

## Teleomorph-anamorph connections in Ascomycetes 3. Three new lignicolous species of *Helminthosphaeria*.

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New lignicolous species *Helminthosphaeria carpathica*, *H. mammillata* and *H. pilifera*, are described and illustrated. *Diplococcium* anamorphs are described and illustrated for the two latter species. No anamorph was observed for *H. carpathica*. These species were collected on decayed wood of deciduous trees in the Czech Republic and Ukraine. A key to species of *Helminthosphaeria* is provided. Relationships of *Helminthosphaeria* with other genera of the Helminthosphaeriaceae are discussed.

Keywords: Ascomycetes, Helminthosphaeriaceae, wood-inhabiting fungi, systematics.

In the course of a study of lignicolous non-stromatic perithecial ascomycetes found in mixed conifer and hardwood, montane and flood plane forests of the Czech Republic and Ukraine, several collections of undescribed species of *Helminthosphaeria* Fuckel emend. Samuels & al. (Samuels & al., 1997) were made. They are described and illustrated here as *H. carpathica*, *H. mammillata* and *H. pilifera*.

*Helminthosphaeria* species are saprobes commonly found on the hymenium of basidiomata of the Aphyllophorales, including Clavariaceae and various corticioid basidiomycetes, but also on decorticated wood (Samuels & al., 1997). *Helminthosphaeria* accommodates species with non-stromatic, subglobose to conical, non-papillate and not collapsing or rarely collapsing perithecia. The perithecial wall is greenish or brown to reddish brown, opaque and two-layered. The outer layer is formed of thick-walled and melanized cells. Cells at the surface of the perithecial wall are usually polyhedral [*H. clavariarum* (Tul. & C. Tul.) Fuckel, *H. mammillata*] and become brick-like towards the interior, but might be also brick-like throughout (*H. corticiorum* Höhnelt), and are usually aggregated into low warts. Setae arise from the entire surface of the perithecial wall. They are stiff, acute, black and opaque (*H. odontiae* Höhnelt, *H. mammillata*, *H. pilifera*) or golden-brown (*H. clavariarum*). The structure of the perithecial apex of *Helminthosphaeria* is character-

istic and identical to that described for the monotypic genus *Tengiomyces* Réblová of the Helminthosphaeriaceae Samuels & al. (Réblová, 1999). The perithecial apex is formed of more or less clavate, thick-walled, dark to mid-brown diverging hyphal elements that grow around the ostiolar opening. Paraphyses are persistent among the asci. Asci have a J- apical annulus that is pronounced or may be lacking. Ascospores are ellipsoidal, non-septate or have several transverse septa, pale brown to greyish to olivaceous translucent and usually have an obscure pore at one or both ends.

A hyphomycetous anamorph, *Diplococcium* Grove, is often found growing around the perithecia. The conidia of the *Diplococcium* are solitary or in short acropetal chains on polytretic conidiogenous cells. Although none of the four described species of *Helminthosphaeria* has ever been grown in artificial culture, the constant, intimate and exclusive relationship between *Helminthosphaeria* and *Diplococcium* led Samuels & al. (1997) to conclude that they were part of a single life cycle. The anamorph of *H. hyphodermatis* Samuels & al. (as *H. hyphodermiae*, Samuels & al., 1997) is not typical of *Diplococcium*, differing in that the conidia are formed through fragmentation of the conidiophore. Two of the three *Helminthosphaeria* species described in the present paper are also associated with *Diplococcium* anamorphs on the natural substratum. A key to the known *Diplococcium* species was provided by Goh & Hyde (1999).

The attempts to culture all three new *Helminthosphaeria* species on cornmeal agar (CMA, Difco) and potato-carrot agar (PCA) were unsuccessful. Ascospore germination was not observed.

*Helminthosphaeria* is currently placed in the Helminthosphaeriaceae (Samuels & al., 1997). *Chaetosphaerella* E. Müll. & C. Booth, with *Oedemium* Link and *Veramycina* Subram. synanamorphs, and *Tengiomyces*, with a *Spadicoides* S. Hughes anamorph, are accepted in the family (Réblová, 1999).

### Material and methods

Herbarium specimens were rehydrated in 3% KOH and studied in lactic acid, water and Melzer's reagent. 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 in the study are indicated in the legends to the illustrations as bright field (BF) and phase contrast (PC). Photographs were taken in lactic acid and Melzer's reagent.

### Key to species of *Helminthosphaeria*

The measurements of ascospores of *Helminthosphaeria clavariarum*, *H. corticiorum*, *H. hyphodermatis* and *H. odontiae* are given according to Samuels & al. (1997).

1. Ascospores remaining non-septate or becoming apiosporous . . . 2
1. Ascospores septate . . . . . 4
2. Ascospores remaining non-septate . . . . . 3
2. Ascospores non-septate or becoming apiosporous, (6–)7.9–10.7 (–12.7) × (2.9–)4.1–6.1(–7.5) µm, anamorph *Diplococcium* . . . . .  
 . . . . . *H. odontiae*
3. Ascospores (10–)11.5–15.6(–19) × (4.5–)5.2–7.2(–8) µm, anamorph *Diplococcium* . . . . . *H. clavariarum*
3. Ascospores (7.2–)8.9–12.1(–16) × (3.3–)4.2–5.4(–6.9) µm, conidia formed through fragmentation of the conidiophore . . . . .  
 . . . . . *H. hyphodermatis*
4. Ascospores developing 1 septum . . . . . 5
4. Ascospores developing 1–3 septa . . . . . 6
5. Septum median, ascospores (8.5–)9.5–12.5(–13.5) × 5–5.5(–7.5) µm, anamorph *Diplococcium*. . . . . *H. pilifera*
5. Septum eccentric in the lower third, ascospores 11.5–12.5 (–13.5) × 5–6 µm, anamorph unknown . . . . . *H. carpathica*
6. Ascospores 1–3-septate, (12.5–)13.5–15.5 × 5–6 µm, anamorph *Diplococcium*, conidia also developing through fragmentation of the conidiophore . . . . . *H. mammillata*
6. Ascospores 1–2(–3)-septate, (10–)13.4–16.6(–20) × (4–)5–6.4(–8.2) µm, anamorph *Diplococcium* . . . . . *H. corticiorum*

### Descriptions of the species

1. ***Helminthosphaeria carpathica*** Réblová, sp. nov. – Figs. 1–6.

Anamorph. – Unknown.

