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Conioscyphascus, a new ascomycetous genus for holomorphs with *Conioscypha* anamorphs

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Abstract: The new genus *Conioscyphascus* is described for the teleomorph of the hyphomycete *Conioscypha varia*, and a second species, *C. gracilis*, based on *Debaryella gracilis*. Species of the new genus produce inconspicuous, superficial or immersed, ostiolate, smooth, subhyaline to pale orange perithecia with two-layered walls, hyaline, septate paraphyses, 8-spored unitunicate asci with a refractive J– apical annulus, and fusiform 3−7-septate ascospores. Phylogenetic analyses of alignments of large- and small-subunit rDNA sequences suggest a strongly supported, close relationship among the three *Conioscypha* species sampled and *Ascotaiwania* and *Carpoligna*, but the family and order relationships of this clade remain uncertain.

Taxonomic novelties: *Conioscyphascus* Réblová & Seifert gen. nov., *Conioscyphascus varius* Réblová & Seifert sp. nov., *Conioscyphascus gracilis* (Munk) Réblová & Seifert comb. nov.
Key words: *Cryptoleptosphaeria*, *Debaryella*, *Diaporthales*, *Glomerellaceae*, life cycles, LSU and SSU rDNA, phylogeny.

INTRODUCTION

Conioscypha Höhn. is a dematiaceous hyphomycete genus with eight terrestrial and freshwater species inhabiting decayed wood, leaves, or bamboo stems; these species have also been isolated from skin scrapings and hair of living animals (Shearer 1973, Matsushima 1975, Udagawa & Toyazaki 1983, Kirk 1984, Matsushima 1993, 1996, Chen & Tzean 2000). The genus is characterized by an unusual mode of conidiogenesis that includes aspects of both phialidic and annellidic ontogeny (Shearer 1973, Shearer & Motta 1973, Cole & Samson 1979, Goh & Hyde 1998). Conidiogenesis occurs at inconspicuous loci along hyphae; a basipetal succession of blastically produced conidia leave behind conspicuous collarettes that are remnants of the initial outer wall of the conidia; these accumulate centripetally to form a multi-layered collarette appearing similar to annellations (Goh & Hyde 1998). To our knowledge, the ultrastructure of the process has not been studied and there is some conjecture as to whether these conidiogenous cells are really phialides, and how the collarettes are produced. The phylogenetic relationships of *Conioscypha* are unknown and no teleomorph connections have been reported.

On specimens of decorticated, strongly decayed wood collected in central Europe, we encountered a nonstromatic, perithecial ascomycete that produced small, pale orange perithecia with an elongate neck and several-layered wall, unitunicate, long-stipitate asci with a J– apical annulus, and fusiform, septate, hyaline ascospores. No anamorph was noted *in vivo*, but isolated ascospores produced a *Conioscypha* anamorph, apparently identical with *C. varia* Shearer (1973).

The teleomorph of this fungus matches the description of the genus *Debaryella* Höhn. (Höhnel 1904), erected for the single species *D. hyalina* Höhn., a parasite with ascomata that develop in perithecial cavities of the lignicolous, stromatic fungus *Eutypa scabrosa* (Bull.) Fuckel. A second species, *Debaryella vexans* Höhn., a parasite on stromata of wood-inhabiting species of *Valsa* Fr. or *Diaporthe* Nitschke, was added later to the genus and is now considered a synonym of *Cryptonectriella biparasitica* (Höhn.) Weese in the *Diaporthales* (Rossman *et al*. 1999). Munk (1957) suggested that *Debaryella* was diaporthaceous and described a third species, the saprobic, lignicolous fungus *D. gracilis* Munk. Subsequent references to *Debaryella* in the literature are based primarily on the original description of *D. hyalina* (Rogerson 1970, Barr 1978, Samuels 1988). Unfortunately, the type material of *D. hyalina* is depauperate (Kohlmeyer *et al*. 1997, Rossman *et al*. 1999) and the protologue lacks diagnostic features that are critical for its assignment to a family or order. Therefore, Kohlmeyer *et al*. (1997), Rossman *et al*. (1999) and Eriksson (2000) considered *Debaryella* a *nomen dubium*, but the name could be resurrected if suitable material is found to serve as an epitype.

Table 1. List of substrates, localities, sources, relevant sexual and asexual states and accession numbers of taxa sequenced in this study.

Teleomorph	Anamorph	Locality and substrate	Source[a]	GenBank	
				LSU	SSU
Conioscyphascus varius	*Conioscypha varia*	Czech Republic, decayed deciduous wood	CBS 113653	AY484512	AY484511
–	*Conioscypha japonica**	Japan, scrapings and hair of dog	CBS 387.84	AY484514	–
–	*Conioscypha lignicola**	Australia, dead leaf base of *Xanthorrhoea preissii*	CBS 335.93	AY484513	–
Chaetosphaeria curvispora	*Chloridium*-like	New Zealand, decayed decorticated wood	CBS 113644	–	AY502933

*Sequences of conidial isolate. [a]CBS = Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Cryptoleptosphaeria Petr., originally described as a monotypic genus in the *Hypocreales* (Petrak 1923), is also relevant to this discussion. The generic diagnosis of *Cryptoleptosphaeria* is similar to that of *Debaryella,* based on *D. hyalina,* i.e. hyaline, thin-walled ascomata, unitunicate cylindrical asci with J– apical annulus, and long-ellipsoidal to fusiform, septate, hyaline ascospores; the species have a similar ecology. *Cryptoleptosphaeria moravica* Petr., the type species, occurs in locular cavities of a foliicolous, *Leptosphaeria*-like ascomycete. Rossman *et al*. (1999) classified *Cryptoleptosphaeria* in the *Diaporthales* and considered it a relative of *Debaryella*, transferring *D. gracilis* to the genus as *C. gracilis* (Munk) Rossman & Samuels. On the basis of its type material (Czech Republic, Moravia: Hranice na Moravě, Skalická near Bečva, in the ascomata of a *Leptosphaeria*-like fungus, on *Phalaris arundinacea*; FH, isotype), *C. moravica* is not congeneric with *C. gracilis*, differing by its thin, single-layered ascomatal wall, disintegrating paraphyses, asci with an obtuse base that lacks a stipe, and by its parasitic habitat. However, *C. gracilis* is morphologically similar to our undescribed fungus with the *Conioscypha* anamorph, differing only by its smaller asci and ascospores, hyaline to subhyaline, short-papillate perithecia and smaller conidia. A different *Conioscypha* anamorph with smaller conidia, attached to the surface of the perithecia and nearby woody substrate, was also found on the type and other herbarium material of *C. gracilis* preserved at C herbarium.

Cryptoleptosphaeria gracilis and our undescribed fungus with the *Conioscypha varia* anamorph also superficially resemble the foliicolous saprobic genus *Juncigena* Kohlm. *et al*., which includes the single species *J. adarca* Kohlm. *et al*. (*Magnaporthaceae*; Kohlmeyer *et al*. 1997). *Juncigena* differs from the teleomorphs of *Conioscypha* by its brownish ascomata, a centrum with pseudoparaphyses attached at both the bottom and the top of the ascomatal cavity, short-stipitate asci and a helicosporous hyphomycete anamorph, classified in *Cirrenalia* Goos.

Despite the characteristic teleomorphs and anamorphs, we were unable to assign *Cryptoleptosphaeria gracilis* and the undescribed fungus with the *Conioscypha* anamorph to any known genus of nonstromatic, perithecial ascomycetes or an ascomycetous order or family. In this study, we introduce the new genus *Conioscyphascus* for holomorphs with *Conioscypha* anamorphs; *Debaryella* (*Cryptoleptosphaeria*) *gracilis* is transferred to this new genus. A key and taxonomic descriptions for the two accepted species are provided, and we explore their systematic position and phylogenetic relationships using phylogenetic analyses of nuclear small (SSU rDNA) and large subunit ribosomal DNA (LSU rDNA).

