



Volutellonectria (Ascomycota, Fungi), a new genus with *Volutella* anamorphs

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Abstract

A new genus, *Volutellonectria* with *Volutella* anamorphs is established, based on and typified by *Cosmospora consors*. It is characterized by small, red perithecia which may collapse laterally when dry, a *Cosmospora*-like perithecial wall structure, clavate asci with an apical ring, fusiform ascospores with smooth surface, and growing on decaying plant debris or woody substrates. Two new species are added to the new genus and described as *Volutellonectria asiana* and *V. ciliata*. Phylogenetic analyses of nuclear ribosomal DNA (ITS and 28S partial) strongly support the separation of *Volutellonectria* from *Cosmospora* and some other species with *Volutella* anamorphs in Nectriaceae, as well as recognition of the new species.

Keywords: *Cosmospora*, morphology, new species, sequence analyses, teleomorph–anamorph connections

Introduction

Cosmospora Rabenh. is a widespread genus and of significant morphological and ecological diversity among members of Nectriaceae. The genus includes fungi with their ascomata on a thin basal stroma or not, small (usually ≤ 300 μm in diam.) and orange to dark red perithecia which are solitary to gregarious, globose to obpyriform, and laterally collapsing or not collapsing when dry. The perithecial wall structure is assumed to be notable among nectriaceous fungi. The walls are thin (usually ≤ 20 μm thick) with 1–2 layers and the wall surface is either glabrous or hairy. Cells of the walls are usually irregular in shape and connected by fine pores where they are adjacent. The asci are cylindrical to clavate, 4–8-spored, and with an apical ring or a simple apex. The ascospores are ellipsoid to ovoid, 1(–3)-septate, uniseriate or biseriate within ascus, yellow-brown or hyaline, and with a smooth, spinulose or striate surface. Species of *Cosmospora* occur on various substrates including fungi, scale insects, and herbaceous or woody plants in tropical and temperate regions. Currently *Acremonium* Link, *Fusarium* Link, *Stilbella* Lindau, *Verticillium* Nees and *Volutella* Fr. are known as its anamorphs, and the genus is polyphyletic (Samuels et al. 1991, Rossman et al. 1999, Schoch et al. 2000, Zhang and Zhuang, 2006, Luo and Zhuang 2008). Towards establishment of a monophyletic *Cosmospora*, *Chaetopsinectria* J. Luo & W.Y. Zhuang was published as a segregate genus (Luo and Zhuang 2010b).

Volutella is characterized by discoid sporodochia with marginal setae, simple to verticillate conidiophores, compact and phialidic conidiogenous cells, and 1-celled, ovoid to oblong conidia (Rossman and Samuels 1993, Barnett and Hunter 1998, Samuels et al. 2006). Several species have been reported linking to genera of nectriaceous fungi. For example, *V. minima*, *V. buxi* and *V. pachysandricola* are connected with *Cosmospora consors*, *Pseudonectria rousieliana* and *P. pachysandricola*, respectively (Rossman and Samuels 1993, Rossman et al. 1999). In this study, we provide morphological and molecular data on *Cosmospora* species that possess *Volutella* anamorphs.

Material and methods

Morphological studies

The methods described by Rossman et al. (1999) and Luo and Zhuang (2010a) were generally followed for morphological examinations. Specimens examined are deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS), The New York Botanical Garden Herbarium, USA (NY), and The US National Fungus Collections (BPI). Cultures are kept in the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences.

Molecular studies

Strains of nectriaceous fungi with *Volutella* anamorphs and related species were from our own laboratory, the United States Department of Agriculture (USDA) and Centraalbureau voor Schimmecultures, Utrecht, The Netherlands (CBS). They were cultured on potato dextrose agar (PDA) and cornmeal dextrose agar (CMD) (Gams et al. 1998). Fungal genomic DNA was extracted using the protocol of Wang and Zhuang (2004). ITS1-5.8S-ITS2 (ITS) and partial 28S nuclear ribosomal DNA were used to solve the phylogenetic problems of selected genera of nectriaceous fungi (Schoch et al. 2000, Crous et al. 2005, Samuels et al. 2009, Luo and Zhuang 2008, 2010b, Zhao et al. 2011). PCR amplification and sequencing of complete ITS and partial 28S nrDNA were conducted with the methods described by Luo and Zhuang (2010b). The reference taxa of DNA sequences, their collection numbers and GenBank accession numbers are listed in TABLE I.