Perithecia superficialia, globosa usque subglobosa, non papillata, 150–200 µm diam, 150–230 µm alta, obscure fusca usque nigra, subrugosa, ostiolata, setosa, setis sensim acutis, simplicibus vel raro ramosis, obscure fuscis, opacis, 22–110 µm longis, basi 5–7 µm latis. Apex peritheci hyphis clavatis 0–1-septatis circumdatus, pariete fusco, parte laterali 24–33 µm crasso, bistratosa, strato externo cellulis poris Munkii instructis. Ostiolum periphysatum. Paraphyses persistentes. Asci cylindrico-clavati, 62–74.5 µm longi (parte sporifera), 8.5–10.5 µm latis, stipite 26–

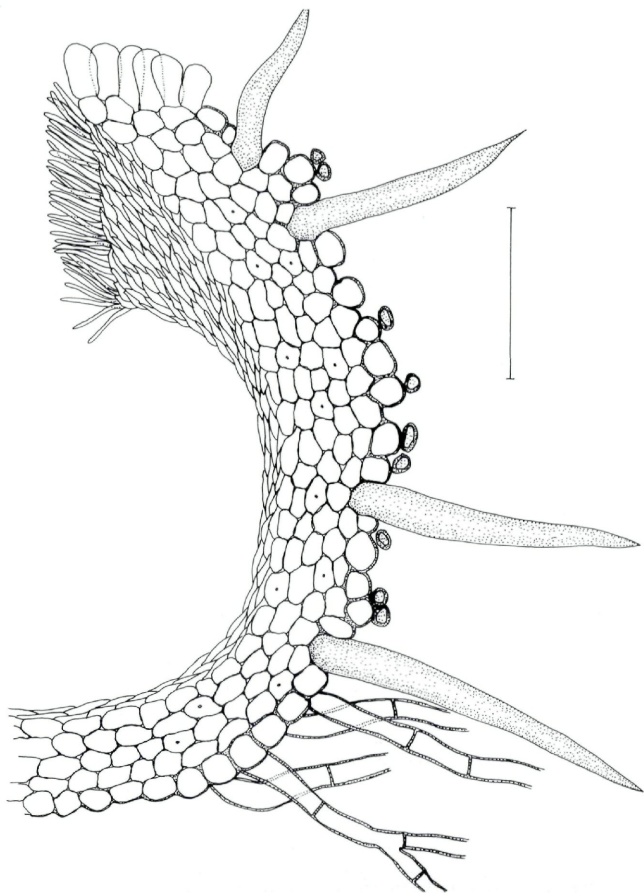


Fig. 1. *Helminthosphaeria carpathica* (PRM 842984). – Median, longitudinal section of perithecium. – Scale bar: 50  $\mu$ m.

36.5  $\mu$ m longo. Ascosporae ellipsoideae, 11.5–12.5(–13.5)  $\times$  5–6  $\mu$ m, in parte inferiore 1-septatae, fuscae.

Holotypus. – Ukraine, Carpati orientales, Montes Boržava, Pilipec, ad lignum putridum dejectum *Fagi sylvaticae*, 25 June 1997, M. Réblová, M.R. 1196/97 (PRM 842984).

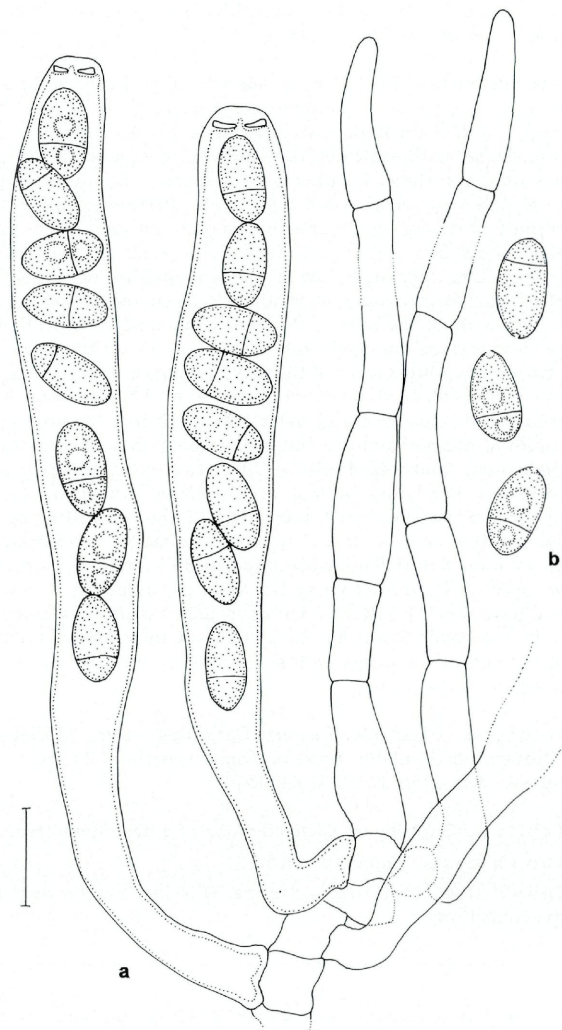


Fig. 2. *Helminthosphaeria carpathica* (PRM 842984). – a. Asci with paraphyses and ascospores. – b. Ascospores. – Scale bar: a, b = 10  $\mu$ m.

**Etymology.** – The epithet 'carpathica' refers to the Carpathian Mts. where the species was collected.

**Teleomorph.** – *Perithecia* superficial, solitary or in small groups of 3–5, globose to subglobose, non-papillate, 150–200  $\mu\text{m}$  wide and 150–230  $\mu\text{m}$  high, dark brown to black, slightly rugose, ostiolate, setose, with scattered dark brown, opaque, oval, protruding cells at the surface. Perithecia in a sparse subiculum; hyphae brown, septate, branched, 4.5–6.5  $\mu\text{m}$  wide. Perithecial apex truncate, formed of diverging, clavate, 0–1-septate, brown, thick-walled hyphal elements arranged around the ostiolar opening. – **Setae** stiff, spine-like, acute, unbranched or rarely branched in the upper part, 0–3-celled, dark brown, opaque, straight to flexuous, 22–110  $\mu\text{m}$  long and 5–7  $\mu\text{m}$  wide at the base. – **Perithecial wall** brown, firm, in surface view *textura angularis*; lateral wall 24–33  $\mu\text{m}$  thick, consisting of two layers. Outer layer of thick-walled, dark brown cells, cells at the surface polyhedral, heavily pigmented in the upper part of the perithecial wall and becoming paler and brick-like toward the interior, with Munk pores in the wall cells. Inner layer of thin-walled, non-pigmented, compressed cells. – **Ostiolar canal** periphysate. – **Paraphyses** persisting among asci, seldom branched, septate, 4–5  $\mu\text{m}$  wide in the lower part, tapering to 3  $\mu\text{m}$ , protruding beyond the tips of the asci. – **Asci** cylindrical-clavate, *pars sporifera* 62–74.5  $\mu\text{m}$  long, 8.5–10.5  $\mu\text{m}$  wide, stipe 26–36.5  $\mu\text{m}$  long, truncate at the top, with a J-, refractive apical annulus, 8-spored. – **Ascospores** ellipsoidal, 11.5–12.5(–13.5)  $\times$  5–6  $\mu\text{m}$ , 1-septate, septum eccentric in the lower third, not constricted at the septum, brown, a pore at the end of the larger cell, smooth, with one oil drop in each cell, 1-seriate in the ascus.

