.85 = UBC 996, ex wood chips, USA, S Ross
CBS 819.85, Canada, E Setliff
CBS 465.86, 466.86, 467.86*, 468.86, 469.86 and 470.86 ex *Tsuga heterophylla*, Canada, M Chung
CBS 135.88, ex *Quercus petraea*, K Skadow

Fresh isolates K Przybyl from Quercus	
Ex necrotic spots on truncs:	Nos 6I, 88A, 162, 203A, 230, 234A, 235 and 238
Ex bark wounds:	Nos 10, 10A, 10I, 10IB, 17, 64IB, 190, 193 and 236
Ex necrotic spots on roots:	Nos 163A, 173B, 229, 233, and 248A
Ex necrotic spot on twig:	No. 195A
Ex necrotic spot on petiole:	No. 198
Ex necrotic spot on leaf:	No. 216

* Cultures lacking synnemata. The last two digits on CBS strain numbers represent the year of accession into the culture collection.

Group A. Aerial mycelium well-developed, floccose or felty, white or greyish white, darkening with age, with or without concentric zones. In a later stage some solitary synnemata may be formed. Representatives (those marked with * lacked synnemata at the onset of our studies): CBS 108.21*, 120.31*, 236.32*, 251.33, 263.35*, 142.37, 126.39*, 252.33, 153.54*, 353.83*, 467.86*, 135.88, 10, 88A, 163A.

Group B. Aerial mycelium mostly scant, at first white or greyish white, later becoming tan to dark brown. Synnemata abundant, arising all over the colony either dispersed or in vague concentric rings, or arising in a broad band near the colony margin, the central portion remaining smooth. In a later stage some floccose aerial mycelium may be formed. Representatives: 143.37, 180.69, 819.85, 466.86, 468.86, 469.86, 470.86, 6I, 10A, 162, 190, 235, 236, 248A.

Group C. A combination of A and B, mostly in the form of vague sectors in which either synnemata or aerial hyphae are preponderant. Representatives: 799.73, 776.85, 465.86,10I, 10IB, 17, 64IB, 173B, 193, 195A, 198, 203A, 216, 229, 230, 233, 234A, 238.

Synnemata

At maturity, two types of synnemata can be distinguished, viz. simple ramified ones. In the simple, unbranched type (Fig. 1) initially an aggregate of irregularly intertwining hyphae is formed from which erect, loose fascicles of variable width, composed of hyaline, undifferentiated hyphae, soon arise (Fig. 1A). The erect hyphae later become septate and branch at the apex (Fig. 1B). Subsequently basal portions of the hyphal bundles become brownish; dark brown, thick-walled enveloping hyphae are formed which remain unbranched and sterile (Fig. 1C). Then with further branching the synnemata become compact, and after repeated dichotomous branching the ultimate cells become conidiogenous (Fig. 1D, E). Profuse conidial slime is soon produced, and several neighbouring synnemata may fuse at the tip.

The ramified type (Fig. 2) is initiated with superficial hyphae which locally form rather straight rhizoids growing downward into the agar. Upward directed, brown, thick-walled branches radiate outwards in a pin cushion-like manner and soon branch repeatedly at the apex (Fig. 2A). The basal portions also form lateral branches which grow parallel to and closely adhering to the first branches, leading to a group of radiating synnemata (Fig. 2B). The ultimate branches are hyaline and finally become conidiogenous (Fig. 2C). Probably parallel hyphae that are produced in a later stage remain sterile and grow out as dark brown, enveloping hyphae (Fig. 2C, D).