All sequences were aligned with ClustalX V.1.8 (Thompson et al. 1997), and the alignments were visually adjusted where necessary with BioEdit 7.0.5 (Hall 1999). Datasets of ITS and 28S were combined for sequence analyses, and *Bionectria ochroleuca* and *Hydropisphaera erubescens* were chosen as outgroup taxa. MrModeltest 2.3 (Nylander 2004) was used to specify a nucleotide substitution model. Bayesian Inference (BI) analysis was performed with the Markov Chain Monte Carlo method in MrBayes 3.04b4 (Huelsenbeck and Ronquist 2001). Four simultaneous Markov chains were run from random starting trees for 5,000,000 generations, and trees were sampled every 100 generations. The first 10,000 trees were discarded as the burn-in phase of the analysis. The remaining 40,000 trees were used for generating a consensus tree and calculating the posterior probabilities of clades. Maximum Parsimony (MP) analysis was conducted by a heuristic search in PAUP* 4.0b10 (Swofford 2002) with the following settings: all characters were equally weighted, gaps were treated as missing characters, starting trees obtained by random taxon addition with 1000 replicates, the branch-swapping algorithm was tree-bisection-reconnection (TBR), steepest descent option not in effect, MulTrees option not in effect. Branch support was assessed by bootstrap method with a full heuristic search. Simple addition sequence in stepwise addition was used and the other settings were the same as those in parsimony analysis.

Results

Phylogenetic analyses

A total of 508 characters were in the ITS alignment containing gaps, and 491 were in the 28S alignment. The combined datasets of 40 strains included 999 characters, of which 310 were parsimony-informative, 57 were variable and parsimony-uninformative, and 632 were constant. MP analysis resulted in 13 most parsimonious trees with the following scores: tree length = 1236, consistency index (CI) = 0.474, homoplasy index (HI) = 0.526, retention index (RI) = 0.701, and rescaled consistency index (RC) = 0.333. The general time reversible model of nucleotide substitution with gamma rate heterogeneity and a proportion of invariance sites (GTR+I+G) was selected as the best evolution model for the combined datasets by hierarchical likelihood

ratio tests (hLRT) and Akaike information criterion (AIC) in MrModeltest. According to the selected model, BI analysis yielded a single phylogenetic tree which had a similar topology to the MP trees. Only the Bayesian consensus tree is shown in FIG. 1.

Two major clades were observed in the phylogenetic analyses (FIG. 1). Clade I contained 24 species belonging to 13 teleomorphic genera and 1 anamorphic genus, i.e. *Cosmospora*, *Volutella*, *Leuconectria* Rossman, Samuels & Lowen, *Calonectria* De Not., *Neonectria* Wollenw., *Nectricladiella* Crous & C.L. Schoch, *Gibberella* Sacc., *Cyanonectria* Samuels & Chaverri, *Albonectria* Rossman & Samuels, *Haematonectria* Samuels & Nirenberg, *Neocosmospora* E.F. Sm., *Chaetopsinectria* J. Luo & W.Y. Zhuang, *Pseudonectria* Seaver and *Lanatonectria* Samuels & Rossman, with 0.93 BI posterior probability (PP) and a weak (< 50%) bootstrap proportion (BP), respectively. Clade II, which included 7 species of *Cosmospora*, *Nectria* (Fr.) Fr. and *Corallomycetella* Henn., received high support (1.00 BIPP; 95% BP).

Cosmospora consors, two *C. consors*-like fungi, the type species of *Volutella* (*V. minima*) and *V. ciliata* constituted a well-supported subclade (1.00 BIPP; 95%BP), which appeared to be monophyletic in Clade I. However, *Cosmospora coccinea* (the type species of the genus) and its associates were together with high support (1.00 BIPP; 91% BP) in Clade II. Our analyses clearly distinguished the *Cosmospora*-like fungi with *Volutella* anamorphs from the typical *Cosmospora*.

Pseudonectria, another genus with *Volutella* anamorphs, was also in Clade I (FIG. 1). Its type species, *P. rousseliana*, was associated with *Lanatonectria* receiving very high support (1.00 BIPP; 99% BP) and distantly related to the *Cosmospora*-like fungi.

Morphological observations

The above mentioned *Cosmospora*-like fungi with *Volutella* anamorphs are similar to the true *Cosmospora* in presence of thin and basal stroma, small-sized and obpyriform perithecia, perithecial wall anatomy, asci with an apical ring, and fusiform ascospores with one septum. Nevertheless, *Cosmospora* usually has cylindrical asci, and uniseriate ascospores with a spinulose or tuberculate surface. True *Cosmospora* grows on fungi, scale insects and plant tissues, and has *Acremonium*, *Fusarium* or *Verticillium* anamorphs (Rossman et al. 1999, Zhang and Zhuang 2006, Luo and Zhuang 2008, 2010b).

