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Discovery of the teleomorph of the hyphomycete, *Sterigmatobotrys macrocarpa*, and epitypification of the genus to holomorphic status

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Abstract: *Sterigmatobotrys macrocarpa* is a conspicuous, lignicolous, dematiaceous hyphomycete with macronematous, penicillate conidiophores with branches or metulae arising from the apex of the stipe, terminating with cylindrical, elongated conidiogenous cells producing conidia in a holoblastic manner. The discovery of its teleomorph is documented here based on perithecial ascomata associated with fertile conidiophores of *S. macrocarpa* on a specimen collected in the Czech Republic; an identical anamorph developed from ascospores isolated in axenic culture. The teleomorph is morphologically similar to species of the genera *Carpoligna* and *Chaetosphaeria*, especially in its nonstromatic perithecia, hyaline, cylindrical to fusiform ascospores, unitunicate asci with a distinct apical annulus, and tapering paraphyses. Identical perithecia were later observed on a herbarium specimen of *S. macrocarpa* originating in New Zealand. *Sterigmatobotrys* includes two species, *S. macrocarpa*, a taxonomic synonym of the type species, *S. elata*, and *S. uniseptata*. Because no teleomorph was described in the protologue of *Sterigmatobotrys*, we apply Article 59.7 of the International Code of Botanical Nomenclature. We epitypify (teleotypify) both *Sterigmatobotrys elata* and *S. macrocarpa* to give the genus holomorphic status, and the name *S. macrocarpa* is adopted for the holomorph. To evaluate the ordinal and familial affinities of *Sterigmatobotrys* and its relationships with the morphologically similar genera *Carpoligna* and *Chaetosphaeria*, phylogenetic relationships were inferred based on aligned sequences of the large subunit nuclear ribosomal DNA (ncLSU rDNA).

Key words: Anamorph-teleomorph connection, *Carpoligna*, ncLSU rDNA, phylogeny, *Pleurothecium*, teleotypification.

INTRODUCTION

Sterigmatobotrys, which originally included *S. elata* and *S. papyrogena* (Oudemans 1886), is a conspicuous, cosmopolitan, dematiaceous hyphomycete genus with species occurring on decaying wood in both terrestrial (Sutton 1973, Hughes 1978, Thomas & Polwart 2003) and freshwater (Eaton & Jones 1971, Eaton 1972, Chang 1991, Hyde & Goh 1999, Kane *et al*. 2002) biotopes. It accommodates fungi with macronematous, irregularly biverticillate to terverticillate conidiophores with stout, septate, darkly pigmented stipes and a penicillus consisting of appressed branches and/or whorls of metulae, terminating in polyblastic conidiogenous cells with minute, sympodially arranged denticles, and hyaline, septate conidia that turn brown at maturity and are aggregated in slime.

Despite its distinctive differentiating characters, *Sterigmatobotrys* was transferred to *Stachybotrys* as a subgenus by Rabenhorst (1907). The transfer was apparently based on a portion of the original illustration of *S. elata* (Saccardo 1881: tab. 899), which depicts brown, globose structures that were possibly spores of a different fungus on the original specimen. Hughes (1958) equated *Graphium macrocarpum* (Corda 1839) with *S. elata* (Oudemans 1886) and re-established *Sterigmatobotrys* as a distinct genus, lectotypified by *S. elata,* with *S. macrocarpa* as the name for its type species. The revision of *Sterigmatobotrys* by Jong & Davis (1971) included a re-examination of Corda's type material of *G. macrocarpum* and a taxonomic review of *Sterigmatobotrys* that reconfirmed its status as a distinct genus.

Salonen & Ruokola (1969) introduced a new genus *Gliodendron*, based on *G. balnicola*, found on decaying wood in an old sauna in Finland. Although the conidia were illustrated as hyaline, they were probably immature. Jong & Davis (1971) and Sutton (1973) listed *Gliodendron* as a possible synonym of *Sterigmatobotrys* and *G. balnicola* is likely identical to *S. macrocarpa*, but type material could not be located.

Two species of *Sterigmatobotrys* are accepted in this study and differ in morphology of their conidia. Conidia of *S. macrocarpa* (= *S. elata*) are usually 2-septate, cylindrical to fusiform, hyaline with a truncate base, and there is a considerably protracted maturation of the middle cell, which turns brown. The other accepted species, *S. uniseptata* (Chang 1991), has 1-septate, hyaline conidia. Other species previously described or classified in the genus are discussed in the taxonomy section below.

Neither known *Sterigmatobotrys* species has a reported teleomorph. In a recent collection of *S. macrocarpa* from the Czech Republic, perithecia were found associated with fertile conidiophores. Identical conidiophores were obtained *in vitro* from single ascospore isolates. The teleomorph produces conical to subglobose, dark brown, opaque, nonstromatic perithecia. The asci are cylindrical, shortly stipitate, truncate at the apex with each ascus having a distinct, inamyloid apical annulus. Mature asci contain eight, hyaline, long-fusiform, 3-septate ascospores. Paraphyses are present but seem to disintegrate with age. Our examination of specimens collected in New Zealand and reported by Hughes (1978) uncovered a single specimen of the teleomorph from that country (DAOM 93821); no teleomorphic specimens were found among the abundant Canadian material accessioned in DAOM.

The teleomorph of *S. macrocarpa* morphologically resembles *Carpoligna pleurothecii* (Fernández *et al*. 1999), the teleomorph of the dematiaceous hyphomycete *Pleurothecium recurvatum*.

These fungi share characters such as macronematous, darkly pigmented conidiophores, cylindrical conidiogenous cells with holoblastic conidiogenesis, denticulate, sympodially arranged, broad and conspicuous denticles, and the morphology of asci and ascospores. The teleomorph of *S. macrocarpa* is also reminiscent of several species of *Chaetosphaeria* that have fusiform, hyaline ascospores, and cylindrical asci, *e.g. Chaet. acutata*, *Chaet. fennica* and *Chaet. ovoidea*. *Chaetosphaeria* is linked with 13 anamorphic genera of dematiaceous hyphomycetes producing phialidic conidia and is phylogenetically classified in the *Chaetosphaeriaceae, Chaetosphaeriales* (Réblová *et al*. 1999, Réblová 2000, Réblová & Winka 2000, Fernández *et al*. 2006). The systematic and phylogenetic position of *Carpoligna* is less certain. Based on the ITS rDNA and ncLSU rDNA sequence data, several hypothetical relationships were suggested and tested by Fernández *et al*. (1999), with discussion of possible relationships of *Carpoligna* with the *Microascales* and *Hypocreales*.

