

## **Teleomorph-anamorph connections in Ascomycetes 2. *Ascochalara gabretae* gen. et sp. nov. and its *Chalara*- like anamorph**

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*Ascochalara gabretae*, the type of the new monospecific genus, is described and illustrated. The associated anamorph is close to the dematiaceous hyphomycete genus *Chalara*. *Ascochalara gabretae* is a saprobe on bare decayed wood of *Abies alba* found in the Czech Republic. On the basis of anatomy of the perithecial wall, asci, paraphyses and ascospores and the *Chalara*-like anamorph, *Ascochalara* is placed in the Chaetosphaeriaceae. The relationships of *Ascochalara* with related taxa of the Chaetosphaeriaceae and Lasiosphaeriaceae are discussed.

Keywords: Chaetosphaeriaceae, Lasiosphaeriaceae, wood-inhabiting fungi, systematics.

A lignicolous, non-stromatic, perithecial ascomycete was collected on bare decayed wood underneath the bark of *Abies alba* in the Czech Republic. The fungus is characterized by the perithecial surface that is covered by a greyish to whitish powder and develops on the outside of the fragile median layer of dark brown, opaque, brick-like cells. Asci have a pronounced, J- apical annulus; paraphyses persist among the asci. Ascospores are long-fusiform to cylindrical, at maturity separating into part-spores within the ascus. The associated anamorph is close to the dematiaceous hyphomycete genus *Chalara* (Corda) Rabenh. On the basis of these features and the *Chalara*-like anamorph, the fungus does not match the concept of any of the hitherto known genera of perithecial ascomycetes. The combination of the characters is unique and suggests the affinity with the Chaetosphaeriaceae Réblová & al. (1999).

The Chaetosphaeriaceae was described for a homogeneous unit of seven closely related genera of perithecial ascomycetes. The perithecial wall is usually two-layered, the outer wall is fragile, of thin-walled, dark brown, opaque, brick-like cells or of cells that form a network (*textura epidermoidea*). In some genera a light-coloured surface layer of different origin is formed on the outside of the dark median layer. Paraphyses are persistent. Asci lack a subapical glo-

bulus and the apex has a pronounced, J- refractive apical annulus. Ascospores are transversely septate, non-fragmenting or fragmenting into part-spores, hyaline, bicolorous or darkly pigmented. Anamorphs with phialidic conidiogenesis belong to diverse genera of the dematiaceous hyphomycetes.

Within the family the fungus can be compared with *Melanopsammella* Höhnelt, which has 2-celled ascospores that fragment into part-spores within the ascus. *Melanopsammella* differs in the lack of a light-coloured surface of the perithecial wall and the *Chloridium* Link : Fr. and *Gonytrichum* C. G. Nees & F. Nees anamorphs (Höhnelt, 1919; Gams & Holubová-Jechová, 1976). *Melanochaeta* E. Müll. & al. possesses three-layered perithecial walls; however, the light-coloured surface layer is not identical with that described for the new fungus, consisting of densely interwoven pale hyphae. The ascospores of *Melanochaeta* are transversely 3-5-septate, bicolorous, becoming dark brown in the middle cells while the end-cells remain hyaline, and the synanamorphs belong to *Sporoschisma* Berk. & Broome and *Chalara* (Müller & al., 1968; Müller & Samuels, 1982). *Porosphaerellopsis* Samuels & E. Müll. is another genus of the Chaetosphaeriaceae described to have a three-layered perithecial wall (Samuels & Müller, 1978), but the surface layer is unlike that known for *Melanochaeta* or the new fungus. The surface layer comprises hyaline to light brown cells that disintegrate. *Porosphaerellopsis* has brown, transversely 3-septate ascospores with a germ pore at each end. Its anamorph belongs to *Sporoschismopsis* Hennebert & Hol.-Jech.

On the basis of the perithecial wall anatomy, ascospores, asci, elements of the hamathecium and the *Chalara*-like anamorph, the new fungus falls within the Chaetosphaeriaceae but cannot be adequately placed in any genus of the family. Therefore, a new genus *Ascochalara* with a single species *Ascochalara gabretae*, is described.