MATERIALS AND METHODS

Herbarium material and cultures

Dried herbarium specimens were rehydrated in 3 % (aq.) KOH and studied in water, Melzer's reagent or 90 % lactic acid. All measurements were made in lactic acid. Means ± standard errors (se) are given for spore and ascus dimensions and are based on 20–25 measurements. Images were captured in Melzer's reagent using differential interference microscopy (DIC) and phase contrast (PC) and processed using Adobe Photoshop 6.0 CE.

Single-ascospore isolates were obtained from fresh material with the aid of a single-spore isolator (Meopta). Cultures were grown on potato-carrot agar (PCA, Gams *et al*. 1998). Colony characters were taken from cultures grown on PCA for 14 d at room temperature (about 25 °C) in incident light. Colour codes in descriptions refer to Kornerup & Wanscher (1978).

Cultures are maintained at the Institute of Botany, Academy of Sciences in Průhonice, the Canadian Collection of Fungal Cultures (DAOM) and the Centraalbureau voor Schimmelcultures (CBS). Strains used in this study and their sources are listed in Table 1.

DNA extraction, amplification and sequencing

Methods for DNA extraction, amplification and sequencing of the first two thirds of the LSU (1600 bp, though only 1260 bp were used for the analysis, in order to match the length of other sequences) were identical to those described by Réblová & Seifert (2004).

For amplification and sequencing of the SSU, methods were similar to those of Hambleton *et al.*

(2003), except that UltraClean Microbial DNA Isolation and UltraClean PCR Purification kits (Mo Bio Laboratories Inc., Solana Beach, CA) were used for DNA extraction and cleaning of PCR products, and an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems, Foster City CA) was used for sequencing.

Sequence data analyses

Phylogenetic relationships were examined using 49 LSU rDNA and 106 SSU rDNA sequences retrieved from GenBank, representing 8 or 9 orders of ascomycetes; accession numbers are given on Figs 1–3. Members of the *Dothideomycetes*, *Taphrinomycetes* and *Saccharomycetes* were used as outgroups in the separate maximum parsimony analyses of the LSU and SSU rDNA. The data sets included new LSU and SSU sequences of the fungus described below as *Conioscyphascus varius* (ascospore isolate), *Conioscypha japonica* Udagawa & Toyazaki and *C. lignicola* Höhn. (conidial isolates) and *Chaetosphaeria curvispora* Réblová & Seifert (SSU only).

All sequences were manually aligned in BioEdit 5.0.9 (Hall 1999). Predicted models of the secondary structure of the LSU (Gutell *et al*. 1993) and SSU (Gutell 1993) rRNA molecules of *Saccharomyces cerevisiae* were used to improve the alignment (HEB alignment, Eriksson 2000). The models of the secondary structure of the LSU and SSU rRNA were highly consistent in all taxa and were comparable with that of *Saccharomyces cerevisiae*. The alignments are available in TreeBase as M2046.

Phylogenetic analyses were performed with PAUP 4.0b10 (Swofford 2002) using maximum parsimony (heuristic search with 100 random sequence additions). All analyses were run with gaps treated as missing data. Support for the branches was tested with 1000 replicates of bootstrap and jackknife analyses using fast step-wise addition. Constraint analyses were run using the Kishino-Hasegawa test as implemented in PAUP, with the *Diaporthales*, *Hypocreales*, *Microascales*, *Glomerella* clade, *Plectosphaerella/Verticillium* clade, *Conioscyphascus*, or various interpretations of these groups forced to be distinct and monophyletic, as explained below.

RESULTS

Phylogenetic analysis of the LSU rDNA sequence data

A first maximum parsimony analysis (PA1) was performed using 386 phylogenetically informative characters in an alignment including 1260 bp from 50 taxa. Two most parsimonious trees (MPTs) were obtained, one of them shown in Fig. 1. The trees differed only in the arrangement of branches for *Lanatonectria flavolanata*. The *Xylariales* (79 % bootstrap support / 78 % jackknife support), *Diaporthales* (98/97), *Microascales* (99/100), *Ophiostomatales* (99/98), *Magnaporthaceae* (100/100) and the *Glomerella* clade (89/90) were well-supported clades at the order or family levels. The *Chaetosphaeriales* was strongly supported (99/99). The *Sordariales* appeared as two major discontinuous lineages separated by the *Chaetosphaeriales*; one lineage (65/63) including the *Chaetomiaceae* (*Sordariales*, chaetomiaceous complex *sensu* Huhndorf *et al.* 2004) and the *Sordariaceae* with the poorly supported *Lasiosphaeriaceae* (*Sordariales*, lasiosphaeriaceous complex *sensu* Huhndorf *et al.* 2004) as a second lineage. The *Hypocreales* were poorly supported (65/65). *Conioscyphascus* and *Conioscypha* were in a well-supported clade of ambiguous ordinal affiliation, here called the *Conioscyphascus/Carpoligna/Ascotaiwania* clade (95/95, abbreviated as CCA clade), which was basal to all sampled unitunicate ascomycetes. *Conioscyphascus* (including the two other *Conioscypha* species) (88/86) and two species of *Ascotaiwania* Sivan. & H.S. Chang (with so-called *Monotosporella* S. Hughes anamorphs) (99/99), were sister groups and strongly supported subclades within the clade. *Carpoligna pleurothecii* was basal to the *Conioscyphascus/Conioscypha* clade, but without bootstrap/jackknife support.

LSU sequences of *Ascotaiwania hughesii* Fallah *et al*. (with a *Helicoon* Morgan anamorph) and *A. persoonii* Fallah *et al*. were excluded from PA1 because they required insertions in the stem parts of the secondary structure to align them with the remainder of the data set. A second analysis, PA2, was performed on a reduced data set to assess the phylogenetic relationships of these two additional *Ascotaiwania* species, including only parts of the sequences that could be unambiguously aligned without disrupting the secondary structure. PA2 included 324 phylogenetically informative characters in an alignment of 1270 bp including 30 taxa (including representatives of the *Hypocreales, Microascales, Glomerella* clade, *Chaetosphaeriales*, the CCA clade, and the *Xylariales* with the *Dothideales* as outgroup). Two MPTs were obtained with similar topologies and support to PA1. One of them is shown in Fig. 2. The *Chaetosphaeriales* and the CCA clade were sister groups but with no bootstrap/jackknife support. Within the CCA clade (90/90) (Fig. 2), the *Conioscyphascus/Conioscypha* clade (83/74) and *Ascotaiwania/Monotosporella* clade (100/100) were monophyletic and well-supported. *Ascotaiwania hughesii* and *A. persoonii* were paraphyletic with the other *Ascotaiwania* spp. at the base of the clade, although the taxa were on a single branch with no support.

Phylogenetic analysis of the SSU rDNA sequence data

A third maximum parsimony analysis (PA3) was performed with 567 phylogenetically informative

characters in an alignment of 1781 bp including 108 taxa (tree length 2815, CI = 0.338, RI = 0.725, HI = 0.662). Forty six MPTs (not shown) were obtained, with *Taphrinomycetes* and *Saccharomycetes* as outgroups. Seven ascomycetous classes were included, i.e. *Pezizomycetes*, *Leotiomycetes*, *Sordariomycetes*, *Dothideomycetes*, *Lecanoromycetes*, *Chaetothyriomycetes* and *Eurotiomycetes*. *Conioscyphascus* formed a monophyletic clade within the *Sordariomycetes* (99/100) with *Plectosphaerella cucumerina* (Lindf.) W. Gams, *Verticillium dahliae* and *Volutella colletotrichoides* J.E. Chilton (in a similar arrangement to Fig. 1). This clade was placed within a larger clade including the monophyletic *Glomerella* clade and its two sisters, the *Microascales* and the *Hypocreales*. Therefore, in subsequent analyses we focused only on the class *Sordariomycetes* (including the same sampling of 38 taxa), with representatives of the *Dothideomycetes* chosen as outgroup.