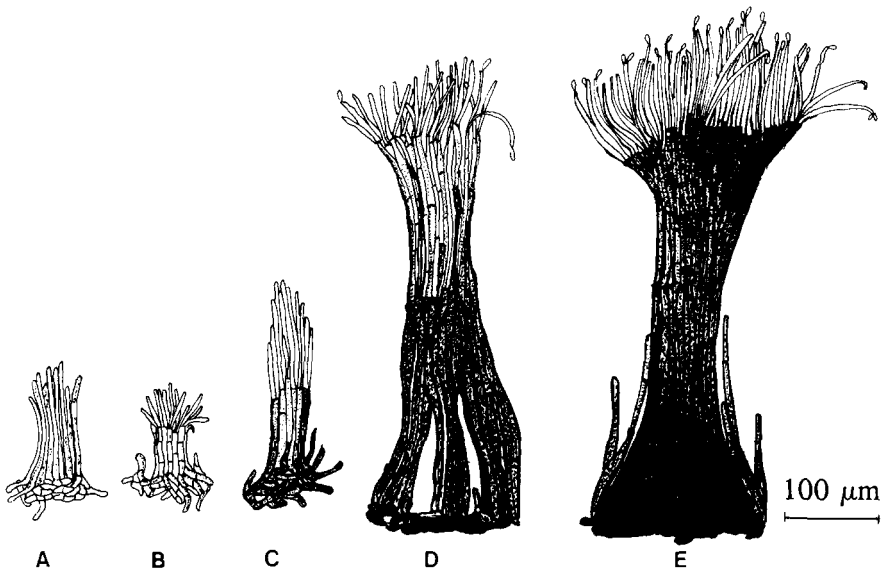

Fig. 1. Development of unbranched synnematal type in culture. (A) Loosely aggregated hyphae arising from intertwining hyphae. (B) Dichotomous branching of fasciculate hyphae. (C) Formation of darkened, enveloping hyphae. (D) Fusing synnemata. (E) Mature, densely compacted synnema.

Micromorphology

Detailed comparison of conidial dimensions and micronematous conidiophores did not reveal any significant discontinuities between strains. The range of variability was covered by the description given by De Hoog (1974). Two types of synnematous conidiophores were found (see below), which produced identical conidia.

Replicate transfer

From the above descriptions it is apparent that cultures often are quite dissimilar, main characters being found in colony appearance and structure of synnemata. Hence initially a necessity to attribute them to various taxa was considered. To verify this, subcultures were made from various parts of sporulating colonies, with the aim to establish whether observed differences were consistent.

When conidial slime was taken with a glass needle from unbranched synnemata, colonies developed which first produced unbranched synnemata

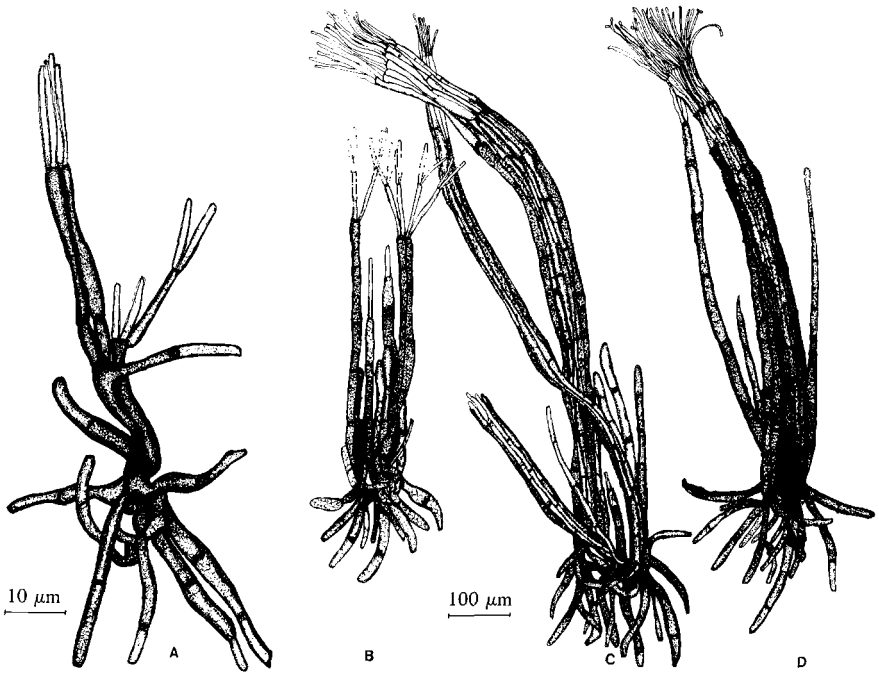

Fig. 2. Development of branched synnematal type. (A) Stiff rhizoids and upward directed hyphae arising from superficial hyphae. (B) Initial stage of radiating synnemata, in part with conidial apparatus. (C, D) Compact, mature synnema with enveloping hyphae.

only, but in a later stage numerous tufts of ramified synnemata were produced from the colony centre towards the marginal parts of the colony. Exactly the same sequence of events was observed when inocula consisting of conidial slime from ramified synnemata were applied.