The link to *Chalara* is suggested on the basis of juxtaposition of perithecia and conidiophores. An attempt to grow this fungus in pure culture was unsuccessful. Neither ascospores nor conidia germinated on cornmeal agar (CMA, Difco) and potato-carrot agar (PCA).

### Material and methods

Fresh material was studied in lactic acid, water and Melzer's reagent. The measurements were taken in Melzer's reagent. The structure of the perithecial wall was studied from freehand sections of dried material cut under the dissecting microscope and mounted in lactic acid. The types of microscopy used are indicated in the legends to the illustrations as bright field (BF), differential inter-

ference contrast (DIC) and phase contrast (PC). Photographs were taken in lactic acid and Melzer's reagent.

### Descriptions

#### *Ascochalara* Réblová, gen. nov.

Perithecia superficialia, subglobosa usque globosa, exsiccata raro lateraliter collabentia, setosa, pulvere grisello vel albido oblecta, papilla nigra glabra exclusa. Paries perithecii fragilis. Canalis ostiolaris periphysatus. Paraphyses copiosae, persistentes. Asci unitunicati, cylindrico-clavati, annulo apicali J-, refractivo, 8-spori. Ascospores longe fusiformes usque cylindricae, septatae, in fragmenta globosa, 0-septata secedentes, hyalinae, leves vel verruculosae.

Anamorphosis *Chalarae* similis.

Perithecia superficial, subglobose to globose, covered by a greyish to whitish powder except for the black, glabrous papilla, non-collapsing or rarely collapsing by lateral pinching when dry, setose. – Perithecial wall fragile. – Ostiolar canal periphysate. – Paraphyses numerous, persist among the asci. – Asci unitunicate, cylindrical-clavate with a J-, refractive apical annulus, 8-spored. – Ascospores when young long-fusiform to cylindrical, transversely septate, at maturity separating into part-spores within the ascus, part-spores rounded, hyaline, smooth or finely verrucose.

Anamorph. – *Chalara*-like.

Type species. – *Ascochalara gabretae* Réblová

Etymology. – From the Latin 'ascus' and 'Chalara', referring to the name of the associated anamorph.

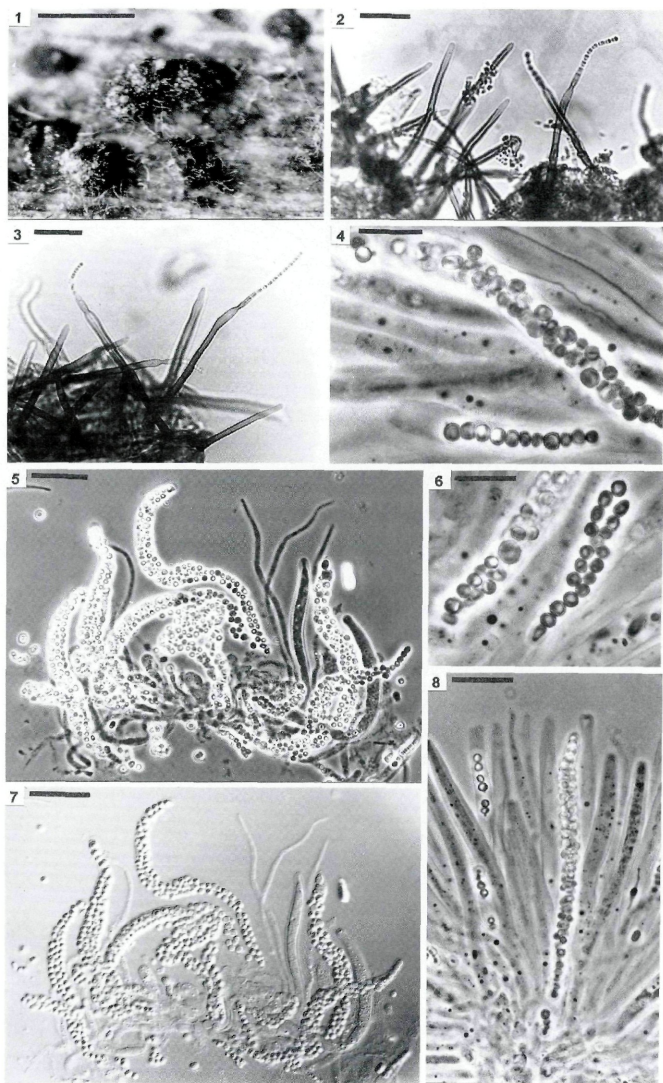
#### *Ascochalara gabretae* Réblová, sp. nov. – Figs. 1–11.