A maximum parsimony analysis (PA4) was performed using the 319 phylogenetically informative characters in an alignment of 1781 bp including 44 taxa. Two MPTs were obtained, which differed only in the grouping of taxa within the *Xylariales*. One of the 2 MPTs is shown in Fig. 3. The well-supported clades were again the *Diaporthales* (100/100), *Microascales* (85/84) and the *Glomerella* clade (97/93). In this analysis, the *Sordariales* (94/93) and *Chaetosphaeriales* (100/100*)* obtained high support. A large monophyletic clade (78/75) contained monophyletic *Hypocreales* (54/54) and *Microascales* (85/84) on one branch, and monophyletic *Glomerella* clade and a clade (84/82) including *P. cucumerina*, *V. dahliae* and *V. colletotrichoides* (100/100) and *Conioscyphascus* on a second branch. This was a sister group to the clade with *Sordariales*, *Diaporthales* and *Xylariales* within the *Sordariomycetes*.

Constraint analyses

The results of the LSU and SSU rDNA analyses provided apparently contradictory hypotheses concerning the sister group and family/order placement of *Conioscyphascus*. In the LSU analysis, the closest relatives to *Conioscyphascus* were *Carpoligna pleurothecii* and two species of *Ascotaiwania* that could not be included in the SSU analysis. These taxa were on a long branch basal to the rest of the unitunicate pyrenomycetes. In the SSU analysis, the closest relatives of *Conioscyphascus* were *Plectosphaerella cucumerina*, and representatives of the *Verticillium dahliae* group, which together were a sister group to the *Glomerella* clade. In other words, when *Carpoligna* and *Ascotaiwania* were present in the data set, they pulled *Conioscyphascus* away from the *Glomerella* clade and the *Plectosphaerella/Verticillium* clade. To determine whether the SSU and LSU analyses were in conflict concerning the relationships of *Conioscyphascus*, we ran several constraint analyses (CA) using monophylies suggested by the SSU to test the robustness of the LSU clades, and *vice versa*.

For the LSU rDNA data set, four CAs were run to test the possible monophyly of the *Conioscyphascus*/*Carpoligna*/*Ascotaiwan*ia clade with *P. cucumerina* and *V. dahliae* (CA1); *P. cucumerina*, *V. dahliae* and the *Glomerella* clade (CA2); *P. cucumerina* and *V. dahliae* with A*scotaiwania* removed (CA3); and *P. cucumerina*, *V. dahliae* and the *Glomerella* clade with *Ascotaiwania* removed (CA4). In CA1, the resulting three trees (not shown) were 12 steps longer and were not considered statistically different from the MPT by the Kishino-Hasegawa (KH) test (P* = 0.3373, 0.3310 and 0.3213). CA2 resulted in two trees (not shown) 10 steps longer than the two MPTs and were not rejected by the KH test (P* = 0.3918 and 0.3620). However, for CA3 and CA4, with the two *Ascotaiwania* species with *Monotosporella* anamorphs excluded from the *Conioscyphascus/Carpoligna* group, the resulting nine and two constraint trees (not shown) were 69 and 63 steps longer; all were rejected by the KH test (P* = 0.0001). Two further CAs were run to test the possible relationship to the *Diaporthales* of *Conioscyphascus* alone (CA5) and to the *Conioscyphascus/Ascotaiwania* clade (CA6), following previous suggestions by Munk (1957) and Rosmann *et al.* (1999) for *C. gracilis*. In CA5 and CA6, the five and seven constraint trees (not shown) were 64 and 73 steps longer than the two MPTs; all were rejected by the KH test (P* = 0.0001).

In the SSU rDNA data set, two CAs were run to assess the inclusion of *Conioscyphascus*, the *Glomerella* clade and the *Plectosphaerella/Verticillium* clade in the *Hypocreales* (CA7), or the *Hypocreales* and *Microascales* (CA8). In CA7, the six trees (not shown) were seven steps longer than the MPT and the KH test did not reject them (P* = 0.4070, 0.3709, 0.3787, 0.3933, 0.3460, 0.3460). In CA8, the fifteen constraint trees (not shown) were 10 steps longer and were also considered acceptable by the KH test (P* values ranged from 0.1492 to 0.2189).

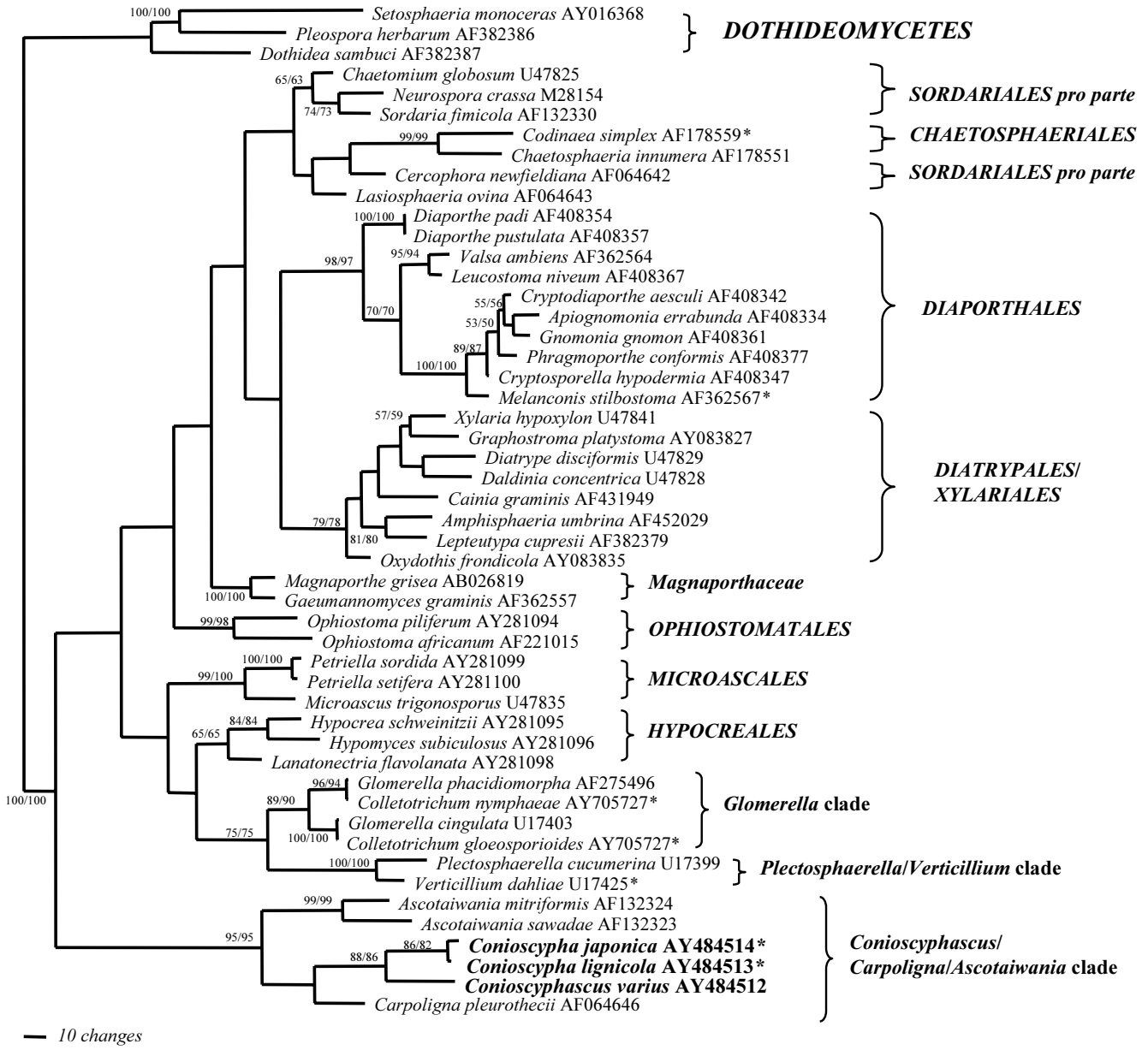


Fig. 1. One of two equally parsimonious trees from a heuristic analysis (PA1) of LSU rDNA sequences from 7 ascomycetous orders. Bootstrap and jackknife values (> 50 %) from 1000 replicates are included at the nodes. The asterisk (*) indicates taxa represented by the anamorph in the phylogeny. Branch lengths are drawn to scale (1821 steps, CI = 0.383, RI = 0.683, HI = 0.617).