**Holotype.** – Ukraine, Eastern Carpathian Mts., Massif Boržava, Pilipec, on decaying wood of *Fagus sylvatica*, 25 June 1997, M. Réblová, M.R. 1196/97 (PRM 842984).

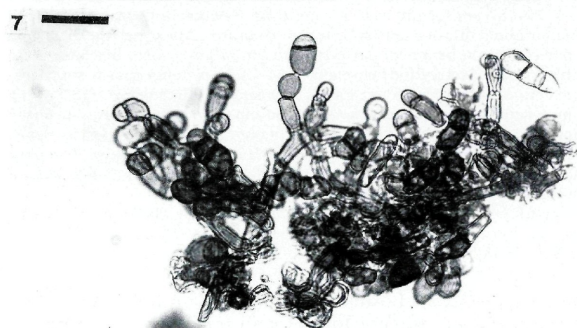
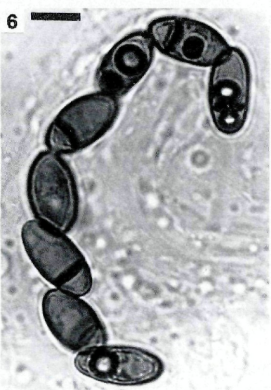
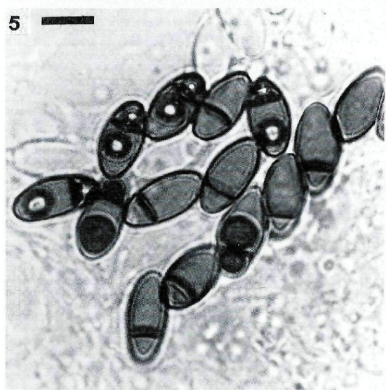
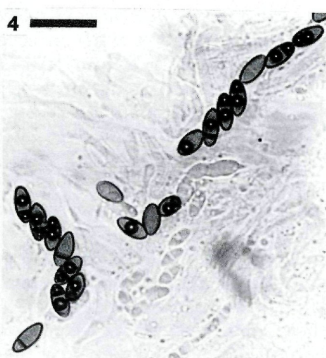
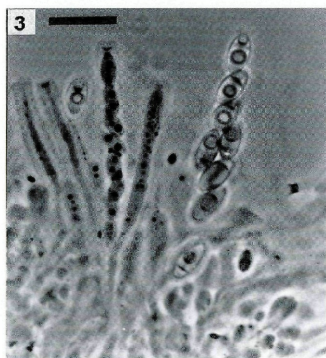
**Habitat.** – Saprobe on decayed wood of a deciduous tree.

**Known host.** – *Fagus sylvatica*.

**Known distribution.** – Europe: Ukraine, known only from the type collection.

Figs. 3–7. 3–6. *Helminthosphaeria carpathica* (PRM 842984). – 3. Young and mature asci containing ascospores. – 4–6. Ascospores. – 7. *Helminthosphaeria mammillata* (PRM 842985). – Conidiophores and conidia of the *Diplococcium* anamorph. –

Figs. 3: PC; 4–7: BF. – Scale bars: 3, 4, 7 = 20  $\mu\text{m}$ ; 5, 6 = 10  $\mu\text{m}$ .



*Helminthosphaeria carpathica* is characterized by 1-septate, ellipsoidal ascospores with an eccentric septum and dark perithecia with only slightly rugose wall. The perithecial apex is formed of brown, clavate, conspicuously diverging hyphal elements growing to the same level around the ostiolar opening. The hyphal elements are of the same colour as the rest of the perithecial wall (Fig. 1).

*Helminthosphaeria carpathica* differs in features of the ascospores from the other two lignicolous species, *H. mammillata* and *H. pilifera*. The two latter species have ascospores with only one or one to three symmetrical transverse septa, respectively. The cells of the perithecial wall of *H. mammillata* lack Munk pores in the walls and form low warts at the surface. The conspicuously diverging hyphal elements at the perithecial apex of *H. mammillata* are unlike those observed in *H. carpathica*. They are rather tapering and obtuse, dark brown to nearly black and distinct from the pale reddish brown perithecial wall. The perithecial wall of *H. pilifera* is only slightly rugose without conspicuous warts and with Munk pores in wall cells, as described for *H. carpathica*. *Helminthosphaeria odontiae*, which occurs commonly on either hymenium of basidiomata of the Aphyllophorales or decayed wood, possesses also apiosporous ascospores but differs in having the smaller ascospores and larger asci.

No anamorph was associated with perithecia in this collection.

## 2. *Helminthosphaeria mammillata* Réblová, sp. nov. – Figs. 7–17.

Anamorph. – *Diplococcium* sp.

Perithecia superficialia, subglobosa usque conica, non papillata, 240–340 µm diam, 240–450 µm alta, obscure rubello-brunnea, apice subnigro excepto, papillis minutis tecta, setosa, setis sensim acutis, simplicibus vel raro ramosis, obscure fuscis, opacis, 20–180 µm longis, basi 5–7 µm latis. Perithecia dense aggregata in subiculo anamorphae dilute rubello-brunneo colorato. Apex perithecii hyphis 0-septatis, pariete dilute brunneo, parte laterali 25–35 µm crasso, bistrato. Ostium periphysatum. Asci cylindrico-clavati, 64–74.5 µm longi (parte sporifera), 10.5–12.5 µm lati, stipite 15.5–26 µm longo. Ascospores ellipsoideae, (12.5–)13.5–15.5 × 5–6 µm, 1-septatae, septo mediano, olivaceo-cinereae usque cinereo-brunneae.

Anamorphosis *Diplococcium* sp.: Conidiophoris macronematosis, mononematosis, raro ramosis, 4–6 µm crassis, fuscis, in fragmenta ellipsoidea, 0-septata secedentibus. Conidia ellipsoidea usque clavata 19–26 × 8.5–9.5 µm, 0–1–2-septata, obscure brunnea.

