ABSTRACT

Title of Document: **SYSTEMATICS OF THE GENUS *COSMOSPORA* (NECTRIACEAE, HYPOCREALES), AND COSPECIATION OF *COSMOSPORA* SPECIES WITH THEIR ASSOCIATED FUNGAL HOSTS**.

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Cosmospora (in the broad sense; Nectriaceae, Hypocreales, Ascomycota) are fungi that parasitize other fungi, particularly fungi in the Xylariales (Ascomycota), or scale insects. Morphologically, these fungi are known for having one of the most simplest and smallest sexual fruiting bodies (<300 μm) among the Nectriaceae. The sexual spores are generally warted. The majority of *Cosmospora* species have acremonium-like or fusarium-like asexual states. The name *Cosmospora* is derived from the ornamentation in the sexual spores (Gr. *kosmos* = ornamented + Gr. *spora* = spore). The main goals of this dissertation were to revise *Cosmospora* sensu stricto, and to determine the evolutionary relationship between *Cosmospora* species and their associated fungal hosts. Additionally, *Corallomycetella* (Nectriaceae, Hypocreales, Ascomycota), a lineage basal to *Cosmospora* sensu lato, was revised as well. Molecular and classical taxonomic tools were used to revise the genera. A genus was

recognized if the clade met the following criteria: 1) the clade was well supported, 2) the clade was associated with a unique asexual state, and 3) the clade was ecologically different. A species was recognized if the clade met the following criteria: 1) the clade was well supported in the majority of single gene trees, 2) the clade was morphologically different, and/or 3) the clade was ecologically different in regards to host. *Cosmospora* species were observed to be highly host specific. Thus, host was recognized as an important character to delineate species, and the host specificity led us to hypothesize that *Cosmospora* species and their associated hosts were cospeciation (i.e., their association was not random). Two new genera, nine new combinations, and eleven new species were described in the taxonomic work included in this dissertation. A significant global congruence was determined between the *Cosmospora* and host phylogenies. However, host-switch events seemed more abundant in the early lineages of the host, while cospeciation events seemed more common in more recent lineages of the host. This phylogenetic signature is consistent with pseudocospeciation, but it could not be confirmed given that divergence estimates could not be estimated.

SYSTEMATICS OF THE GENUS *COSMOSPORA* (NECTRIACEAE, HYPOCREALES), AND COSPECIATION OF *COSMOSPORA* SPECIES WITH THEIR ASSOCIATED FUNGAL HOSTS.

By

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Preface

This dissertation contains an introductory chapter, four original articles/chapters, and a concluding chapter. Each original article is presented in manuscript format. The first three original articles represent taxonomic work for *Pseudocosmospora* C. Herrera & P. Chaverri, *Corallomycetella* Henn., and *Cosmospora* Rabenh., respectively, and have similar methodologies. The fourth original article is a coevolutionary study between cosmospora-like fungi and their associated fungal hosts. A single reference section containing all the cited literature throughout the dissertation is presented at the end.

Dedication

To my dad, Alfonso Herrera, who despite his humble upbringing and the countless adversities he faced as an immigrant, was able to support his sons' dreams.

Acknowledgements

I am grateful to many people for their support. I am particularly grateful to my advisor Dr. Priscila Chaverri for her guidance in completing my research. Drs. Priscila Chaverri, Amy Y. Rossman, and Gary J. Samuels collectively taught me how to collect specimens of the Nectriaceae. Additionally, Gary J. Samuels showed me how handle fresh samples of the Nectriaceae once we had them in the lab as well as how to handle herbarium specimens. Dr. Amy Y. Rossman's comments on my manuscripts have made me a better writer. I could not be here at this stage in my life without the mentoring of Dr. Chun-Juan Wang at the State University of New York: College of Environmental Science and Forestry (Syracuse, NY) as an undergraduate student. I am grateful to her for sharing her knowledge about the fungi, and teaching me new skills that would allow me to be successful in the mycological world.

