

Life-history of Ascomycetes: Two new species of *Chaetosphaeria* with *Chloridium* and *Chloridium-Dictyochaeta* anamorphs

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Two new species of *Chaetosphaeria*, *Chaet. dilabens* and *Chaet. hebetiseta*, with *Chloridium* and *Chloridium-Dictyochaeta* anamorphs, are described and illustrated on material from Japan and Ukraine, respectively. The life-histories of both species were confirmed by cultural studies.

Key Words—Ascomycota; Chaetosphaeriaceae; conidiogenesis; life-history; systematics.

The genus *Chaetosphaeria* Tul. & C. Tul. encompasses saprobic taxa commonly encountered in terrestrial habitats on decaying wood and bark of angiosperms and gymnosperms. The generic concept is delimited to species with nonstromatic, dark perithecia, persistent paraphyses, unitunicate asci with a nonamyloid (J-), refractive apical annulus and hyaline, transversely 1- to multiseptate ascospores. Ascospores are nonfragmenting or rarely fragmenting into part-spores. Anamorphs belong to several phialidic genera of dematiaceous hyphomycetes (Réblová, 2000; Réblová and Winka, 2000).

A species of *Chaetosphaeria* and its *Chloridium* Link: Fr. anamorph were collected by W. Gams several times on old bamboo stems in rather progressed stage of decay in Japan; the *Chloridium* anamorph was also isolated by L. Pfenning from soil below *Theobroma cacao* in Brazil (Pfenning, 1993). The ascospores are hyaline, 3-septate and fragmenting at an early stage within the ascus. The phialides have darker campanulate collarettes and produce pale brown conidia on a single conidiogenous locus. This species does not match any of the six *Chaetosphaeria* species documented from bamboo by Sawada (1942), Dôke (1947), Hino and Katumoto (1961), Eriksson and Yue (1988, 1998), Petrini et al. (1989) and Candoussau et al. (1996). The tendency of ascospores to fall apart was so far known only in a few *Chaetosphaeria* species with 2-celled ascospores having *Chloridium* and *Gonytrichum* C. G. & F. Nees anamorphs, viz. *Chaet. inaequalis* (Grove) W. Gams & Hol.-Jech., *Chaet. chloroconia* W. Gams & Hol.-Jech., *Chaet. preussii* W. Gams & Hol.-Jech. and *Chaet. vermicularioides* (Sacc. & Roum.) W. Gams & Hol.-Jech.

The second undescribed species of *Chaetosphaeria* has an anamorph intermediate between *Chloridium* and *Dictyochaeta* Speg. It was collected by M. Réblová on

decaying wood of *Fagus sylvatica* in Ukraine. Its perithecia are covered with conspicuous setae, which are dark brown near the base, paler upwards with a subhyaline to hyaline, broadly rounded end-cell; the ascospores are hyaline, 3-septate and nonfragmenting; the conidiophores have terminal and lateral phialidic openings and conidia are hyaline and ellipsoidal, formed on a single conidiogenous locus within the collarette.

The two as yet undescribed species of *Chaetosphaeria* are described and illustrated here as *Chaetosphaeria dilabens* and *Chaet. hebetiseta*, respectively. Their life-histories were confirmed by cultural studies.

The position of *Chaet. dilabens* and *Chaet. hebetiseta* was analysed by Réblová and Winka (2000) using molecular tools and both were referred to the Gongromeriza-group by Réblová (2000). This group is one of four groups distinguished in *Chaetosphaeria* on morphological and molecular grounds, particularly by the structure of the phialide and conidium and ascospore morphology. The Gongromeriza-group accommodates species of *Chloridium* formerly classified in sections *Gongromeriza* (Preuss) W. Gams & Hol.-Jech. and *Psilobotrys* (Sacc.) W. Gams & Hol.-Jech. (Gams and Holubová-Jechová, 1976), and some species of *Dictyochaeta* sensu stricto with nonsetulose conidia. It is confined to species with a single conidiogenous locus within the collarette, with either percurrently or sympodially proliferating conidiophores, and hyaline or light-pigmented, nonseptate conidia without setulae. The teleomorphs belong exclusively to *Chaetosphaeria* having either fragmenting or nonfragmenting ascospores.

Fragmenting ascospores are characteristic of several perithecial nonstromatic ascomycete genera including *Ascochalara* Réblová (Réblová, 1999), *Chaetosphaeria* (as *Melanopsammella* Höhn., Höhnel, 1919; Réblová et al., 1999), *Neorehmia* Höhn. and *Trichosphaerella* Bomm

et al. (Samuels and Barr, 1997, Rossman et al., 1999). The inclusion in *Chaetosphaeria* of *Melanopsammella inaequalis* (Grove) Höhn., the type of the genus, and several other species with 1-septate, fragmenting ascospores having *Gonytrichum* and *Chloridium* anamorphs, by Gams and Holubová-Jechová (1976) has caused some expansion of the generic concept. Retention of *Melanopsammella* on this feature was suggested by Réblová et al. (1999) but found to be less appropriate by Réblová and Winka (2000).

Materials and Methods

Dry herbarium specimens were rehydrated in 3% (aq.) KOH and subsequently studied in water, Melzer's reagent and cotton blue in lactic acid.

Mass- and single-ascospore isolates of *Chaet. dilabens* and *Chaet. hebetisetata*, respectively, were obtained. Colonies were grown on cornmeal agar (CMA, Difco), malt extract agar (2% MEA, Difco), oatmeal agar (OA) and potato-carrot agar (PCA), colony characters were taken from cultures grown for 10 d in darkness and 10 d in cool white fluorescent light at 24°C. The cultures are maintained at the Institute of Botany, Academy of Sciences in Průhonice, Czech Republic and the Centraalbureau voor Schimmelcultures (CBS) in Baarn, the Netherlands.

