

A new species of *Chaetosphaeria* with *Menispora ciliata* and phialophora-like anamorphs

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A new species of *Chaetosphaeria* with a *Menispora ciliata* anamorph is described from decayed wood of *Acer campestre* collected in the Czech Republic. A phialophora-like synanamorph was produced *in vitro* on potato carrot agar medium. *Chaetosphaeria ciliata* is clearly distinguished from other *Chaetosphaeria* species by its *Menispora* anamorph with its aseptate conidia with polar setulae, phialides with a tapering apex that recurves downwards towards the main stipe of the conidiophore and the dimensions of its asci and ascospores. The ascospores and asci of *Ch. ciliata* are comparatively smaller than those of *Ch. ovoidea*, *Ch. pulviscula* and *Ch. tortuosa*, the other three *Chaetosphaeria* species with *Menispora* anamorphs. The ITS rDNA data clearly confirms the placement of *Ch. ciliata* in the *Menispora* group. Based on ascus and ascospore morphology and similarity of ITS sequences, *Ch. ciliata* is most closely related to *Ch. pulviscula*.

Key words: anamorph-teleomorph relationships, hyphomycetes, *Chaetosphaeriaceae*, cultivation, systematics.

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Introduction

Menispora is a dematiaceous, phialidic hyphomycete genus forming conspicuous hairy colonies on decayed wood, the inner side of bark or on decayed leaves and is one of the 16 anamorph genera linked with the life cycles of *Chaetosphaeria* Tul. & C. Tul. species (*Chaetosphaeriaceae*, *Chaetosphaeriales*). The macronematous, brown conidiophores, which bear phialides in a lateral or terminal position with a tapering, strongly recurved apex, indistinct shallow collarete and hyaline conidia without or with polar setulae, clearly distinguish *Menispora* from other dematiaceous hyphomycetes with enteroblastic conidiogenesis. We accept 11 *Menispora* species (Réblová *et al.*, 2006), which exhibit two morphological patterns of conidiophores and setae, i.e. a) lacking setae that grow

independently of conidiophores; the conidiophores terminate in sterile, whip-like extensions that branch laterally in their lower part; phialides borne on short metulae along the main axis of the conidiophore or its branches; phialides arise singly or in groups, terminally or laterally, or b) with setae occurring independently of conidiophores; the conidiophores terminating in a mono- or polyphialide; phialides are rarely lateral. To date, only three of the five *Menispora* species assigned to the first morphological group are linked with teleomorphs, all species of *Chaetosphaeria* (Booth, 1957; Holubová-Jechová, 1973; Constantinescu *et al.*, 1995; Réblová *et al.*, 2006), while other *Menispora* spp. lack known teleomorphs. A phialophora-like synanamorph (produced *in vitro*) was experimentally proven for *Chaetosphaeria ovoidea* (Fr.) Constant., K. Holm & L. Holm (anamorph: *Menispora*