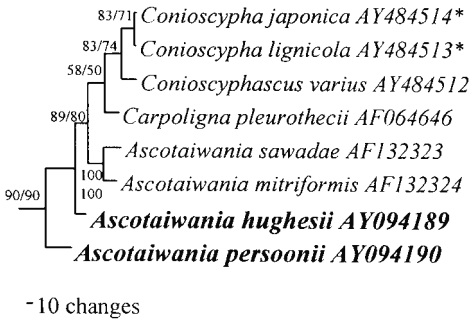


Fig. 2. Part of one of two equally parsimonious trees from a heuristic analysis (PA2) of LSU rDNA sequences from 7 ascomycetous orders, showing the *Conioscyphascus*/*Carpoligna*/*Ascotaiwania* clade (CCA clade). Bootstrap and jackknife values (> 50 %) from 1000 replicates are included at the nodes. The asterisk (*) indicates taxa represented by the anamorph in the phylogeny. Branch lengths are drawn to scale (1225, CI = 0.442, RI = 0.664, HI = 0.558).

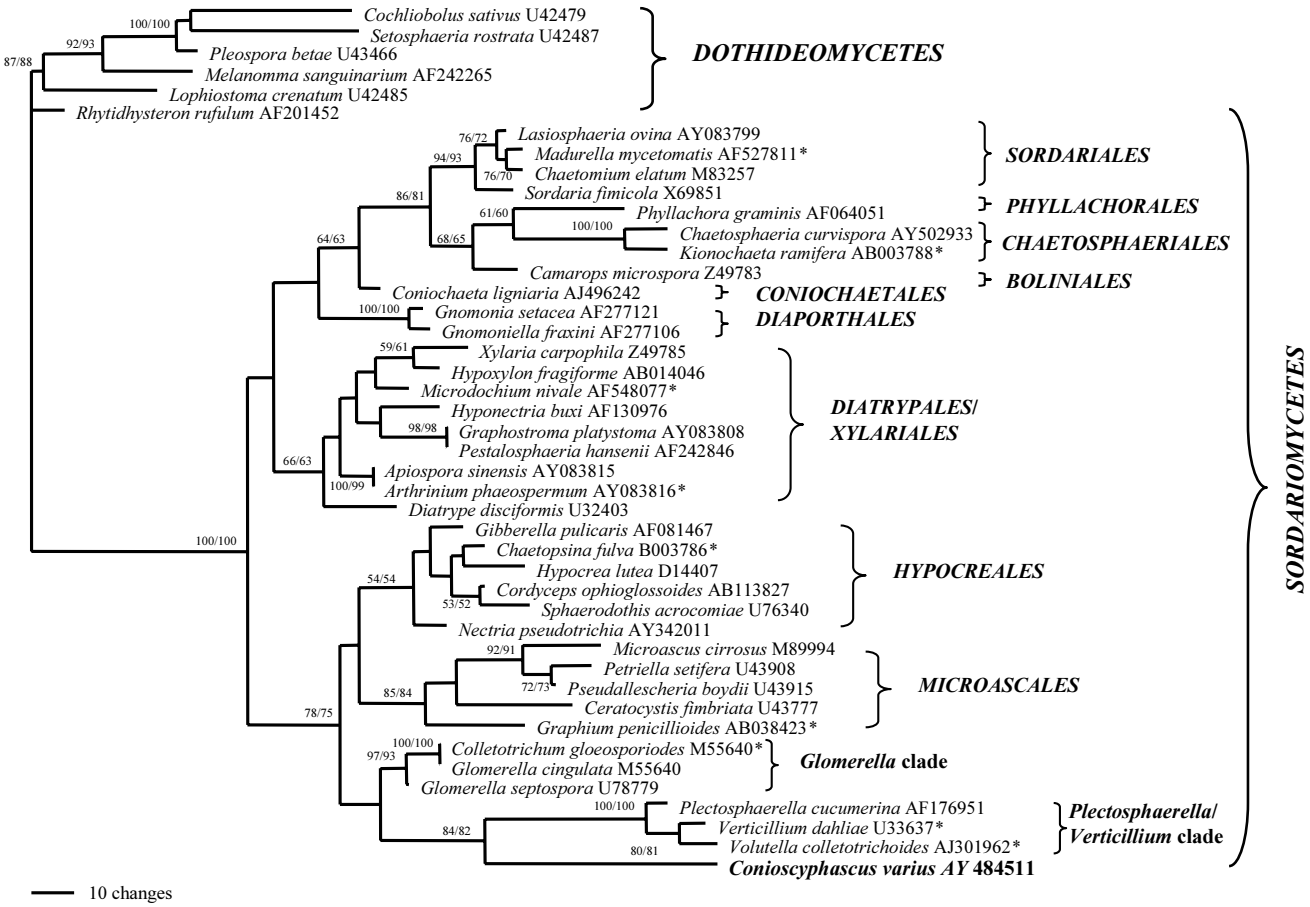


Fig. 3. One of two equally parsimonious trees from a heuristic analysis (PA4) of SSU rDNA sequences from 6 ascomycetous orders. Bootstrap and jackknife values (> 50 %) from 1000 replicates are included at the nodes. The asterisk (*) indicates taxa represented by the anamorph in the phylogeny. Branch lengths are drawn to scale (912 steps, CI = 0.464, RI = 0.738, HI = 0.536).

TAXONOMY

The undescribed perithecial teleomorph of *Conioscypha varia* and two other species, *C. japonica* and *C. lignicola*, the type species of the genus, formed a well-supported, monophyletic clade in all our phylogenetic analyses. This clade could not be assigned unambiguously to a known order or family, and constraint analyses suggested the *Hypocreales*, *Microascales, Glomerellaceae* or the *Plectosphaerella/Verticillium* clade as equally possible closely related taxa. Furthermore, no particular relationship with members of the *Diaporthales* was evident. Because neither mature authenticated material nor other herbarium material of *Debaryella hyalina* are available to confirm the identity and taxonomic position of *Debaryella*, the new genus *Conioscyphascus* is proposed for holomorphs with *Conioscypha* anamorphs. One new species is described and *D. gracilis* is transferred to the new genus.

Conioscyphascus Réblová & Seifert, **gen. nov.** MycoBank MB500024.

Anamorph: *Conioscypha* Höhn., Ann. Mycol. 2: 58. 1904. emend. Shearer, Mycologia 65: 128. 1973.

Etymology: *Conioscypha*, the generic name of the anamorph; *-ascus* (Gk) refer to the part of the organism that reproduces sexually.

Ascomata immersa vel superficialia, singula, conica vel globosa vel subglobosa, subhyalina vel armeniaca, papillata usque ostiolo centrali elongato, cylindraceo, praedita. Paraphyses copiosae, septatae, hyalinae. Paries perithecii coriaceus, bistratosus. Asci cylindraceo-clavati, unitunicati, 8-spori. Ascosporae fusiformes usque naviculares, septatae, non constrictae, hyalinae.

Anamorphe *Conioscypha*. Conidiophora micronematosa, mononematosa. Cellulae conidiogenae hyalinae, intercalares vel ex apice hypharum ortae; conidia liberata collaria multiplicia relinquentia. Conidia brunnea, continua.

Species typica: *Conioscyphascus varius* Réblová & Seifert sp. nov.

Perithecia superficial or immersed, solitary, glabrous, hyaline to subhyaline to pale orange, globose to subglobose with subcylindrical papilla or cylindrical elongated neck, ostiole periphysate. *Perithecial wall* leathery, waxy, two-layered, cells in the outer wall *ca*.

2–2.5 µm diam, thick-walled. *Paraphyses* present, hyaline, septate, partly disintegrating at maturity, broader at the base, tapering towards the tip, apically free, longer than the asci, arising from the ascogenous hyphae. *Asci* unitunicate, cylindrical-clavate, stipitate, apical annulus distinct, refractive, J–. *Ascospores* fusiform to fusiform-navicular, hyaline, 3–7-septate, smooth-walled. *Conidiophores* micronematous, mononematous. *Conidiogenous* cells terminal or intercalary, hyaline, cyathiform to doliiform, with multilayered, hyaline, cup-like collarettes. *Conidia* formed singly and successively by percurrent proliferation of the conidiogenous cell and seceding by apical rupture of the outer wall. Conidia variable in shape, 1-celled, dark brown, sometimes with a central pore at the base.

