Teleomorph of *Rhodoveronaea* (Sordariomycetidae) discovered and re-evaluation of *Pleurophragmium*

Martina Réblová[1*]

[1]Department of Plant Taxonomy, Institute of Botany, Academy of Sciences, CZ-252 43 Průhonice, Czech Republic

Réblová, M. (2009). Teleomorph of *Rhodoveronaea* (Sordariomycetidae) discovered and re-evolution of *Pleurophragmium*. Fungal Diversity 36: 129-139.

An undescribed teleomorph was discovered for *Rhodoveronaea* (Sordariomycetidae), a previously known anamorph genus with the single species *R. varioseptata* characterized by pigmented, septate conidia and holoblastic-denticulate conidiogenesis. The teleomorph is a lignicolous perithecial ascomycete with nonstromatic, dark perithecia, consisting of a globose immersed venter and stout conical emerging neck; cylindrical long-stipitate asci, nonamyloid apical annulus and fusiform, septate, hyaline ascospores. The fertile conidiophores of *R. varioseptata* were associated with the perithecia on the natural substratum and they were also obtained *in vitro*. Because no teleomorph was designated in the protologue of *Rhodoveronaea*, the new Article 59.7 of the International Code of Botanical Nomenclature is applied in this case and *Rhodoveronaea* is expanded to holomorphic application by teleomorphic epitypification of the type species *R. varioseptata*. The name *R. varioseptata* is fully adopted for the discovered teleomorph. On the basis of detailed morphology, cultivation studies and phylogenetic analyses of ncLSU rDNA sequences, *Rhodoveronaea* is segregated from the morphologically similar genera *Lentomitella* and *Ceratosphaeria*; its relationship with *Ceratosphaeria fragilis* and *C. rhenana* is discussed. The relationship of *Rhodoveronaea* with the morphologically similar *Dactylaria parvispora* is investigated using morphological features and molecular sequence data. Phylogenetic findings based on ncLSU rDNA sequence data indicate that *Dactylaria* is polyphyletic. The genus *Pleurophragmium* is excluded from the synonymy of *Dactylaria* and is re-evaluated for *P. bicolor* (= *P. parvisporum*), the type species of the genus.

Key words: anamorph-teleomorph relationships, *Ceratosphaeria*, *Dactylaria*, *Ramichloridium*, *Rhamphoria*, systematics

Article Information
Received 13 October 2008
Accepted 15 February 2009
Published online 31 May 2009
*Corresponding author: Martina Réblová; e-mail: Martina.reblova@ibot.cas.cz

Introduction

During a survey of lignicolous perithecial ascomycetes, a fungus morphologically similar to *Lentomitella* Höhn. and *Ceratosphaeria* Niessl was collected on decayed wood of angiosperms in temperate zones of the Northern hemisphere. It possesses dark, nonstromatic perithecia with a globose to subglobose immersed venter and dark stout conical neck emerging above the substratum; the hamathecium consists of persistent paraphyses and periphyses; unitunicate asci have a long dissolving stipe, a distinct nonamyloid apical annulus and eight uniseriately arranged, 4-celled, hyaline, fusiform ascospores. The conidiophores and conidia of *Rhodoveronaea varioseptata* Arzanlou *et al*. (2007) were formed on the natural substratum and *in vitro*. The anamorph is a dematiaceous hyphomycete with holoblastic-denticulate conidiogenesis recently segregated for a veronaea-like isolate phylogenetically related to the *Annulatascaceae* and morphologically distinct from other known hyphomycetes in the *Ramichloridium* complex (Arzanlou *et al*., 2007). *Rhodoveronaea* was described as a monophyletic asexually reproducing taxon and no teleomorph was designated in the protologue.

The unknown perithecial ascomycete, which was found to be the teleomorph of *R. varioseptata*, resembles species of *Lentomitella* in the appearance of the perithecia, morphology of the hamathecium and hyaline ascospores, but the latter taxon can be clearly distinguished from it by short-stipitate asci

with a shallow nonamyloid apical annulus and 1−several-celled, ellipsoidal, uni- or biseriately arranged ascospores. The phaeoisaria-like anamorph was experimentally linked to *L. crinigera* (Cooke) Réblová (Réblová, 2006). Among other perithecial ascomycetes, the unknown fungus also resembles *Ceratosphaeria fragilis* Wilberf. and *Ceratosphaeria rhenana* (Auersw.) Berl. & Voglino, Syll. Fung., addit. 1–4: 164. 1886, *Ceratosphaeria lampadophora* (Berk. & Broome) Niessl, the type species of the genus, is a lignicolous perithecial ascomycete with dark ascomata and elongated neck, cylindrical unitunicate asci with distinct apical annulus, septate, cylindrical to cylindrical-fusiform to cymbiform bi- or triseriately arranged ascospores and a harpophora-like anamorph with phialidic conidiogenesis (Réblová, 2006). *Ceratosphaeria* was confirmed by the ncLSU ribosomal DNA sequence data (ncLSU rDNA) to be accommodated in the *Magnaporthaceae* (Huhndorf *et al*., 2004). Cultural studies or sequence data of any of the other 43 species attributed to *Ceratosphaeria* are, however, not yet available. Comparison of the type material of *Ceratosphaeria fragilis* and the protologue and original illustration of *C. rhenana* confirm that they differ from the undescribed teleomorph of *R. varioseptata* in morphology of perithecia, asci and ascospores.

The taxonomic placement of the undescribed fungus was determined through cultivation studies and a phylogenetic analysis of ncLSU rDNA sequences, which included *Lentomitella*, *Ceratosphaeria*, *Rhodoveronaea* and several other morphologically similar ascomycetes. In order to study the phylogenetic relationship of *R. varioseptata* and the morphologically similar *Dactylaria parvispora* (Preuss) de Hoog & Arx, ncLSU rDNA sequence data were obtained from GenBank. *Pleurophragmium* Costantin, based on its type species, *P. bicolor* Costantin 1888 [= *P. parvisporum* (Preuss 1852) Hol.-Jech.], was transferred to *Dactylaria* Sacc. and accepted as its generic synonym by de Hoog & Arx (1973), but this synonymy was not recognized by other authors. On the basis of LSU phylogeny, the genus *Pleurophragmium* is re-evaluated for its type species; its relationship with *Rhodoveronaea* and other genera of the *Dactylaria* complex is discussed.