Results and Discussion

Chaetosphaeria dilabens Réblová & W. Gams, sp. nov.

Figs. 1–3

Anamorph: *Chloridium* sp. (described here).

Perithecia superficialia, stromati basilari tenui insidentia, globosa vel subglobosa, 160–240 µm alta, 130–190 µm diam, fusca ad atra, glabra. Peritheciolorum paries fragilis, 12–14 µm crassus, strato amorpho hyalino tenui, ca. 2–2.5 µm crasso obtectus. Paraphyses parce ramosae, anastomosantes. Asci cylindrici vel clavati, 58–74 × 5–6.5 µm. Ascosporae fusiformes, 10–11.5(–12) × 2–2.5 µm, 3-septatae, cellulis (2–)2.5–3 × 2–2.5 µm longis cito in ascis dilabentibus, leves.

Conidiophora macronematosa, mononematosa, fusca ad basim, sursum pallidiora, ad 180(–200) µm longa, 3.5–4.5 µm lata in parte media. Collare conspicue fuscus, campanulatum, 4–4.5(–5) µm longum. Conidia in capitulis mucidis adhaerentia, ellipsoidea vel modice asymmetrica, 3–4.5 × 1.7–2.5 µm, non-septata, dilute brunnea, levia. Chlamydo sporae absentes.

Teleomorph: Perithecia superficial, sitting on a thin basal stroma, gregarious to solitary, globose to subglobose, 160–240 µm high and 130–190 µm diam, dark brown to black, papillate, ostiolate, glabrous. Ostiolar canal periphysate. Perithecial wall brittle, carbonaceous, 12–14 µm thick, consisting of two regions; outer region of thin-walled, opaque, brown, brick-like cells with some tendency to form a textura epidermoidea, with a thin, ca. 2–2.5 µm thick layer of an amorphous hyaline matrix on the surface; inner region of thin-walled, hyaline, compressed cells. Paraphyses sparsely branching,

anastomosing, 2–3.5 µm wide near the base, tapering to 2–2.5 µm, protruding beyond the asci. Asci unitunicate, cylindrical to clavate, 58–74 × 5–6.5 µm, slightly truncate at the top, short-stipitate, with a nonamyloid (J-), refractive, 2–2.5 µm diam and 1 µm deep apical annulus, 8-spored. Ascospores fusiform, 10–11.5(–12) × 2–2.5 µm, 3-septate, the cells separating at an early stage within the ascus, part-spores (2–)2.5–3 × 2–2.5 µm, when young with thick, refringent septa, smooth, hyaline, 2-seriate in the ascus.

Anamorph in nature (Fig. 2): Setae absent. Conidiophores macronematous, mononematous, solitary, erect, straight or curved, cylindrical, unbranched, dark brown near the base, paler upwards, up to 180(–200) µm long, 3.5–4.5 µm wide in the middle and 4.5–6 µm wide above the base, 7–10(–16)-septate, proliferating percurrently, smooth-walled. Phialides terminal, integrated, monoblastic, subhyaline, 20–30(–33) µm long and 4.5–5 µm wide, gradually tapering in the upper half to 1.5–2 µm. Collarettes conspicuously darker, flaring, campanulate, 4–4.5(–5) µm deep and 3–4.5 µm wide. Conidia adhering in slimy heads, formed on a single conidiogenous locus within the collarette, ellipsoidal to somewhat asymmetrical, 3–4.5 × 1.7–2.5 µm, nonseptate, nonsetulose, pale brown, smooth-walled. Chlamydo sporae absent.

Characteristics in culture (Fig. 3): Colonies on PCA slow-growing, reaching 15–17 mm diam in 10 d at 24°C in darkness; when grown for another 10 d at 24°C in cool white fluorescent light, reaching 25–28 mm diam; aerial mycelium very thin, pale grey, above a black layer of conidiophores and conidia arising from the agar surface, margin 1–2 mm wide, pale grey; conidia abundantly produced throughout the colony, conidial heads appearing black; reverse dark grey to black. On CMA, OA and MEA 15–17 mm diam after 10 d and 28–35 mm after another 10 d; on CMA and OA slightly more aerial mycelium than on PCA, on MEA even more abundant and felty; conidial sporulation abundant throughout the colony except on MEA. Mycelium superficial or immersed; hyphae branched, septate, pale to mid brown, smooth-walled to finely verruculose, 1.5–2.5 µm wide. Setae absent. Conidiophores as on the natural substratum, occasionally sympodially proliferating, 35–72 µm long and (2.5–)3–3.5 µm wide in the middle, 3–6-septate. Phialides as on the natural substratum, 9.5–19 µm long and 3–3.5(–4) µm wide. Conidia 3–4 × 2–2.5 µm. Chlamydo sporae absent.

Etymology: Latin *dilabor* = to fall apart, referring to the characteristics of the ascospores that fragment into part-spores.

Habitat: Saprobic on decaying bamboo culms.

Known hosts: bamboo, *Bambusa* sp.

Known distribution: Asia: Japan; South America: Brazil.

Holotype: Japan, Kyoto, Daitokuji Temple, on *Bambusa* sp., 28 Aug. 1983, M. Tsuda, W. Gams (CBS-H 3524), culture CBS 735.83.