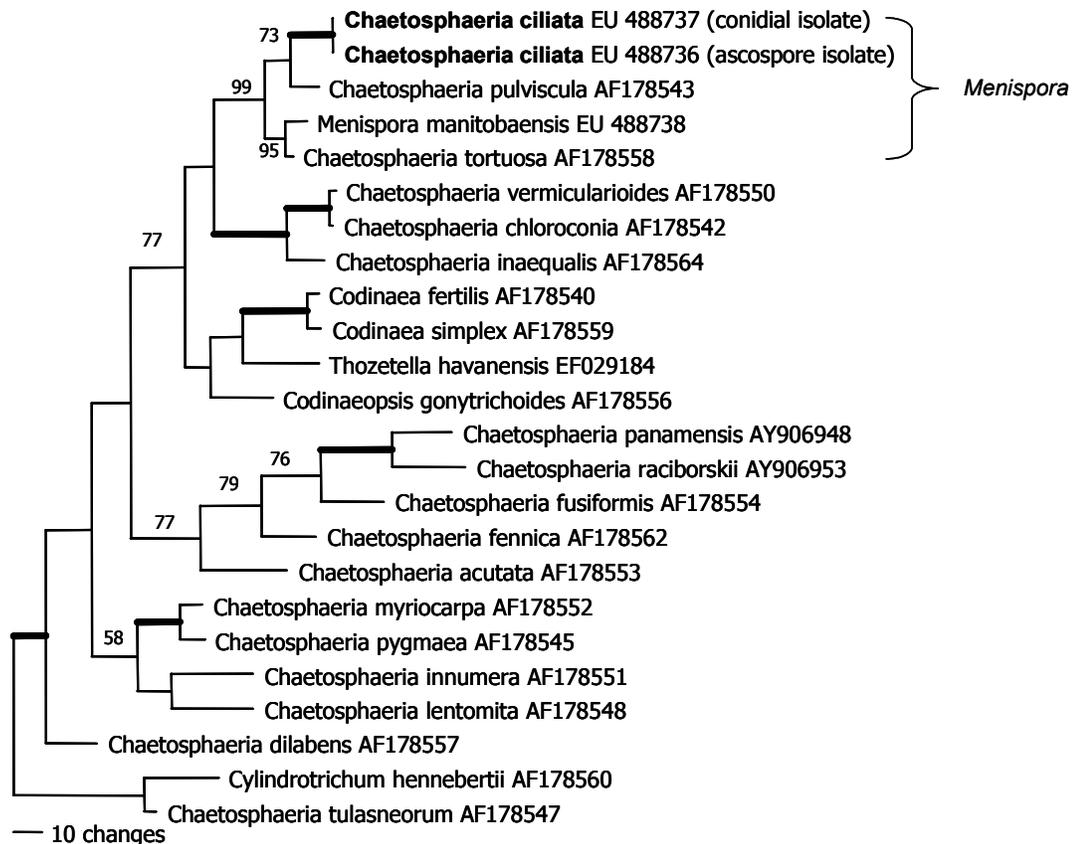


Fig. 1. One of the two most parsimonious trees from a heuristic analysis of ITS rDNA sequences from *Chaetosphaeria* species. Bootstrap values $\geq 50\%$ from 1000 replicates are included at the nodes. Thickened branches indicate bootstrap support of 100%. Branch lengths are drawn to scale.

glauca Pers. and *Ch. pulviscula* (Curr.) C. Booth (anamorph: *Menispora caesia* Preuss) (Réblová, 1998).

On a sample of decayed wood of *Acer campestre* from the Czech Republic, we observed a conspicuous colony of *Menispora ciliata* Corda associated with an inconspicuous pyrenomycete. The perithecia were not mixed with the colony, but were nonstromatic, superficial, globose to subglobose, dark brown to black; the asci were cylindrical-fusiform with a shallow apical ring and contained eight fusiform, 1(-3)-septate, hyaline, finely verruculose ascospores. The morphological characters of the unknown fungus are in perfect agreement with those of the genus *Chaetosphaeria* (Réblová, 2000). In PCA culture, the ascospore and conidial isolates yielded sporulating colonies of *M. ciliata* and its phialophora-like synanamorph after 9 months.

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Because no teleomorph has been described for *M. ciliata* and the link between the two morphs was confirmed *in vitro*, *in vivo* and by using ITS rDNA sequence data, a new holomorph species and a new anamorph-teleomorph connection in the genus *Chaetosphaeria* is proposed. An ITS nrDNA phylogenetic analysis is performed to estimate the relationship of *Ch. ciliata* among other *Chaetosphaeria* species with *Menispora* anamorphs.

Materials and methods

Dried herbarium specimens were rehydrated in water and studied in water, Melzer's reagent or 90% lactic acid. Single-ascospore isolates were obtained from fresh material with the aid of a single-spore isolator (Meopta, Czech Republic). Cultures were grown on potato-carrot agar (PCA, Gams *et al.*,

2007). Colony characters were derived from cultures grown on PCA and placed at 25°C in the dark for 14d. Cultures are maintained in the Canadian Collection of Fungal Cultures, Ottawa, Canada (DAOM) and the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS).

All measurements were made in Melzer's reagent. Means \pm standard errors (S.E.) are based on 25 measurements and are given for ascospore, ascus and conidial dimensions. The length/width ratios (L/W) for asci, ascospores and conidia are given. Images were captured in Melzer's reagent using differential interference microscopy (DIC) and phase contrast (PC) and processed using Adobe Photoshop 6.0 CE.

DNA extraction, amplification and sequencing

Methods for DNA extraction, amplification and sequencing of the ITS rDNA (ITS1-5.8S-ITS2) were identical to those described by Réblová and Seifert (2004).