Because the teleomorph of *S. macrocarpa* is apparently undescribed and because no teleomorph was described in the protologue of *Sterigmatobotrys* (Oudemans 1886), we emend the generic name *Sterigmatobotrys* by the epitypification of both *S. elata* and *S. macrocarpa* with our teleomorphic specimen from the Czech Republic, applying ICBN Art. 59.7 (McNeill *et al.* 2006). The name *S. macrocarpa* is adopted for the holomorph and the recent herbarium material documenting both morphs designated as an epitype (teleotype) below. With our epitypification, the genus *Sterigmatobotrys* becomes holomorphic with one remaining anamorph-only species included, namely *S. uniseptata*.

The phylogenetic relationships of *Sterigmatobotrys* to other ascomycetes can only be vaguely inferred based on morphological characters of the anamorph, *e.g.* holoblastic conidiogenesis, in combination with the rather undiagnostic teleomorph. The aim of our phylogenetic study is to elucidate the relationship of *Sterigmatobotrys* with the morphologically similar *Carpoligna pleurothecii* and other representative taxa in relevant orders of *Ascomycota*. To evaluate such relationships, phylogenetic analyses were performed based on ncLSU rDNA sequences of ascospore and conidial isolates of terrestrial and freshwater strains of *S. macrocarpa*.

MATERIAL AND METHODS

Morphological observations

Dried herbarium specimens were rehydrated in water. Sections of perithecia, asci, ascospores, paraphyses, conidia, conidiophores, and conidiogenous cells were studied in microscope slide preparations mounted in water, Melzer's reagent, or 90 % lactic acid. Sections of the perithecial wall were made by hand. All measurements were made in Melzer's reagent. Means ± standard errors (s.e.) based on 25 measurements are given for dimensions of asci, ascospores, and conidia. Images were captured in Melzer's reagent using differential interference (DIC) or phase contrast (PC) microscopy using an Olympus DP70 camera operated by Imaging Software Cell* on an Olympus BX51 compound microscope and Olympus SZX12 stereomicroscope. Images were processed with Adobe Photoshop CS4 Extended.

Single ascospores were isolated from fresh material with the aid of a single-spore isolator (Meopta, Prague, Czech Republic). Isolates were grown on potato-carrot agar (PCA) and malt extract agar (2 % MEA) (Gams *et al*. 1998). Colonies were examined at 7, 21, and 30 d after incubation at 25 °C in the dark and under near UV/fluorescent light (12 h light/12 h dark). Cultures are maintained at CBS Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS), and the Canadian Collection of Fungus Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada (DAOM).

Table 1. Sources and accession numbers of isolates sequenced for this study.

Taxon	Source*	Substrate and Locality	GenBank accession numbers LSU
Sterigmatobotrys macrocarpa	DAOM 230059 CBS 113468	Canada, decayed wood in a stream	GU017316
Sterigmatobotrys macrocarpa	PRM 915682	Czech Republic, decayed wood of *Abies alba*	GU017317
Carpoligna pleurothecii	CBS 101581	Czech Republic, decayed wood of *Carpinus betulus*	AF261070
Carpoligna pleurothecii	CBS 101580	Czech Republic, decayed wood of *Carpinus betulus*	GU017318

*CBS = Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; DAOM = Agriculture and Agri-Food Canada Collection, Ottawa, Canada; PRM = Mycological Herbarium National Museum Prague, Czech Republic.

DNA extraction, amplification and sequencing

DNA was isolated with an UltraClean Microbial DNA Kit (MoBio Laboratories, Inc., Canada) using mycelium removed from PCA or MEA cultures following the manufacturer's protocol for filamentous fungi. All PCR experiments were carried out using a PTC-200 thermocycler (MJ Research). PCR reactions containing 2–4 mM $MgSO_4$ were performed using Platinum *Taq* DNA polymerase High Fidelity (Invitrogen) in 25.0 mL volumes. PCR conditions were as follows: 2 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 55–60 °C, and 165–270 s at 68 °C; 10 min at 68 °C. Amplicons were purified using UltraClean PCR Clean-up Kit (MoBio Laboratories, Inc., Canada) following the manufacturer's directions. All nucleotide sequences were obtained by the dideoxy chain-terminating method using ABI PRISM 3100 or ABI PRISM 3130xl automated DNA sequencers (Applied Biosystems). For PCR reactions the following primer pairs were used: ITS5 with LR0R or LR8 (Vilgalys unpubl. data: www.botany.duke.edu/fungi/mycolab, White *et al*. 1990). For sequencing reactions, the primers LR0R, LR3R, LR6, LR7, LR16, LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994, Vilgalys & Sun 1994), JS7 and JS8 (Landvik 1996) were used. Sequences were edited using Sequencher v. 4.9 software (Gene Codes Corporation, Ann Arbor, MI, USA).

Phylogenetic analyses

New ncLSU rDNA sequences of two strains of *S. macrocarpa* and two strains of *Carpoligna pleurothecii* were obtained from ascospore and conidial isolates. New sequences, their sources, and GenBank accession numbers are listed in Table 1; other homologous sequences retrieved from GenBank are given on Fig. 1, mostly from the studies of Huhndorf *et al.* (2004), Réblová & Seifert (2004), Spatafora *et al*. (2006), and Zhang *et al.* (2006).