Anamorph. – *Chalara*-like.

Perithecia superficialia, subglobosa usque globosa, exsiccata raro lateraliter collabentia, papillata, setosa, 275–300 µm diam, 300–360 µm alta, pulvere grisello vel albido oblecta, papilla nigra glabra exclusa. Paries perithecii fragilis, 2-stratosus: stratum exterius e cellulis tenuitunicatis, fuscis, lateriformibus constans, 26–31 µm crassum; stratum interius e cellulis tenuitunicatis, compressis, subhyalinis. Canalis ostiolaris periphysatus. Paraphyses copiosae, persistentes. Asci cylindrico-clavati, 112–129 × (7.5–)8–9 µm, annulo apicali J-, refractivo, 8-spori. Ascospores longe fusiformes usque cylindricae, (8–)9–11(–12)-septatae, in fragmenta globosa, 0-septata secedentes, hyalinae, leves vel verruculosae.

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1–8. *Ascochalara gabretae* (PRM 842987). – 1. Perithecia on natural substratum. – 2, 3. Conidiophores with conidia in chains of the *Chalara*-like anamorph on natural substratum. – 4, 6. Ascospores after separating into part-spores. – 5, 7, 8. Asci containing ascospores separating into part-spores and with persistent tapering paraphyses. – Figs. 1–3: BF; 4–6, 8: PC; 7: DIC. – Scale bars: 1 = 500 µm; 2, 3, 5, 7, 8 = 20 µm; 4, 6 = 10 µm.



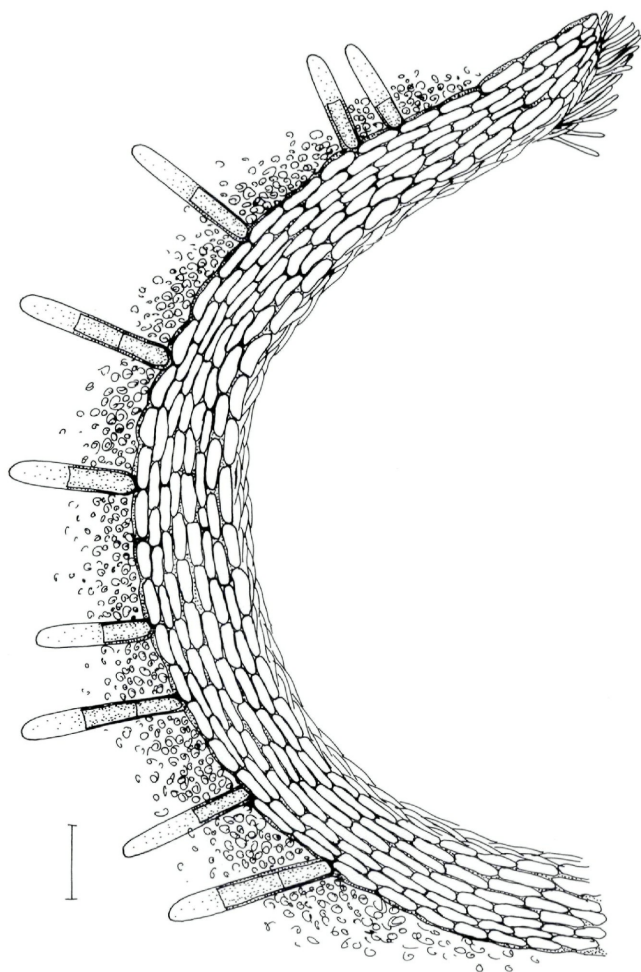


Fig. 9. *Ascochalara gabretae* (PRM 842987). Median longitudinal section of perithecium. – Scale bar: 20  $\mu\text{m}$ .