Because no legitimate name exists for the holomorph and because no teleomorph was designated in the protologue of *Rhodoveronaea*, the generic name *Rhodoveronaea* is expanded here to holomorphic application and the type species *R. varioseptata* is epitypified (Art. 59.7). The name *R. varioseptata* is fully adopted for the teleomorph and the material documenting both morphs is designated as an epitype (teleotype) in this study.

Materials and Methods

Herbarium material and fungal strains

Dried herbarium specimens were rehydrated in water. Sections of perithecial wall, asci, ascospores and paraphyses were studied in microscope slide preparations mounted in water, Melzer's reagent or 90% lactic acid. All measurements were made in Melzer's reagent. Means ± standard errors (S.E.) based on 25 measurements are given for ascospores, asci and conidia dimensions. Images were captured in Melzer's reagent using differential interference microscopy (DIC) and phase contrast (PC) and processed using Creative Suite 3 Photoshop Extended.

Ascospore isolates (Table 1) were obtained from fresh material with the aid of a single-spore isolator (Meopta, Prague, Czech Republic). Cultures were grown on potato-carrot agar (PCA) and malt extract agar (MEA) (Gams *et al*., 1998). Colonies were examined after 7, 21 and 30 d at 25 C in the dark and under near UV/fluorescent light (12 h light: 12 h dark). Cultures are maintained at CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, and in the Institute of Botany, Academy of Sciences, Průhonice, Czech Republic.

DNA extraction, amplification and sequencing

DNA was isolated with the aid of 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

Table 1. Sources and accession numbers of ascospore isolates used in this study**.**

Taxon	Source*	Substrate and Locality	GenBank accession numbers LSU sequences
Ceratostomella cuspidata	ICMP 17629	New Zealand, decayed wood of *Nothofagus* sp.	FJ617558
Rhodoveronaea varioseptata	CBS 123472	Czech Republic, wood of *Carpinus betulus*	FJ617559
Rhodoveronaea varioseptata	CBS 123473	Sweden, Öland, deciduous wood	FJ617560
Rhamphoria sp.	-	Czech Republic, deciduous wood	FJ617561

*CBS = Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands

carried out using a PTC-200 thermal cycler (MJ Research). PCR reactions containing 2–4 mM $MgSO_4$ were performed using Platinum *Taq* DNA polymerase High Fidelity (Invitrogen) and primer pairs NS5 + LR8 (standard PCR on the chromosomal DNA template) or ITS5 + LR8 (semi-nested 2nd PCR on the dsDNA amplicon obtained by the standard 1st PCR) in 25.0 µL 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 automated DNA sequencers ABI PRISM 3100 or ABI PRISM 3130xl (Applied Biosystems). A portion of the large ribosomal subunit DNA was cycle-sequenced using primers LROR, LR3R, LR7, LR16, LR6 and JS7. Sequences were edited and assembled into larger consensus sequences using Sequencher 4.8 software (Gene Codes Corporation, Ann Arbor, MI).

Sequence data analyses

Two new ncLSU rDNA sequences of the two strains of the *Rhodoveronaea* teleomorph were obtained from the ascospore isolates (Table 1) to investigate its position in the Sordariomycetes; new ncLSU rDNA sequences of *Ceratostomella cuspidata* and *Rhamphoria* sp. (both ascospore isolates) were obtained in this study. Other homologous LSU sequences were retrieved from GenBank: *Amphisphaeria umbrina* AF452029, *Aniptodera chesapeakensis* AF279374, *Annulusmagnus brunneisporus* AY094185, *Annulusmagnus triseptatus* AY59 0287, *Aquaticola ellipsoidea* AY316356, *Aquaticola hyalomura* AY590291, *Arthrobotrys entomopaga* AY965774, *Ascitendus austriacus* AF261067, *Barbatosphaeria barbirostris* EF577059, *Barrina polyspora* AY346261, *Bertia moriformis* AY695261, *Buergenerula spartinae* DQ341492, *Calosphaeria pulchella* AY761075, *Camarops microspora* AY083821, *Camarops ustulinoides* DQ470941, *Cataractispora receptaculorum* AF132327, *Ceratostomella pyrenaica* DQ07 6323, *Ceratosphaeria lampadophora* AY76 1084, *Ceriosporopsis halima* AY490788, *Chaetomium globosum* U47825, *Chaetosphaeria innumera* AF178551, *Chaetosphaerella phaeostroma* AY346274, *Colletotrichum boninense* DQ286167, *Colletotrichum gloeosporioides* AY705727, *Codinaea simplex* AF178559, *Coniochaeta discoidea* AY346297, *Cornipulvina ellipsoides* DQ231441, *Corollospora luteola* DQ104809, *Cryptadelphia groenendalensis* AY281104, *Cryptadelphia polyseptata* AY281102, *Dactylaria monticola* EU107289, *Dactylaria purpurella* EU107301, *Dactylella clavata* EU107319, *Daldinia concentrica* U47828, *Diaporthe pustulata* AF408357, *Diatrype disciformis* U47829, *Dothidea sambuci* AY544681, *Endomyces scopularum* AF268488, *Fragosphaeria purpurea* AF096191, *Gaeumannomyces graminis* AF362557, *Gnomonia gnomon* AF408361, *Glomerella cingulata* DQ286199, *Graphostroma platystoma* AY083827, *Halosphaeria appendiculata* AY090892, *Hypocrea schweinitzii* AY281095, *Hypomyces subicu-losus* AY281096, *Kohlmeyeriella tubulata* AF49 1265, *Lanatonectria flavolanata* AY281098, *Lasiosphaeria ovina* AF064643, *Lentomitella*