In Nectriaceae, the genus *Pseudonectria* is also connected with *Volutella* anamorphs, and has poorly developed basal stroma, *Cosmospora*-like perithecia and wall structure, clavate asci, and biseriate ascospores. *Pseudonectria*, however, differs obviously from the *Cosmospora*-like fungi in possessing pale yellow, yellow to scarlet, rarely orange or grayish yellow-green perithecia, asci with a simple apex or inconspicuous ring, ascospores aseptate, and growing on Buxaceae (Rossman et al. 1999).

These morphological distinctions of the *Cosmospora*-like fungi are fully supported by our phylogenetic analyses based on ITS and 28S sequences (FIG. 1). A new monophyletic genus in Nectriaceae is therefore proposed.

Taxonomy

Volutellonectria J. Luo & W.Y. Zhuang, gen. nov.

Mycobank MB561605

Etymology: The generic name refers to its connection with *Volutella* anamorphs, and its similarity to *Nectria*.

Type: *Dialonectria consors* Ellis & Everh.

Ascomata perithecial, solitary, on a thin basal stroma, superficial, obpyriform, with an acute apex, usually less than 300 µm diam., laterally collapsing or not collapsing when dry, turning dark red in 3% KOH and yellow

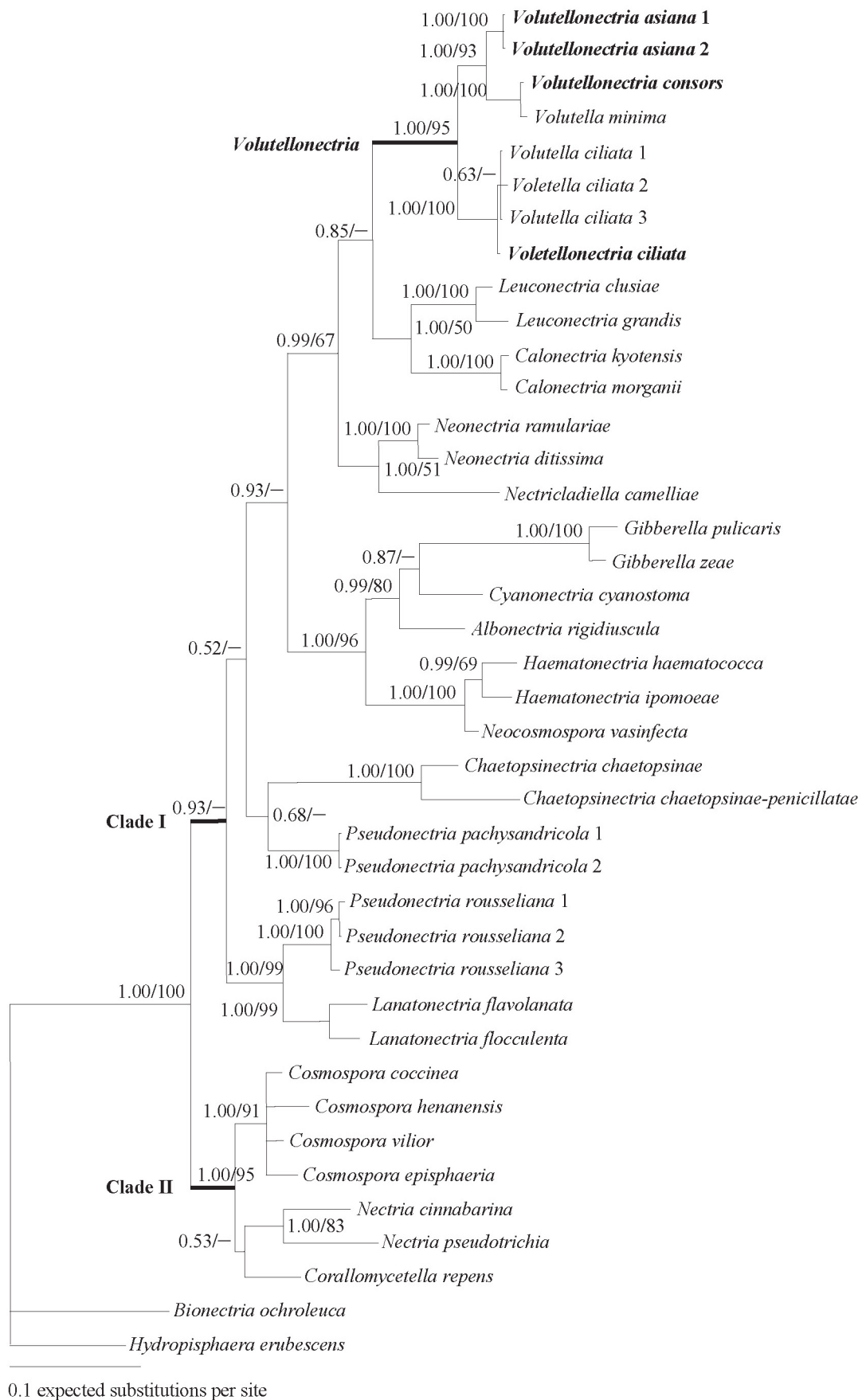


FIGURE 1. The BI consensus tree inferred from combined ITS and 28S nrDNA datasets. BI posterior probability values/MP bootstrap support values $\geq 50\%$ are shown at internodes. The dashes indicate the values $< 50\%$.