Additional specimens and isolates examined: Japan, Kyoto, Arashiyama, on dead bamboo, Aug. 1988, W. Gams (CBS-H 4389), culture CBS 712.88. Brazil, Pará,

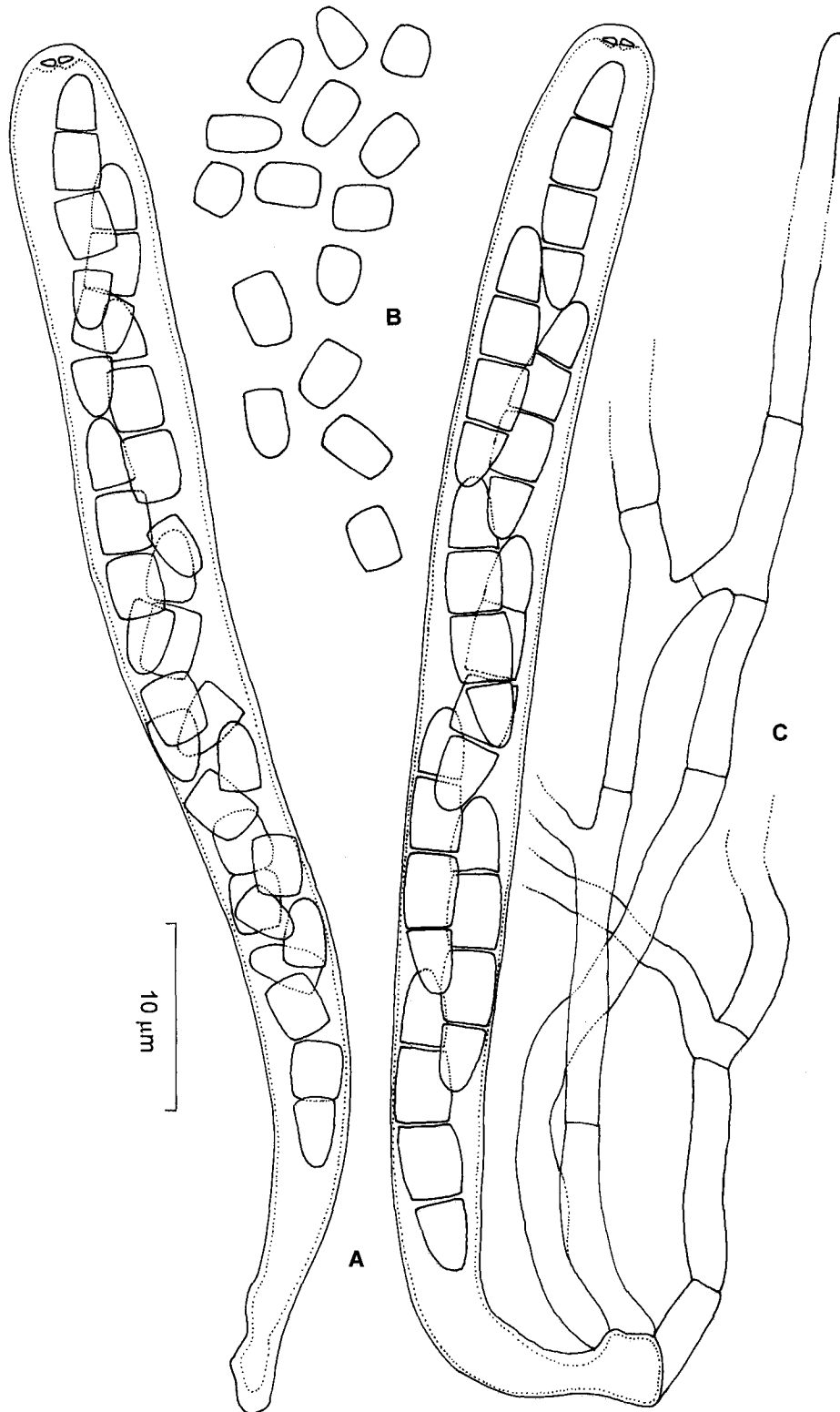


Fig. 1. *Chaetosphaeria dilabens* (CBS-H 3524).
A. Asci containing ascospores. B. Part-spores. C. Paraphyses.