I thank the many people that helped us with collecting trips: A. Romero and R. Sanchez in Argentina; O. Liparini Pereira, G. Barata, D. Lustosa in Brazil; C. Mendez in Costa Rica; P. Johnston in New Zealand; Luis Mejia in Panama, and T. Iturriaga in Venezuela. I also thank many people that have generously provided us with fresh specimens and/or isolates: Paul Diederich, Yuuri Hirooka, Christian Lechat, Kadri Põldmaa, and Keith A. Seifert. I gratefully acknowledge the assistance of the curators and their staff of the herbaria from which specimens were generously loaned. These herbaria include: U.S. National Fungus Collection (BPI); Royal Botanic Gardens, Kew (K); William and Lynda Steere Herbarium, New York Botanical Garden (NY);

the New Zealand Fungal and Plant Disease Collection (PDD); and Herbarium of the Botany Department, Swedish Museum of National History (S).

I thank the Latin American Studies Center (LASC) at the University of Maryland and the North American Mycological Association (NAMA) for the funding of a collecting trip to Panama. I also thank the American Society of Naturalists (ASN) for the partial support to attend the 2013 Workshop on Molecular Evolution at Woods Hole, MA; the Mycological Society of America (MSA), the Fungal Environmental Sampling and Informatics Network (FESIN), and The Graduate School (University of Maryland) for the funding to travel and present at the annual Mycological Society of America meetings. My research was funded by the National Science Foundation (NSF) PEET grant DEB-0731510 and the Department of Plant Science and Landscape Architecture (University of Maryland).

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Introduction

This dissertation is a contribution to fungal systematics. Systematics is the science of biological diversity, specifically the science that discovers, describes, and classifies all organisms. Taxonomy and nomenclature are part of systematics. Taxonomy deals with the classification of species, and nomenclature provides the principles and rules to name species and other taxa (reviewed in Schuh & Brower 2009). In this dissertation, a classification reflecting phylogenetic relationships (phylogeny) was sought.

Fungi remain a poorly known group of organisms. Fungal diversity is estimated conservatively at 1.5 million species; however, only 5% of fungal species have been identified and studied (Hawksworth 1991, 2001). Although the number of described species has risen exponentially since the first edition of the Dictionary of the fungi (reviewed in Blackwell 2011), there is much fungal diversity to be discovered. Hence, taxonomic work, such are the first three articles in this dissertation, is important to discover new species and to increase our knowledge of the world's biota.

The fungal system studied in this dissertation was *Cosmospora* Rabenh. (sensu lato; Nectriaceae, Hypocreales, Ascomycota). Fungi in the Hypocreales are characterized by brightly colored, soft-textured, ostiolate ascomata (sexual fruiting bodies); unitunicate asci; and hyaline to golden-yellow/golden-brown ascospores (sexual spores; Rogerson 1970; Rossman et al. 1999). Hypocrealean asexual states are

numerous, but they generally have phialidic conidiogenesis (or conidium development; Samuels and Seifert 1987). There are seven families in the Hypocreales: Bionectriaceae, Clavicipitaceae, Cordycipitaceae, Hypocreaceae, Nectriaceae, Niessliaceae, and Ophiocordycipitaceae. The Nectriaceae have generally superficial, red to purple, uniloculate ascomata that change color in 3% KOH and lactic acid and have non-disarticulating ascospores. Members of the Nectriaceae are unlike species of other major hypocrealean families such as the Hypocreaceae that have disarticulating ascospores and from the Bionectriaceae that have white to brown ascomata that do not change color in 3% KOH and lactic acid (Rossman et al. 1999). Higher-level phylogenetic studies have confirmed the Nectriaceae to be a distinct monophyletic family (Rehner & Samuels 1995; Rossman et al., 2001; Castlebury et al. 2004).

Cosmospora (sensu lato) have reddish, small-sized (<300 microns), and pear-shaped sexual fruiting bodies (perithecia). The asci are unitunicate, cylindrical to clavate, and usually have an apical ring. Each ascus contains eight, uniseriately arranged sexual spores (ascospores). The ascospores are ellipsoid to ellipsoid-fusiform, one-septate, yellow-brown and warted at maturity (Samuels et al. 1991; Rossman et al. 1999). The morphology of the sexual state is highly conserved, and it is the main reason that at one time there were 73 species classified under *Cosmospora* (sensu lato; http://www.indexfungorum.org). However, *Cosmospora* was thought to be polyphyletic given the range of asexual states (anamorphs). With the use of molecular data, the genus was restricted to cosmospora-like fungi with an acremonium-like asexual state (Gräfenhan et al. 2011). Many monophyletic genera were revived or

created to accommodate the remaining species: *Chaetopsina* Rambelli, *Nectricladiella* Crous & Schoch, *Fusicolla* Bonord., *Kryptocosmospora* Hirooka et al., *Macroconia* (Wollenw.) Gräfenhan et al., *Microcera* Desm., *Pseudocosmospora* C. Herrera & P. Chaverri,, *Stylonectria* Höhn., and *Volutella* Fr. (Schoch et al. 2000, Gräfenhan et al. 2011, Luo and Zhang 2010, 2012, Herrera et al. 2013).