in lactic acid, smooth or hairy. Ascomatal walls of 1 or 2 layers, cells usually irregular in shape. Asci unitunicate, clavate, with an apical ring. Ascospores uniseptate, hyaline, often smooth-walled. Anamorph *Volutella*.

***Volutellonectria consors* (Ellis & Everh.) J. Luo & W.Y. Zhuang, comb. nov.**

MycoBank MB561606

- ≡ *Dialonectria consors* Ellis & Everh., J. Mycol. 4: 122. 1888.
- ≡ *Nectriella consors* (Ellis & Everh.) Saccardo, Syll. Fung. 9: 941. 1891.
- ≡ *Nectria consors* (Ellis & Everh.) Seaver, Mycologia 1: 61. 1909.
- ≡ *Cosmospora consors* (Ellis & Everh.) Rossman & Samuels, Stud. Mycol. 42: 119. 1999.

Anamorph: *Volutella minima* Höhn.

Description and illustrations: Samuels (1977).

***Volutellonectria asiana* J. Luo, X.M. Zhang & W.Y. Zhuang, sp. nov.**

FIGS. 2A–B, 3A–H

MycoBank MB561607

Etymology. The specific epithet refers to the locality of the fungus.

Ascomata on old sporodochia or thin basal stromata, perithecial, solitary or 2–5 in a group, superficial, obpyriform, 110–210 µm high, 85–190 µm diam, with a small and red papilla, not collapsing or laterally collapsing when dry, red-orange when fresh and orange to red-orange when dry, turning dark red in 3% KOH and orange-yellow in lactic acid, smooth. *Ascomatal wall* 7.5–12 µm thick, of two layers; outer layer 6–9.5 µm thick, cells angular, 4–7.5 × 2.5–5 µm, cell wall 0.5–1.5 µm thick; inner layer 1.5–3.5 µm thick, cells flattened, 5–11 × 1–2 µm, cell wall 0.5–1 µm thick. *Asci* subcylindrical to clavate, 8-spored, with an apical ring, 35–47 × 4–6 µm (n = 50). *Ascospores* subfusoid, uniseptate, not constricted at the septum, hyaline, smooth, irregularly biseriolate, 9.5–15 × 2–3 µm (n = 50).

Sporodochia solitary or gregarious on substrate, usually stipitate, with a small basal stroma, 75–145 µm diam. *Seta* arising from sporodochial base and surrounding the conidiophores, 175–325 µm long, 3–5.5 µm wide at base, tapering to a round apex, 1–4-septate, smooth, hyaline, walls 0.5–1.5 µm thick. *Conidiophores* branched, 2540 µm long, 2–3 µm wide at base, closely aggregated. *Conidiogenous cells* in whorls of 2–4, adpressed, straight, cylindrical, slightly tapering towards the tip, 6.5–13 µm long, 1.5–2.5 µm wide at base, 0.5–1.5 µm near aperture (n = 50). *Conidia* rod-shaped, distally rounded, with a median displaced hilum, straight, 1-celled, hyaline, smooth, 4–8.5 × 1.5–2.5 µm (n = 50).

Colonies on PDA 3 cm diam after 5 d in the dark at 24 C, pale pinkish cinnamon, surface velvety, aerial mycelium white to yellowish, reverse light pinkish cinnamon. Colonies on (CMD) reaching 2.5 cm diam after 5 d in the dark at 24 C, pale pinkish buff, surface felty, aerial mycelium absent to sparse, reverse light buff. *Conidiophores* simple, unbranched, erect, septate. *Conidiogenous cells* cylindrical, slightly tapering towards the tip, 15–52 µm long, 1–1.5 µm wide at base, 0.51 µm near aperture (n = 50). *Conidia* subellipsoid to subfusoid, distally rounded, with a median displaced hilum, straight, 1-celled, hyaline, smooth, 4–11.5 × 1.5–2.5 µm (n = 50). *Sporodochia* not observed.

Holotype. CHINA. HAINAN, Bawanling, 1100 m, on leaves of a palm, 6 July 2000, Zhuang W.Y., Wu W.P. and Zhang X.M., H17, HMAS 76861, ex type culture HMAS 188475.