cirrhosa AY761085, *Lentomitella crinigera* AY761086, *Leptographium procerum* AY7891 63, *Lepteutypa cupressi* AF382379, *Leucostoma niveum* AF408367, *Lindra thalassiae* DQ470947, *Lulworthia grandispora* DQ5228 56, *Magnaporthe grisea* AB026819, *Melanospora brevirostris* AY015627, *Melanospora zamiae* AY046579, *Microascus trigonosporus* U47835, *Mycosphaerella flexuosa* DQ246232, *Myrmecridium flexuosum* EU041825, *Myrmecridium schulzeri* EU041826, *Neurospora crassa* M38154, *Nitschkia grevillei* AY346294, *Ophiostoma piliferum* AY281094, *Ophiostoma protearum* AF221014, *Orbilia delicatula* AY 261178, *Orbilia luteorubella* DQ480728, *Papulosa amerospora* DQ470950, *Petriella setifera* AY281100, *Pleospora herbarum* AF382386, *Pleurophragmium parvisporum* EU107296, *Pleurostoma ootheca* AY761079, *Pleurostomophora richardsiae* AY249089, *Rhamphoria delicatula* AF261068, *Rhodoveronaea varioseptata* EU041870, *Saccharomyces cerevisiae* J01355, *Setosphaeria monoceras* AY016368, *Sordaria fimicola* AF132330, *Striatosphaeria codinaeophora* AF178546, *Togninia minima* AY761082, *Togninia vibratilis* DQ649065, *Togniniella acerosa* AY761076, *Valsa ambiens* AF362564, *Vanderwaltozyma polyspora* AY048169, and *Xylaria hypoxylon* U47841.

All sequences were manually aligned in BioEdit 7.0.9.0 (Hall, 1999). Predicted models of the secondary structure of the LSU (Gutell *et al*., 1993) molecules of *Saccharomyces cerevisiae* Meyen ex E.C. Hansen were used to improve the alignment. The models of the secondary structure of the LSU were highly consistent in all taxa and were comparable with that of *S. cerevisiae*.

Phylogenetic analyses

Phylogenetic relationships were examined using 91 ncLSU rDNA sequences of taxa from 25 different orders or families of the Pezizomycotina. Members of the Saccharomycotina were used as an outgroup. Maximum parsimony and Bayesian analysis were used to estimate phylogenetic relationships of *Rhodoveronaea* among all taxa investigated. The final alignment included 1221 bp after the introduction of gaps; it is deposited in TreeBase (SN4172). For all analyses, the first 75 characters were excluded from the alignment because of the incomplete 5′-end in most available sequences.

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

Bayesian analysis was performed in a likelihood framework as implemented by MrBayes v3.0b4 software package to reconstruct phylogenetic trees (Huelsenbeck and Ronquist, 2001). Because ModelTest 3.5 (Posada and Crandall, 1998) indicated that the GTR+I+G substitution model best fits the model of DNA evolution for our data, the parameters in MrBayes were set as follows: "lset nst = 6," and "rates = gamma". Multiple Bayesian searches using Metropolis-coupled Markov chain Monte Carlo sampling were conducted. One cold and three heated Markov chains were used in the analysis. The Bayesian analysis was run for 5 000 000 generations, with trees sampled every 100 generations. The first 20 000 trees, which represented the burn-in phase of the analysis, were discarded. To estimate posterior probabilities (pP) of recovered branches (Larget and Simon 1999) 50% majority rule consensus trees were created from the remaining trees using PAUP.

Results

Phylogenetic analysis of the ncLSU rDNA sequence data

A maximum parsimony analysis (MPA) was performed using 488 phylogenetically informative characters. Two most parsimonious trees (MPTs) were obtained; one is shown in Fig. 1. In the MPA, *Rhodoveronaea varioseptata* strains from ascospore and conidial isolates (100% bootstrap support/1.0 posterior probability) grouped in a monophyletic clade (99/1.0) with two *Rhamphoria* species (100/1.0). The clade is shown as a part

of a larger cluster containing also the *Ophiostomatales* (95/1.0) and two *Myrmecridium* Arzanlou *et al.* species (100/1.0). The species presently included in *Dactylaria* are polyphyletic; *D. purpurella* (Sacc.) Sacc., the type species of the genus, resided on a separate position among all sampled ascomycetes, while D. *parvispora* (as *Pleurophragmium parvisporum*) is shown as a sister of *Papulosa* (without branch support) and *Dactylaria monticola* R.F. Castañeda & W.B. Kendr. grouped in the *Xylariales* (both within the Sordariomycetes) (99/1.0).

In the Bayesian analysis (BAY, details not shown) the two *Myrmecridium* species are in a basal position to the *Ophiostomatales* but they form a separate clade with *Papulosa* and *P. parvisporum* (0.6).

Taxonomy

Rhodoveronaea Arzanlou *et al*., Stud. Mycol. 58: 89. 2007.

Description of the teleomorph

Perithecia non stromatica, gregaria vel solitaria, fusca usque atra, venter subglobosus vel conicus, submersus; ostiolum e substrato protrudens, subincurvum, periphysatum. Paries perithecii coriaceus, bistratosus. Paraphyses septatae, hyalinae, sursum angustatae, ascos superantes. Asci unitunicati, cylindracei, 8-spori, apice rotundato, longe stipitati. Ascosporae fusiformes, hyalinae, 3-septatae.

Perithecia nonstromatic, gregarious or solitary, dark brown to black, venter subglobose to conical, immersed; neck conical, emerging above the substratum, straight or slightly curved, ostiolum periphysate. *Perithecial wall* leathery, two-layered. *Paraphyses* septate, hyaline, tapering towards the tip, longer than the asci. *Asci* unitunicate, cylindrical, 8-spored, broadly rounded at the apex, long-stipitate. *Ascospores* fusiform, hyaline, 3-septate.