Sequence data analysis

The phylogenetic relationships of *Ch. ciliata* were examined using ITS and LSU rDNA sequences. *Chaetosphaeria tulasneorum* and *Cylindrotrichum hennebertii*, which are phylogenetically distinct from the core of *Chaetosphaeria* (Réblová and Winka, 2000) were used as outgroups. Three new ITS sequences were obtained for ascospore and conidial isolates of *Chaetosphaeria ciliata* (EU 488736, EU 488737) and from a conidial isolate of *Menispora manitobaensis* (EU 488738). Other homologous sequences were retrieved from GenBank; accession numbers are given on Fig. 1 and in Table 1. All sequences were manually aligned in BioEdit 5.0.9 (Hall, 1999). The alignment is available in TreeBase as SN 3808.

Phylogenetic analysis

Phylogenetic analysis of ITS nrDNA sequences was performed using Maximum parsimony to estimate the relationship of *Ch. ciliata* among other *Chaetosphaeria* species with *Menispora* anamorphs. Maximum parsimony analysis was conducted with

PAUP* 4.0b10 (Swofford, 2002). A heuristic search was performed with the stepwise-addition option with 1000 random taxon addition replicates and TBR branch swapping. All characters were unordered and given equal weight. Gaps were treated as missing data. Branch support was assessed on the recovered topologies by performing 1000 bootstrap replicates with a full heuristic search, consisting of ten random-addition replicates for each bootstrap replicate.

Results

Phylogenetic analysis of the ITS nrDNA sequence data

Maximum parsimony analysis was performed using 192 phylogenetically informative characters in an alignment including 564 characters from 24 taxa. Two most parsimonious trees (Fig. 1) were obtained [tree length 722, consistency index (CI) = 0.524, retention index (RI) = 0.613, homoplasy index (HI) = 0.476]. The two MPTs differed in the placement of *Codinaeopsis gonytrichoides*, which is shown either as a sister to the *Menispora*-clade and *Chloridium sensu str.*-clade (*Ch. vermicularioides*, *Ch. chloroconia*, *Ch. inaequalis* in Fig. 1) or as a sister to the *Codinaea-Thozetella*-clade (tree not shown).

The ascospore and conidial isolates of *Ch. ciliata* (100% bootstrap support) formed in a strongly supported clade (99) including all sampled *Menispora* species, *i.e.* *Menispora manitobaensis*, *Ch. tortuosa* and *Ch. pulviscula*. *Chaetosphaeria ciliata* is shown as a sister (75) to *Ch. pulviscula*.

***Chaetosphaeria ciliata* Réblová & Seifert, sp. nov. (Figs 2-18)**

MycoBank: 511267

Etymology: Named so that the epithet corresponds with that of its anamorph, *Menispora ciliata*.

Perithecia gregaria, non stromatica, fusca vel atra, globosa vel subglobosa, glabra, 180-250 μ m alta, 200-250 μ m diam; ostiolum periphysatum. *Paries perithecii* fragilis, 28-33 μ m crassus, bistratosus. Paraphyses persistentes, copiosae, septatae, 3-3.5 μ m latae prope basim, sursum ad 1.5-2 μ m, ascus superantes. Asci unitunicati, cylindrico-fusiformis, 78-94(-98) \times 7.5-9(-9.5) μ m, longit.:latit. 10.6:1. Ascospores fusi-

Table 1. Sources and accession numbers of own isolates and sequences used in this study.

Taxon		Source*	Substrate and Locality	GenBank accession numbers	
Teleomorph	Anamorph			Ascospore isolate	Conidial isolate
<i>Chaetosphaeria ciliata</i> Réblová & Seifert	<i>Menispora ciliata</i> Preuss	CBS 122131	decayed wood of <i>Acer campestre</i> , Czech republic	EU 488736	EU 488737
	<i>Menispora manitobaensis</i> B. Sutton	KAS 1603	unidentified decayed wood, Ontario, Canada	-	EU 488738

*KAS = Keith A. Seifert culture collection; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

formes, 14.5-17(-18) × (2.5-)3-4 µm, longit.:latit. 4.5:1, hyalinae, 1(-3)-septatae, verruculose.

Anamorphes *Menispora ciliata* et *Phialophorae similis*.

Anamorph:

A-Anamorph. *Menispora ciliata* Corda, Icon. Fung. 1:16. 1837.

≡ *Eriomene ciliata* (Corda) Maire, Ann. Mycol. 4:329. 1906.