Type species: *Conioscyphascus varius* Réblová & Seifert sp. nov.

Commentary: *Conioscyphascus* is distinguished from other perithecial ascomycetes by a unique combination of characters, including subhyaline to pale orange perithecia, unitunicate, stipitate, cylindrical-clavate asci with a distinct, J– apical annulus, apically free paraphyses, fusiform, septate, hyaline ascospores, and its *Conioscypha* anamorphs.

The perithecia of *Conioscyphascus* species are subhyaline to pale-coloured, very inconspicuous and scarcely visible on the natural substratum after drying. This might explain the few reports of these fungi (Munk 1957, Minoura & Muroi 1978, Romero 1994).

In both specimens of *C. varius,* we saw evagination of the apical ring outside the apical part of the ascus at maturity, and the ascospores were released through it (Figs 6, 7, 24c). In material of *Debaryella gracilis,* Munk (1957: fig. 72d) observed that the distal part of the ascus broke off along a circular zone to liberate the ascospores. Our study of the type and other material of *D. gracilis* from Munk's herbarium (C) did not confirm this observation. The apical ring of *D. gracilis* has the same structure as the ring of *C. varius* and seems to be fully functional, although evagination was not observed.

The *Conioscypha varia* anamorph obtained *in vitro* from both collections of *C. varius* frequently produced conidia with basal arms (Figs 12, 13). Normally, delimitation of the conidia occurs at the base of the collarette, allowing continued production of conidia. In some conidiogenous apertures, however, delimitation of the conidia occurs in the subtending hypha, effectively locking the conidium into place and preventing any further formation of conidia from that locus (W. Gams, pers. comm.).

The central basal pore of the conidia (Fig. 16) may function during germination, but we did not observe conidia of *C. varia* germinating in culture. Similar pores were also reported for *C. japonica* and *C. taiwaniana* J.L. Chen & S.S. Tzean (Chen & Tzean 2000). The pore on natural material of *C. gracilis* was eccentric, occurring on one side of the conidium at the base.

Key to species of *Conioscyphascus* [For a key to *Conioscypha* species refer to Goh & Hyde (1998).]

1. Perithecia pale orange, long-beaked; ascospores longer than 35 µm; asci 120–160 µm long in *pars sporifera* ..... ***C. varius***
1. Perithecia subhyaline or dirty wood-colour, short papillate; ascospores usually up to 36 µm long; asci 85–120(–150) µm long in *pars sporifera* ..... ***C. gracilis***

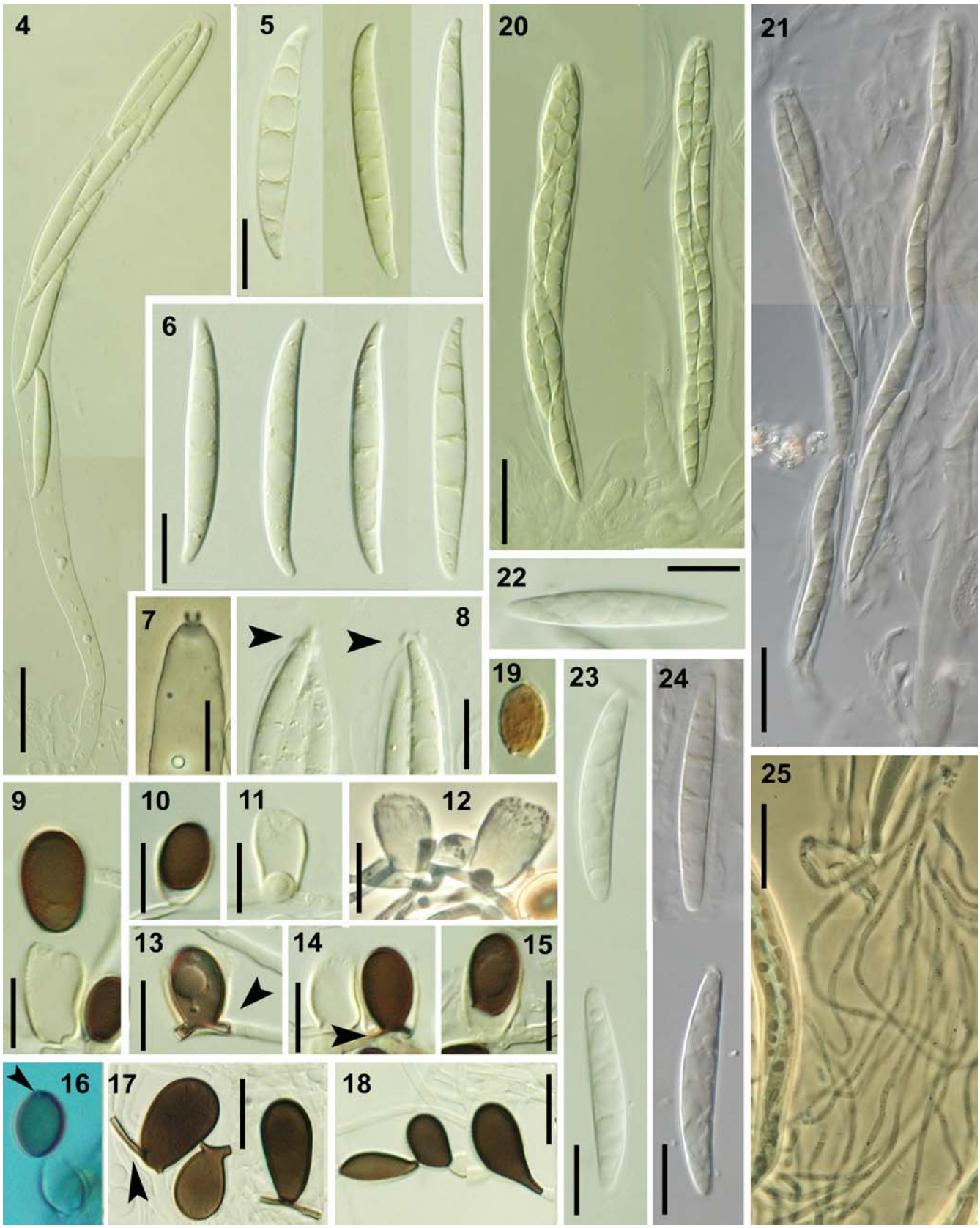
Conioscyphascus varius Réblová & Seifert, **sp. nov.** MycoBank MB500025. Figs 4–18, 26, 27.

Anamorph: *Conioscypha varia* Shearer, Mycologia 65: 133. 1973.

= *Cylicogone regenerans* Emden & Veenb.-Rijks, Acta Bot. Neerl. 22: 637. 1973.

Ascomata collo excepto immersa, singula, globosa vel subglobosa, armeniaca, 350–450 µm diam, 350–460 µm alta, ostiolo centrali elongato, cylindraceo 300–350 µm longo praedita. Paraphyses copiosae, hyalinae. Asci cylindraceo-clavati, 120–160 (mean ± se = 146.5 ± 5) µm longi in parte sporifera, 10–12(–13) (mean ± se = 11.5 ± 0.2) µm lati, stipite (40–)52–80(–95) µm longo, 8-spori. Ascosporae fusiformes usque naviculares, (34–)36–41(–42) (mean ± se = 38.4 ± 0.4) × (3.5–)4–4.5(–5) (mean ± se = 4 ± 0.3) µm, 5–7-septatae, non constrictae, hyalinae.

Anamorphe *Conioscypha varia*. Conidiophora micronematosa, mononematosa. Cellulae conidiogenae hyalinae, intercalares vel ex apice hypharum ortae collis multiplicibus, 11–18 (mean ± se = 14.8 ± 1) µm longis, (6–)8–10 (mean ± se = 8.3 ± 0.5) µm latis. Conidia ellipsoidea, brunnea, ad basim truncata, 10–18(–19) (mean ± se = 14.5 ± 0.7 × 6–10(–11) (mean ± se = 8 ± 0.4) µm, continua, laevia.