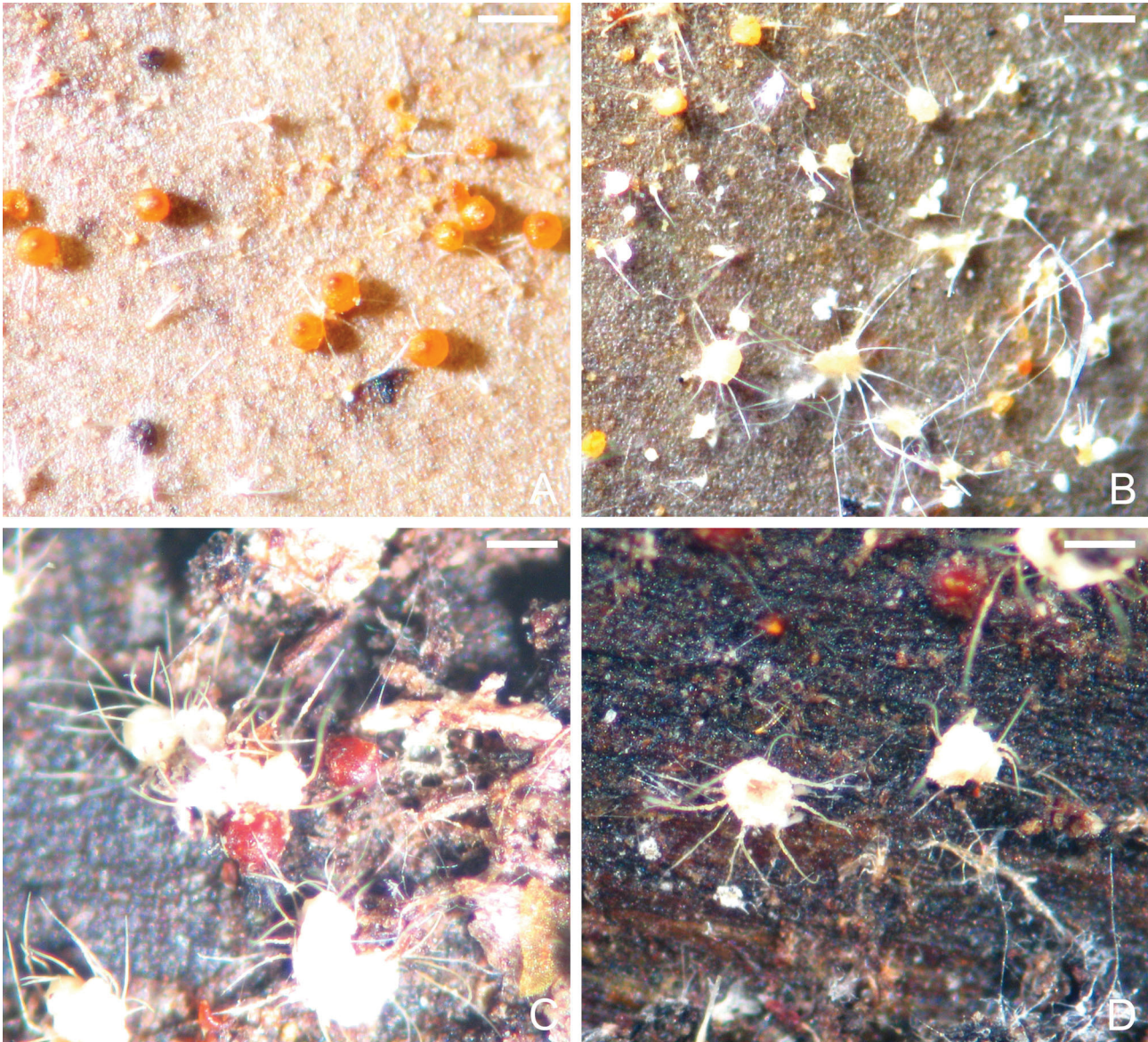


FIGURE 2. Ascomata (left) and sporodochia (right) on natural substrates. A–B. *Volutellonectria asiana* (HMAS 76861). C–D. *Volutellonectria ciliata* (GJS85.205). Bars = 200 μ m.

Paratype. Thailand. Saraburi, Khao Yai National Park, Phaedeodai, 1100 m, on *Pandanus* sp., 12 August 1997, *Samuels G.J.* and *Chaverri P.*, 8410, BPI 745740.

Notes. *Volutellonectria asiana* is most similar to *V. consors* in having red-orange and obpyriform perithecia with a red papilla, subcylindrical to clavate asci with an apical ring, hyaline ascospores with a smooth surface, smooth-walled sporodochial setae, 1-celled conidia, and light-colored colonies. *V. consors*, however, differs in having larger perithecia 210–270 μ m high and 150–220 μ m diam, a hairy perithecial surface, thicker perithecial walls 15–25 μ m thick, somewhat longer asci 40–55 μ m long, wider ascospores 3–4 μ m wide, ellipsoid conidia, and red sclerotia formed in culture (*Samuels* 1977).