B-Anamorph. *Phialophora*-like.

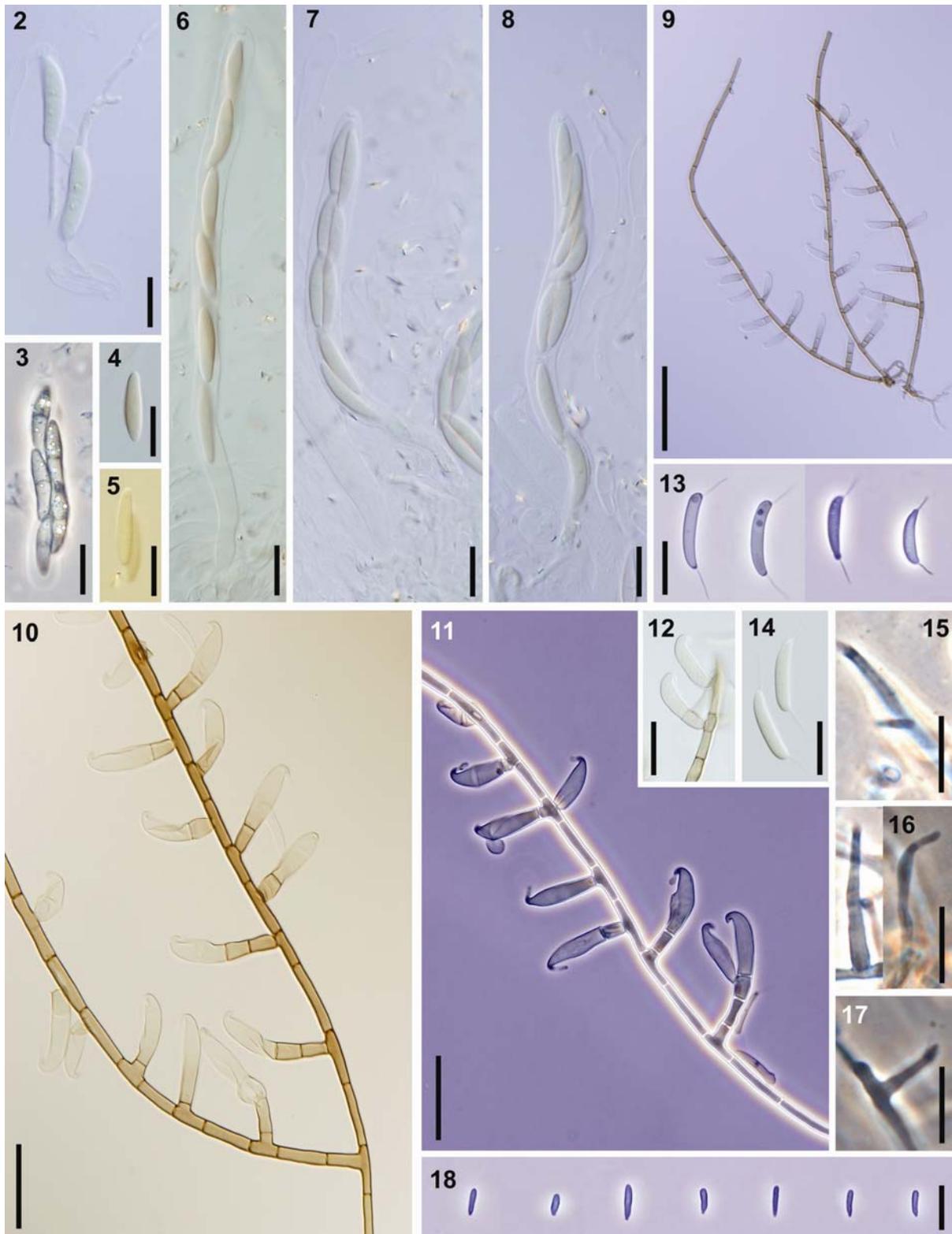
Colonies *in vivo* spreading, inconspicuous to conspicuous, grayish-brown becoming olivaceous after the conidia have dispersed, comprising a dense carpet of independent conidiophores with a mass of slimy conidia in the lower third. No setae are produced independent of the conidiophores.