Figs 4–25. *Conioscyphascus* spp. 4–18. *Conioscyphascus varius*. 4. Ascus. 5, 6. Ascospores. 7, 8. Apical annulus (arrows indicate evagination of annulus). 9–18. *Conioscypha varia* anamorph, conidia and conidiogenous cells (arrows indicate: 13, 14, 17 conidial arms; 16 basal circular pore), from culture on PCA. 19–25. *Conioscyphascus gracilis.* 19. Conidium of the *Conioscypha* sp. anamorph, from nature. 20, 21. Asci. 22–24. Ascospores. 25. Paraphyses. DIC: 4–6, 8–11, 13–24; PC: 7, 12, 25. *Conioscyphascus varius*: 4–7 from PRM 900537 (holotype), 8 from PRM 901091; 9–11, 13–15, 17, 18 from CBS 113653; 12, 16 from CBS 113654. *Conioscyphascus gracilis*: 20, 22, 23, 25 from C 24673; 21, 24 from C (holotype). Scale bars: Figs 4, 20, 21 = 20 μm; 5–18, 22–25 = 10 μm.

Perithecia completely immersed with only the neck protruding above the surface, solitary, pale orange,

indistinct, collapsing laterally upon drying, globose to subglobose, 350–450 µm diam, 350–460 µm high, glabrous, neck 300–350 µm high, 110–120 µm wide, central, cylindrical, upright. *Perithecial wall* (27–)37–50 µm thick; outer wall of thick-walled, brick-like cells that become thinner towards the interior, inner layer of thin-walled, hyaline, elongated cells; wall in the neck formed of slightly diverging rows of elongated and thick-walled cells, *textura porrecta* to *epidermoidea* in surface view. *Paraphyses* abundant, hyaline, septate, 4–6 µm wide near the base, tapering to 2–2.5 µm, partly disintegrating at maturity. *Asci* cylindrical-clavate, 120–160 (mean ± se = 146.5 ± 5) µm long in *pars sporifera*, 10–12(–13) (mean ± se = 11.5 ± 0.2) µm wide, long-stipitate, stipe (40–)52–80(–95) µm long, L/W 12.8:1 (for asci in *pars sporifera*), truncate to broadly rounded at the apex, apical annulus refractive, J–, distinct, evaginate at maturity outside the ascus, with ascospores discharged through the evaginated ring. Ascospores fusiform-navicular, tapering towards the ends and slightly curved, (34–)36–41(–42) (mean ± se = 38.4 ± 0.4) × (3.5–)4–4.5(–5) (mean ± se = 4 ± 0.3) µm, L/W 9.6:1, hyaline, narrowly rounded at both ends, 5–7-septate, not constricted a the septa, smooth-walled.

Characteristics in culture: *Colony* 12–13 mm diam, whitish to pale yellowish, having a slimy appearance, with a marginal greyish ring because of the dark brown conidia. *Mycelium* usually immersed in the substrate, aerial mycelium scarcely developed and tightly appressed to the surface of the colony; hyphae hyaline, 2–2.5 µm wide, septate, smooth. Sporulation copious, at first limited to a marginal ring, after 1–2 mo sporulation widespread throughout the colony. Margin entire, discrete. Reverse whitish to pale yellowish. *Conidiophores* micronematous, mononematous, arising terminally or laterally, hyaline, reduced to a minute protuberance on the hyphae. *Conidiogenous cells* terminal or intercalary, hyaline, cyathiform, with multilayered, hyaline, cup-like collarette 11–18 (mean ± se = 14.8 ± 1) µm long, (6–)8–10 (mean ± se = 8.3 ± 0.5) µm wide in the flaring distal part, composed of layers of previously ruptured outer walls of conidiogenous cell. *Conidia* ellipsoidal, slightly truncate at the base, apically rounded, 10–18(–19) (mean ± se = 14.5 ± 0.7) µm long, 6–10(–11) (mean ± se = 8 ± 0.4) µm wide, L/W 1.8:1, basal scar 3–4 µm wide, conidia frequently with basal arms, dull brown, aseptate, smooth-walled, with a central, round pore at the base, formed singly and successively by percurrent proliferation of the apex of the conidiogenous cell, separating by apical rupture of the outer wall of the conidiogenous cell. *Chlamydospores* abundant, terminal or intercalary on hyphae, ellipsoidal to obpyriform to irregularly shaped, 6–17 (mean ± se = 12.3 ± 1.1) µm long, 6–10 (mean ± se = 7.4 ± 0.4) µm wide, aseptate, dull brown, smooth-walled.

Known distribution: Czech Republic, U.S.A. (Maryland).

Habitat: Saprobic on decayed wood.

Holotype: **Czech Republic**, Southern Bohemia, Šumava Mts. National Park, Povydří National Nature Reserve, Modrava, brook Zhůřský potok close to Turner's hut, decayed deciduous wood, 27 Oct. 2001, M. Réblová 1890-01 (PRM 900537, **holotype**).

Additional specimen examined: **Czech Republic**, Southern Bohemia, Šumava Mts. National Park, Povydří National Nature Reserve, Čeňkova Pila, decayed wood of *Ulmus glabra*, 27 Aug. 2000, M. Réblová 1676-00 (PRM 901091).

Cultures: CBS 113653 (ex-holotype PRM 900537), CBS 113654 (ex-PRM 901091).

Commentary: The anamorph of *C. varius* formed *in vitro* is identical to *Conioscypha varia,* originally isolated from submerged balsa wood. In the type culture of *C. varia* (Shearer 1973)*,* the conidia varied greatly in shape from ovoidal to flame-shaped to navicular to subellipsoidal and were slightly shorter than those of our strain.