When small parts of aerial mycelium from synnematal cultures, without synnematal conidia but with an abundance of conidia from the *Sporothrix* synanamorph, were used as inoculum, resultant colonies consisted of an even mat of aerial mycelium, mostly without synnemata. Initially all synnemata, when present, were of the ramified type, but from their conidial slime dripping onto the medium unbranched synnemata were produced. Inoculations using these conidial slimes led to purely synnematal colonies.

When small parts of brown, submerged, undifferentiated hyphae were used as inoculum, resultant colonies consisted of whitish, aerial mycelium and brown, submerged hyphae; synnemata remained absent. In contrast to colonies from aerial hyphae these colonies soon became brown in appearance.

When single spore isolations were made from the mononematous *Sporothrix* synanamorph, cultures grew with a mat of whitish, aerial mycelium, after

5 days mostly still lacking synnemata. After 10 days synnemata were produced in most cultures, except the ones that were without synnemata at the onset of our studies (marked with * in Table 1).

Conclusion

Although the aspects of cultures may be markedly different, it frequently proved to be possible to produce one type using inocula from the other and *vice versa*. In main traits only two types can be distinguished, viz. synnematal cultures *versus* mycelial cultures. From the fact that cultures displaying the two colony types (Group C) display these as sectors, it may be deduced that a degenerative mutation is concerned. Mycelial cultures are found particularly among the strains that have been maintained in artificial culture over a prolonged period (Table 1). All strains should therefore be regarded as representing a single, pleomorphic species, *Ophiostoma piceae*.

Nearly all strains examined lacked perithecia, and we failed to reproduce these using media with oak branchlets under near-UV light. Only two strains, 173B, from necrotic spots of *Quercus* in Poland, and CBS 135.88, from *Quercus petraea,* GDR (Skadow & Traue 1986) produced scattered perithecia, which enabled us to confirm the identity of our taxon with *O. piceae*.

Nomenclatural consequences

Münch (1907) introduced *Ceratostomella piceae* and described it as having *"Cladosporium"* and *"Graphium"* anamorphs. The authentic culture, CBS 108.21, however, now only has a *Sporothrix* conidial state. The synnematous synanamorph could not be reproduced by any of our methods. The strain CBS 108.21 did not deviate significantly from hyphal strains obtained from other synnematous strains.

Possibly the oldest name for the *O. piceae* complex is *Sphaeria dryina* Pers. : Fr. Two authentic specimens at the Leiden Herbarium, L 910.267–769 and –779, comprise some black perithecia on decorticated *Quercus* wood. The ascospores were allantoid, 2.9–4.8 × 1.5–2.0 μm, subhyaline to pale yellowish, indistinguishable from those of *O. piceae*. However, no ostiolar hyphae were found, and hyphae with possible anamorphs were lacking; we therefore regard this taxon as of doubtful identity.

Ceratostomella quercus Georgévitch (as *"querci"*) was described from *Quercus pedunculata* in Yugoslavia. It was described (Georgévitch 1926) as having asci 6–8 × 2–4 μm. Hunt (1956) supposed it to be different from *O. piceae* only by the colour of its wood stain, which would be taxonomically

insufficient. No authentic material was available at the herbaria BEO and SARA; we consider it as of doubtful identity.

The authentic strain of *Ceratostomella fagi* Loos, CBS 236.32 has lost the synnematal anamorph as described by Loos (1932). For this reason, Melin & Nannfeldt (1934) classified the species in a mononematous *pilifera* group of *Ophiostoma*. The species was introduced as differing from *O. piceae* in having shorter ostiolar hyphae and wider ascospores, but the deviations mentioned are too close to the dimensions of 173B to be regarded as significant.

Ceratostomella catoniana Goid., from *Pyrus* in Italy, was synonymized with *O. piceae* by De Hoog (1974) on the basis of an authentic strain, CBS 263.35. As in nearly all older strains, the synnematal synanamorph is now lost. It was originally characterized by relatively short perithecial necks.

