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 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 differences 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 confirmed its status as a distinct genus.

Salonen & Ruokola (1969) introduced a new genus Gliodendron, based on G. binalicola, 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 ascus 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 ascii 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. fenneri and Chaet. ovoidea. *Chaetosphaeria* is linked with 13 anamorphic genera of dematiaceous hyphomycetes producing phialidic conidia and is phylogenetically classified in the *Chaetosphaeriaceae*, *Chaetosphaeria* (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 teleomorph specimen from the Czech Republic, applying ICBN Art. 59.7 (McNeill et al. 2016). 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 pleurotheci* 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 contrast (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).

**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 MgSO4 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 LROR or LR8 (Vilgalys unpubl. data: www.botany.duke.edu/fungi/mycolab, White et al. 1990). For sequencing reactions, the primers LROR, 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 Sequencer 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 pleurotheci* 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).

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

| Taxon             | Source* | Substrate and Locality | GenBank accession numbers |
|-------------------|---------|------------------------|---------------------------|
| Sterigmatobotrys  |         |                        |                           |
| macrocarpa        | DAOM    | Canada, decayed wood in a stream | GU017316 |
|                  | CBS     | Czech Republic, decayed wood of Abies alba | GU017317 |
| Sterigmatobotrys  |         |                        |                           |
| macrocarpa        | PRM     | Czech Republic, decayed wood of Carpinus betulus | AF261070 |
| Carpoligna pleurotheci | CBS 101581 | Czech Republic, decayed wood of Carpinus betulus | GU017318 |
| Carpoligna pleurotheci | CBS 101580 | Czech Republic, decayed wood of Carpinus betulus | GU017318 |
**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.

| Order/Genus                      | Bootstrap Support | Posterior Probability |
|----------------------------------|-------------------|-----------------------|
| **Melanosporales**               |                   |                       |
| **Coronophorales**               |                   |                       |
| **Lulworthiales**                |                   |                       |
| **Helminthosphaeriaceae**        |                   |                       |
| **Chaetosphaeriaceae**           |                   |                       |
| **Boliniales**                   |                   |                       |
| **Sordariales**                  |                   |                       |
| **Coniochaetales**               |                   |                       |
| **Ophiostomatales**              |                   |                       |
| **Annullatascaeae**              |                   |                       |
| **Papulosaceae**                 |                   |                       |
| **Magnaporthaceae**              |                   |                       |
| **Calosphaeriales**              |                   |                       |
| **Jobellisiasceae**              |                   |                       |
| **Togniniaceae**                 |                   |                       |
| **Diaporthales**                 |                   |                       |
| **Microascales**                 |                   |                       |
| **Glomerellales**                |                   |                       |
| **Pleiotrophaleaceae**           |                   |                       |
| **Hypocreales**                  |                   |                       |
| **Xylariales**                   |                   |                       |

- 10 changes

Strain abbreviations are given in the table above. The phylogenetic tree is rooted with **Leotia lubrica** AY544644, **Microglossum rufum** DQ470981.
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 nclLSU 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 (Leotiacaeae, 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 (Swoford 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 Stachybotrys elata Sacc., lectotype chosen by Hughes 1958, p. 814. One species is characterized by large, some 200–450 μm high, 380–500 μm diam, nonstromatic, solitary, dark brown to black, venter conical to subglobose, superficial, ostiole periphysate. Perithecial wall leathery to fragile, two-layered. Paraphyses present, septate, hyaline, tapering towards apex, longer than asci. Ascii unistromatic, 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).

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, ascus 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.
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.
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
Sterigmatobotrys macrocarpa

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 1983, 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 both 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. 3H, 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, monovercillate 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 | Conidia 1-septate, rarely 2-septate, at maturity, hyaline, cylindrical to subclavate, 13–17 × 4.5–5.5 μm | S. macrocarpa | S. uniseptata |
|---|---|---|---|
| | | | |

**DISCUSSION**

Although Sterigmatobotrys macrocarpa has rather nondescript teleomorphic characters such as dark, non-stromatic perithecia, ununiticate, 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 Carrolpina or Chaetosphaeria. Carpoligna pleurotheci, 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 Carrolpina and Sterigmatobotrys.

Our ncLSU phylogeny confirms that Sterigmatobotrys is closely related to Carrolpina and its Pleurothecium anamorph and to the anamorphic species Pleurothecium obovoideum. Pleurothecium recurvatum (telemorph Carrolpina pleurotheci) and Sterigmatobotrys share similar patterns of conidial ontogeny and conidigenous cell morphology, but differ in conidiphore 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 branches and metulae terminating in polyblastic conidiogenous loci. The denticles of S. macrocarpa are more conspicuous and conidiogenous cells bearing several conspicuous denticles produced in a sympodial pattern, are typical 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 nudis and T. longissima (Hughes 1980, Jones et al. 2002). In both species, thick-walled, dark brown, multisepitate 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. nudis is closely related phylogenetically to S. macrocarpa.