Perithecia solitary or gregarious, usually near the anamorphic part of the colony but not among the conidiophores, dark brown, opaque, smooth to slightly roughened, superficial, globose to subglobose, 180-250 µm high, 200-250 µm diam, not collapsing when dry, papillate; ostiolum periphysate. *Perithecial wall* fragile, 28-33 µm thick, two-layered. Outer wall *textura angularis*, composed of brown, thin-walled, polyhedral cells with opaque walls. Inner layer of thinner-walled, subhyaline to hyaline elongated and compressed cells. *Paraphyses* persistent, septate, copious, not constricted at the septa, branched, anastomosing, 2.5-3.5 µm wide near the base, tapering to 1.5-2 µm, longer than the asci. *Asci* unitunicate, 8-spored, cylindrical-fusiform, rounded to obtuse at the apex, 78-94(-98) × 7.5-9(-9.5) µm ($\bar{x} \pm S.E. = 89.2 \pm 2.7 \times 8.5 \pm 0.2$), L:B 10.6:1, shortly stipitate, apical annulus distinct, shallow, non-amyloid, 1-1.5 µm high, 2.5-3.5 µm wide. *Ascospores* fusiform, 14.5-17(-18) × (2.5-)3-4 µm ($\bar{x} \pm S.E. = 15.6 \pm 0.1 \times$

3.5 ± 0.1), L:B 4.5:1, hyaline, 1(-3)-septate, finely verruculose, 2-seriate in the ascus.

Conidiophores of A-anamorph (*M. ciliata*) macronematous, septate, brown, paler towards the apex, smooth-walled, up to 800 µm long, 3.5-4.5 µm wide near the base, unbranched, flexuous or irregularly coiled apically, terminating in a sterile subhyaline cell 1.5-2.5 µm wide. *Phialides* develop in the lower portion of the main axis on one or both sides of the conidiophore, 15-27 × 3.5-5.5 µm, cylindrical, hyaline, subhyaline toward the base, with an abruptly tapering apex that is strongly recurved towards the main stipe, conidiogenous aperture 1-1.5 µm wide; arising from 1-3-septate, subhyaline to pale brown metulae (7-)10-25 × 4.5-5 µm, *collarette* shallow, indistinct. *Conidia* falcate 15-18 × 2-3.5 µm ($\bar{x} \pm S.E. = 16.6 \pm 0.3 \times 3.2 \pm 0.1$), hyaline, aseptate, asymmetrical, slightly tapered and pointed at the basal end and obtuse at the other, each polar cell with a single straight or slightly curved setulum, 6-9 µm long, subterminally or terminally inserted on the concave side.

Colonies *in vitro* after 14 d on MEA at 25°C 21-25 mm in diam, convex or cushion-like in the middle, flat at the margin, with felty whitish to pale gray dense aerial mycelium, surrounded by a grey ring of substrate mycelium 3-4 mm wide, margin entire, sometimes gnawed. Colonies sterile (sporulation on PCA after 9 months at 13°C in darkness), reverse inconspicuous. The morphology of conidiophores, phialides and conidia of *M. ciliata* is identical to that observed on the natural substratum. *Conidiophores* up to 700 µm long, 3-4.5 µm diam, rarely branching in the lower part. *Phialides* cylindrical, 16-28 × 5-6.5(-7) µm, hyaline,



Figs 2-18. *Chaetosphaeria ciliata* (from holotype PRM 858075). **2-5.** Ascospores. **6-8.** Asci. **9-11.** Conidiophores of A-anamorph *Menispora ciliata* (*in vitro*, PCA). **12-14.** Conidia of the A-anamorph *M. ciliata* (*in vitro*, PCA). **15-17.** Conidiophores of the B-anamorph phialophora-like (*in vitro*, PCA). **18.** Conidia of the B-anamorph phialophora-like (*in vitro*, PCA). Bars: 2-8, 12-17 = 10 μ m; 9 = 50 μ m; 10, 11 = 20 μ m.

subhyaline towards the base, conidiogenous aperture 1-1.5 μm wide, with a shallow, indistinct collarete, metulae, 1-3-septate, subhyaline, 7-22 \times 4-4.5(-5.5) μm . *Conidia* 10-16.5(-17) \times 2.5-3 μm ($\bar{x} \pm \text{S.E.} = 15.3 \pm 0.5 \times 2.7 \pm 0.1$), setulae 3.5-9 μm long. B-anamorph (phialophora-like): *Phialides* borne directly on aerial mycelium, straight, 0-1-septate, cylindrical towards the base, tapering slightly towards to tip, subhyaline, 8-25 \times 2-2.5 μm , collarete shallow, 1-1.5 μm long, 1-1.6 μm wide. *Conidia* formed in slimy heads, hyaline, ellipsoidal, straight or slightly curved, rounded at distal end, apiculate at proximal end, 5.5-7(-7.5) \times 1-1.5 μm ($\bar{x} \pm \text{S.E.} = 6.3 \pm 0.3 \times 1.5 \pm 0.1$) μm .