Holotypus. – Bohemia meridionalis, Montes Silva Gabreta, Medvědice apud Volary, ad lignum putridum *Aceris pseudoplatani*, 18 Sept. 1997, M. & K. Réblová, M.R. 1008/97 (PRM 842985).

Etymology. – From Latin ‘*mammillatus*’ = nipple-shaped, referring to the surface of the perithecial wall formed of low warts.



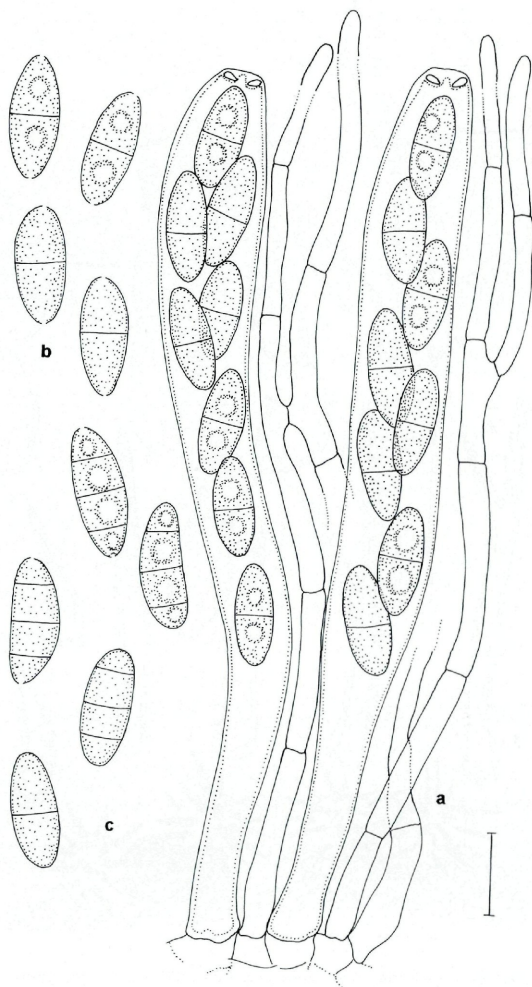


Fig. 8. *Helminthosphaeria mammillata* (PRM 842985). – a. Asci with paraphyses and ascospores. – b, c. Ascospores. – Figs. a, b from PRM 842985; c from M.R. 879/96. – Scale bar: a-c = 10  $\mu$ m.

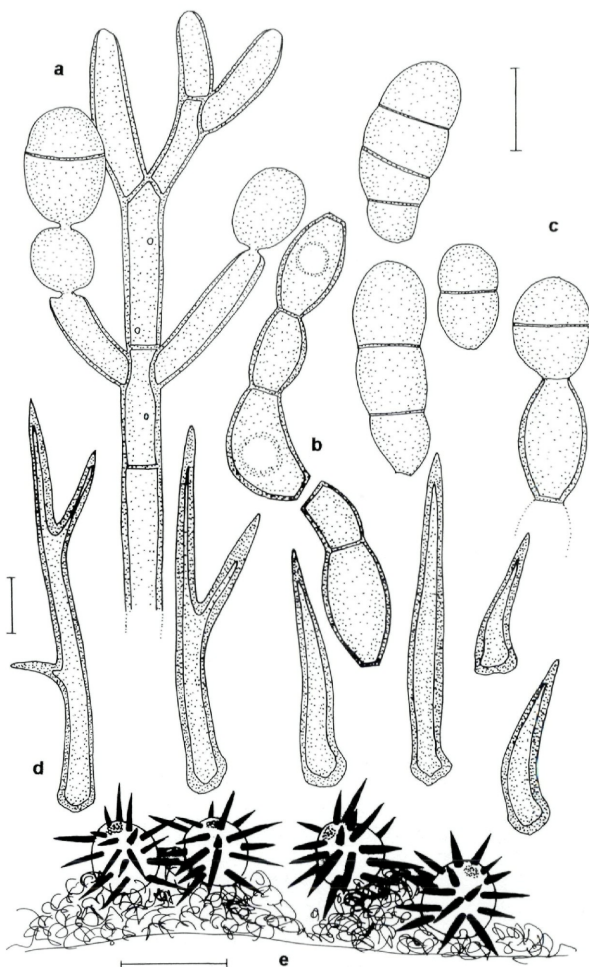


Fig. 9. *Helminthosphaeria mammillata* (PRM 842985). – a. Conidiophore with conidia of the *Diplococcium* anamorph. – b. Conidia of the *Diplococcium* anamorph formed through fragmentation of the conidiophore. – c. Conidia of the *Diplococcium* anamorph. – d. Perithecial setae. – e. Habit sketch of a group of perithecia on a subiculum. – Scale bars: a–c = 10  $\mu$ m; d = 20  $\mu$ m; e = 500  $\mu$ m.

Teleomorph. – *Perithecia* superficial, aggregated in small groups of 3–7, subglobose to conical, not collapsing or rarely collapsing by lateral pinching when dry, non-papillate, flattened at the top, 240–340  $\mu\text{m}$  diam and 240–450  $\mu\text{m}$  high, dark reddish brown except for the dark brown to black apex, roughened by low warts, ostiolate, setose, with scattered dark brown, opaque, protruding cells at the surface. *Perithecia* densely aggregated on a subiculum; hyphae reddish brown, septate, branched, ca. 4  $\mu\text{m}$  wide. Perithecial apex formed of the conspicuously diverging, non-septate, dark brown to black, thick-walled, tapering and obtuse hyphal elements arranged around the ostiolar opening. – Setae stiff, spine-like, acute, unbranched or seldom branched, non-septate, dark brown, opaque, straight or flexuous, 20–180  $\mu\text{m}$  long and 5–7  $\mu\text{m}$  wide at the base. – Perithecial wall pale reddish brown, firm, in surface view *textura angularis*, lateral wall 25–35  $\mu\text{m}$  thick, consisting of two layers. Outer layer of thick-walled, pale reddish brown cells, cells at the surface polyhedral, heavily pigmented, aggregated in low warts and becoming brick-like towards the interior. Inner layer of thin-walled, non-pigmented, compressed cells. – Ostiolar canal periphysate. – Paraphyses persisting among the asci, branching, anastomosing, septate, 3–4  $\mu\text{m}$  wide, not tapering, slightly protruding beyond the tips of the asci. – Asci cylindrical-clavate, *pars sporifera* 64–74.5  $\mu\text{m}$  long, 10.5–12.5  $\mu\text{m}$  wide, stipe 15.5–26  $\mu\text{m}$  long, truncate to broadly rounded at the top, with a J-, refractive apical annulus, 8-spored. – Ascospores ellipsoidal, straight or inequilateral, (12.5–)13.5–15.5  $\times$  5–6  $\mu\text{m}$ , 1-septate, septum median, not constricted at the septum, olivaceous-grey to grey-brown, smooth, an obscure pore at both ends of the ascospore, with one oil drop in each cell, 2-seriate in the ascus.