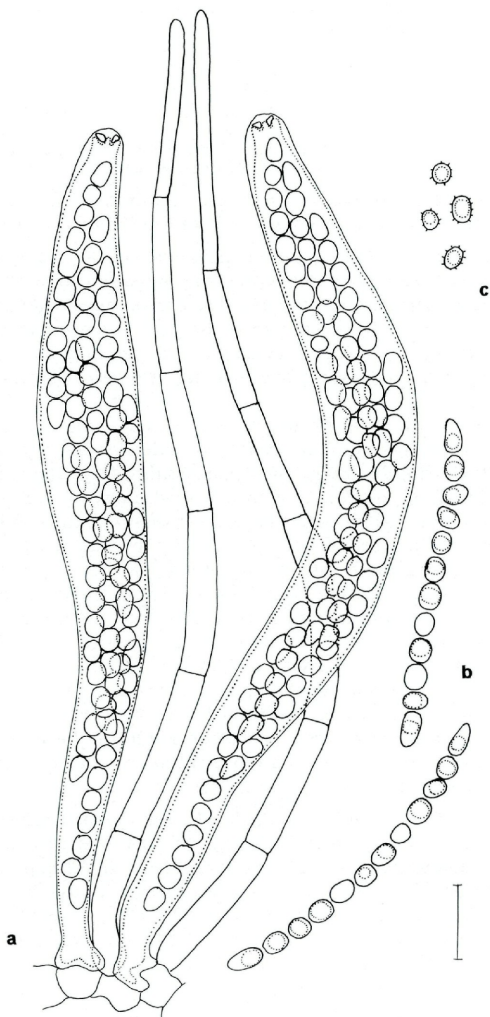


Fig. 10. *Ascochalara gabretae* (PRM 842987). – a. Asci with fragmenting ascospores and paraphyses. – b. Ascospores after separating into part-spores. – c. Part-spores with slightly verrucose wall. – Scale bar: 10  $\mu$ m.

Anamorphosis *Chalarae* similis: Coloniae effusae, fuscae, pilosae, conidiis albidiae. Conidiophoris macronematosis, mononematosis, fuscis, sursum pallidioribus, 46–82  $\mu\text{m}$  longis, in medio 3–3.5  $\mu\text{m}$  latis, septatis. Phialides integrae, subcylindricae, 20–26  $\mu\text{m}$  longae, in medio 3–3.5  $\mu\text{m}$  latae, sub collari modice angustatae ad 1.5–2  $\mu\text{m}$ , collare 7–10.5  $\mu\text{m} \times 2$ –2.5  $\mu\text{m}$ . Conidia subcylindrica usque clavata, basi truncata, sursum rotundata, (2.5–)3–5  $\times$  1.5–2  $\mu\text{m}$ , hyalina, levia, in brevibus vel longis catenis connexa.

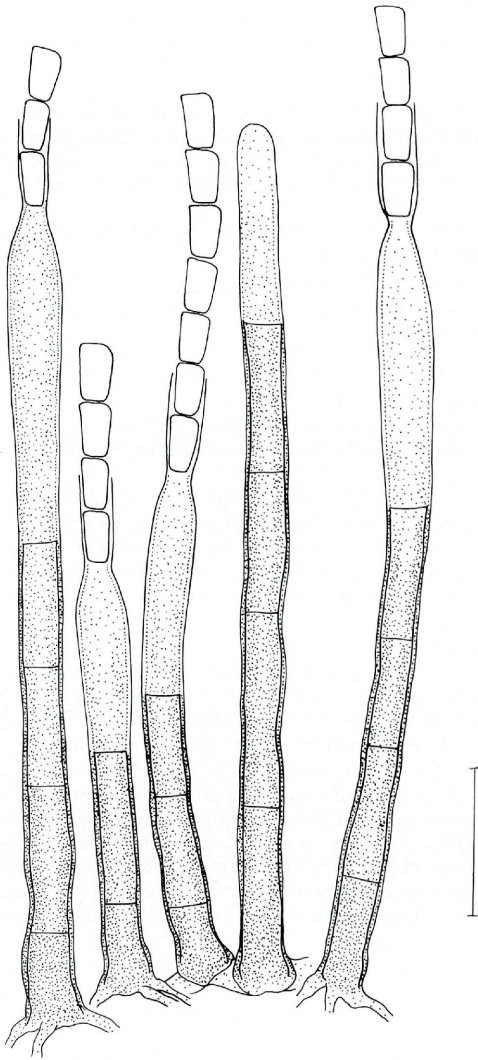
Holotypus. – Bohemia, Montes Silva Gabreta, ad lignum putridum *Abietis albae*, 27 Aug. 1997, M. Řeblová, M.R. 989/97 (PRM 842987).