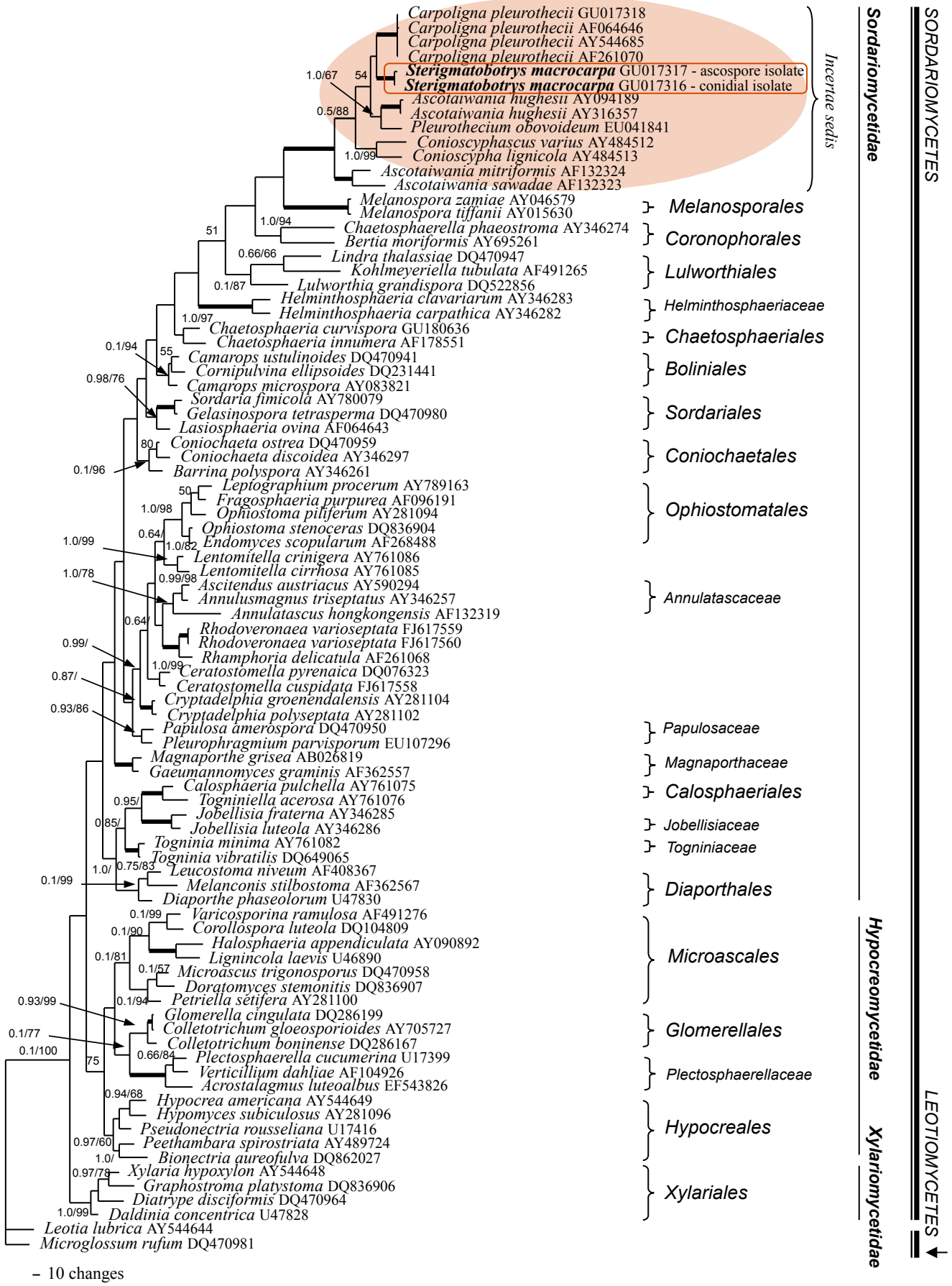

Fig. 1. One of the four most parsimonious trees from a heuristic analysis of ncLSU rDNA sequences from 21 ascomycetous orders and families. Bootstrap support values ≥ 50 % from 1000 replicates of full heuristic search are included at the nodes. Thickened branches indicate posterior probability values = 1.0 pP and 100 % bootstrap support. Posterior probability values < 0.95 pP are shown at the nodes. Branch lengths are drawn to scale.

All sequences were manually aligned in BioEdit v. 7.0.9.0 (Hall 1999). Predicted models of the secondary structure of the LSU rRNA molecules of *Saccharomyces cerevisiae* (Gutell *et al*. 1993) were used to improve decisions on homologous characters.

Phylogenetic relationships were examined using the ncLSU sequences of taxa from 21 orders or families of *Sordariomycetes,* using the outgroup method (Nixon & Carpenter 1993) with two outgroup species, *Leotia lubrica* and *Microglossum rufum* (*Leotiaceae*, *Helotiales*, *Leotiomycetes*). Bases 1–75 were excluded from the analysis because of incompleteness of the 5'-end of most available sequences. The final alignment is deposited in TreeBase (10527).

Maximum parsimony analyses were conducted with PAUP v. 4.0b10 (Swofford 2002). A heuristic search was performed with the stepwise-addition option with 1 000 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 estimated by performing 1 000 bootstrap resamplings using heuristic searches, each consisting of ten random-addition replicates.

Bayesian analysis was performed in a likelihood framework as implemented by MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). The program MrModeltest2 v. 2.3. (Nylander 2008) was used to infer the appropriate substitution model, which would best fit the model of DNA evolution for our sequence data set. Bayesian searches using Metropolis-coupled Markov chain Monte Carlo sampling were conducted. One cold and three heated Markov chains were used. Bayesian analyses were run for 5 million generations, with trees sampled every 1 000 generations. The first 20 000 trees, representing the burn-in phase, were discarded. To estimate posterior probabilities (PP) of recovered branches (Larget & Simon 1999), 50 % majority rule consensus trees were produced from the remaining trees using PAUP.

RESULTS

The phylogenetic analysis was performed on an alignment consisting of the first 2/3 of the ncLSU region for 87 isolates representing 81 species from 21 ascomycetous families or orders and 1299 total characters: 588 constant, 151 unique, and 485 parsimony informative. A maximum parsimony (MP) heuristic search produced four most parsimonious trees (MPTs) with a length of 3661 steps (CI = 0.297, RI = 0.639, HI = 0.703), one of which is shown in Fig. 1. For the Bayesian analysis, the GTR+I+G substitution model was inferred.