Anamorph in nature (Figs. 7, 9). – Colonies cushion-like beneath groups of perithecia, pale reddish brown. – Conidiophores macronematous, mononematous, cylindrical, sparingly branched, 4–6  $\mu\text{m}$  wide, septate, brown, smooth. Conidiophores were old and fragmented into 1-celled, more or less ellipsoidal, straight or geniculate segments with truncate ends, 11.5–17  $\times$  7.5–9.5  $\mu\text{m}$ . – Conidiogenous cells terminal or intercalary, polytretic, conidia formed through minute pores. – Conidia ellipsoidal to clavate, 19–26  $\times$  8.5–9.5  $\mu\text{m}$ , 0–1–2-septate, dark brown with darker septa, slightly constricted or non-constricted at the septa, truncate at the base.

Holotype. – Czech Republic, Southern Bohemia, Šumava Mts. National Park, Medvědice near Volary, on rotten wood of *Acer pseu-*

*doplatanus*, 18 Sept. 1997, M. & K. Réblová, M.R. 1008/97 (PRM 842985).

Habitat. – Saprobe on decayed wood of deciduous trees.

Known hosts. – *Acer pseudoplatanus*, *Fraxinus excelsior*, *Quercus robur*.

Known distribution. – Europe: Czech Republic.

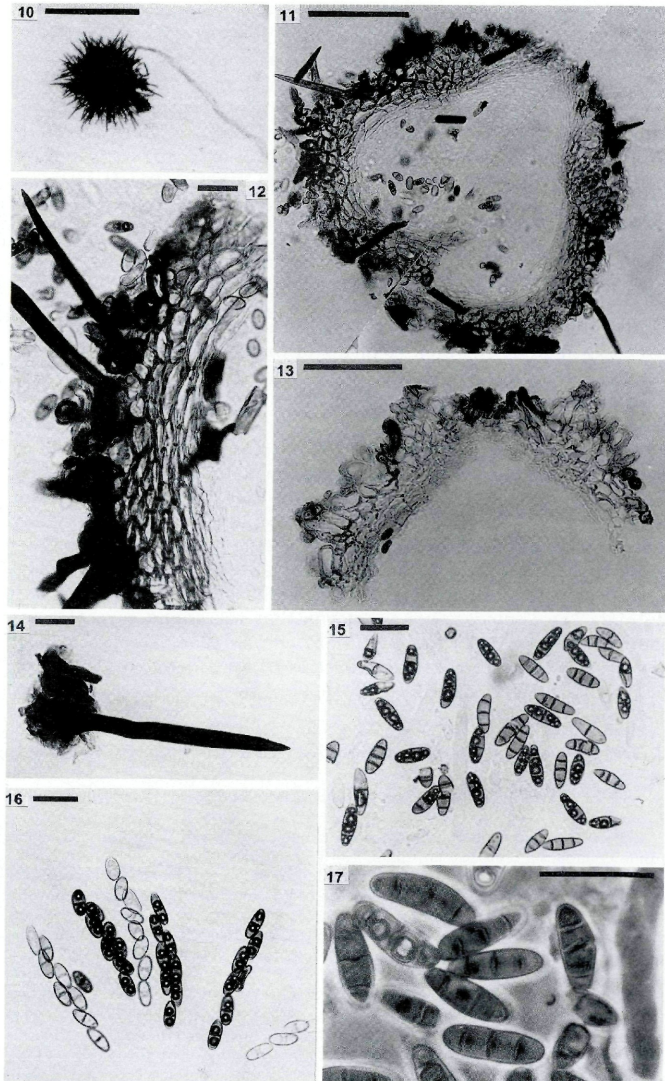
Additional material examined. – Czech Republic, Southern Moravia, flood plane forest Cahnov near Břeclav, on rotten wood of *Quercus robur*, 28 Aug. 1974, V. Holubová-Jechová, M.R. 879/96; Southern Bohemia, Šumava Mts. National Park, Spáleníště, on rotten wood of *Fraxinus excelsior*, 16. Aug. 1999, M. Réblová, M.R. 1460/99, M.R. 1462/99.

*Helminthosphaeria mammillata* is characterized by not collapsing or rarely collapsing perithecia, pale reddish brown perithecial wall with a dark, flat apex and 1–3-septate olivaceous-grey to grey-brown ascospores. Perithecia are aggregated in small groups on a reddish brown mycelium and associated with conidiophores of the *Diplococcium* anamorph. Conspicuously diverging hyphal elements arise from the flat perithecial apex around the ostiolar opening. The hyphal elements are thick-walled, dark brown to nearly black and opaque, unlike other species the hyphal elements are not clavate but taper from the base to an obtuse tip. They are clearly distinct from the rest of the perithecial wall, which is formed of pale reddish brown polyhedral cells (Fig. 13).

The perithecia of the type material contained only 1-septate ascospores; no delayed secondary septa were observed in released ascospores. The perithecia of the collection M.R. 879/96 made by V. Holubová-Jechová, however contained mature released ascospores that were predominantly 3-septate and seldom 1-septate. Ascospores of many species of non-stromatic lignicolous perithecial ascomycetes typically develop septa after discharge from the asci, e.g. *Chaetosphaeria* Tul. & C. Tul. of the Chaetosphaeriaceae Réblová & al. or *Lasiosphaeria* Ces. & De Not. of the Lasiosphaeriaceae Nannf. In all other features of perithecial wall, asci, paraphyses and the anamorph, both collections of *H. mammillata* are identical and belong to one species. The type material of *H. mammillata* is in excellent condition while the specimen M.R. 879/96 is rather old.

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Figs. 10–17. *Helminthosphaeria mammillata* (PRM 842985). – 10. Perithecium. – 11. Longitudinal section of perithecium. – 12. Longitudinal section of perithecial wall. – 13. Longitudinal, off center, section of the perithecial apex. – 14. Perithecial seta. – 15–17. Ascospores. – Figs. 10–16: BF; 17: PC. – Figs. 10–12, 14, 16 from PRM 842985; 13, 15, 17 from M.R. 879/96. – Scale bars: 10 = 500  $\mu$ m; 11, 13 = 100  $\mu$ m; 12, 14 = 10  $\mu$ m; 15–17 = 20  $\mu$ m.