Rhodoveronaea varioseptata Arzanlou *et al*., Stud. Mycol. 58: 91. 2007. (Epitypified by Réblová) (Figs. 2−18)

Epitype: CZECH REPUBLIC, Southern Moravia: Protected landscape area Pálava, Klentnice, on decayed wood of a stump of *Carpinus betulus* (*Lentomitella cirrhosa* is present on this specimen), 1 Oct. 1999, M. Réblová M.R. 1501 (PRA; ex-type culture deposited as CBS 123472). **Epitype designated here!**

Additional material examined: SWEDEN: Öland, Kastlösa Parish, Västerstads woodland, 4 km S of Kastlösa, decayed deciduous wood, 2 June 2001, M. Réblová, M.R. 1818 (culture deposited as CBS 123473).

Perithecia non stromatica, gregaria vel solitaria, fusca usque atra, venter subglobosus vel conicus, submersus; ostiolum e substrato protrudens, subincurvum, periphysatum. Paries perithecii coriaceus, bistratosus. Paraphyses septatae, hyalinae, sursum angustatae, ascos superantes. Asci unitunicati, cylindracei, 8-spori, apice rotundato, longe stipitati, 115−130 μm (*pars sporifera*) × 8–9 μm. Ascosporae fusiformes, hyalinae, 3-septatae, 14–16.5(−20) × 5–6.5(−7) μm.

Perithecia nonstromatic, immersed in the substratum, solitary to gregarious, dark brown to black, venter subglobose to conical, 350−450 μm high, 300−400 μm diam, neck conical, tapering, straight or slightly flexuous, 300−450 μm long, 180−200 μm wide near the base tapering to ca. 150 μm. *Perithecial wall* leathery, two-layered, 60−80 μm thick; outer layer of *textura prismatica* to *epidermoidea*, composed of brown cells that become darker towards the surface; inner layer of *textura prismatica*; cells hyaline, thin-walled, and flattened. Ostiolum periphysate. *Paraphyses* septate, branched, constricted at the septa, wider near the base *ca* 3.5−4.5 μm, tapering to *ca* 2.5 μm towards the tip, longer than the asci, partially dissolving. *Asci* unitunicate, arising from croziers, cylindrical, relatively thick-walled, 115−130 μm long in *pars sporifera*, 8–9 μm wide (mean ± S.E. = 124.9 ± 2.1 × 8.72 ± 0.17 μm), stipe 40−60 μm long; ascal apex rounded with a distinct refractive apical annulus 2.8−3 μm wide and 1.6−1.9 μm high; stipe at maturity dissolving along the sides, base bulbose. *Ascospores* fusiform to narrowly ellipsoidal, tapering at the ends, 14–16.5(−20) × 5–6.5(−7) μm (mean ± S.E. = 15.4 ± 0.12 × 5.97 ± 0.11 μm), hyaline, smooth, 3-septate, sometimes slightly flattened at one side, usually with 3–4 large guttules.

Colonies *in vivo* spreading, inconspicuous, brown, hairy. *Conidiophores* arising in small fascicles in irregular rows, macronematous, straight or flexuous, simple,