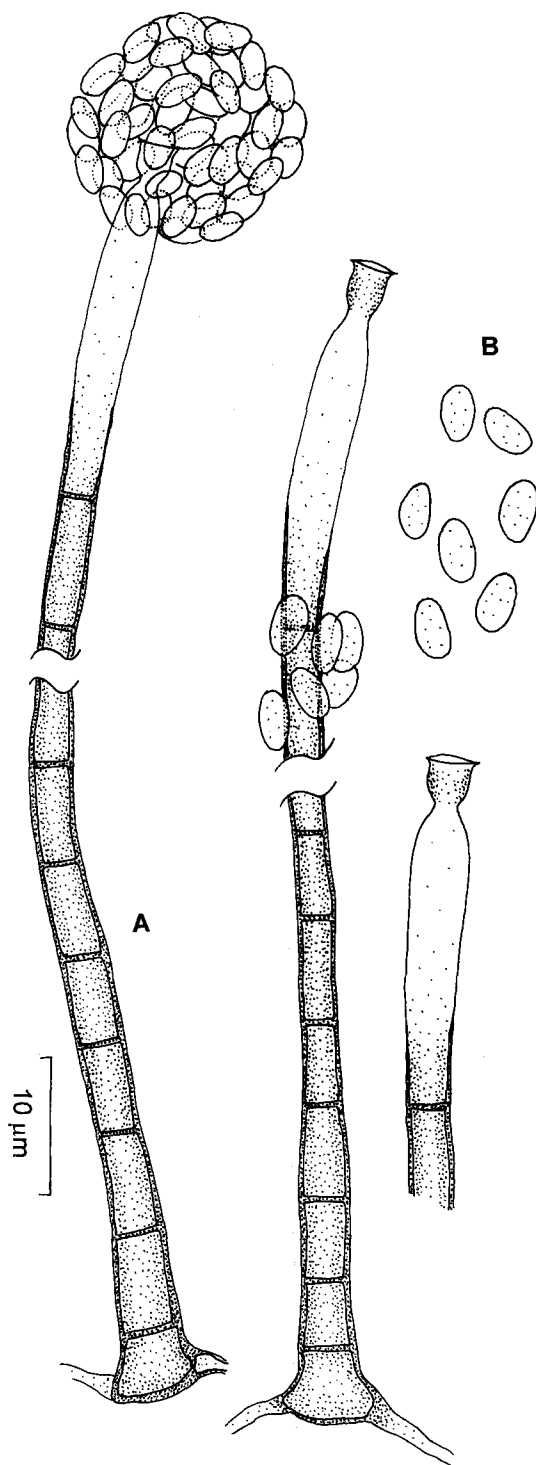


Fig. 2. *Chaetosphaeria dilabens* (CBS-H 3524).
A. Conidiophore with conidia adhering in a slimy head. B. Conidia. On the natural substratum.

200 km south-east of Belém, ex washed root of *Theobroma cacao*, L. Pfenning, culture CBS 551.89.

Note: *Chaetosphaeria dilabens* is characterized by 3-septate, fragmenting ascospores and a *Chloridium* anamorph having a conspicuously darker, campanulate

collar and ellipsoidal, pale brown conidia formed on a single conidiogenous locus within the collar. The hitherto described *Chaetosphaeria* with fragmenting ascospores, including *Chaet. chloroconia*, *Chaet. inaequalis*, *Chaet. vermicularioides* and *Chaet. preussii*, have 1-septate and short-fusiform ascospores, and the associated anamorphs belong to the related genera *Chloridium* and *Gonytrichum*. The three former species of *Chaetosphaeria* having *Chloridium* and *Gonytrichum* anamorphs with conidia formed on multiple conidiogenous loci within the collar belong to the *Chloridium*-group (Réblová, 2000; Réblová and Winka, 2000). They would represent the genus *Melanopsammella* sensu stricto, if generic separation were required (Réblová et al., 1999; Réblová and Winka, 2000). The remaining *Chaetosphaeria* species with fragmenting ascospores, viz. *Chaet. dilabens* and *Chaet. preussii*, together with others having nonfragmenting ascospores and *Chloridium* anamorphs with conidia formed on a single conidiogenous locus are placed in the *Gongromeriza*-group. The interrelationships among the individual groups distinguished in *Chaetosphaeria* are discussed in detail in Réblová and Winka (2000) and Réblová (2000).

The presence of pale brown conidia is not unusual among species of *Chloridium*. Brown conidia occur in *Chloridium carpathicum* Hol.-Jech & Révay (as '*carpathicum*', Holubová-Jechová and Révay, 1987), *Chl. phaeosporum* W. Gams & Hol.-Jech., with var. *phaeosporum* (Gams and Holubová-Jechová, 1976) and var. *cubense* Hol.-Jech. (Holubová-Jechová, 1987), and *Chaetosphaeria fennica* (P. Karsten) Réblová & W. Gams (Réblová and Gams, 1999). *Chloridium phaeosporum* var. *phaeosporum*, though having similarly percurrently proliferating conidiophores and ellipsoidal conidia formed on a single conidiogenous locus, differs in having basally truncate conidia and a conspicuously darker basal hilum. The var. *cubense* has ellipsoidal conidia with rounded ends and a conspicuous darker basal hilum. Both varieties have longer conidia than those of *Chaet. dilabens*. A weak tendency to sympodial proliferation of conidiophores was only observed in the type variety (Gams and Holubová-Jechová, 1976), while in var. *cubense* sympodially proliferating conidiophores were not observed by Holubová-Jechová (1987). However, *Phialophora taiwanensis* Matsush. (Matsushima, 1983), regarded as a synonym of *Chl. phaeosporum* var. *cubense* by Matsushima (1993), possesses conspicuously sympodially proliferating conidiophores. This species is probably a synonym of *Phialophora geniculata* van Emden (van Emden, 1975); its pigmentation suggests affinity with the *Pseudogliomastix* anamorph of *Wallrothiella subiculosae* Höhn. (Gams and Boekhout, 1985); the species was commonly isolated from alkaline soils (Nagai et al., 1995).

Chloridium carpathicum and *Chaet. fennica* possess similar pale brown, ellipsoidal conidia but differ significantly from *Chaet. dilabens* in the structure of the phialides that produce conidia successively on multiple conidiogenous loci within the collar. In both species the apical part of the phialide proliferates conspicuously