No authentic material of *Ophiostoma roboris* Georgescu & Teodoru was available at herb. BUCF or at the Poplar Research Institute at Novi Sad, Yugoslavia. The fungus originated from *Quercus robur* in Romania. The description of the anamorph seems to fall within the range of variability of *O. piceae* as established above. However, synnematal conidia were mentioned to occasionally cohere in short chains, which would suggest a percurrent rather than a sympodial conidiogenesis. The synonymy of *O. piceae* and *O. roboris* therefore remain doubtful. The maximal described lengths of perithecial necks were larger than observed by Münch (1907) for *O. piceae.* Primary conidia were occasionally 3-septate and up to 4.5 μm. Potlajchuk & Schekunova (1985) mentioned *O. roboris* to be common on *Quercus* and *Malus* in the Soviet Union, as opposed to *O. piceae* which would occur on *Picea* and *Pinus*. *Ophiostoma piceae* was supposedly characterized by the presence of a *"Cephalosporium"* anamorph. Potlajchuk (1957) distinguished three basic types within the mononematous anamorph referred to in the present article as *Sporothrix,* viz. a *Rhinotrichum* anamorph, with lateral conidia, a *Hyalodendron* anamorph, with catenulate conidia, and a *Cephalosporium* anamorph, with dense conidial heads. On this basis, species and genera within the *Ophiostoma* complex were distinguished. However, the present studies have shown, that the *Sporothrix* anamorph produces short chains of conidia (*"Hyalodendron"*) in the aerial mycelium, lateral conidia (*"Rhinotrichum"*) mostly on creeping hyphae, and dense, sympodial clusters without secondary conidia (*"Cephalosporium"*) in submersion. The taxonomic value of these features is therefore doubtful.

Of *Ophiostoma valachicum* Georgescu and Teodoru, from *Quercus* in Romania, no material was available at BUCF. The species was erected because of the presence of a *Rhinotrichum* anamorph; for the validity of this character, see under *O. roboris.* In addition, it would have smaller (base 85–220 μm), somewhat lighter, immersed perithecia, with short (360–370 μm) necks. Earlier studies, however, have shown that perithecial dimensions in *Ophiostoma*

species may vary rather considerably (Griffin 1968; Von Arx 1973). the ascospore shape and size fitted those of *O. piceae;* synonymy with that taxon therefore seems likely. *Ophiostoma valachicum* was mentioned by Potlajchuk & Schekunova (1985) as an etiologic agent of oak dieback in the Soviet Union; no material was sent upon request.

Ophiostoma floccosum Mathiesen, from pine and spruce in Sweden, was earlier synonymized with *O. piceae* by De Hoog (1974) on the basis of authentic material (CBS 799.73); this conclusion was confirmed in the present study.

Ophiostoma kubanicum Sherbin-Parfenenko was also described from *Quercus* dieback in the Soviet Union; no authentic material was available for study at LE or LEP. It was characterized by long ostiolar hyphae and milky white ascospore slime. Potlajchuk & Schekunova (1985) described one of the anamorphs as *Verticillium;* this probably refers to an earlier paper by Sherbin-Parfenenko, which was not available for study. Ivanchenko (1957) treated this anamorph as identical to the *"Cephalosporium"* anamorph, a name which has often been used for submerged *Sporothrix* conidial heads; see above.

***Ophiostoma piceae* (Münch) Syd. and P. Syd.**

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?Ophiostoma roboris Georgescu and Teodoru *apud* Georgescu et al. – Anal.

Inst. Cerc. exp. For., Ser. 1, 11: 207. 1948 = *Ceratocystis roboris* (Georgescu and Teodoru) Potlajchuk – Novosti Sist. niz. Rast. 22: 154. 1985.

?Ophiostoma valachicum Georgescu and Teodoru *apud* Georgescu et al. – Anal. Inst. Cerc. exp. For., Ser. 1, 11:198. 1948 = *Ceratocystis valachicum* (Georgescu and Teodoru) Potlajchuk – Novosti Sist. niz. Rast. 22: 155. 1985.

Ophiostoma floccosum Mathiesen – Svenka bot. Tidskr. 45: 219. 1951 = *Ceratocystis floccosa* (Mathiesen) Hunt – Lloydia 19: 36. 1956.

?Ophiostoma kubanicum Sherbin-Parfenenko – Goslesbumizgat p. 49. 1953 = *Ceratocystis kubanicum* (Sherbin-Parfenenko) Potlajchuk & Schekunova – Novosti Sist. niz. Rast. 22: 153. 1985.