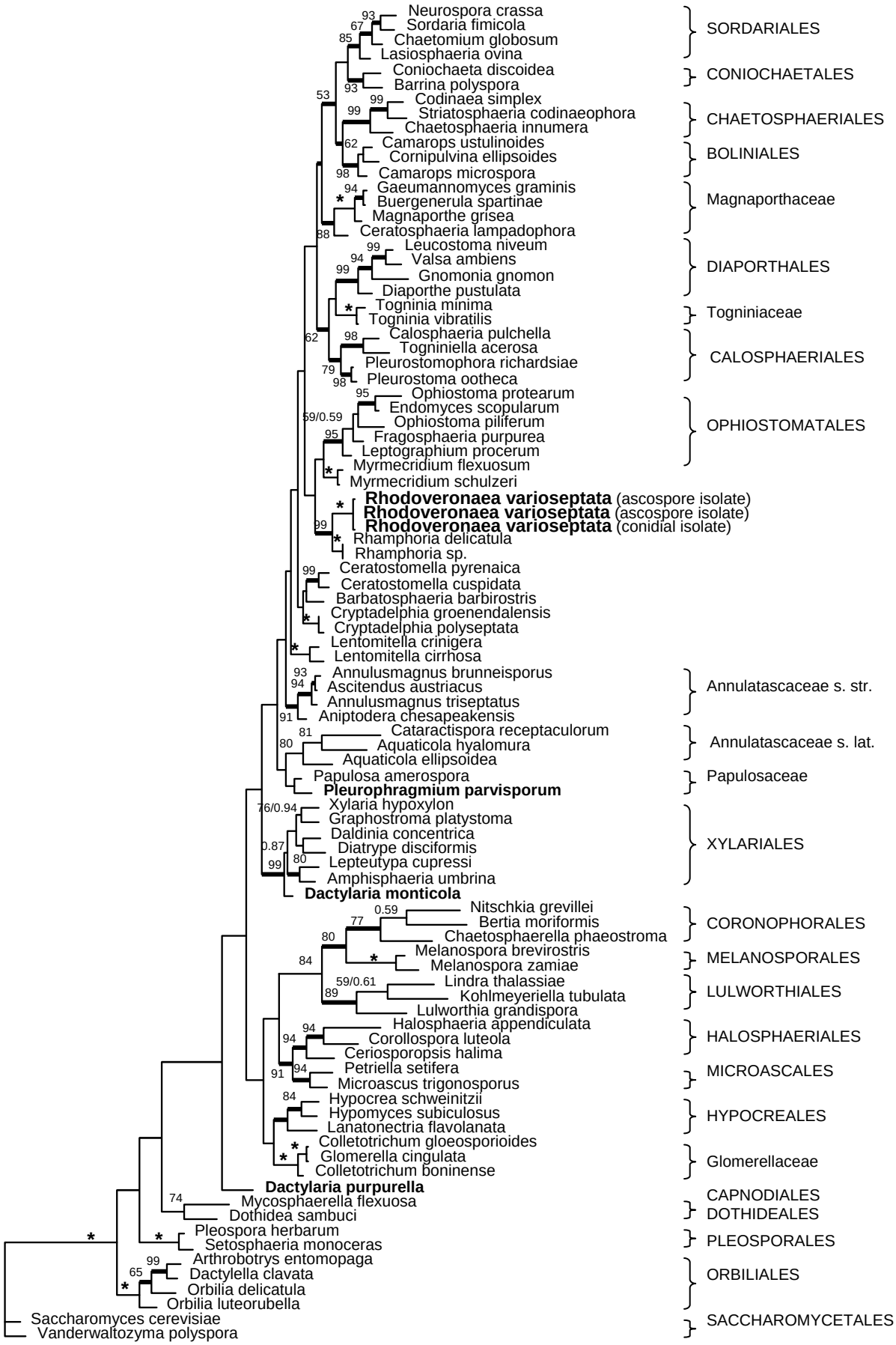


Fig. 1. One of the two most parsimonious trees from a heuristic analysis of ncLSU rDNA sequences from 21 ascomycetous orders and families [tree length 3778, consistency index (CI) = 0.297, retention index (RI) = 0.595,

homoplasy index (HI) = 0.703]. Bootstrap support values ≥ 50 % from 1000 replicates are included at the nodes. An asterisk (*) indicates bootstrap support values of 100 % and posterior probability values of 1.00 obtained for a node. Thickened branches indicate posterior probability values ≥ 0.95 pP. Posterior probability values < 0.95 pP are shown at the nodes. Branch lengths are drawn to scale.

brown to red-brown, cylindrical, 80−93 × 4–5 μm. *Conidiogenous cells* integrated, polyblastic, sympodial, smooth, pale brown to subhyaline, bearing up to 15 sympodially produced denticles producing conidia holoblastically. *Conidia* ellipsoidal to obovoidal, apically rounded, with a flat basal scar and marginal frill (at least *in vitro*), 14–16 × 5−6.5 μm (mean ± se = 15.18 ± 0.26 × 5.76 ± 0.24 μm), 0−3(−4)-septate, pale brown, smooth, marginal basal frill not observed.

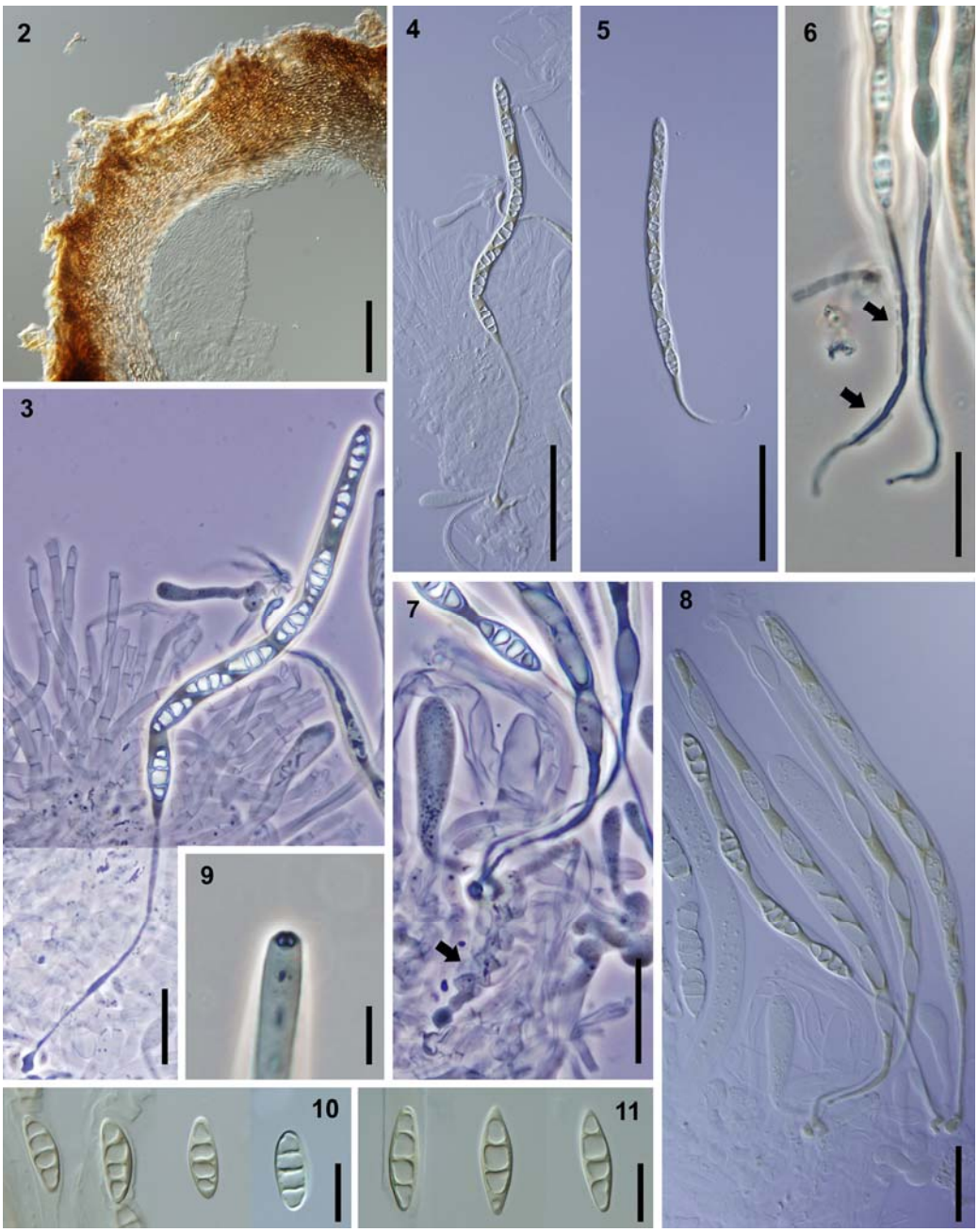
Colonies *in vitro* after 30 d on MEA at 25°C 12–14 mm in diam, velvety; surface olivaceous-gray to olivaceous-pale brown with a pale gray zone at the margin, aerial mycelium dense, whitish to pale gray, margin entire; reverse olivaceous-black. *Conidiophores* scarcely developed at the margin of the colony, macronematous, straight or flexuous, simple, red-brown, cylindrical, 60−80 × 4–4.5 μm. *Conidiogenous cells* integrated, polyblastic, sympodial, pale brown to subhyaline, smooth, bearing up to 15 denticles producing conidia holoblastically. *Conidia* ellipsoidal to obovoidal, rounded at the terminal end, at the base protruding with a flat scar and a minute marginal frill 11.5–13 × (4.5−)5−6 μm (mean ± S.E. = 12.5 ± 0.27 × 5.35 ± 0.17 μm), 0−3-septate, pale brown, smooth. *Chlamydospores* not observed.