The ascospore (terrestrial) and conidial (freshwater) isolates of *Sterigmatobotrys macrocarpa* (1.0 posterior probabilities/100 % bootstrap support) are shown in a sister relationship to *Carpoligna* (1.0/100) (Fig. 1). Two other holomorphic genera, *Conioscyphascus* with its *Conioscypha* anamorphs (1.0/99) and the paraphyletic genus *Ascotaiwania,* group with the previously mentioned taxa in a robust clade (1.0/100) labeled as *incertae sedis* in the phylogram.

This robust clade is a sister group to the large group consisting of several well-defined orders or families, *viz. Melanosporales* (1.0/100), *Coronophorales* (1.0/94), *Lulworthiales* (0.1/87), *Helminthosphaeriaceae* (1.0/100), *Chaetosphaeriales* (1.0/97), *Coniochaetales* (1.0/96), *Boliniales* (1.0/94), and *Sordariales* (0.98/76).

TAXONOMY

Sterigmatobotrys Oudem., Nederl., Kruidk. Arch. Ser. II, 4: 548. 1886.

Type species: *Stachybotrys elata* Sacc., lectotype chosen by Hughes 1958, p. 814.

= *Stachybotrys* Corda subgenus *Sterigmatobotrys* Oudem., Krypt. Fl. Deutsch. Oesterr. Schweiz, Band I, Abt. 8: 631. 1907.

= *Gliodendron* Salonen & Rucuola, Mycopath. Mycol. Appl. 38: 332. 1969.

The following description of the teleomorph supplements the previous generic concept based on the anamorph (*cf.* Ellis 1971), to provide a holomorphic generic concept:

Perithecia nonstromatic, solitary, dark brown to black, papillate, venter conical to subglobose, superficial, ostiole periphysate. *Perithecial wall* leathery to fragile, two-layered. *Paraphyses* present, septate, hyaline, tapering towards apex, longer than asci. *Asci* unitunicate, cylindrical, 8-spored, truncate at apex, short-stipitate. *Ascospores* fusiform to cylindrical-fusiform, hyaline, 3-septate.

Sterigmatobotrys macrocarpa (Corda) S. Hughes, Canad. J. Bot. 36: 814. 1958. Figs 2, 3.

Basionym: *Graphium macrocarpum* Corda, Icon. Fung. 3: 13. 1839 (**holotype** PRM 155517; **epitype** PRM 915682 designated here).

[≡ *Graphium macrocarpum* Sacc., Mycol. Veneta, p. 187. 1873. *nom. illeg.*, *non G. macrocarpum* Corda 1839].

≡ *Harpographium macrocarpum* (Corda) Sacc., Syll. Fung. 4: 620. 1886.

= *Acrothecium bulbosum* Sacc., Michelia 1: 74. 1877 (**holotype** PAD, examined by us and Hughes 1958).

= *Stachybotrys elata* Sacc., Michelia 2: 560. 1882 (**lectotype** *Fungi Italici Autographice Delineati*, Fascs 17-28, Tab. 899. 1881, designated here and reproduced as Fig. 4, excluding the five dark globose spores in the centre of the figure; **epitype** PRM 915682 designated here).

≡ *Sterigmatobotrys elata* (Sacc.) Oudem., Nederl. Kruidk. Arch. Ser. II, 4: 548. 1886.

≡ *Phragmostachys elata* (Sacc.) Costantin, Les Mucédinées Simples: 97. 1888, as '*Phragmostachys atra*'. A *lapsus calami* for the species epithet *fide* Bisby (1943).

= *Atractina biseptata* Höhn., Hedwigia 43: 298. 1904 (holotype not examined by us or Hughes 1958).

= *Gliodendron balnicola* Salonen & Ruokola, Mycopath. Mycol. Appl. 38: 332. 1969. (holotype not traced).

Synonymy adapted from Hughes (1958) and Sutton (1973).

Perithecia 300–450 µm high, 380–500 µm diam, nonstromatic, solitary to gregarious, semi-immersed to superficial, dark brown to black, venter conical to subglobose, with a beak or short obtuse neck, with dark brown to black *ca.* 3–4 µm wide hairs at base, attached tightly to substratum. *Perithecial wall* 25–30 µm thick, leathery to fragile, two-layered, outer layer of *textura prismatica*, composed of dark brown cells, inner layer of *textura prismatica*; cells hyaline, thin-walled, flattened. Ostiole periphysate. *Paraphyses* septate, branched, slightly constricted at septa, *ca.* 4−5 µm wide tapering to *ca.* 2 µm, longer than asci, partially disintegrating with age. *Asci* 165–188 × 10–11 µm (mean ± S.E. = 176.9 ± 2.3 × 10.6 ± 0.1 µm), unitunicate, arising from croziers, cylindrical, ascal apex truncate with a distinct refractive, inamyloid apical annulus *ca.* 3 µm diam and 1.5 µm high. *Ascospores* 29–34.5(–36) × 4–5(–5.5) µm (mean ± S.E. = 32.6 ± 0.4 × 4. 7 ± 0.1 µm), fusiform to cylindrical-fusiform, narrowly rounded at ends, sometimes slightly flattened at one side, often curved, hyaline, smooth, 3-septate.