Most cosmospora-like fungi are parasites of other fungi (mycoparasites) including *Cosmospora* sensu stricto. Tsuneda (1982) first described the attack by cosmospora-like mycoparasites. Species of *Cosmospora* penetrate the fruiting bodies of the host, and slowly consume the fleshy insides of the fungal host with its vegetative hyphae. This growth of *Cosmospora* on their hosts is slow, perhaps to ensure an extended period of nutrient uptake. The fungal host is able to mature, but is prevented from releasing its ascospores. Ultimately, the host's fleshy insides are completely replaced by vegetative hyphae of the *Cosmospora* species. The mycoparasitic attack ends with the formation of perithecia directly on the surface of the host's fruiting bodies, while simultaneously consuming its own vegetative hyphae for the production of perithecia (Tsuneda 1982).

An exception to the fungicolous habit of cosmospora-like fungi is *Microcera*, which is entomo-parasitic on scale insects (Gräfenhan et al. 2011). Porcelli & Frisullo (1998) suggested that insect dispersal might be the primary dispersal mechanism for this group of fungi; asexual spores (conidia) are dispersed via crawlers. It suggests that the attack by *Microcera* species is slow, but ultimately it will kill the scale insect carrying conidia. Species such as *M. larvarum* only affects female scale insects, which are not allowed to reproduce. The bodies of infected scale insects are

completely colonized with hyphae, and appear as “waxy mummies.” Sporodochia (fructifications of the asexual state) and perithecia are formed on the dead mummified scale insects; the sporodochia appear as a whitish cylindrical stalk and an orange globular head (Porcelli & Frisullo 1998). Additionally, some species of *Microcera* have been shown to be lichenicolous (Bills et al. 2009; unpublished data).

Species of cosmospora-like fungi are of economic importance. Species of *Microcera* have the potential to become biocontrol agents against scale insects, and have been shown to contain pharmaceutically active secondary metabolites. Studies have shown high mortality rates of scale insects caused by isolates of *Microcera* in vitro conditions (Ganassi et al. 2000; Cozzi et al. 2002). An antidiabetic secondary metabolite (aquastatin A), which could be useful against type 2 diabetes and obesity, was extracted from a species of *Microcera* isolated from intertidal sediments (Seo et al. 2009). Antifungal metabolites called parnafungins were extracted from the *M. larvarum* species complex. Parnafungins have been shown to have potent and broad-spectrum growth inhibitory activity against important clinical fungal pathogens such as *Aspergillus fumigatus* and *Candida albicans* (Bills et al. 2009). It is possible that other cosmospora-like fungi could have similar properties to *Microcera*. Species of *Pseudocosmospora* are parasites of species of *Eutypa* Tul. & C. Tul. and *Eutypella* (Nitschke) Sacc. (Herrera et al. 2013), which include some important plant pathogens. For example, *Eutypa* dieback of grapevine is caused by *Eutypa lata* (Pers.) Tul. & C. Tul., and is responsible for significant economic losses in the wine industry (Siebert 2001). Finding a *Pseudocosmospora* species that could be used as a biocontrol agent against plant diseases caused by *Eutypa* and *Eutypella* species is likely.