Comments: Ascospores in the collection from Sweden (M.R. 1818) were somewhat longer (14−20 × 5−6 μm). Conidia of the *R. varioseptata* anamorph formed *in vivo* did not possess a minute marginal frill at the base; the frill was only observed in conidia produced *in vitro* (MEA).

Discussion

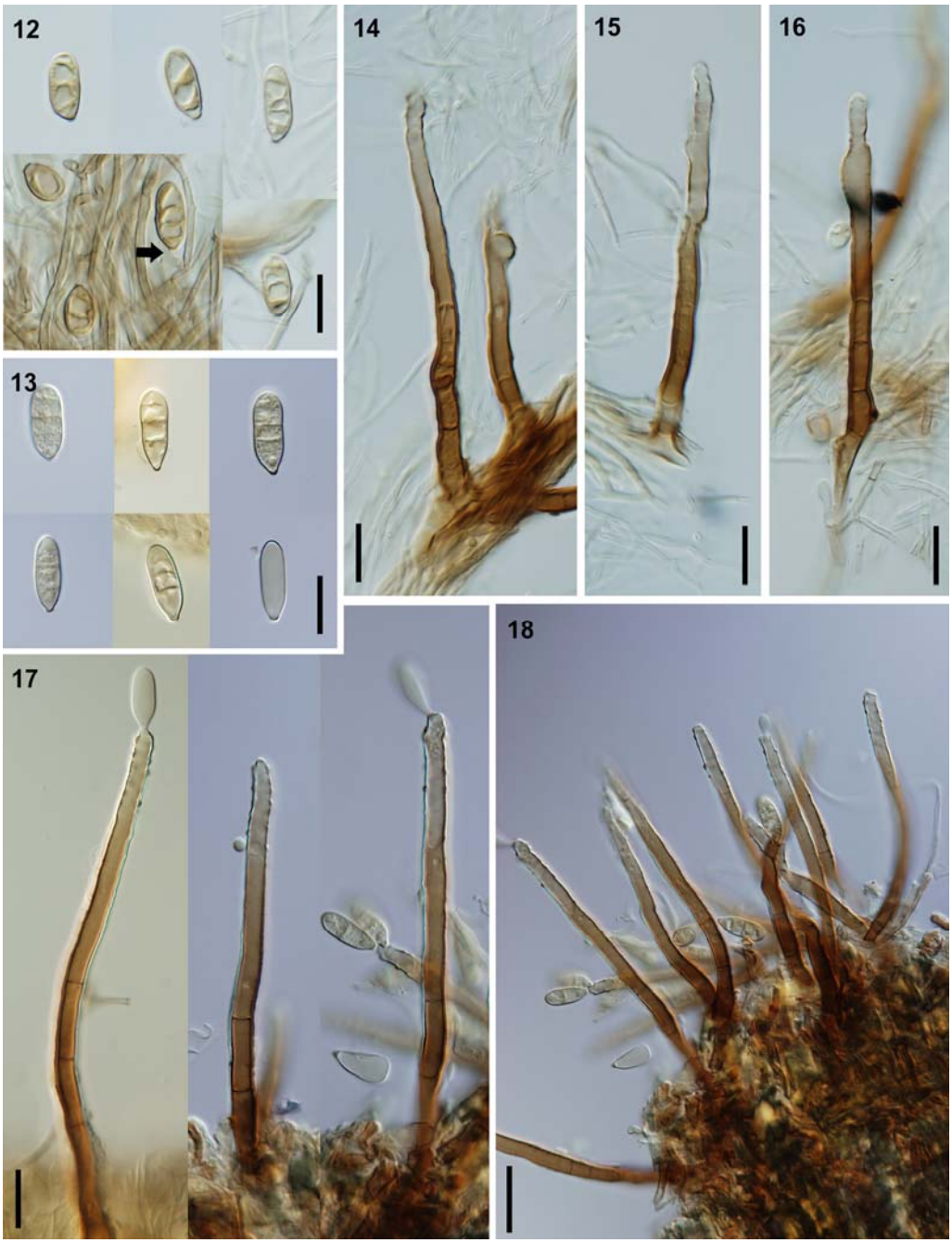
In the phylogenetic analysis, the closest relative to *Rhodoveronaea varioseptata* (100/1.0) are two *Rhamphoria* species (100/1.0) within a single clade (99/1.0). *Rhamphoria* accommodates species with minute, nonstromatic, usually pyriform perithecia containing short-stipitate asci with or without visible discharge mechanism and ascospores, which are clavate to ellipsoidal to irregularly shaped, hyaline, aseptate when young and becoming transversely septate with several longitudinal septa, slightly constricted. The ascospores may produce a large amount of microscopic hyaline conidia filling the ascus. The dematiaceous hyphomycetes *Phaeoisaria* Höhn. sensu de Hoog & Papendorf (1976) and *Idriella* P.E. Nelson & S. Wilh. were linked to *Rhamphoria* by Müller & Samuels (1982) *in vitro*. A phaeoisaria-like anamorph was also confirmed for *Lentomitella* (Réblová 2006). In the present ncLSU rDNA phylogeny, *Rhodoveronaea* is clearly separated from the morphologically similar genera *Lentomitella* and *Ceratosphaeria*. *Lentomitella* can be distinguished from *Rhodoveronaea* by short-stipitate asci, ellipsoidal ascospores rounded at the ends and globose, apiculate, hyaline conidia of the phaeoisaria-like anamorph. *Ceratosphaeria*, based on the type species *C. lampadophora*, differs from *Rhodoveronaea* by cylindrical asci with a shorter and persistent stipe, long-fusiform to cylindrical to cymbiform ascospores arranged 2−3-seriately within the ascus and harpophora-like anamorph with phialidic conidiogenesis (Réblová 2006).