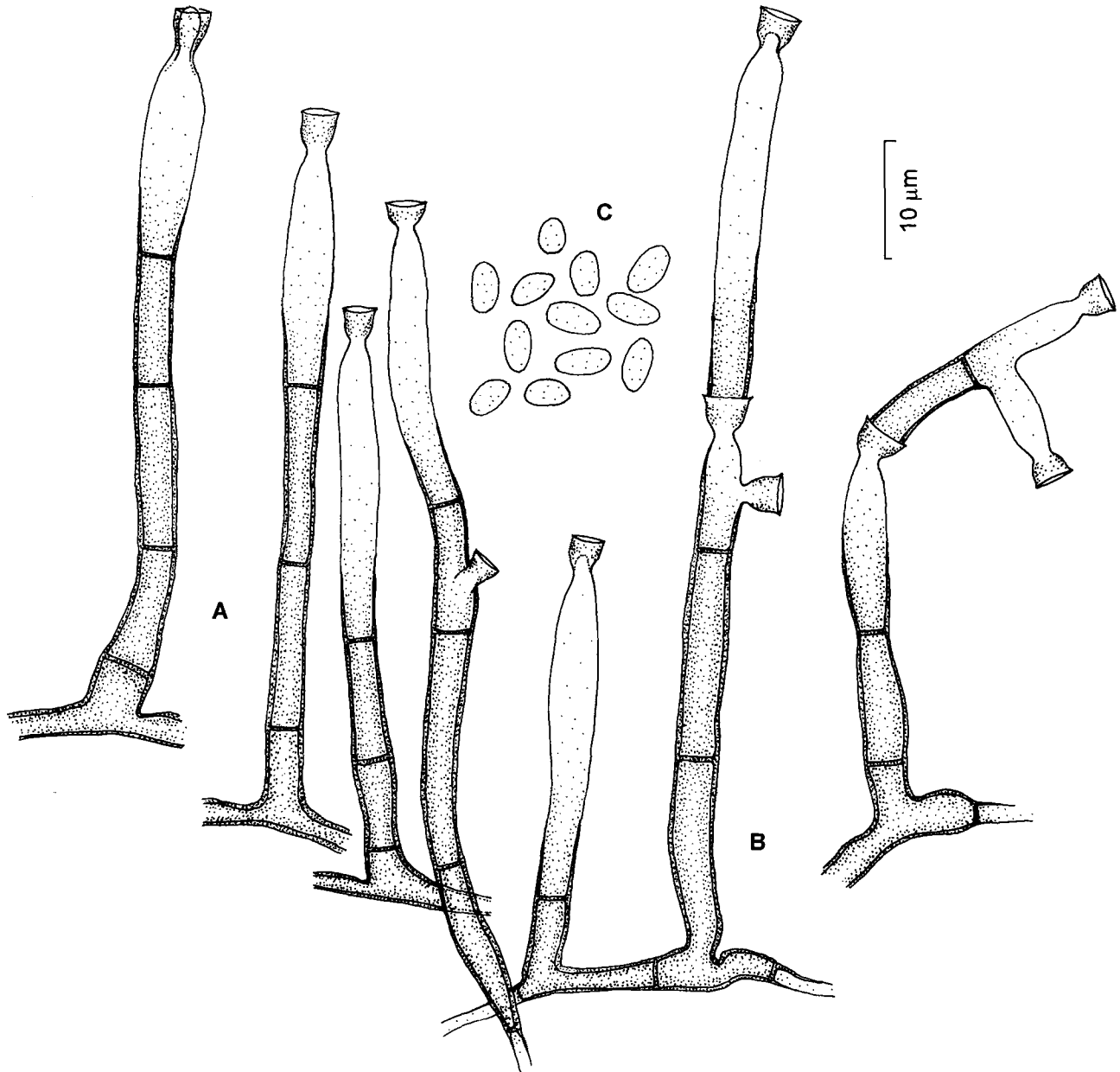


Fig. 3. *Chaetosphaeria dilabens* (CBS 735.83).

A. Conidiophores (OA, 27 d old). B. Conidiophores (PCA, 7 mo old). C. Conidia (OA, 27 d old). In culture.

beyond the primary collarette. Despite the similarity in conidial shape and colour, *Chl. carpathicum*, *Chaet. fennica* and *Chaet. dilabens* differ sufficiently in the structure of the phialide to warrant their specific distinction.

Chaetosphaeria hebetiseta Réblová & W. Gams, sp. nov.

Figs. 4–6

Anamorph: *Chloridium-Dictyochoeta* sp. (described here).

Perithecia superficialia, globosa vel subglobosa, 275–310 μm alta, 270–290 μm diam, fusca, dense setosa. Setae ad 140 μm longae, 2.5–4.5 μm latae in parte media, ad basim fuscae, sursum pallidiores. Perithecio-

rum paries fragilis, 18–22 μm crassus. Paraphyses ramosae, anastomosantes. Asci cylindrici vel clavati, 96.5–123(–126) \times 10.5–11.5 μm . Ascosporae fusiformes, (15–)17–21(–22.5) \times 4–5(–7) μm , 3-septatae, verruculosae.

Conidiophora macronematosa, mononematosa, fusca ad basim, sursum pallidiora, ramos nonnullos simplices ferentia, plerumque ostiola phialidica singula, raro bina in quoque ramo formantia, ad 80 μm longa, 3–4.5 μm lata in parte media. Collare hyalinum, angustum, 2–3 μm latum. Conidia in cultura capitulis mucidis adhaerentia, ellipsoidea vel modice asymmetrica, 4–4.5(–5) \times 2–2.5 μm , non-septata, hyalina, levia. Chlamydo-