*Helminthosphaeria mammillata* produced two kinds of conidia. The conidia either arose through minute pores of the conidiogenous cells or through fragmentation of the conidiophores. Samuels & al. (1997) described the formation of conidia through fragmentation of the conidiophore in the fungicolous *Helminthosphaeria hyphodermatis*. The authors observed the anamorph of *H. hyphodermatis* to be associated with the perithecia on the natural substratum in several collections but no other conidia were seen. *Helminthosphaeria hyphodermatis* differs from *H. mammillata* in having smaller and non-septate ascospores.

The closest species to *H. mammillata* is the lignicolous *H. pilifera*, which differs in having deeply collabent perithecia, non-warted and only slightly roughened dark brown perithecial walls, 1-septate, smaller, broadly ellipsoidal ascospores and much shorter asci in the *pars sporifera*. The hyphal elements of the perithecial apex are clavate and less conspicuous than those observed in *H. mammillata* or *H. carpathica* and do not differ in colour from the rest of the perithecial wall.

The fungicolous *H. corticiorum* is similar to *H. mammillata* in having ellipsoidal, 0–1–2(–3)-septate ascospores and in the anatomy of perithecial apex. It differs in that the ascospores are longer and when 1-septate develop an eccentric septum or commonly only 2 septa after discharge from the ascus, longer and narrower non-stipitate asci, greenish perithecial walls, absence of reddish brown cushions of subiculum, size of the conidia and conidiophores and habitat. The *Diplococcium* anamorph that was found several times jointly with the perithecia of *H. corticiorum* (Samuels & al., 1997) was recently described as a new species *Diplococcium varieseptatum* Goh & K. D. Hyde (Goh & Hyde, 1999).

### 3. *Helminthosphaeria pilifera* Réblová, sp. nov. – Figs. 18–28.

Anamorph. – *Diplococcium* sp.

Perithecia superficialia, subglobosa usque conica, non papillata, sicca profunde collabentia, 160–170 µm diam, 180–200 µm alta, obscure fusca, subrugosa, ostiolata, setosa, setis sensim acutis, simplicibus vel raro ramosis, obscure fuscis, opacis, 15–140 µm longis, basi 5.5–7.5 µm latis. Apex perithecii hyphis clavatis 0-septatis instructus, circumdatus, pariete fusco, parte laterali 28–33(–44) µm crasso, bistratoso, strato externo cellulis poris Munkii instructis. Ostiolum periphysatum. Paraphyses persistentes. Asci cylindrico-clavati, (31.5–)42–52.5 µm longi (parte sporifera), 9.5–10.5 µm lati, stipite 21–52 µm longo. Ascosporae ellipsoideae usque suballantoideae (8.5–)9.5–12.5(–13.5) × 5–5.5(–7.5) µm, 1-septatae, septo mediano, fuscae.

Anamorphosis *Diplococcium* sp.: Conidiophoris macronematosi, mononematosi, fuscis, 130 µm longis, 4–5(–6) µm latis. Conidia cylindrica usque ellipsoidea, (16.5–)17.5–26(–29) × (6–) 7.5–9.5 µm, 2–3-septata, obscure brunnea.

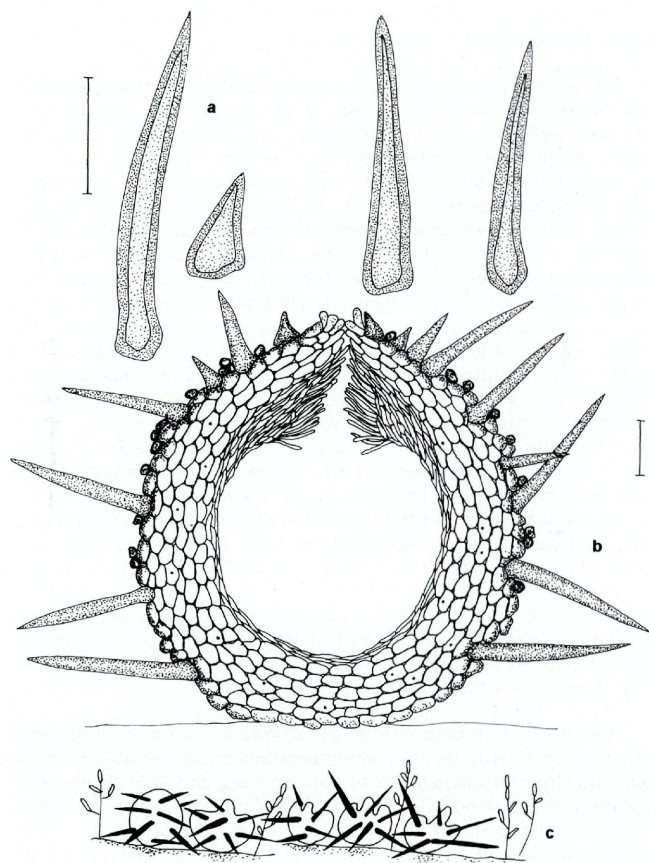


Fig. 18. *Helminthosphaeria pilifera* (PRM 842986). - a. Perithecial setae. - b. Median, longitudinal section of perithecium. - c. Habit sketch of perithecia and conidiophores. - Scale bars: a = 10  $\mu$ m; b = 20  $\mu$ m.

**Holotypus.** – Bohemia meridionalis, Montes Silva Gabreta, Ždánidla apud Prášily, ad lignum putridum *Fagi sylvaticae*, 26 Aug. 1998, M. Réblová, M.R. 1408/98 (PRM 842986).