Habitat: Saprobic on decayed wood and bark of deciduous trees.

Known distribution: So far known from temperate zone of Northern (Holubová-Jechová, 1973) and Southern Hemispheres (New Zealand, Hughes and Kendrick, 1968).

Material examined: CZECH REPUBLIC, Southern Moravia: Mikulčice, natural reserve Skařiny, on decayed wood of *Acer campestre*, 22 October 2006, M. Réblová (PRM 858075; **holotype**; ex-type culture deposited as CBS 122131); Southern Moravia: Mikulčice, natural reserve Skařiny, on decayed deciduous wood, 24 October 2004, M. Réblová (M.R. 2903/04); obora Soutok near Lanžhot, decayed deciduous wood, 23 October 2004, M. Réblová (M.R. 2909/04 (PRA)); Southern Bohemia: Šumava Mts., Volary district, Mt. Spáleníště near Stožec, decayed wood of a twig of *Acer pseudoplatanus*, 28 August 2000, M. Réblová (M.R. 2441/00 (PRA)); Southern Bohemia: Modrava district, Pytlácký roh, on decorticated wood of a trunk of *Picea abies*, 27 August 2000, M. Réblová (M.R. 1769/00 (PRA)).

Discussion

Chaetosphaeria ciliata is distinguished from the other three species of *Chaetosphaeria* with *Menispora* anamorphs by its aseptate conidia with polar setulae, phialides with the apex recurving downwards towards the conidiophores stipe, and the size of asci and ascospores. Both the asci and ascospores of *Ch. ciliata* are comparatively smaller than those of the other three *Chaetosphaeria* species with *Menispora* anamorph [viz. asci (100-)115-150 \times 8.5-11.5 μm and ascospores 21-29 \times 4-5.5(-6) μm of *Ch. ovoidea*; asci 90-115 \times 7-8.5 μm and ascospores 19-23(-25) \times 3-3.5(-4) μm of

Ch. pulviscula; asci (110-)120-133(-145) \times 12-14 μm and ascospores 19-24 \times 5-6 μm of *Ch. tortuosa*; Réblová *et al.*, 2006]. The finely verruculose ornamentation of the ascospore walls of *Ch. ciliata* was not observed in the other three *Chaetosphaeria* species with *Menispora* anamorphs, which are smooth-walled.

Within *Menispora*, *M. ciliata* belongs to the morphological group of species typically not developing setae independent from conidiophores. On natural substrate, the conidiophores of *M. ciliata* were not observed to branch. On PCA conidiophores sometimes formed simple branches at the lower part of conidiophore, terminated in a sterile subhyaline cell.

Sporulation of *Ch. ciliata* was delayed *in vitro* and conidiophores and conidia of *M. ciliata* and its phialophora-like synanamorph were formed on PCA after 9 months at 13°C in darkness. Of the other three *Chaetosphaeria* species linked with *Menispora*, both *Ch. ovoidea* and *Ch. pulviscula* are known to produce the phialophora-like synanamorph *in vitro* (Réblová, 1998).

Our ITS sequence data clearly show *Ch. ciliata* as a member of the *Menispora*-clade. In our preliminary phylogenetic analysis of *Menispora* and *Chaetosphaeria* with *Menispora* anamorphs based on LSU nrDNA sequence data, all five sampled species form a strongly supported clade (100%); *Ch. ciliata* resides on one branch together with *Ch. pulviscula* (100%) as a sister to a another subclade (100%) containing *Ch. ovoidea* and *Menispora manitobaensis* (100%) together with *Ch. tortuosa* (Réblová and Seifert, unpublished). The *Menispora*-clade is placed within a large clade (100%) containing representatives of *Codinaea*, *Codinaeopsis*, *Dictyochoetopsis*, *Menisporopsis* and *Thozetella*, most of which lack links to teleomorphs.

A key to accepted species of *Menispora* and their teleomorphs was published in Réblová *et al.* (2006).

Acknowledgements

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