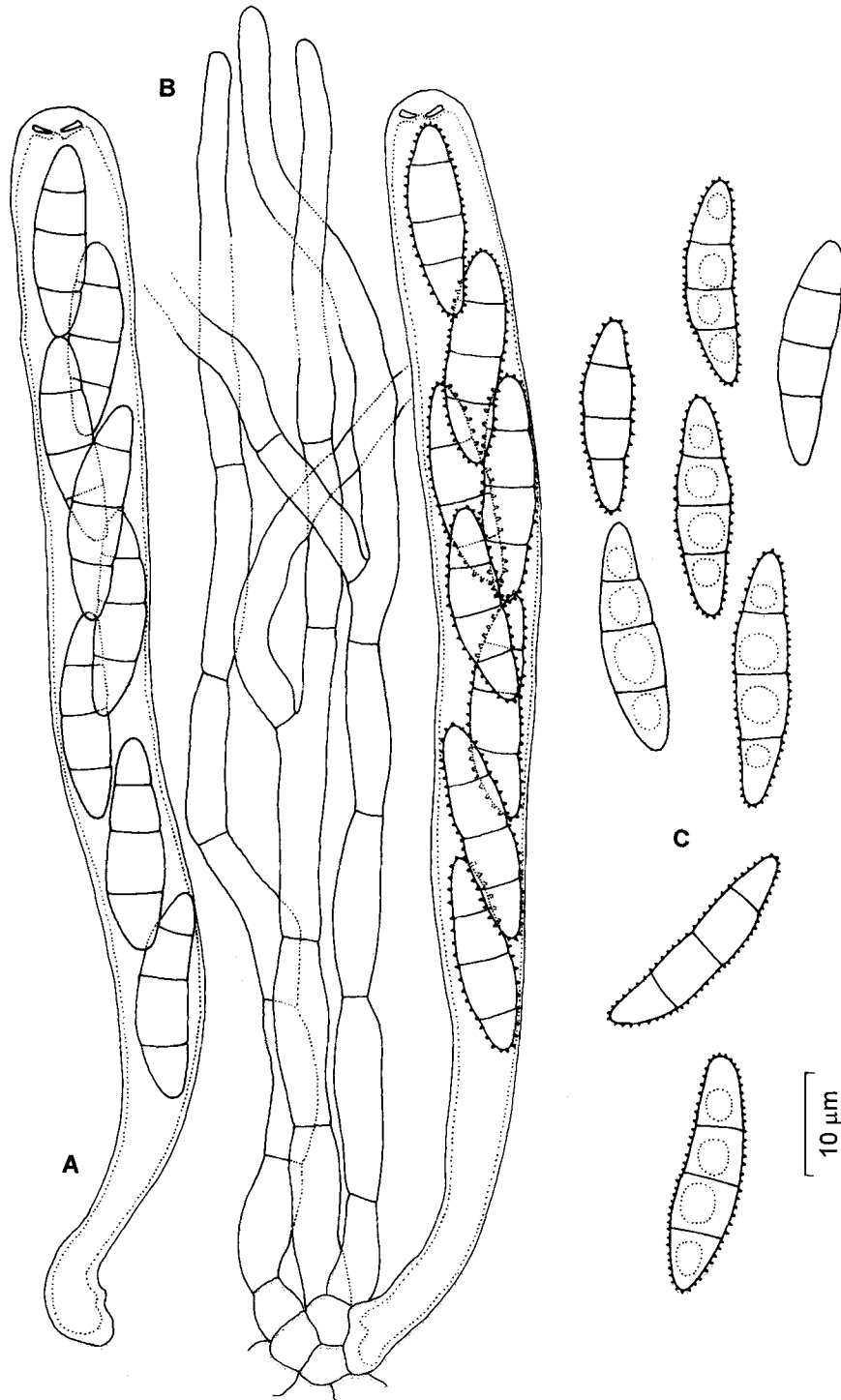


Fig. 4. *Chaetosphaeria hebetiseta* (CBS-H 6566).
A. Asci containing ascospores. B. Paraphyses. C. Ascospores.

sporaes absentes.

Teleomorph: Perithecia superficial, densely gregarious, globose to subglobose, 275–310 μm high and 270–290 μm diam, dark brown, papillate, ostiolate, densely setose. Setae erect, unbranched, straight or gently curved, arising singly from the perithecial wall or the sur-

face of the natural substratum, up to 140 μm long and 2.5–4.5 μm wide in the middle, up to 6-septate, thick-walled, dark brown near the base, paler upwards; end-cell 14.5–27 μm long and 4–6.5 μm wide, thin-walled, subhyaline to hyaline, cylindrical to clavate, broadly rounded at the top. Ostiolar canal periphysate. Perithe-

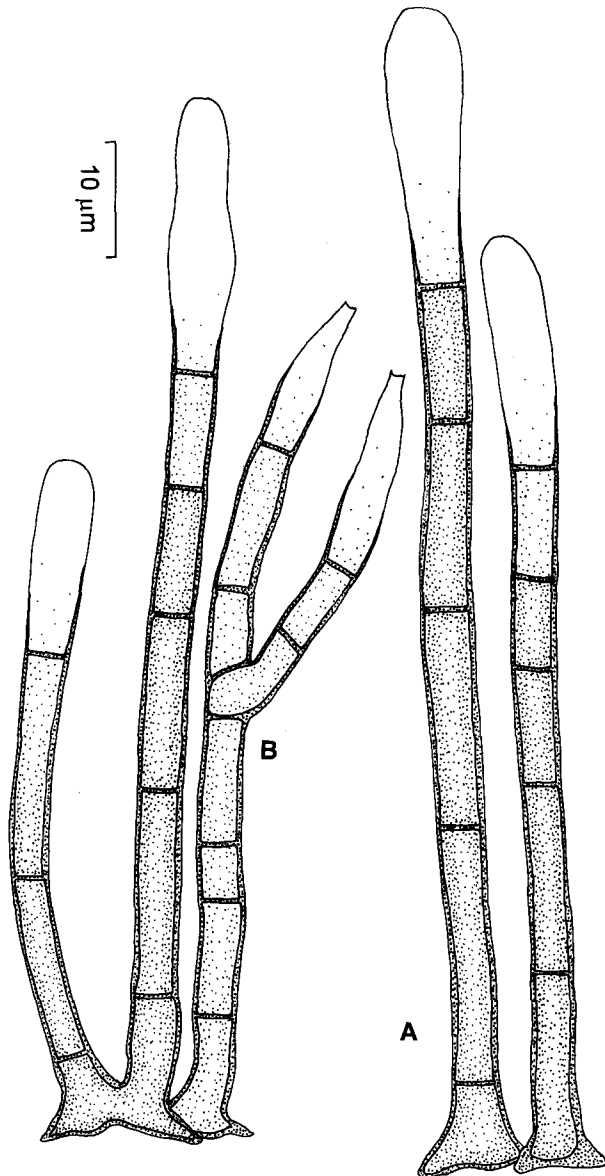


Fig. 5. *Chaetosphaeria hebetiseta* (CBS-H 6566).
A. Setae. B. Conidiophore and setae. On the natural substratum.