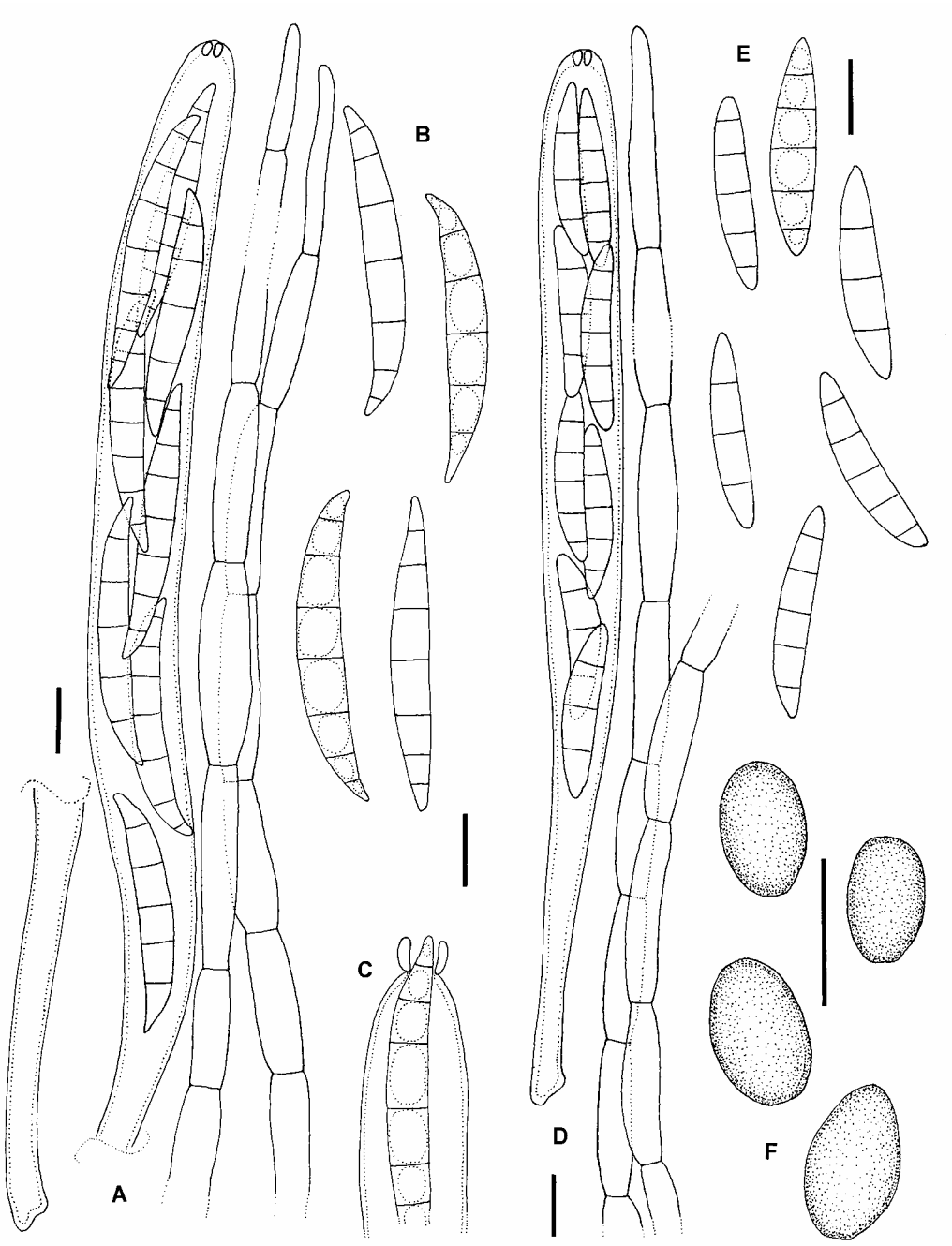


Fig. 26. A–C. *Conioscyphascus varius*. A. Ascus with paraphyses. B. Ascospores. C. Ascal apex with apical annulus during the discharge of ascospore. D–F. *Conioscyphascus gracilis*. D. Ascus with paraphyses. E. Ascospores. F. Conidia of *Conioscypha* anamorph, from nature. A–C from PRM 900537 (holotype); D–F from C (holotype). Scale bars = 10 μm.

Conioscyphascus gracilis (Munk) Réblová & Seifert, **comb. nov.** MycoBank MB500026. Figs 19–25, 26, 28.

≡ *Debaryella gracilis* Munk, Bot. Tidsskr. 51: 226. 1957.

≡ *Cryptoleptosphaeria gracilis* (Munk) Rossman & Samuels, Stud. Mycol. 42: 185. 1999.

Anamorph: *Conioscypha* sp.

Perithecia usually superficial, with the base slightly immersed, rarely completely immersed, solitary, subhyaline to pale yellowish brown to dirty wood-colour, indistinct, collapsing laterally upon drying, subglobose, 170–200 μm diam, 180–250 μm high, glabrous, papilla subcylindrical, broadly rounded to obtuse at the apex. *Perithecial wall* 42–50 μm thick, outer wall of yellowish, thick-walled, polyhedral cells that become more elongated towards the interior; inner layer of thin-walled, hyaline, elongated cells; wall in the papilla formed of slightly diverging rows of elongated, thick-walled cells, *textura porrecta* in surface view. *Paraphyses* abundant, hyaline, filiform, septate, 2.5–3 μm wide near the base, tapering to 1.5–2 μm, longer than the asci. *Asci* cylindrical-clavate, 85–120(–150) (mean ± se = 111 ± 2.7) μm long in *pars sporifera*, 7–10 (mean ± se = 11 ± 0.2) μm wide, stipe 12–27 μm long, L/W 10:1, truncate at the apex,

apical annulus refractive, J–, 3–3.5 µm diam, 1.5–2 µm high. *Ascospores* fusiform, (24–) 26–36(–37) (mean ± se = 30.4 ± 0.6) × (3.5–)4–5 (mean ± se = 4.4 ± 0.06) µm, L/W 7:1, hyaline, straight or slightly curved, narrowly rounded at both ends, 3–5-septate, not constricted at the septa, smooth-walled.

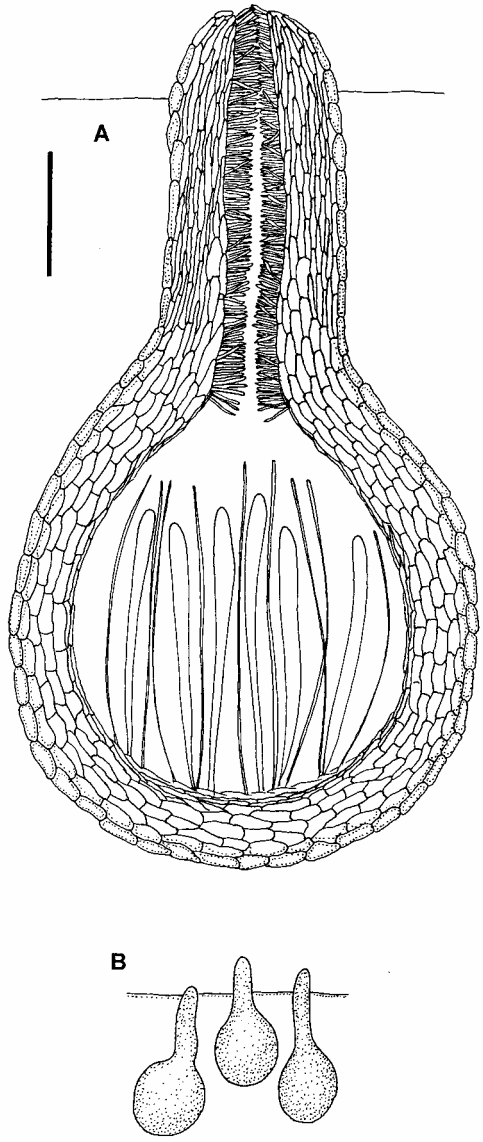


Fig. 27. *Conioscyphascus varius*. A. Median, longitudinal section of perithecium. B. Habit sketch of perithecia. From PRM 900537 (holotype). Scale bar = 100 µm.

Anamorph: Several conidia of a putative *Conioscypha* anamorph were attached to the outer wall of the perithecia. *Conidia* ellipsoidal, slightly truncate at the base, apically rounded and slightly tapering, 8–10 (mean ± se = 9 ± 0.4) µm long, 6–7 (mean ± se = 6.4 ± 0.4) µm wide, L/W 1.3:1, basal scar *ca*. 3 µm wide, dull brown with a pore near the base. Mycelium or conidiogenous cells not observed.

Illustrations and descriptions: Munk (1957: 194, fig. 72), Shearer (1973: 128, figs 3, 7a–d), Minoura & Muroi (1978: 129, fig. 2), Romero (1994: 76, fig. 8a–c, plate IX–1, 2).

Known distribution: Argentina, Denmark, Japan.

Habitat: Freshwater and terrestrial saprobe on decayed wood.

Holotype: **Denmark**, Lunden park in Silkeborg, decayed wood of a stump (?*Viburnum lantana*), associated with *Nectria* sp., 2 Apr. 1954, A. Munk (C).

Additional specimens examined: **Denmark**, Sjælland, Bernstorffsparken, decayed wood of decorticated branch, 23 Mar. 1965, A. Munk 15 (C); Sjælland, Tokkenkøb Hegn, decayed decorticated wood, 20 Jun. 1993, T. Læssøe (C 32138); North Jylland, Høstemark Skov, decorticated wood of twigs and branches of *Ribes rubrum*/*nigrum*, associated with *Nectria* sp., 10 Apr. 1995, T. Læssøe (C 24673).

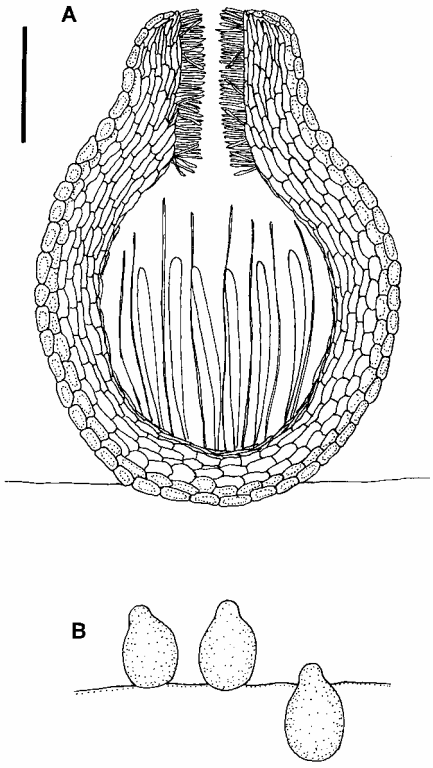


Fig. 28. *Conioscyphascus gracilis*. A. Median, longitudinal section of perithecium. B. Habit sketch of perithecia. From C (holotype). Scale bar = 100 µm.