**Etymology.** – From Latin '*piliferus*' = pilose, piliferous, referring to the cover of the perithecial wall.

**Teleomorph.** – *Perithecia* superficial, solitary to gregarious, subglobose to conical, collapsing deeply by lateral pinching when dry, non-papillate, 160–170  $\mu\text{m}$  diam and 180–200  $\mu\text{m}$  high, dark brown, slightly roughened, ostiolate, setose, at the surface with scattered dark brown, opaque, oval, protruding cells. Perithecial apex broadly rounded, formed of diverging, clavate, 0-septate, brown, thick-walled hyphal elements arranged around the ostiolar opening. – Setae stiff, spine-like, acute, unbranched, 0-septate, dark brown, opaque, straight to flexuous, 15–140  $\mu\text{m}$  long and 5.5–7.5  $\mu\text{m}$  wide at the base. – Perithecial wall brown, firm, in surface view *textura angularis*, with distinct Munk pores in wall cells, lateral wall 28–33(–44)  $\mu\text{m}$  thick, consisting of two layers. Outer layer of thick-walled, mid-brown cells, cells at the surface polyhedral, heavily pigmented and becoming more brick-like towards the interior, with Munk pores in the wall cells. Inner layer of thin-walled, non-pigmented compressed cells. – Ostiolar canal periphysate. – Paraphyses persisting among the asci, branching, septate, some cells swollen, 3–4  $\mu\text{m}$  wide in the lower part, tapering to 2.5–3  $\mu\text{m}$ , protruding beyond the tips of the asci. – Asci cylindrical-clavate, *pars sporifera* (31.5–)42–52.5  $\mu\text{m}$  long, 9.5–10.5  $\mu\text{m}$  wide, stipe 21–52  $\mu\text{m}$  long, truncate to broadly rounded at the top, with a J-, refractive apical annulus, 8-spored. – Ascospores ellipsoidal to suballantoid, (8.5–)9.5–12.5(–13.5)  $\times$  5–5.5(–7.5)  $\mu\text{m}$ , 1-septate, septum symmetrical, not constricted at the septum, brown, a very indistinct pore at both ends of the ascospores, smooth, with one oil drop in each cell, 1–2-seriate in the ascus.

**Anamorph** in nature (Figs. 20; 23–28). – Colonies hairy, dark brown. – Conidiophores macronematous, mononematous, cylindrical, sparingly branched, up to 130  $\mu\text{m}$  long and 4–5(–6)  $\mu\text{m}$  wide, septate, brown, smooth. – Conidiogenous cells terminal or intercellar, polytretic, conidia formed through minute pores. – Conidia cylindrical to ellipsoidal, (16.5–)17.5–26(–29)  $\times$  (6–) 7.5–9.5  $\mu\text{m}$ , 2–3-septate, dark brown with darker septa, slightly constricted or not constricted at the septa, truncate at the base.

**Holotype.** – Czech Republic, Southern Bohemia, Sumava Mts. National Park, Ždánidla Mt. near Prášily, on rotten wood of *Fagus sylvatica*, 26 Aug. 1998, M. Réblová, M. R. 1408/98 (PRM 842986).



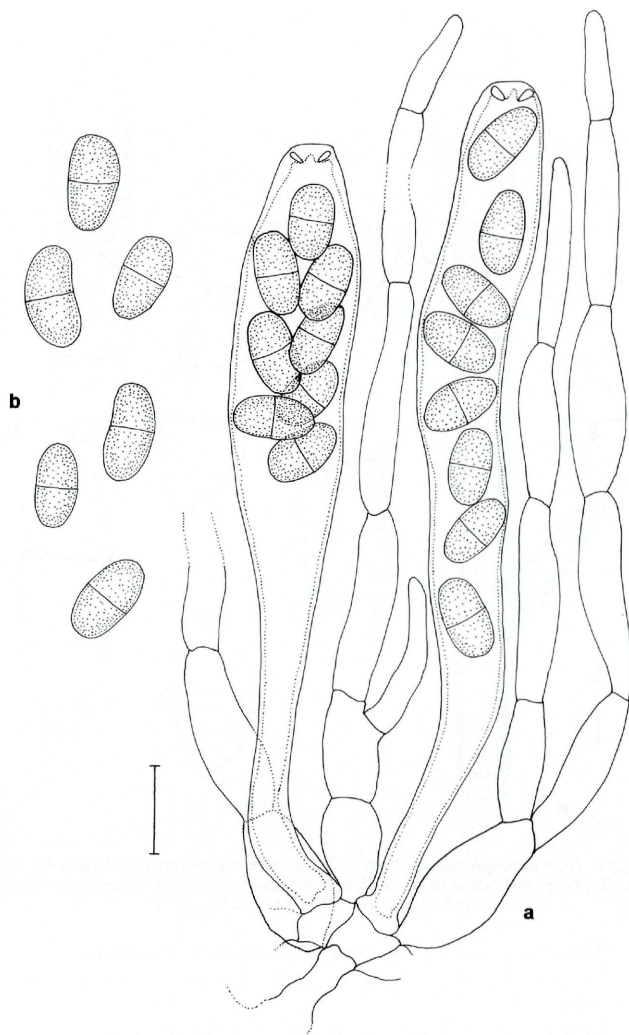


Fig. 19. *Helminthosphaeria pilifera* (PRM 842986). - a. Asci with paraphyses and ascospores. - b. Ascospores. - Scale bar: a, b = 10  $\mu$ m.