Colonies in vivo effuse, brown, hairy. *Conidiophores* up to 325 µm long, 10–13 µm wide, solitary, macronematous, mononematous, arising

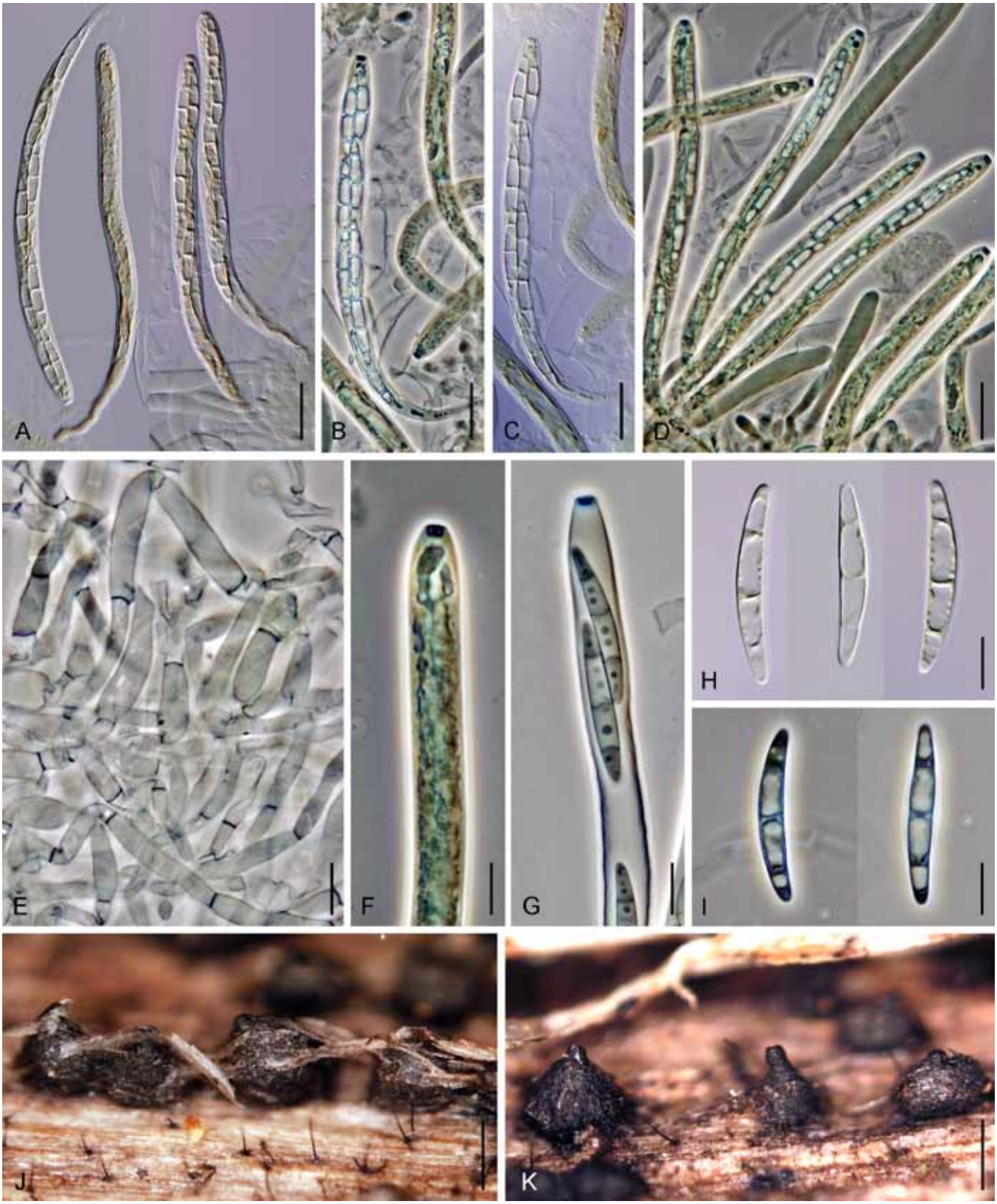

Fig. 2. *Sterigmatobotrys macrocarpa*. A–D. Asci with ascospores. E. Paraphyses. F, G. Asci with a distinct apical annulus. H, I. Ascospores. J, K. Perithecia of the teleomorph associated with conidiophores on the host. A–I from PRM 915682. Scale bars: A–D = 20 µm; E–I = 10 µm; J, K = 250 µm.

from stromatic cells, consisting of a well-defined stipe, terminating in an irregularly biverticillate to terverticillate head. Stipe straight, stout, septate, dark brown, slightly tapering and paler at apex. *Penicillate head* consisting of 2–4 brown branches, sometimes absent, then 2–4 brown to subhyaline metulae with terminally arranged conidiogenous cells. *Metulae* 6.5–13.5 × (2.5–) 3 µm. *Conidiogenous cells* 5–22 × 1.5–3.5 µm (mean ± S.E. = 12.3 ± 3.8 × 2.2 ± 0.6 µm), terminal, more or less parallel, polyblastic, smooth, cylindrical, hyaline, bearing 2–6 sympodially produced denticles from which conidia develop holoblastically. *Conidia* 17–20.5 × 4.5–5.5 µm (mean ± S.E. = 19.2 ± 0.2 × 5 ± 0.06 µm), ellipsoidal to ellipsoidal–fusoid to ellipsoidal–clavoid, apically rounded, with a flat basal scar, 2–3-septate, smooth, hyaline when young, at maturity the middle cell becomes larger and turns brown, often seen anastomosing; aggregated in a hyaline, slimy head.

