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 collarette 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
sporulating colonies of ascospore and conidial isolates yielded *Sphaeria* 100 phialophora-like synanamorph after 9 months. Agreement with those of the genus characters of the unknown fungus are in perfect verruculose ascospores. The morphological fusiform, 1(-3)-septate, hyaline, finely with a shallow apical ring and contained eight to black; the asci were cylindrical-fusiform superficial, globose to subglobose, dark brown with the colony, but were nonstromatic, pyrenomycete. The perithecia were not mixed connection in the genus *Sphaeria* observed a conspicuous colony of *Campestre* (Réblová, 1998). *Menispora caesia* Booth (anamorph: *Menispora 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 *Sphaeria* (Réblová, 2000). In PCA culture, the ascospore and conidial isolates yielded sporulating colonies of *M. ciliata* and its phialophora-like synanamorph after 9 months. 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 *Sphaeria* is proposed. An ITS nrDNA phylogenetic analysis is performed to estimate the relationship of *Ch. ciliata* among other *Sphaeria* 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.,
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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 Centraal-bureau voor Schimmelcultures, Utrecht, The Netherlands (CBS).

All measurements were made in Melzer’s reagent. Means ± 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 Codinaeae-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 µm alta, 200-250 µm diam; ostiolum peripheryatum. Paries peritheci fragments, 28-33 µm crassus, bistratatus. Paraphyses persistentes, copiosae, septatae, 3-3.5 µm latae prope basim, sursum ad 1.5-2 µm, ascos superantes. Asci uniloculi, cylindrico-fusiformes, 78-94(-98) × 7.5-9(-9.5) µm, longit. latit. 10.6:1. Ascospores fusi-
Table 1. Sources and accession numbers of own isolates and sequences used in this study.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Teleomorph</th>
<th>Anamorph</th>
<th>Source*</th>
<th>Substrate and Locality</th>
<th>GenBank accession numbers</th>
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<td><em>Chaetosphaeria ciliata</em></td>
<td>Réblová &amp; Seifert</td>
<td><em>Menispora ciliata</em> Preuss</td>
<td>CBS 122131</td>
<td>decayed wood of <em>Acer campestre</em>, Czech republic</td>
<td>EU 488736 EU 488737</td>
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<td><em>Menispora manitobaensis</em></td>
<td>B. Sutton</td>
<td><em>Menispora ciliata</em></td>
<td>KAS 1603</td>
<td>unidentified decayed wood, Ontario, Canada</td>
<td>EU 488738</td>
</tr>
</tbody>
</table>

*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 *Phialophora similis*.


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 hylaline 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 ascus. 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 (\(\bar{x} \pm S.E. = 15.6 \pm 0.1 \times 3.5 \pm 0.1\)), 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 to pale brown towards the base, with an abruptly tapering apex that is strongly recurved towards the main stipe, conidiogenous aperture 1.1-1.5 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,
subhyaline towards the base, conidiogenous aperture 1-1.5 µm wide, with a shallow, indistinct collar, metulae, 1-3-septate, subhyaline, 7.22 × 4.45(-5.5) µm. Conidia 10-16.5(-17) × 2.5-3 µm (± S.E. = 15.3 ± 0.5 × 2.7 ± 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 × 2.25 µm, collarette 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) × 1-1.5 µm (± S.E. = 6.3 ± 0.3 × 1.5 ± 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 Škaršiny, 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 Škaršiny, 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. Spáleniště 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 asceptate 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 × 8.5-11.5 µm and ascospores 21-29 × 4.5-5(-6) µm of *Ch. ovoidea*; asci 90-115 × 7-8.5 µm and ascospores 19-23(-25) × 3-3.5(-4) µm of *Ch. pulviscula*; asci (110-)120-133(-145) × 12-14 µm and ascospores 19-24 × 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 another subclade (100%) containing *Ch. ovoidea* and *Menispora mantobaeensis* (100%) together with *Ch. tortuosa* (Réblová and Seifert, unpublished). The *Menispora*-clade is placed within a large clade (100%) containing representatives of *Codinaea*, *Codinaceopsis*, *Dictyochaetopsis*, *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).

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References