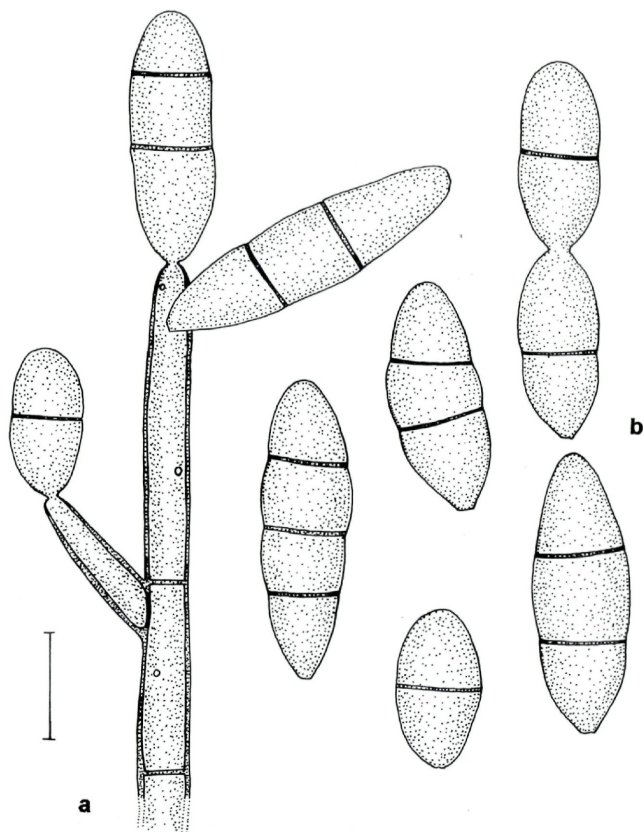


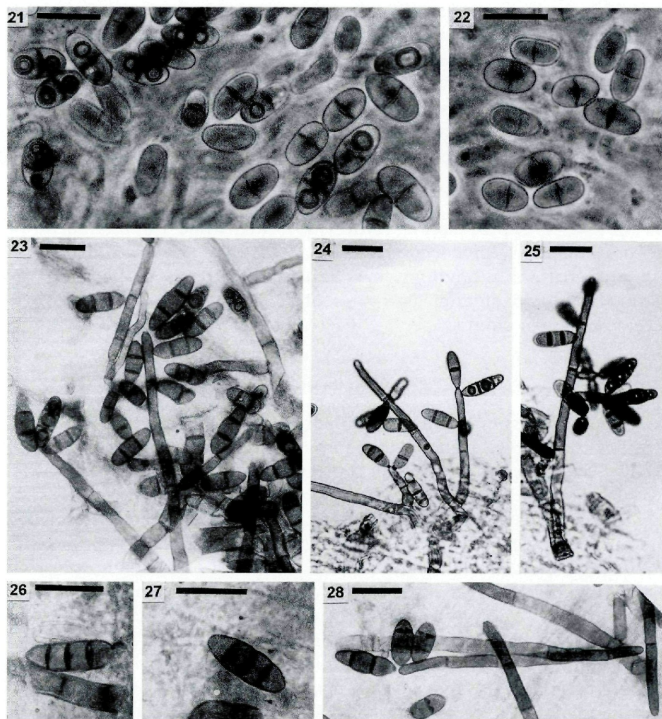
Fig. 20. *Helminthosphaeria pilifera*, *Diplococcium* anamorph (PRM 842986). – a. Conidiophore with conidia of *Diplococcium* anamorph. – b. Conidia of *Diplococcium* anamorph. – Scale bar: a, b = 10 µm.

Habitat. – Saprobe on decayed wood of deciduous trees.

Known hosts. – *Fagus sylvatica*, *Fraxinus excelsior*, *Ulmus montana*.

Known distribution. – Europe: Czech Republic.

Additional material examined. – Czech Republic, Southern Bohemia, Šumava Mts. National Park, Medvědice near Volary, on decayed wood of



Figs. 21–28. *Helminthosphaeria pilifera* (PRM 842986). – 21, 22. Ascospores. – 23.–28. Conidiophores and conidia of the *Diplococcium* anamorph. – Figs. 21, 22: PC; 23–28: BF – Scale bars: 23–28 = 20  $\mu$ m; 21, 22 = 10  $\mu$ m.

*Ulmus montana*, 31 Aug. 1998, M. Réblová & J. Čermák, M.R. 1300/98; Spálenště, on rotten wood of *Fraxinus excelsior*, 16. Aug. 1999, M. Réblová, M.R. 1461/99.

*Helminthosphaeria pilifera* is characterized by deeply collapsing perithecia, 1-septate, broadly ellipsoidal to suballantoid brown ascospores with a median septum, and slightly roughened, dark brown perithecial walls with Munk pores in the cells. Perithecia of *H. pilifera* grew on the surface of soft, decorticated wood in colonies that were “hairy” because of the *Diplococcium* conidiophores. Perithecia of other *Helminthosphaeria* species do not collapse or rarely collapse, e.g. *H. clavariarum* or *H. mammillata*.

*Helminthosphaeria pilifera* is similar to *H. mammillata*, which differs in having larger 1–3-septate, ellipsoidal ascospores, warty, pale reddish brown perithecial walls without Munk pores in the cells, conspicuously diverging dark hyphal elements at the flat perithecial apex, not collapsing or rarely collapsing larger perithecia sitting in reddish brown compact hyphal cushions with *Diplococcium* conidiophores.

The *Diplococcium* anamorph of *H. odontiae* is very similar to that of *H. pilifera* but the conidia are longer and narrower and develop 1–3(–4) septa. Goh & Hyde (1999) identified the anamorph of *H. odontiae* as *Diplococcium clarkii* M. B. Ellis. *Helminthosphaeria odontiae* differs in having non-collapsing perithecia, larger, non-stipitate asci and smaller 0(–1–2)-septate ascospores; the septum is eccentric when single.

### Discussion

*Helminthosphaeria* has previously been restricted to fungicolous species that rarely occur on wood apart from any conspicuous fungal host. Such an apparently strong preference for a fungal substratum would seem to be a strong generic character. Although the presence of a fungal host cannot be ruled out for the three species described herein, none was observed in the neighbourhood in the rotting wood. When anatomical and morphological details of the teleomorphs and the associated anamorphs are considered, the only consistent difference between the lignicolous and fungicolous species of *Helminthosphaeria* was that the asci of the lignicolous species have a longer stipe than those of the fungicolous species. Moreover, conidia of fungicolous species have a conspicuous germ pore whereas conidia of the lignicolous *H. mammillata* and *H. pilifera* lack pores or the pores are obscure.

Samuels & al. (1997) described two types of anamorphs for the respective *Helminthosphaeria* species. Conidia of the type species *H. clavariarum*, *H. corticiorum* and *H. odontiae* are typical of *Diplococcium*. Conidia of *H. hyphodermatis* formed by fragmentation of the conidiophore. The two types of anamorphs did not intergrade, nor were they formed together. Samuels & al. (1997) did not assign the apparent anamorph of *H. hyphodermatis* to a genus. The finding in *H. mammillata* of conidiophores both typical of *Diplococcium* and fragmenting as in *H. hyphodermatis* indicates that all are *Diplococcium* anamorphs and that the concept of *Diplococcium* must be expanded to include fragmentation of the conidiophores as a means of conidiogenesis.

The genera *Helminthosphaeria*, *Chaetosphaerella* and *Tengomyces* are placed in the Helminthosphaeriaceae on the basis of

similar anatomy of the perithecial wall and conidiogenesis of the associated anamorphs. The relationships between *Chaetosphaerella* and *Tengiomyces* were discussed by Réblová (1999). Their conidia are solitary or form acropetal chains and are produced through a minute pore of polytretic conidiogenous cells. The type of conidiogenesis of *Diplococcium*, *Oedemium* and *Spadicoides*, whether it is holoblastic or enteroblastic, has been discussed many times, e.g. Ellis (1971), Campbell (1968, 1969), Brotzman & al. (1975), Carroll & Carroll (1974) or Cole & Samson (1979). Here and in Réblová (1999) I accept the explanation of Cole & Samson (1979), who based their observation on ultrastructural data, that the poroconidium is a special kind of holoblastic propagule. Of particular interest is the case of *Chaetosphaerella phaeostroma* (Durieu & Mont.) E. Müll. & C. Booth which has both *Oedemium* and *Veramycina* synanamorphs. While the former is characterized by holoblastically formed poroconidia, the latter produces conidia from a head of terminally arranged phialides (Hughes & Hennebert, 1963; Réblová, 1999). The presence of two synanamorphs produced by the same teleomorph, each with a different kind of conidiogenesis, is unusual.

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