Synanamorphose

Graphium pirinum Goid. – Boll. Staz. Patol. veg. Roma, n. Ser. 15: 132. 1935.

?Graphium roboris Georgescu and Teodoru *apud* Georgescu et al. – Anal. Inst. Cer. exp. For., Ser. 1, 11: 213. 1948.

?Graphium kubanicum Potlajchuk and Schekunova – Novosti Sist. niz. Rast. 22: 153. 1985.

Synanamorphose

Hyalodendron pirinum Goid. – Boll. Staz. Patol. veg. Roma, n. Ser. 15: 136. 1935.

Rhinotrichum valachicum Georgescu and Teodoru *apud* Georgescu et al. – Anal. Inst. Cer. exp. For., Ser. 1, 11: 201. 1948.

?Hyalodendron roboris Georgescu and Teodoru *apud* Georgescu et al. – Anal. Inst. Cer. exp. For., Ser. 1, 1: 209. 1948

?Verticillium kubanicum Potlajchuk and Schekunova – Novosti Sist. niz. Rast. 22: 153. 1985.

The description of the teleomorph is based on 173B on OA at 20–22° C. Ascomata scattered, partly immersed; basal part globose, 70–145 μm diam, hyphae up to 85 μm long; neck black at the base, pale brown near the apex, cylindrical, straight or curved, rarely slightly nodose, 380–1000 μm long, 25–35 μm wide at the base and 7–12 μm at the apex; necks bearing 6–20 radiating, ostiolar hyphae which are hyaline, cylindrical, up to 62 μm long, with 0–4 thin

septa and rounded tips. Ascospores hyaline, 1-celled, allantoid, 2–6 × 1–2 μm, without sheath, collecting in subhyaline to pale yellowish mucous.

The description of the anamorphs is based on CBS 466.86 after 10 d on OA at 20–22° C. Ranges of variability are given between (), data of further strains between [].

Colonies attaining a diam of 15–20 [–35] mm; aerial mycelium at first scant, farinose, soon becoming floccose, mostly whitish grey, locally pale to dark brown, forming vague concentric zones, particularly near the margin. Reverse with same but more intense pigmentation. Synnemata initially arising in a broad band near the colony margin, later produced all over the colony. Submerged hyphae mostly pale brown, straight or flexuose, smooth- or occasionally rough-walled, and thick-walled, 1.5–4.0 μm wide. Chlamydospores thick-walled, (sub)globose, 4–8 × 4–6 μm [absent in many strains]. Aerial hyphae 1.0–2.5 μm wide, loose or fasciculate. Conidiogenous cells of *Sporothrix* synanamorph arising terminally or laterally, orthotropically from undifferentiated hyphae, [10–]20–55[–70] μm, mostly slightly tapering towards the tip. Conidiogenesis sympodial from a swollen or unswollen, apical cluster of denticles, heads measuring up to 6[–12] μm in length; clusters later often becoming irregular due to conidium production lower down in a later stage. Denticles (sub)cylindrical, 0.5–1.5[–2] μm long. Conidia arising in short, often branched chains, hyaline, thin- and smooth-walled; primary conidia mostly clavate, 0–1(–2)-septate, [7–]10–25[–34] μm long and 2.0–3.5 μm wide. Secondary conidia 1-celled, ellipsoidal, 3.0–10.5 × 1–3 μm. Lateral blastoconidia [absent in many strains] hyaline, thin-walled, (sub)globose to ovoidal, 2–4 × 1–3 μm. Synnemata of two types:

1. Unbranched, single or fusing, arising from a clew of dark brown, interwoven hyphae, [150–]450–600[–700] μm long, stipe consisting of densely compacted, thick-walled, septate, fertile hyphae, dark brown at the base, paler near the apex, surrounded by thick-walled, dark brown, enveloping, sterile hyphae. Fertile hyphae branched repeatedly at the apex, ultimate series of branches being a palissade of conidiogenous cells which splays out during maturation. Conidiogenous cells slender, with a short, sympodial rachis with few, inconspicuous scars. Conidia produced in mucous masses, subhyaline, oblong, 2.5–5.5 × 2.0–2.5 μm.
2. Profusely branched at the basis and becoming shrub-shaped, individual stipes as in type 1 but 600–800[–1000] μm long.

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