Commentary: The conidial dimensions of the putative anamorph of *C. gracilis* do not match any of the presently known species of *Conioscypha.* The asci in the type collection of *C. gracilis* were somewhat larger (115–150 µm in *pars spori-fera*) than the asci of other specimens examined (85–106 µm in *pars sporifera*). Larger asci were reported also by Romero (1994, 120–140 µm for the whole ascus) on material from Argentina. Munk (1957) did not report larger asci in the type and reported an ascus size of 80–95 µm in *pars sporifera*.

DISCUSSION

The phylogenetic affinities of the monophyletic clade including *Conioscyphascus*, *Ascotaiwania* and *Carpoligna* (CCA clade) to the other sampled unitunicate ascomycetes remain unclear and the high degree of DNA divergence between the *Conioscyphascus* clade and the other taxa included in this study was unexpected. Despite different grouping of *Conioscyphascus* in the LSU and SSU rDNA analyses, the constraint analyses testing the monophyly of *Conioscyphascus* with the *Hypocreales*, *Microascales*, *Glomerellaceae* and the *Plectosphaerella* clade, resulted in trees that were accepted by the KH test as possible alternative phylogenetic hypotheses.

There are a few common morphological features that unite the genera of the CCA clade: thin-walled unitunicate asci with a distinct, J– apical annulus; true, apically free paraphyses; symmetrical, transversely septate ascospores; absence of stromatic tissue or clypeus and similar anatomies of the perithecial walls. The genera are distinguished by the hyaline ascospores of *Conioscyphascus* and *Carpoligna* versus the concolorous (pale brown) or versicolorous ascospores (brown middle cells, hyaline polar cells) of *Ascotaiwania*. The colour of the perithecial wall also differs, being hyaline or pale orange in *Conioscyphascus* and dark or opaque in *Ascotaiwania* and *Carpoligna.* The anamorphs of these genera represent two different modes of conidiogenesis. The *Conioscypha* anamorphs of *Conioscyphascus* species exhibit a unique mode of conidiogenesis with multiple, conspicuous collarettes forming a multilamellar structure around the blastic (presumed to be phialidic) conidiogenous locus. The other species exhibit variations of holoblastic conidiogenesis. The *Pleurothecium recurvatum* (Höhn.) Morgan anamorph of *Carpoligna pleurothecii* F.A. Fern. *et al*. (Fernández *et al*. 1999) and the *Helicoon farinosum* Linder anamorph of *Ascotaiwania hughesii* Fallah *et al*. (Fallah *et al*. 1999) have rhexolytic conidial secession on denticulate, sympodially proliferating conidiogenous cells. The so-called *Monotosporella* anamorphs of *Ascotaiwania mitriformis* (Ranghoo & Hyde 1998) and *A. sawadae* (Sivichai *et al*. 1998) have a single terminal conidiogenous locus or lateral loci lacking denticles. These latter phragmoconidial anamorphs are aleurioconidium-like, and borne singly on erect, monoblastic conidiogenous cells. They are a poor fit in *Monotosporella*, which otherwise includes species with percurrently proliferating conidiogenous cells, and includes presumed teleomorphs in the *Hysteriaceae* (Hughes 1979). *Acarocybiopsis* J. Mena *et al*. (1999) may be a better fit for these anamorphs, although there are still deviating characters.

Fernández *et al*. (1999) discussed the possible affinities of *Carpoligna* F.A. Fern. & Huhndorf with the *Hypocreales* and *Microascales* on the basis of partial LSU rDNA sequence data. *Carpoligna* has relatively few, rather simple morphological characters, which makes its recognition as a distinct taxon difficult in absence of knowledge of its anamorph. It mimics *Chaetosphaeria* Tul. & C. Tul. in particular, but the *Pleurothecium* Höhn. anamorph differs significantly from the phialidic anamorphs accepted in *Chaetosphaeria* (Réblová & Winka 2000, Réblová 2000).

The structure of the apical annulus of *Conioscyphascus* species is reminiscent of the annulus of some members of the *Diaporthales*. However, a similarly well-developed annulus also occurs in species of *Ascotaiwania* and *Carpoligna,* which are sister groups to *Conioscyphascus* in the phylogenetic analyses. Based on the presently available LSU and SSU rDNA sequence data, *Conioscyphascus* has obviously no affinity to the *Diaporthales* (Figs 1, 3). Members of the *Diaporthales* usually have short-stipitate asci that float free, the centrum is aparaphysate or has paraphysoid tissues that deliquesce early in development, and conidiogenesis is enteroblastic. Rossman *et al*. (1999) placed *Cryptoleptosphaeria* in the *Diaporthales* based on features of the asci and the apical annulus of the type species of *C. moravica*. Our transfer of *C. gracilis* to *Conioscyphascus* leaves *Cryptoleptosphaeria* monotypic; its morphology is fully documented by Rossman *et al*. (1999) based on the type material of *C. moravica.*

Ceratosphaeria Niessl, based on *C. lampadophora* (Berk. & Broome) Niessl, shares hyaline, long-fusiform, septate ascospores with *Conioscyphascus*, as well as unitunicate asci with a well-developed, J– apical annulus, true, apically free paraphyses, and globose to subglobose perithecia with elongated central necks occurring on strongly decayed wood. It differs by its perithecial wall, composed of the darkly pigmented cells with opaque walls, the *Harpophora*-like anamorph, and its phylogenetic affinity (LSU rDNA) with members of the *Magnaporthaceae* (Réblová, unpublished).

Presently, *Ascotaiwania* is considered "*incertae sedis*" on the basis of LSU rDNA analyses (Ranghoo *et al*. 1999, Réblová & Winka 2001). The genus was classified in the *Amphisphaeriaceae sensu lato* by Sivanesan & Chang (1992). Our analysis (Fig. 1) does not support the relationship of *Ascotaiwania* with the *Amphisphaeriaceae* (*Xylariales*), represented in our LSU rDNA analysis by *Amphisphaeria umbrina* (Fr.) De Not., *Lepteutypa cupressi* (Nattrass *et al*.) H.J. Swart and *Oxydothis frondicola* K.D. Hyde. The apical annulus of the ascus of *Ascotaiwania* species was later used as a critical feature to accommodate the genus in the *Annulatascaceae* (Wong *et al*. 1998), a family erected mainly on the basis of a single feature, i.e. a large apical annulus. According to our LSU rDNA analyses, the *Annulatascaceae* are polyphyletic, with the core species having affinities with the

Trichosphaeriales (Réblová & Seifert 2004). Although the *Monotosporella*-like and *Helicoon* anamorphs of the three *Ascotaiwania* species discussed above were obtained *in vitro*, the anamorphs of eight other *Ascotaiwania* species remain unknown (Sivanesan & Chang 1992, Hyde 1995, Chang *et al*. 1998, Fallah *et al*. 1999, Hyde & Goh 1999, Dulymamode *et al*. 2001, Wong & Hyde 2001). The occurrence of two morphologically distinctive types of conidia, conidiophores and conidiogenesis in *Ascotaiwania* suggest that the genus might not be monophyletic as currently circumscribed. However, our parsimony analysis (Fig. 2) supported a relationship of *A. hughesii* (with a *Helicoon* anamorph) and *A. persoonii* (anamorph unknown) with the *Ascotaiwania/Monotosporella* subclade (100/100), although they were basal to that group and *Ascotaiwania* was then paraphyletic with the other taxa in the clade. Sequences of the type species, *A. lignicola* Sivan & H.S. Chang, are unavailable.

It is difficult to find an appropriate group among existing ascomycete orders to accommodate *Conioscyphascus*, *Ascotaiwania* and *Carpoligna*. Because of differences in teleomorph morphologies and in the mode of conidiogenesis associated with certain types of peridial morphology, especially the degree of pigmentation, we can only hypothesize that these three genera may finally represent distinct groups at the family or order level. However, such a hypothesis needs to be tested with sequence data for additional taxa.

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