In the ncLSU phylogeny presented here, Sterigmatobotrys falls in a robust clade (1.0/100) as a sister to Carrolpina pleurotheci and its anamorph Pleurothecium recurvatum (1.0/100). Two other holomorph genera, Conioscyphascus and its Conioscypha anamorphs (1.0/99) and the paraphyletic genus Ascutaiwania, group in this clade. Ascotaiwania Hughesi with a helicosporous anamorph group with the asexually reproducing Pleurothecium obovoideum (1.0/67), with a sister relationship to Sterigmatobotrys, while Ascotaiwania mitriformis and A. saywadeae (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 rcsSU 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 with a distinct, inamyloid apical annulus; and symmetrical, transversely septate ascospores, which are hyaline in Carrolpina, Conioscyphascus and Sterigmatobotrys species but concolourous (pale brown) or bicolorous (brown middle cells, hyaline polar cells) in Ascutaiwania 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 conical conidiospore locus producing ellipsoidal to ovoid dark pigmented conidia (Shearer 1973, Réblová & Seifert 2004). Conidiogenesis of the Pleurothecium anamorphs of Carrolpina and Sterigmatobotrys represents a variation of a holoblastic theme. Pleurothecium recurvatum and S. macrocarpa have rheoxylotal conidal saccation 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 Lentomittella, Rhamphoria or Rhodoveronaea. Although P. parvisporum can be placed in the family Papulosacaeae (Réblová 2009) and Rhodoveronaea is sister to the Annulatasaceae, other morphologically similar anamorphs apparently do not belong in known families, as shown in Fig. 1.

Ascotaiwania Hughesi 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 Orbiliaceae (Orbiliomycetes) (Pfister 1997). Other known phylogenetic affinities are with the Tubellaceae (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 Sterigmatobrytras 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. hughesi and H. farinosum needs to be reconfirmed.

By accepting Sterigmatobrytras as a separate genus morphologically and genetically closely related to Pleurothecium obvoideum 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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REFERENCES

Arzaniou M, Groenewald JZ, Gams W, Braun U, Shin HD, Crous PW (2007). Phylogenetic and morpho-reproductive variation of Ramichondrium and allied genera. Studies in Mycology 58: 57–93.

Bisby GR (1943). Stachybrytras. Transactions of British Mycological Society 26: 133–143.

Corda ACJ (1839). Fungi growing on wood in water-cooling towers. II. Fungi growing on wood in different positions in a water cooling system. Material und Organismen 6: 81–92.

Ellis MB (1971). Fungi Canadenses No. 185. National Museum of Canada, Agriculture Canada, Ottawa.

Fernández FA, Lutzoni FM, Huhndorf SM (1999). Teleomorph-anamorph transitions: the new gyromyceorous genus Carpoligna and its Pleurothecium anamorph. Mycologia 91: 251–262.

Fernández FA, Miller AN, Huhndorf SM, Lutzoni FM, Zoller S (2006). Systematics of the genus Chaetosphaeria and its allied genera: morphological and phylogenetic diversity in north temperate and neotropical taxa. Mycologia 98: 121–130.

Gams W, Hoekstra ES, Aptroot A (eds) (1998). CBS Course of Mycology: Central bureau voor Schimmelmilities, Baarn, The Netherlands.

Goos RD, Abdullah SK, Fisher P.J, Webster J (1986). The anamorph genus Helicocon. Transactions of the British Mycological Society 87: 115–122.

Gutell RR, Gray MW, Schirane MN (1993). A compilation of large subunit (23S and 23S-like) ribosomal RNA structures. Nucleic Acids Research 21: 3055–3074.

Hall TA (1999). BioEdit 5.0.9: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Huelsenbeck JP, Ronquist F (2001). MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Huhndorf SM, Miller AN, Fernández FA (2004). Molecular systematics of the Sordariales: the order and the family Lasiostrachyaeaeaeaeae. Mycologia 96: 369–387.

Hughes SJ (1978). Revisions Hymphycomycetum alcyonum sp aperiments de nominibus revisandis. Canadian Journal of Botany 36: 727–836.

Hughes SJ (1978). New Zealand Fungi 25. Miscellaneous fungi. New Zealand Journal of Botany 16: 311–370.

Hyde KD, Goh TK (1999). Fungi on submerged wood from the River Coln, England. Mycological Research 103: 1561–1574.

Jones EBG, Eaton RA, Smolthepol S (2002). Taeniolella rudis and Taeniolella longissima sp. nov. with secondary sympodioconidia from freshwater habitats. Mycologia 43: 201–206.

Jong SC, Davis EE (1971). The genus Sterigmatobrytras. Norwegian Journal of Botany 18: 177–181.

Kane DF, Tam WY, Jones EBG (2002). Fungi colonizing and sporulating on submerged wood in the River Severn, UK. Fungal Diversity 10: 45–55.

Landvik S (1996). Neolecotia, a fruit-body-producing genus of the basidiomycetes, as shown by SSU and LSU DNA sequences. Mycological Research 100: 199–202.

Larget B, Simon DL (1999). Markov Chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Molecular Biology and Evolution 16: 750–759.