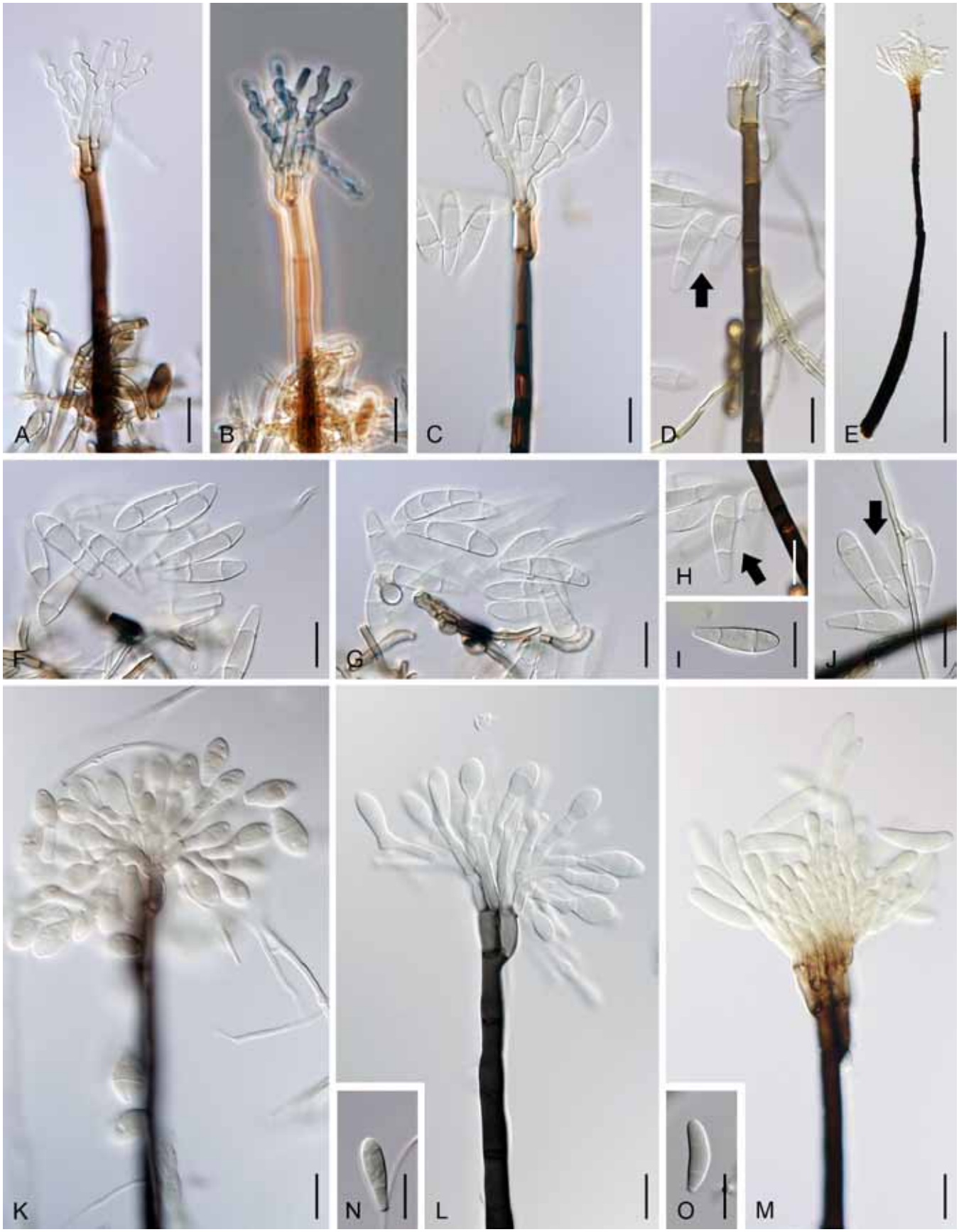

Fig. 3. *Sterigmatobotrys macrocarpa*. A–E, K–M. Conidiophores . F–J, N, O. Conidia. A–D, F–J from culture (PCA, 14 d old, DAOM 230059); E, M, O from the host *Abies alba* (PRM 915682); K, L, N from culture (MEA, 14 d old). Scale bars: A–D, F–O = 20 µm; E = 100 µm.

Colonies in vitro after 30 d on MEA at 25 °C 11–13 mm diam, convex in middle with abundant grayish-brown aerial mycelium, surrounded by a planar zone of sparse black aerial mycelium, margins subsurface; reverse black. Sporulating conidiophores develop throughout colony, more frequently at margins. *Conidiophores* 160–230 × 5.5–7 µm, morphologically identical to those *in vivo* but shorter and thinner. *Conidiogenous cells* 10–16(–25) × 2–3 µm (mean ± S.E. = 15.9 ± 1.5 × 2.7 ± 0.08 µm), identical in shape to those observed *in vivo*. *Metulae* 8–12(–14) × (2.5–)3–4 µm. *Conidia* 13–18 × 5.5–6(–7) µm (mean ± S.E. = 14.9 ± 0.6 × 6.4

Fig. 4. *Sterigmatobotrys macrocarpa*. Illustration of *Stachybotrys elata* (Saccardo, *Fungi Italici Autographice Delineati*, Fascs 17–28, Tab. 899. 1881); selected as a lectotype in this study.

± 0.2 µm), ellipsoidal to obovoidal, apically rounded, truncate at base with a flat basal scar, 1−2-septate, hyaline, smooth. On PCA *conidia* 19–23(–25) × 5–6 µm (mean ± S.E. = 22.2 ± 0.3 × 5.5 ± 0.07 µm), ellipsoidal, ellipsoidal-fusoid to ellipsoidal-clavoid, often slightly curved, apically rounded, truncate at base with a flat basal scar. *Chlamydospores* not observed.

Specimens examined: **Canada**, Ontario, Madawaska Highlands, Morrow Creek Trail, 9 May 2001, on submerged decayed wood in a stream (developing in a damp chamber), K.A. Seifert no. 1421, culture deposited as DAOM 230059, CBS 113468. **Czech Republic**, Šumava Mts. National park, Jilmová skála near Zátoň, 1 Oct. 2007, decaying wood of *Abies alba*, M. Réblová no. 2973, PRM 915682, **epitype designated here** of the holotype of *Graphium macrocarpum* Corda and **lectotype** of *Stachybotrys elata* Sacc.; Prague, Lobkowitz Garden, on a shingle made of pine wood, leg. A.C. Corda, PRM 155517, **holotype** of *Graphium macrocarpum*. **Italy**, Padova, on decaying wood of a trunk, PAD, **holotype** of *Acrothecium bulbosum*. **New Zealand**, North Island, North Auckland, Puketi Forest, 20 June 1963, on *Agathis australis*, leg. S.J. Hughes no. 898, DAOM 93821; Westland Province, Jackson River valley, a track to the Lake Ellery, 33 km SW of Haast, 11 Mar. 2003, on decayed wood, leg. M. Réblová, M.R. 2793, PDD 94360.