cial wall brittle, carbonaceous, 18–22 μm thick, consisting of two regions; outer region of thin-walled, opaque, dark brown, brick-like cells; inner region of thin-walled, subhyaline to hyaline, compressed cells. Paraphyses copious, branching, anastomosing, hyaline, septate, 3–4 μm wide near the base, tapering to 2 μm , protruding beyond the asci. Asci unitunicate, cylindrical to clavate, 96.5–123(–126) \times 10.5–11.5 μm , rounded at the top, short-stipitate, with a nonamyloid (J-), refractive, 2.5–3 μm diam and 1 μm deep apical annulus, 8-spored. Ascospores fusiform, (15–)17–21(–22.5) \times 4–5(–7) μm , one of the middle cells sometimes slightly inflated, 3-septate, not constricted or slightly constricted, finely verrucose, hyaline, 2-seriate in the ascus.

Anamorph in nature (Fig. 5): Setae also arising from vegetative hyphae, identical to those covering the perithecia, never observed to end in a monophialide or polyphialide. Conidiophores sparse among the setae, macronematous, mononematous, solitary, erect, straight or gently curved, cylindrical, sparsely simply branched, mid-brown near the base, paler upwards, up to 80 μm long and 3–4.5 μm wide in the middle, septate, usually with 1(–2) phialidic openings per branch. Collarettes hyaline, narrow, 2–3 μm wide. Conidia not observed.

Characteristics in culture (Fig. 6): Colonies on PCA slow-growing, reaching 10 mm diam in 10 d at 24°C in darkness; when grown for another 10 d at 24°C in cool white fluorescent light, reaching 16 mm diam; felty, whitish to ivory, dark grey in the middle due to crowded conidiophores, aerial mycelium scarce, margin fimbriate; conidia produced centrally, scarce, conidial masses whitish; reverse whitish. On CMA, OA and MEA reaching 12–14 mm diam; on CMA, OA felty, whitish, aerial mycelium scarce, conidia produced centrally, copious; on MEA having a moist appearance, whitish, aerial mycelium scarce, no sporulation. Mycelium superficial or immersed; hyphae branched, septate, subhyaline, smooth-walled, 1.5–2.5 μm wide. Setae absent. Conidiophores macronematous, mononematous, solitary, erect, straight or gently curved, cylindrical, simply branched or unbranched, mid brown to pale brown near the base, paler upwards, (57–)88–240 μm long and 4–5 μm wide in the middle, tapering to 1–1.5 μm immediately below the apical collarette, 4–8-septate, smooth-walled. Phialides terminal, integrated or intercalary, monoblastic, with 3–10 lateral phialidic openings arising from sympodial proliferation. Collarettes hyaline, narrow, 2–3 μm wide and 1.5–2.5 μm deep. Conidia adhering in slimy heads, formed on a single conidiogenous locus, ellipsoidal to somewhat asymmetrical, rounded at the tip, slightly tapering towards the base, 4–4.5(–5) \times 2–2.5 μm , nonseptate, nonsetulose, hyaline, smooth-walled. Chlamydospores absent.

Etymology: Latin *hebes*=obtuse, referring to the obtuse setae.

Habitat: Saprobic on decaying wood of a deciduous tree.

Known host: *Fagus sylvatica* L.

Known distribution: Europe: Ukraine, known only from the type locality.

Holotype: Ukraine, Eastern Carpathian Mts., Kvasi near Rachiv, on the right bank of the Tisa river, on decayed wood of *Fagus sylvatica*, 29 June 1997, M. Réblová (CBS-H 6566); culture M. Réblová 938–97, CBS 102340.

Note: *Chaetosphaeria hebetiseta* is characterized by conspicuous setae that are dark brown near the base with a subhyaline to hyaline, gently swollen and bluntly rounded end-cell, 3-septate, nonfragmenting, hyaline ascospores and a *Chloridium-Dictyochaeta* anamorph. Setae identical to those covering the perithecia, were observed in association with conidiophores on the natural substratum but were lacking in pure culture.