Our molecular data also support the establishment of *V. asiana* as a new species (FIG. 1). The two isolates are grouped together with high statistical support (1.00 BIPP; 100% BP) and separated from the morphologically similar species, *V. consors*.

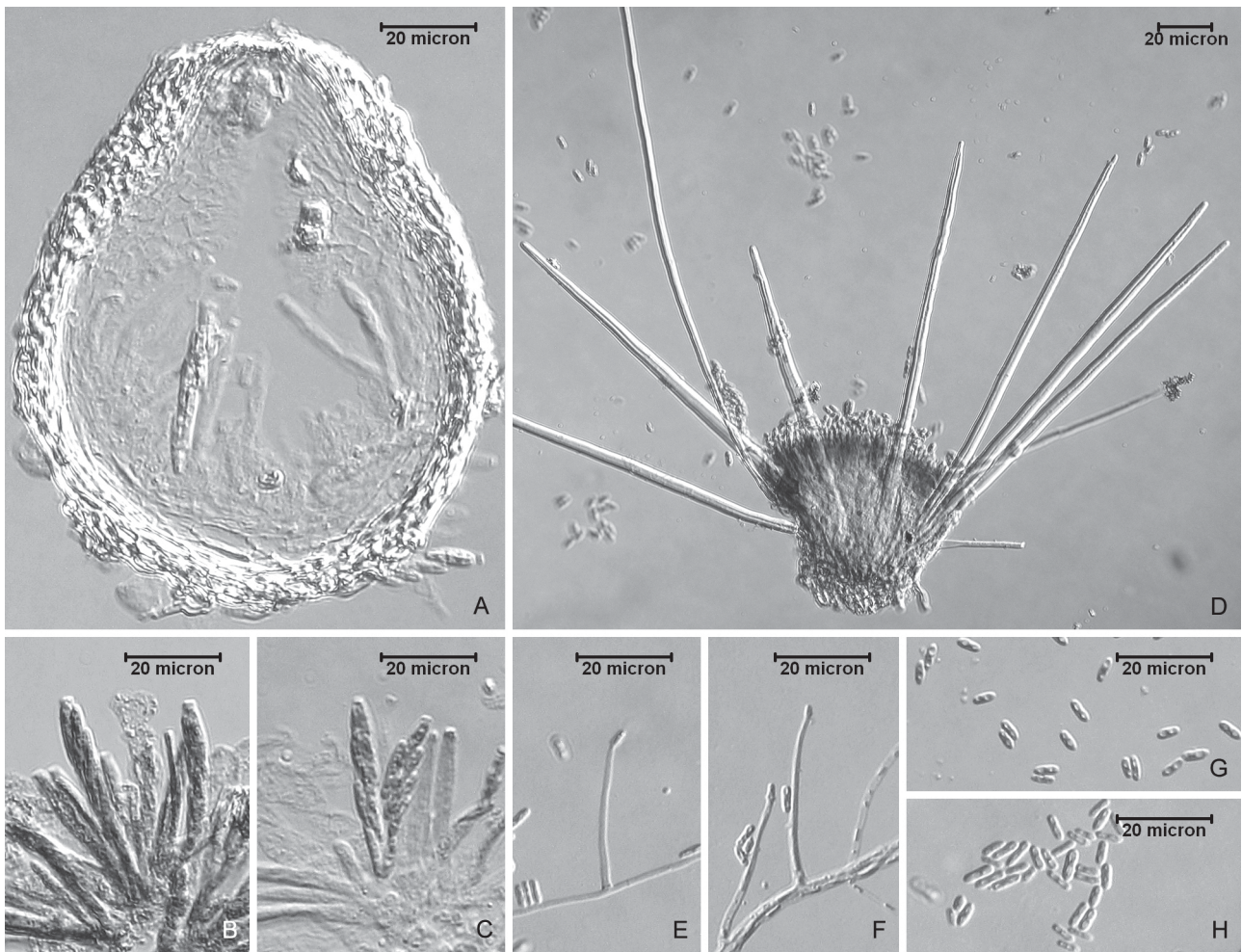


FIGURE 3. A–H. Morphology of *Volutellonectria asiana* (HMAS 76861). A. Median section of an ascoma. B–C. Asci with ascospores. D. Sporodochium from natural substrate. E–F. Conidiophores bearing conidia from aerial mycelium in culture. G. Conidia from sporodochia on natural substrate. H. Conidia from conidiophores in culture.