Notes: Fertile conidiophores of *Sterigmatobotrys macrocarpa* occur worldwide on wood of coniferous trees, *e.g. Abies*, *Picea,* and *Taxus* in Asia (Taiwan), Europe, North America, and New Zealand (Ellis 1971, Hughes 1978). Although the anamorph is reported from both terrestrial and freshwater biotopes, the teleomorph is known so far only from terrestrial material of *Abies alba* collected in the Czech Republic and *Agathis australis* from New Zealand.

Conidia of *S. macrocarpa* undergo a protracted maturation. The middle cell eventually turns brown, but often only hyaline conidia are observed on the substrate. Mature conidia were not seen either *in vitro* or on recently collected herbarium material. This aspect of conidial maturation was illustrated by Saccardo (1881: tab. 899) and Ellis (1971: 369; fig. 251). Conidia on PCA (Figs 3F–J, M, O) were identical to those found in nature, while conidia observed on MEA (Figs 3K, L, N) were rather obovoidal to obpyriform and significantly shorter, often slightly wider in the middle and usually with one septum; the second septum developed later. Conidia were observed to anastomose in culture (Fig. 3D, H, J, MEA and PCA, DAOM 230059). Conidiophores of *S. macrocarpa* produced in culture were shorter than those on the natural substrate.

No type material of *Stachybotrys elata* is available. The illustration (Saccardo 1881, tab. 899, reproduced here as Fig. 4) accompanying the original description of *S. elata* (Saccardo 1882: 560) is the only surviving original element. Therefore, the illustration of *S. elata* is designated as lectotype. Because the five globose brown structures in the centre of the figure are possibly spores of a different fungus on the original specimen, as noted in the Introduction, we explicitly exclude these from the lectotypification.

Unfortunately our culture of *S. macrocarpa* derived from ascospores (PRM 915682) is no longer viable.

Other species of *Sterigmatobotrys*

Sterigmatobotrys elongata (Peck) Pound & Clem., Minnesota Bot. Stud. 1: 667. 1896.
Basionym: *Stachybotrys elongata* Peck, Annual Rep. New York State Mus. 43: 29. 1890.

Notes: The protologue shows a macronematous, monoverticillate hyphomycete unlikely to be related to *Sterigmatobotrys;* it is perhaps better placed in *Aspergillus*, *Memnoniella,* or *Stachybotrys*.

Sterigmatobotrys papyrogena (Sacc.) Oudem., Ned. Kruidk. Arch., ser. 2, 4: 549. 1886.
Basionym: *Periconia papyrogena* Sacc. Michelia 1: 273. 1878.
≡ *Stachybotrys papyrogena* (Sacc.) Sacc., Syll. Fung. 4: 269. 1886.

Note: This species was considered a synonym of *Memnoniella echinata* by Smith (1962).

Sterigmatobotrys uniseptata H.S. Chang, Mycol. Res. 95: 1142. 1991.

Note: For a description and illustrations, see Chang (1991). The type is on an unidentified decaying twig submerged in a stream from Taiwan and is the only record of *S. uniseptata*.

KEY TO ACCEPTED SPECIES OF *STERIGMATOBOTRYS*

Conidia 2-septate at maturity, middle cell eventually turning brown, ellipsoidal, ellipsoidal-fusoid to ellipsoidal-clavoid, often gently curved, 17–20.5 × 4.5−5.5 µm *in vivo*, slightly smaller *in vitro* ..... *S. macrocarpa*
Conidia 1-septate, rarely 2-septate, at maturity, hyaline, cylindrical to subclavate, 13–17 × 4.5–5.5 µm ..... *S. uniseptata*

DISCUSSION

Although *Sterigmatobotrys macrocarpa* has rather nondescript teleomorphic characters such as dark, non-stromatic perithecia, unitunicate, short-stipitate asci with a distinct apical inamyloid annulus, septate, tapering paraphyses, and fusiform, hyaline, 3-septate ascospores, the experimentally proven connection with its distinctive anamorph makes the holomorph easily identifiable among other perithecial ascomycetes. If the morphologically poorly differentiated teleomorph of *Sterigmatobotrys* was found without its anamorph, it could be easily confused with species of *Carpoligna* or *Chaetosphaeria*. *Carpoligna pleurothecii*, the type and only species of its genus, differs from *S. macrocarpa* by setose papillate perithecia, shorter and wider asci, and shorter and slightly wider ascospores. Distinguishing *Chaetosphaeria* species from *S. macrocarpa* is more difficult, but generally the apical annulus of *Chaetosphaeria* is discoid and less conspicuous than the pronounced apical annulus of species of *Carpoligna* and *Sterigmatobotrys*.

Our ncLSU phylogeny confirms that *Sterigmatobotrys* is closely related to *Carpoligna* and its *Pleurothecium* anamorph and to the anamorphic species *Pleurothecium obovoideum*. *Pleurothecium recurvatum* (teleomorph *Carpoligna pleurothecii*) and *Sterigmatobotrys* share similar patterns of conidial ontogeny and conidiogenous cell morphology, but differ in conidiophore morphology. Cylindrical, prolonged, hyaline, polyblastic conidiogenous cells bearing several conspicuous denticles produced in a sympodial pattern, are typical of *P. recurvatum* (Fernández *et al.* 1999: 256; figs 15–23) and to some extent also *P. obovoideum* (Arzanlou *et al*. 2007: 83; fig. 28). The conidiophore apex of *Sterigmatobotrys* is more complex but could be interpreted as a branched, penicillate derivation of the basic pattern seen in *Pleurothecium*. *Sterigmatobotrys* species form several series of branches and metulae terminating in polyblastic conidiogenous cells that extend sympodially, resulting in a zig-pattern of opened conidiogenous loci. The denticles of *S. macrocarpa* are rather minute and rudimentary compared to those of *P. recurvatum*. In *P. recurvatum* and *S. macrocarpa*, macronematous, dematiaceous conidiophores regularly occur in axenic culture; however, the conidiophores of *P. obovoideum* are reduced to a conidiogenous cell bearing up to three denticles (CBS 209.95; Arzanlou *et al*. 2007).