Among the known *Ceratosphaeria* species, *C. fragilis* and *C. rhenana* (≡ *Gnomonia rhenana* Auersw. 1869) can be compared with the teleomorph of *R. varioseptata*. *Ceratosphaeria fragilis* differs from *R. varioseptata* by paler brown perithecia with greatly elongated, slender and sometimes nodulose necks, which are apically pale-colored; longer asci and longer ellipsoidal ascospores (Wilberforce 1987; IMI 302517 holotype). *Rhodoveronaea varioseptata* differs from *C. rhenana* in having shorter, conical perithecial necks, long-stipitate asci and shorter, fusiform ascospores tapering towards the ends, arranged uniseriately within the ascus. The type material of *C. rhenana* is lost (it is not present in B and it was apparently lost during the Second World War; pers. comm. B. Hein, Curator of the Berlin herbarium). In the protologue of *C. rhenana* (as *Gnomonia rhenana*, Auerswald 1869: Mycologia



Figs 2-11. *Rhodoveronaea varioseptata* (teleomorph). **2.** Longitudinal section of the perithecial wall. **3-5, 8.** Asci with ascospores. **6.** Long stipe, arrows indicate places where the stipe dissolves at ascus maturity. **7.** Ascogenous hypha with remnants of asci and asci still attached arising from crosiers (arrow). **9.** Ascal apex with the annulus. **10, 11.** Ascospores. **Figs 2-10.** from PRA (epitype), **11**. from M.R. 1818. Scale bars: 2, 4, 5 = 50 µm; 3, 6-8 = 20 µm; 9-11 = 10 µm.

Europaea 5−6: 23, Table 10, Fig. 1139), a lignicolous fungus is described with immersed globose venter *ca* 300 µm diam and a long cylindrical neck; cylindrical 8-spored asci 154 × 12 µm with a briefly tapering stipe, containing biseriately arranged ascospores, 24 × 7 µm, which are elongate-oblong, rounded at the ends, aseptate, and hyaline. Von Höhnel (1906: 1214), in his revision of Feltgen's herbarium material, identified a part of the collection of *Trichosphaeria tetraspora* Feltg. as *C. rhenana*; but this fungus differed from the protologue of *C. rhenana* by longer asci (180−190 × 6−7 µm) and shorter ascospores (14−16 × 5 µm). Winter (1885: 257) based his description of *C. rhenana* on a part of Fuckel's exsiccate collection (Fungi rhenani no. 1804, *pro parte*). Revision of the exsiccate Fungi rhenani no. 1804 from Fuckel's herbarium in Genève by the present author resulted in the identification of *Lentomitella cirrhosa* (Pers.: Fr.) Réblová in the specimen; perithecia of



Figs 12-18. *Rhodoveronaea varioseptata* (anamorph). **12.** Conidia, arrow indicates the marginal basal frill (from culture, MEA, 1 mo). **13.** Conidia (from nature substratum). **14-18.** Conidiophores with terminal conidiogenous cells with minute hyaline denticles. **Figs 12, 14−16** from culture (MEA, 1 mo) CBS 123472; **13, 17, 18.** from PRA (epitype). Scale bars: **12−17** = 10 μm; **18** = 25 μm.

C. rhenana sensu Winter were not found. However, a specimen of Fungi rhenani no. 1804 from Kew (K 84430) contained the fungus sensu Auerswald and Winter (1885) as *C. rhenana*, which is in agreement with the protologue of *C. rhenana*. The material deposited in Kew is rather old and contains only few perithecia with elongate-oblong ascospores with rounded ends (18−)20−23 × 5−6 μm; each ascospore contains 4 large guttules, septa indistinct; asci were not observed. Munk (1957) reported a collection of *C. rhenana* from the herbarium of P. Larsen in C, but of which only the description and the illustration are left. This fungus with asci 90−120 *pars sporifera* × 9−12 μm and 4-celled, hyaline 18−25 × 5.5−6.5 μm ascospores could represent a collection of *C. rhenana* from Denmark; however, the narrowly ellipsoidal ascospores deviate from the elongate-oblong ascospores with rounded ends from the protologue of *C. rhenana*. Unfortunately, the

material of *C. rhenana* in Kew (K 84430) is not suitable for typification due to its poor condition and lack of asci. Other exsiccate collections distributed under Fungi rhenani no. 1804 need to be studied and fresh material of this fungus needs to be recollected to redescribe the species and to investigate its relationship with other *Ceratosphaeria* species and other morphologically related taxa.

The fungus collected by P. Larsen in Denmark and reported by Munk (1957) as *C. rhenana* can be compared with the teleomorph of *R. varioseptata*; the characteristics of the perithecia with short dark tapering necks, long-stipitate asci and fusiform 4-celled ascospores match well the description of *R. varioseptata*; with the exception of the ascospore size; they are somewhat larger in the Danish collection.