***Volutellonectria ciliata* J. Luo & W.Y. Zhuang, sp. nov.**

FIGS. 2C–D, 4A–F

Mycobank MB561608

Etymology. The specific epithet refers to its correlated anamorph.

Ascomata around old sporodochia, covered with hairs, perithecial, solitary or 2–6 in a group, superficial, obpyriform, 180–240 μm high, 160–215 μm diam, with an acute and red papilla, not collapsing when dry, orange-red when fresh and red when dry, turning dark red in 3% KOH and yellow in lactic acid, smooth. *Ascomatal wall* 14–21.5 μm thick, of two layers; outer layer 11–16 μm thick, cells 6–10 \times 3–5.5 μm , angular, cell wall 1–2 μm thick; inner layer 2–5.5 μm thick, cells flattened, 9–13 \times 2–4.5 μm , cell wall 0.5–1 μm thick. *Asci* subcylindrical to clavate, 8-spored, with an apical ring, 45–60 \times 4–6.5 μm (n = 50). *Ascospores* subellipsoid, uniseptate, not or slightly constricted at the septum, hyaline, smooth, irregularly biseriate, 7.5–11 \times 2.5–3.5 μm (n = 50).

Sporodochia solitary or gregarious on substrate, generally stipitate, with a small and basal stroma, 90–200 μm diam. *Setae* arising from stromatic base and surrounding the conidiophores, 215–435 μm long, 5–7.5 μm wide at base, tapering to a round apex, 3–8-septate, spinulose, hyaline to yellowish, walls 0.5–1.5 μm thick. *Conidiophores* branched, 60–95 μm long, 1.5–2.5 μm wide at base, closely aggregated. *Conidiogenous cells*

in whorls of 1–3, adpressed, straight, cylindrical, slightly tapering towards the tip, 5–12 μm long, 1–2 μm wide at base, 0.5–1 μm near aperture ($n = 50$). *Conidia* ellipsoid, distally rounded, with a median displaced hilum, straight, 1-celled, hyaline, smooth, 3–5.5 \times 1.5–2.5 μm ($n = 50$).

Holotype. INDONESIA. NORTH SULAWESI, Eastern Dumoga-Bone National Park, Gn muajat, Danau Alia, 1400 m, on herbaceous stem, 26 October 1985, *Samuels G.J.*, 2400A (NY), ex type culture GJS 85.205.

Notes. *Volutellonectria ciliata* is similar to *V. consors* and *V. asiana* in having obpyriform perithecia with a small and red papilla, subcylindrical to clavate asci with an apical ring, smooth ascospores, smooth and 1-celled conidia, and light-colored colonies. However, *V. consors* differs from *V. ciliata* in having laterally collapsing perithecia when dry, a hairy perithecial surface, smooth-walled sporodochial setae, somewhat longer conidia (4.5–)5–7(–13) μm long, and red sclerotia formed in culture (Samuels 1977). *Volutellonectria asiana* differs in having smaller perithecia 110–210 \times 85–190 μm , thinner perithecial walls 7.5–12 μm thick, shorter asci 35–47 μm long, smaller ascospores 4–8.5 \times 1.5–2.5 μm , smooth-walled sporodochial setae, and subellipsoid to subfusoid conidia 4–11.5 \times 1.5–2.5 μm .