Similarly complex apical conidiophore branching was reported for synanamorphs of the hyphomycetes *Taeniolella rudis* and *T. longissima* (Hughes 1980, Jones *et al.* 2002). In both species, thick-walled, dark brown, multiseptate macroconidia arise in acropetal chains and produce penicillate conidiophores on a hyaline extension of the apical cell of the terminal macroconidium; the head consists of several metulae with terminally arranged conidiogenous cells that produce hyaline 1- or 2-septate conidia. Preliminary ITS data (Seifert, unpubl. data) suggests that *T. rudis* is closely related phylogenetically to *S. macrocarpum*.

In the ncLSU phylogeny presented here, *Sterigmatobotrys* falls in a robust clade (1.0/100) as a sister to *Carpoligna pleurothecii* and its anamorph *Pleurothecium recurvatum* (1.0/100). Two other holomorphic genera, *Conioscyphascus* and its *Conioscypha* anamorphs (1.0/99) and the paraphyletic genus *Ascotaiwania*, group in this clade. *Ascotaiwania hughesii* with a helicosporous anamorph groups with the asexually reproducing *Pleurothecium obovoideum* (1.0/67), with a sister relationship to *Sterigmatobotrys*, while *Ascotaiwania mitriformis* and *A. sawadae* (1.0/100) with *Monotosporella*-like anamorphs (Ranghoo & Hyde 1998, Sivichai *et al*. 1998) occur at the basal position of the whole *incertae sedis* clade (1.0/100). *Pleurothecium obovoideum* was recently segregated from *Ramichloridium* by Arzanlou *et al.* (2007). In our ncLSU analysis *P. obovoideum* causes paraphyly of *Pleurothecium*; it is clearly segregated from the type species *P. recurvatum*.

The clade labeled *incertae sedis* (Fig. 1) includes four holomorphs described during the last two decades (Sivanesan & Chang 1992, Fernández *et al.* 1999, Réblová & Seifert 2004, Arzanlou *et al.* 2007). Part of this group was discussed by Réblová & Seifert (2004) when the genus *Conioscyphascus* was proposed. They performed a series of constraint analyses based on ncLSU and ncSSU rDNA sequences to test the monophyly of *Conioscyphascus* with the *Glomerellales*, *Hypocreales* and *Microascales,* which were indicated as possible alternative hypotheses. All four teleomorph genera of this clade share similar morphological characters such as nonstromatic perithecia, which are hyaline to pale orange in *Conioscyphascus* or darkly pigmented and opaque in other genera; similar anatomy of the perithecial wall, consisting of several layers of polyhedral cells; apically free, septate paraphyses; unitunicate asci with a distinct, inamyloid apical annulus; and symmetrical, transversely septate ascospores, which are hyaline in *Carpoligna*, *Conioscyphascus* and *Sterigmatobotrys* species but concolourous (pale brown) or bicolorous (brown middle cells, hyaline polar cells) in *Ascotaiwania* species.

The four holomorphic genera of this clade are experimentally linked with anamorphs, but with two different modes of conidiogenesis. The *Conioscypha* anamorphs of *Conioscyphascus* species have an unusual mode of conidiogenesis with multiple, conspicuous collarettes forming a multilamellar structure around a blastic conidiogenous locus producing ellipsoidal to ovoid dark pigmented conidia (Shearer 1973, Réblová & Seifert 2004). Conidiogenesis of the *Pleurothecium* anamorphs of *Carpoligna* and *Sterigmatobotrys* represents a variation of a holoblastic theme. *Pleurothecium recurvatum* and *S. macrocarpa* have rhexolytic conidial secession on polyblastic, denticulate, sympodially proliferating conidiogenous cells. The holoblastic conidiogenesis on wide, conspicuous denticles of *P. recurvatum* is reminiscent of several other hyphomycetes, such as species of *Brachysporium*, in which denticles often remain attached to the conidium, and the tiny denticles of, for example, species of *Dactylaria* or *Pleurophragmium*. *Cryptadelphia* with its *Brachysporium* anamorph and the anamorphic species *Pleurophragmium parvisporum*, recently reinstated and separated from *Dactylaria* by Réblová (2009), grouped near other perithecial ascomycetes that produce anamorphs with holoblastic-denticulate conidiogenesis of phaeoisaria-, ramichloridium- or sporothrix-type, *e.g.* species of *Lentomitella*, *Rhamphoria* or *Rhodoveronaea*. Although *P. parvisporum* can be placed in the family *Papulosaceae* (Réblová 2009) and *Rhodoveronaea* is sister to the *Annulatascaceae*, other morphologically similar anamorphs apparently do not belong in known families, as shown in Fig. 1.

Ascotaiwania hughesii was experimentally linked with a *Helicoön* anamorph identified as conspecific with *H. farinosum* (Fallah *et al*. 1999). The genus *Helicoön* includes about 17 species (Linder 1929, Goos *et al.* 1986, Zhao *et al*. 2007); based on a molecular analysis, it is polyphyletic (Tsui & Berbee 2006). The type species, *H. sessile*, was connected with *Orbilia* of the *Orbiliales* (*Orbiliomycetes*) (Pfister 1997). Other known phylogenetic affinities are with the *Tubeufiaceae* (*H. gigantisporum*), *Pleosporales* (*H. richonis*) or *Dothideomycetes s. lat*. (*H. fuscosporum*). Conidiogenesis in *Helicoön* species is generally monoblastic, but some have conidiogenous cells that extend sympodially once or twice, leaving broad conidiogenous denticles. Their conidium

development suggests that the coiled conidia may have been derived from structures that were originally chlamydospores or aleurioconidia. In this light, we could conclude that *Helicoön*, *Pleurothecium,* and *Sterigmatobotrys* are not homologous anamorphs and that taxonomic evaluations based on direct comparison of these characters may be inappropriate (Seifert & Samuels 2000). However, the connection between *A. hughesii* and *H. farinosum* needs to be reconfirmed.

By accepting *Sterigmatobotrys* as a separate genus morphologically and genetically closely related to *Pleurothecium obovoideum* and *P. recurvatum,* we acknowledge the existence of a characteristic pattern of conidium and conidiogenous cell development in this fungal clade.

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