Etymology. – From Latin ‘Silva Gabreta’ = Šumava Mts. National Park, referring to the area where the fungus was collected.

Teleomorph. – Perithecia superficial, solitary, subglobose to globose, not collapsing or rarely collapsing by lateral pinching when dry, papillate, ostiolate, setose, 275–300  $\mu\text{m}$  diam and 300–360  $\mu\text{m}$  high, black, covered by a whitish to greyish powder except for a black glabrous papilla. The powdery cover is ca. 15–20  $\mu\text{m}$  thick, disappearing with increasing age, leaving the perithecia dark and glabrous. – Setae septate, thick-walled, 15–47  $\mu\text{m}$  long and 3–3.5  $\mu\text{m}$  wide in the middle, mid-brown at the bottom, paler towards the apex, apical cell hyaline and broadly rounded at the tip. – Perithecial wall fragile, lateral wall consisting of two layers. Outer layer fragile, 26–31  $\mu\text{m}$  thick, of thin-walled, dark brown to nearly black, opaque, brick-like cells; inner layer of subhyaline to hyaline, thin-walled, compressed cells. – Ostiolar canal periphysate. – Paraphyses abundant, persisting among the asci, septate at 11–20  $\mu\text{m}$  intervals, 130–142  $\mu\text{m}$  long, 3–4  $\mu\text{m}$  wide in the lower part and tapering to 1.5  $\mu\text{m}$ , protruding beyond the tips of the asci. – Asci cylindrical-clavate, 112–129  $\times$  (7.5–)8–9  $\mu\text{m}$ , truncate at the top, short-stipitate, ascus apex with a J-refractive apical annulus arranged ca. 1–1.5  $\mu\text{m}$  beneath the apex. – Ascospores when young long-fusiform to cylindrical, with (8–)9–11(–12) transverse septa, at maturity cells separating into part-spores within the ascus; part-spores almost rounded, 3–4  $\times$  3–4  $\mu\text{m}$ , hyaline, smooth or finely verrucose, ascospores 2–3-seriate within the ascus.

Anamorph in nature (Figs. 2, 3, 11). – Colonies effuse, hairy, brown, whitish grey when sporulating. – Conidiophores macronematous, mononematous, erect, unbranched, 46–82  $\mu\text{m}$  long including the phialide, ca. 3.5  $\mu\text{m}$  wide, brown at the base, paler apically, 2–4-septate in the lower part. – Phialides terminal, subcylindrical, pale brown to subhyaline, 27–36.5  $\mu\text{m}$  long, venter subcylindrical, 20–26  $\mu\text{m}$  long, 3–3.5  $\mu\text{m}$  wide, tapering to 1.5–2  $\mu\text{m}$  just below the collarette, collarette cylindrical, subhyaline to hyaline, 7–

Fig. 11. *Ascochalara gabretae*, *Chalara*-like anamorph (PRM 842987). – a. Conidiophores and conidia adhering in chains, on natural substratum. – Scale bar: 10  $\mu\text{m}$ .





10.5  $\mu\text{m}$  long and 2–2.5  $\mu\text{m}$  wide. – Conidia subcylindrical to wedge-shaped, slightly rounded at the apex, truncate at the base, (2.5–)3–5  $\times$  1.5–2  $\mu\text{m}$ , hyaline, smooth, adhering in short or long chains.

**Holotype.** – Czech Republic, Southern Bohemia, Šumava Mts. National Park, glacial cirque of the lake Černé jezero near Železná Ruda, on bare wood beneath the bark of a trunk of *Abies alba*, 27 Aug. 1997, M. Réblová, M.R. 989/97 (PRM 842987).