Linder HD (1929). A monograph of the helicosporous fungi imperfecti. Annals of the Missouri Botanical Garden 16: 227–348.

McNeil J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, McKee J, Nicolson DH, Prado J, Silva PC, Skog JE, Wiershma JA, Turland NJ (eds.) (2006). International code of botanical nomenclature (Vienna Code): Adopted by the Seventeenth International Botanical Congress Vienna, Austria, July 2005.

Gantner Verlag, Ruggell, Liechtenstein.

Nixon KC, Carpenter JM (1993). On ourcospores. Cladistics 9: 413–426.

Nylander J (2008). MrModeltest v. 2.3. Program distributed by the author. Evolutionary Biology Centre, Uppsala, Sweden.

Oudemans CAJE (1986). Contribution à la flore mycologique des Pays-Bas. XI. Nederlandsch Kruidkundig Archief serie 2, 4: 502–562.

Pfister DH (1997). Castor, Pol lux and life histories of fungi. Mycologia 89: 1–23.

Rabenhorst L (1897). Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. 20. Auflage, Band I. Plte, Abt. 8. Verlag von Eduard Kummer, Leipzig, Germany: 626–631.

Ranghoo VM, Hyde KD (1998). Ascomycetes from freshwater habitats: Ascoscloria aquatica gen. et sp. nov. and a new species of Ascoascia from wood submerged in a reservoir in Hong Kong. Mycologia 90: 1055–1062.

Réblová M (2000). The genus Chaetosphaeria and its anamorphs. Studies in Mycology 45: 149–168.

Réblová M (2000). Teleomorph of Rhodoveronaea (Sordariomycetidae) discovered and re-evaluation of Pleurophagrium. Fungal Diversity 36: 129–139.

Réblová M, Winka K (2000). Phylogeny of Sterigmatobrytras and its anamorphs based on morphological and molecular data. Mycologia 92: 939–954.

Réblová M, Seifert KA (2004). Conioscyphoccus, a new ascomycetous genus for holomorphs with Conioscypha anamorphs. Studies in Mycology 59: 95–108.

Réblová M, Barr ME, Samuels GJ (1999). Chaetosphaeriaceae, a new family for Chaetosphaeria and its allies. Sydowia 51: 49–59.

Rhoder S, Samuels GJ (1994). Taxonomy and phylogeny of Gloidiocadium analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98: 625–634.

Saccardo PA (1881). Fungi italici Autographi. Lieuten. Fascs 17–28: Tabs 641–1120. Patavii, Italy.

Saccardo PA (1882). Fungi Veneti novi vel critici v. Mycologiae Venetiae addendi (adjectis nonnullis extra-Venetia). Series VIII. Michelia 2 (no. 8): 528–563.

Salama A, Rukola AL (1969). Mycoflora of the Finnish “sauna” (bath-house). Mycological Papers 5: 305–307.

Seifert KA, Samuels GJ (2000). How should we look at anamorphs? Studies in Mycology 45: 5–18.

Shearer CA (1973). Fungi of the Chesapeake Bay and its tributaries II. The genus Conioscyphus. Mycologia 65: 128–136.

Sivanesan A, Chang HS (1992). Ascoascidia, a new amphibiphyaeceous ascomycete genus on wood from Taiwan. Mycologia 84: 481–484.

Smith G (1962). Some new and interesting species of microfungi. Transactions of the British Mycological Society 45: 387–394.

Spatafora JW, Sung GH, Johnson D, Hesse C, O’Rourke B, et al. (2006). A five-gene phylogeny of Puccinomycotina. Mycologia 98: 1019–1028.

Sutton BC (1973). Hyphcomycetes from Manitoba and Saskatchewan, Canada. Mycological Papers 132: 1–143.

Swofford DL (2002). PAUP*: Phylogenetic Analysis Using Parsimony (and other methods). Version 4. Sinauer Associates, Sunderland, MA, USA.

Thomas PA, Polwart A (2003). Biological flora of the British Isles. Taxus baccata L. Journal of Ecology 49: 489–524.

Tsui CK, Berbee ML (2000). Phylogenetic relationships and convergence of helicosporous fungi inferred from ribosomal DNA sequences. Molecular Phylogenetics and Evolution 9: 587–597.

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4238–4246.
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Vilgalys R, Sun BL (1994). Ancient and recent patterns of geographic speciation in the oyster mushroom Pleurotus revealed by phylogenetic analysis of ribosomal DNA sequences. *Proceedings of National Academy of Sciences* 91: 4599–4603.

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: A guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). Academic Press, San Diego, California, USA: 315–322.

Zhang N, Castlebury LA, Miller AN, Huhndorf SM, Schoch C, Seifert KA, Rossman AM, Rogers JD, Kohlmeyer J, Volkmann-Kohlmeyer B, Sung GH (2006). An overview of the systematics of the Sordariomycetes based on four-gene phylogeny. *Mycologia* 98: 1077–1088.

Zhao G, Liu X, Wu W (2007). Helicosporous hyphomycetes from China. *Fungal Diversity* 26: 313–524.