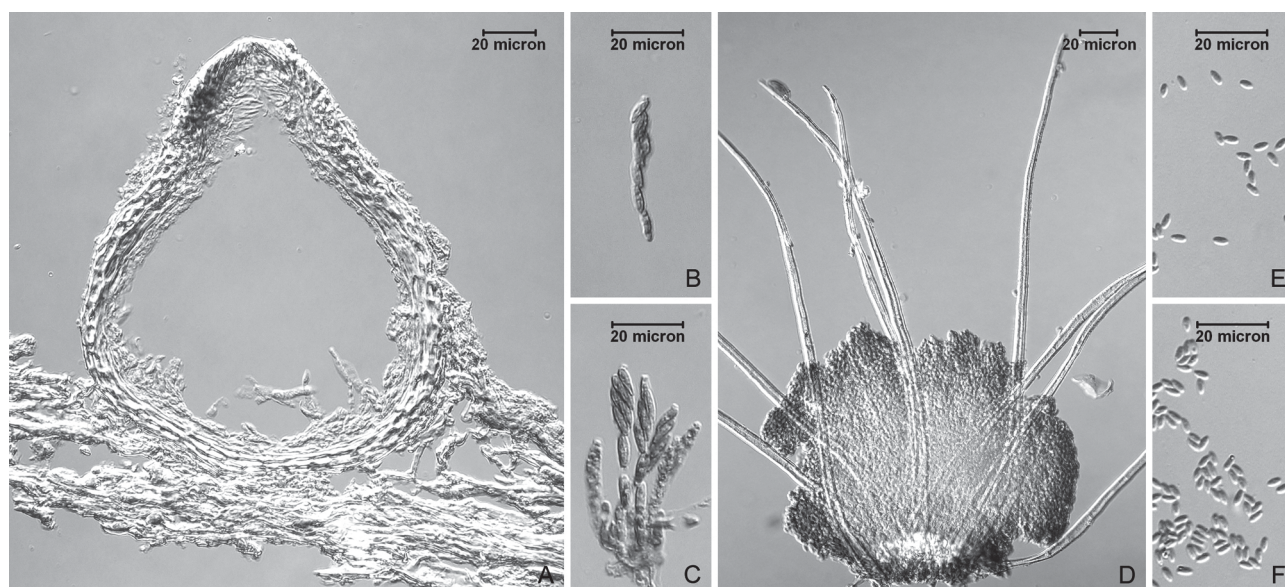


FIGURE 4. A–F. Morphology of *Volutellonectria ciliata* (GJS85.205). A. Median section of an ascoma. B–C. Asci with ascospores. D. Sporodochium from natural substrate. E–F. Conidia from sporodochia on natural substrate.

Except for the slightly shorter conidia (3–5.5 \times 1.5–2.5 μm vs. 5–7 \times 2 μm), morphology of the anamorph of *Volutellonectria ciliata* fits well the original description of *Volutella ciliata* (Saccardo 1886). This difference is treated as infraspecific variation and thus a teleomorph and anamorph connection is established, which is supported by our molecular data (FIG. 1). In the phylogenetic tree, *Volutellonectria ciliata* and *Volutella ciliata* formed a well supported subclade (1.00 BIPP; 100% BP), which was distinguishable from the other two species of the genus.

Discussion

Understanding the whole fungus has long been a task of mycologists (Kendrick 1979). As to nectriaceous fungi, small-sized perithecia and a *Cosmospora*-like perithecial wall structure are common in several genera like *Chaetopsinectria*, *Cosmospora*, *Cyanonectria*, *Nectricladiella*, *Pseudonectria*, *Volutellonectria*, etc. (Rossman et al. 1999, Schoch et al. 2000, Samuels et al. 2009, Luo and Zhuang 2010b). It is clear that teleomorphic morphology alone provides insufficient information to outline a genus within these fungi. Mycologists aim to establish holomorphic and monophyletic genera and a natural fungal classification

system. Increasing numbers of holomorphic genera are being proposed on the basis of morphological and molecular analyses, such as *Cyanonectria/Fusarium*, *Leuconectria/Gliocephalotrichum*, *Glionectria* Crous & S.L. Schoch/*Gliocladiopsis* S.B. Saksena, and *Xenocalonectria* Crous & C.L. Schoch/*Xenocylindrocladium* Decock, Hennebert & Crous (Schoch et al. 2000, Zhuang et al. 2007, Zhuang and Luo 2008, Samuels et al. 2009). *Chaetopsinectria/Chaetopsina* Rambelli, *Nectricladiella/Cylindrocladiella*, and *Volutellonectria/Volutella* are all segregates of *Cosmospora* and represent well-established holomorphic genera.

Ideally a teleomorphic genus should be linked to only one anamorphic genus (Rossman et al. 1999, Schoch et al. 2000). However, both *Pseudonectria* and *Volutellonectria* have a *Volutella* anamorph. In our phylogenetic tree *Pseudonectria rousseliana* (type species of the genus) was closely related to *Lanatonectria* (1.00 BIPP; 99% BP), and *P. pachysandricola* was weakly associated with *Chaetopsinectria* (0.68 BIPP; < 50% BP) in a second subclade. *Volutellonectria* appears to be a sister group to *Leuconectria* and *Calonectria*. The fact that *Volutellonectria* is distantly related to *Pseudonectria* species gives a hint that the anamorphic genus *Volutella* as currently circumscribed is unlikely to be monophyletic. All *Volutella* anamorphs of *Volutellonectria* share salmon-colored sporodochia with hyaline and thick-walled setae, compact conidiophores, and slimy, ellipsoid to fusoid conidia. However, *Volutella* species associated with *Pseudonectria* differ in producing sparse setae from sporodochia, much more diffuse sporodochia, and absence of sporodochial stipe (Samuels 1977, Rossman and Samuels 1993, Rossman et al. 1999, Samuels et al. 2006). These differences, accompanied with their teleomorphic variations, suggest heterogeneity within *Volutella*. Since the anamorphs of *Volutellonectria* include the type species of *Volutella* it is obvious that these represent a true and typical *Volutella*.

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