**Habitat.** – Lignicolous saprobe on decayed wood underneath the bark of fallen trunk.

**Known host.** – *Abies alba*.

**Known distribution.** – Czech Republic, known only from the type locality.

Freshly collected material of *Ascochalara gabretae*, contained only immature perithecia with young asci and long-fusiform to cylindrical, septate ascospores but asci with ascospores separating into part-spores within the ascus were observed in two weeks incubation in a moist chamber.

The perithecial setae could also represent immature conidiophores. Similar young conidiophores with subhyaline and sterile tips were seen growing among fertile conidiophores on the natural substratum. Nevertheless, the perithecial setae diverge in all directions, remain shorter and do not appear to develop into phialides at their apices.

The *Chalara*-like anamorph is related to the *Chalara brevispora* Nag Raj & W. B. Kendr. the anamorph of *Chaetosphaeria bramleyi* C. Booth (Gams & Holubová-Jechová, 1976; M. Réblová, pers. observation of the type material IMI 67848, and IMI 27368). It differs from *Chalara brevispora* in having larger conidia and longer phialides, but the two anamorphs are very similar.

Of the species of *Chalara* that have not yet been linked to any known teleomorph, *Chalara longipes* (Preuss) Corda is morphologically similar. It grows on dead needles of coniferous trees, including *Picea abies*, *Pinus mugo* and *Pinus* sp. (Holubová-Jechová, 1984) and on petioles of *Serenoa serrulata* (Sutton, 1978). Holubová-Jechová (1984) collected *Chalara longipes* twice on needles of *Picea abies* and *Pinus mugo* in the Šumava Mts. National Park, where the collection of *A. gabretae* and its *Chalara*-like anamorph was made. However, *Chal. longipes* slightly differs in having longer conidiophores (42–138  $\mu\text{m}$  long) and larger and narrower, cylindrical conidia (3.5–7.5  $\times$  1–1.5  $\mu\text{m}$ ) (Holubová-Jechová, 1984). The occurrence of *Chal. longipes* on coniferous wood has not yet been reported.

## Discussion

On the basis of the light-coloured perithecial surface that becomes a whitish to greyish powder, the anatomy of the dark outer perithecial layer; persistent paraphyses; hyaline, long-fusiform to cylindrical ascospores that fragment into part-spores within the ascus and the *Chalara*-like anamorph, the affinity of *Ascochalara gabretae* lies with genera of the Chaetosphaeriaceae.

The Chaetosphaeriaceae represent a monophyletic group of genera within the Sordariales Chadeff. ex D. Hawksw. & O. E. Erikss. They were segregated from the Lasiosphaeriaceae Nannf. (Lundqvist, 1972; Barr, 1990; Eriksson & Hawksworth, 1998) on the basis of the anatomy of perithecial wall, asci, elements of hamathecium, ascospores and the associated anamorphs (Réblová & al., 1999). The Lasiosphaeriaceae were then restricted to the core genera *Apiosordaria* von Arx & W. Gams, *Lasiochaeria* Ces. & De Not., *Cercophora* Fuckel, *Bombardia* (Fr.) P. Karsten and *Eosphaeria* Höhnelt. Other genera that were placed in the Lasiosphaeriaceae require further revision and possibly also reclassification. At present, the Chaetosphaeriaceae accommodate seven genera, viz. *Ascocodinaea* Samuels & al., *Chaetosphaeria* Tul. & C. Tul., *Melanochaeta*, *Melanopsammella*, *Porosphaerella* E. Müll. & Samuels, *Porosphaerellopsis* and *Striatosphaeria* Samuels & E. Müll. The anamorphs belong to several related genera of dematiaceous hyphomycetes that show a similar degree of organization of conidiophores and conidiogenous cells, viz. *Cacumisporium* Preuss, *Catenularia* Grove, *Cordana* Preuss, *Cylindrotrichum* Bonord., *Chalara*, *Chloridium*, *Custingophora* Stolk & al., *Dictyochaeta* Speg., *Gonytrichum*, *Menispora* Pers.: Fr., *Phialophora* Medlar, *Sporoschisma*, *Sporoschismopsis* and *Zanclospora* S. Hughes & W. B. Kend.