The relationship of *R. varioseptata* and *Dactylaria* was investigated using ncLSU rDNA sequences. Both possess erect pigmented conidiophores with terminal, polyblastic conidiogenous cells with several minute denticles and pigmented obovoidal conidia. De Hoog (1985) in his revision of *Dactylaria* complex classified accepted species into four sections based on the characteristics of conidia and conidiophores. Goh & Hyde (1997) revised *Dactylaria* based on a comparison of conidial morphology; they accepted only 37 of the 41 species attributed to *Dactylaria* by de Hoog (1985), for which they provided a key and added a species from submerged wood. Results of the ITS (Bussaban, 2005) and the LSU phylogeny (this study) indicate that *Dactylaria* is polyphyletic. *Dactylaria purpurella*, the type species of the genus, resided in a position with unclear ordinal affiliation among all sampled taxa; it was clearly separated from *D. parvispora* (*Dactylaria* sect. *Pleurophragmium*), which grouped as a sister to *Papulosa* Kohlm. & Volkm.-Kohlm. (*Papulosaceae*, Sordariomycetes), and from *D. monticola*, which takes a basal position to the *Xylariales* (99/1.0). However, *Pleurophrag-mium* and *Dactylaria* are morphologically similar dematiaceous hyphomycetes dis-tinguished mainly by longer, erect, thick-walled and many-septate conidiophores in *Pleurophragmium*. Detailed description and illustration of *P. parvisporum* was prepared by Ellis (1968, 1971), Matsushima (1975) and Holubová-Jechová (1972). Based on the recent findings, *Pleurophragmium* must be re-evaluated based on *P. parvisporum*; its exclusion from *Dactylaria* is supported and justified by molecular and morphological characters, although both strains *D. purpurella* and *P. parvisporum* were obtained from GenBank without possibility to verify the original material. *Pleurophragmium parvisporum* differs from *R. varioseptata* by longer dark-brown conidiophores (100−300 μm long) and longer conidia (10−20 μm long), which do not form a marginal basal frill (Arzanlou *et al*. 2007; this study Fig. 12) in culture (comparison of size of conidia and conidiophores *in vivo fide* de Hoog 1985). Till recently, 40 species were attributed to *Pleurophragmium*, some were transferred to *Dactylaria*, *Spiropes* Cif. or *Pseudospiropes* M.B. Ellis (de Hoog & von Arx, 1973) but most of them remain to be placed in *Pleurophragmium*.

Acknowledgements

This study was supported by the Project of the National Foundation of the Czech Republic (206/09/0547) and Institutional Research Concept No. AV0Z60050516 (Institute of Botany of the ASCR). I am grateful for help of Dr Václav Štěpánek, who undertook the DNA sequencing of ascospore isolates of *Rhodoveronaea* and other sequences newly presented in this study. I wish to thank Prof. Walter Gams for his careful reading of the manuscript and further suggestions. I wish to thank Prof. Walter Gams and Dr Scott Redhead for discussion on epitypification and the interpretation of the Article 59.7 of the Code of Botanical Nomenclature.

References

Arzanlou, M., Groenewald, J.Z., Gams, W., Braun, U., Shin, H.D. and Crous, P.W. (2007). Phylogenetic and morphotaxonomic revision of *Ramichloridium* and allied Genera. Studies in Mycology 58: 57-93

Bussaban, B., Lumyong, S., Lumyong, P., Seelanan, T., Park, D.C., McKenzie, E.H.C. and Hyde, K.D. (2005). Molecular and morphological characterization of *Pyricularia* and allied genera. Mycologia 97: 1002-1011.

Ellis, M.B. (1968). Dematiaceous hyphomycetes. IX. *Mycological Papers* 114: 1-44.

Ellis, M.B. (1971). Dematiaceous hyphomycetes. Commonwealth Mycological Institute: Kew, Great Britain.

Gams, W., Hoekstra, E.S. and Aptroot, A. (1998). In: *CBS Course of Mycology*, Centraalbureau voor Schimmelcultures (eds. W. Gams, E.S. Hoekstra and A. Aptroot). Baarn, The Netherlands: p. 165.

Goh, T.K. and Hyde, K.D. (1997). A revision of *Dactylaria*, with description of *D. tunicata* sp. nov. from submerged wood in Australia. Mycological Research 101: 1265-1272.

Gutell, R.R., Gray, M.W. and Schnare, M.N., (1993). A compilation of large subunit (23S and 23S-like) ribosomal RNA structures. Nucleic Acids Research 21: 3055-3074.

Hall, T.A., (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.

Holubová-Jechová, V. (1972). A note on *Pleurophragmium simplex* (Hyphomycetes). Česká Mykologie 26: 223-225.

Hoog, G.S. de. and Papendorf, M.C. (1976). The genus *Phaeoisaria*. Persoonia 8: 407-414.

Hoog, G.S. de. and von Arx (1973). Revision of *Scolecobasidium* and *Pleurophragmium*. *Kavaka* 1: 55-60.

Hoog, G.S. de. and von Arx. (1985). Taxonomy of the *Dactylaria* complex, IV−V. Studies in Mycology 26: 1-122.

Huelsenbeck, J.P. and Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754-755.

Huhndorf, S.M., Miller, A.N. and Fernández, F.A. (2004). Molecular systematics of the *Sordariales*: the order and the family *Lasiosphaeriaceae* redefined. Mycologia 96: 368-387.

Larget, B. and Simon, D.L. (1999). Markov Chain Monte Carlo Algorithms for the Bayesian Analysis of Phylogenetic Trees. Molecular Biology and Evolution 16: 750-759.

Matsushima, T. (1975). In: *Icones microfungorum a Matsushima lectorum*. Kobe, Japan: p.209.

Müller, E. and Samuels, G.J. (1982). Anamorphs of pyrenomycetous Ascomycetes I. *Rhamphoria* Niessl and *Trichosphaerella* Bommer, Rousseau & Saccardo. Sydowia 35: 143-149.

Munk, A. 1957. Danish Pyrenomycetes. A Preliminary Flora. Dansk Botanisk Arkiv 17(1): 1-491.

Posada, D. and Crandall, K.A. (1998). Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Réblová, M. (2006). Molecular systematics of *Ceratostomella* sensu lato and morphologically similar fungi. Mycologia 98: 63-93.

Swofford, D.L. (2002). *PAUP**. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sunderland, MA. Sinauer Associates.

Wilberforce, P.W. (1987). A new *Ceratosphaeria* species on *Fagus* from east Kent. Transactions of British Mycological Society 88: 132-133.

Winter, G. (1885 “1887”). Die Pilze Deutschlands, Oesterreichs und der Schweiz. 2. Abth. Ascomyceten: Dr. L. Rabenhorst’s Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz, 2. Aufl. Leipzig. Gymnoasceen und Pyrenomyceten, Lief. 18: 257–320.