Chaetosphaeria hebetiseta resembles *Chaet. calli-*

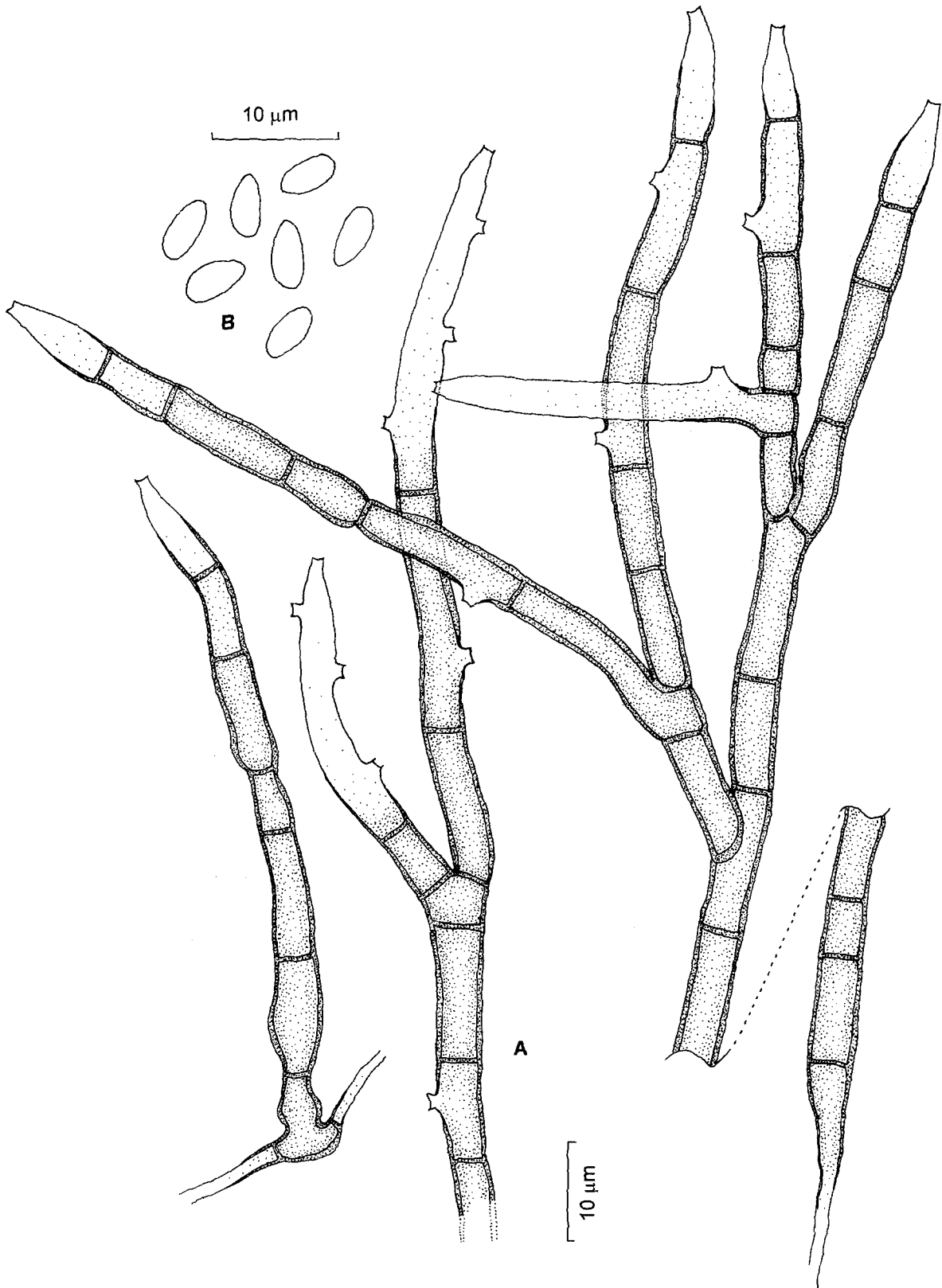


Fig. 6. *Chaetosphaeria hebetiseta* (CBS 102340).
A. Conidiophores. B. Conidia. In culture (OA, 40 d old).

morpha (Mont.) Sacc. in many respects. The close phylogenetic relationship between these two species was confirmed by molecular studies (Réblová and Winka, 2000). *Chaetosphaeria callimorpha* possesses similar but much longer (up to 280 μm , Hughes and Kendrick, 1968) and more-septate (up to 16-septate) setae growing among the conidiophores and asymmetrical, narrowly clavate, larger conidia (10–14.5 \times 2–2.9 μm , Hughes and Kendrick, 1968; 10–16 \times 2–2.5 μm , Booth, 1957). The asci and ascospores of *Chaet. callimorpha* (60–80 \times 6–8 μm and 11–16 \times 3–4 μm , Hughes and Kendrick, 1968; 65–80 \times 7–8.5 μm and 12–15 \times 3.5–4 μm , Booth, 1957) are smaller, the ascospores are generally 1-septate for a long time with a delayed formation of two additional septa.

Chaetosphaeria hebetiseta resembles *Chloridium smithiae* Sinclair & Eicker (Sinclair and Eicker, 1985) in having conidiophores with numerous lateral phialidic openings, but the latter has flaring collarettes, smaller, globose conidia and lacks setae.

Chaetosphaeria montana Réblová (Réblová, 1998) and several *Dictyochoaeta* species, including *D. fuegiana* Speg. (Gamundí et al., 1977; Holubová-Jechová, 1984), *D. querna* P. M. Kirk (Kirk, 1982) and *D. tumidiseta* Kuthubutheen & Nawawi (as '*tumidoseta*', Kuthubutheen and Nawawi, 1991), possess similar nonseptate, hyaline conidia and setae, which are dark brown near the base and paler upwards, with subhyaline to hyaline, sometimes swollen end-cells. The relationship and identity of *D. fuegiana* and *D. querna* with *Chaet. callimorpha* have been addressed several times (e.g., Holubová-Jechová, 1984; Kuthubutheen and Nawawi, 1991). These species possess larger and narrowly clavate conidia that clearly separate them from *Chaet. hebetiseta*. *Dictyochoaeta tumidiseta* differs from the remaining *Dictyochoaeta* species and *Chaet. hebetiseta* in having monophialides, falcate conidia and setae with conspicuously inflated and much wider end-cells than found in the other species mentioned. *Chaetosphaeria montana* produces typically falcate conidia with short subulate setulae at either end and is most closely related to *Chaet. pulchriseta* S. Hughes et al. (Hughes and Kendrick, 1968).

Chaetosphaeria innumera Berk. & Broome ex Tul. & C. Tul., to which *Chaet. hebetiseta* also should be compared, differs clearly in its *Chloridium botryoideum* (Corda) S. Hughes anamorph, the absence of setae and smaller 3-septate ascospores [(10–)12–16 \times 3.5–4(–4.5) μm] and smaller asci (65–90 \times 7–9 μm) (Gams and Holubová-Jechová, 1976).

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