In the Chaetosphaeriaceae only *Melanopsammella* has ascospores that fragment into part-spores within the ascus. *Melanopsammella* differs from *Ascochalara* in that the ascospores are bicellular and that the two-layered perithecial wall lacks a powdery surface at the surface layer. Moreover, perithecia are glabrous or setose but perithecial setae are acute, dark brown and opaque unlike those of *Ascochalara*. The anamorphs of *Melanopsammella* belong to *Chloridium* and *Gonytrichum*. The septate ascospores of both *Melanopsammella* and *Ascochalara* fragment into part-spores including a portion of the primary ascospore wall and mimic true polyspory as described by Barr (1991) for members of the Herpotrichiellaceae Munk.

The character of fragmenting ascospores was considered to have diagnostic value at the generic level in the Chaetosphaeriaceae. On the basis of fragmenting ascospores, *Melanopsammella* was sepa-

rated from *Chaetosphaeria*, though both genera show marked similarity in teleomorphs and anamorphs (Réblová & al., 1999).

The *Chalara* is very heterogeneous. Most links of *Chalara* species are with *Ceratocystis* Ellis & Halstead and rarely to *Cryptendoxylla* Malloch & Cain (Nag Raj & Kendrick, 1975), *Pyxidiphora* Brefeld & Tavel (Lundqvist, 1980; Blackwell & Malloch, 1989), *Cyathicula* De Not. (Gams & Philippi, 1992), *Quasiconcha* M. E. Barr & M. Blackw. (Blackwell & Gilbertson, 1985) and members of the Chaetosphaeriaceae (Gams & Holubová-Jechová, 1976; Holubová-Jechová, 1984; Müller & Samuels, 1982). However, the majority of the hitherto known *Chalara* species have not yet been linked to any teleomorph. Of the Chaetosphaeriaceae, *Chaet. chalaroides* Hol.-Jech., *Chaet. bramleyi*, *Melanochaeta aotearoae* (S. Hughes) E. Müll. & al. and *Ascochalara gabretae* have been linked as teleomorphs to a *Chalara* anamorph or synanamorph.

Association of all these teleomorph genera with *Chalara* anamorphs might suggest an interrelationship. *Chalara* is anatomically rather simple. Because of the simplicity of the characters of conidiogenous cells and conidiophores they could also appear have arisen independently in many unrelated taxa in the course of evolution. Müller & Samuels (1982) discussed three groups of *Chalara* represented by their teleomorphs, i) *Ceratocystis*, *Cryptendoxylla* and *Pyxidiphora*, ii) *Chaetosphaeria* and *Melanochaeta* and iii) two inoperculate cup fungi. *Ascochalara* would belong to the group that includes *Chaetosphaeria* and *Melanochaeta*.

A light-coloured surface layer of the perithecial wall of different origin is found in several genera of the Chaetosphaeriaceae or the related Lasiosphaeriaceae. Within the Lasiosphaeriaceae the surface layer of interwoven, pale hyphae forming a weft is diagnostic of *Lasiochaeta ovina* (Pers. : Fr.) Ces. & De Not., *L. chrysentera* Carroll & Munk, *Bombardia macrocarpa* Carroll & Munk, *Cercophora gossypina* Lundq. or *C. coprophila* (Fr.) Lundq., among others.

In the Chaetosphaeriaceae a light-coloured perithecial surface of different origin is observed in species of *Melanochaeta* and *Porosphaerellopsis* and in *Chaetosphaeria*. Among the *Chaetosphaeria* species only *Chaet. lentomita* W. Gams & Hol.-Jech. (Gams & Holubová-Jechová, 1976) possesses the surface layer (ca. 1.5–2 µm thick, M.R. 1265) of hyaline, amorphous matrix. *Melanochaeta* has a surface layer formed of densely interwoven greyish to whitish hyphae that grow out of the median, fragile and dark brown layer. Samuels & Müller (1978) described the surface layer of *Porosphaerellopsis* as consisting of hyaline to light brown cells that disintegrate to leave an amorphous perithecial surface. The perithecial surface of *Ascochalara gabretae* is covered by a pale powder. The powder is easily separable from the perithecial wall and disappears

with increasing age leaving the perithecia black and glabrous. So far, this kind of the perithecial surface have not been observed in the Chaetosphaeriaceae.

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