Chapter Summaries

The goal of the first chapter is to determine the identity of *Cosmospora vilior* (Starbäck) Rossman & Samuels. *Cosmospora vilior* was a confused species as result of the conservative morphology of *Cosmospora* species. The name was applied to *Cosmospora* species that grow on xylariaceous fungi (fungi growing on the fruiting bodies the Xylariaceae) and had a dark-green colony on cornmeal-dextrose agar (CMD; Samuels et al. 1991). However, the holotype specimen of *C. vilior* was determined to grow on a *Eutypella* species. A recently collected specimen resembling the holotype specimen of *C. vilior* was selected as epitype. An epitype is a specimen that supplements the holotype specimen (i.e., it provides information on missing characters such as DNA barcodes). *Cosmospora vilior* clustered with other *Cosmospora* species that grew on *Eutypa* and *Eutypella* species. The clade was determined to be distinct from other cosmospora-like genera, and described as a new genus *Pseudocosmospora* C. Herrera & P. Chaverri. The following characters unite *Pseudocosmospora* species: the asexual state (acremonium-like), the colony (salmon-pink in potato-dextroxe agar), and the hosts. Ten species were included in *Pseudocosmospora* including *P. vilior* (Starbäck) C. Herrera & P. Chaverri (≡ *C. vilior*). The first chapter has been published as Herrera et al. (2013a).

The second chapter is a taxonomic revision of *Corallomycetella* Henn. (Nectriaceae, Hypocreales, Ascomycota). *Corallomycetella* is basal to *Cosmospora* (in the broad sense, excluding *Chaetopsina* and *Volutella*; Hirooka et al. 2011). Two species of *Corallomycetella* were included in Rossman et al. (1999), and are plant pathogens of tropical trees such as rubber trees (*Hevea brasiliensis* Müll.Arg.). *Corallomycetella*

species are characterized by the root-like structures (rhizomorphs) produced in culture and in nature. The ascospores are reported to be smooth in *C. repens* (Berk. & Broome) Rossman & Samuels and roughened in *C. jatrophae* (Möller) Rossman & Samuels. However, a recent collection had striated ascospores, but otherwise was similar to *Corallomycetella* species. This collection led us to re-examine *Corallomycetella*, and determine if this unusual specimen represented a new species. Striated ascospores (surface view) were determined to be an overlooked character in *C. repens*. *Corallomycetella repens* was determined to comprise two species, and *C. jatrophae* was determined to be unrelated to *Corallomycetella*. *Corallonectria* C. Herrera & P. Chaverri was described to accommodate *C. jatrophae*. Additionally, we found sequences in GenBank that have been labeled as "*Nectria mauritiicola*," a synonym of *C. repens* (sensu Rossman), but are actually sequences of *Sarocladium kiliense* (Grütz) Summerb. The second chapter has been published as Herrera et al. (2013b).

The third chapter is taxonomic revision of *Cosmospora viliuscula* species complex. *Cosmospora viliuscula* grows on other fungi, particularly on the fruiting bodies of the Xylariaceae. It was previously confused with *C. vilior*. Apart from the host, *C. viliuscula* is characterized by the dark-green colony produced on CMD (Samuels et al. 1991). Up to now, it has been thought to consist of a single species, but the phylogenetic results revealed that *Cosmospora viliuscula* was a species complex. Each well-supported clade was host specific and regarded as a species. *Cosmospora viliuscula* was restricted to *Cosmospora* species growing on *Kretzschmaria* cf. *deusta* (in tropical forests). Seven new species and one new combination were described.

Additionally, the sexual states of *C. arxii* a (W. Gams) Gräfenhan & Schroers and *C. khandalensis* (Thirum. & Sukapure) Gräfenhan & Seifert were described for the first time.

The fourth chapter seeks to determine the evolutionary relationship between *Cosmospora* species and their associated fungal hosts. During the taxonomic revision of these fungi (chapter three), it was observed that these species demonstrated a high degree of host-specificity, which suggested that their association could not be random. It was hypothesized that *Cosmospora* species and their associated fungal hosts had cospeciated. The phylogenies of *Cosmospora* and their hosts were determined to be congruent. However, there was only a global congruence. The only host-parasite links that could be considered cospeciation events occurred in more recent evolutionary lineages of the host. Host-switches seemed to be occur more frequently in the early lineages of the host. This suggests that the host-switch events must have been very conservative (i.e., host-switches between closely related hosts) that mimic the phylogenetic signal of cospeciation.

Chapter 1: *Pseudocosmospora*, a new genus to accommodate *Cosmospora vilior* and related species

C.S. Herrera, A.Y. Rossman, G.J. Samuels, and P. Chaverri. 2013. *Mycologia* 05(5): 1287–1305. *Reprinted with permission of* Mycologia.

ABSTRACT

Cosmospora sensu Rossman accommodated nectroid fungi with small, reddish, smooth, thin-walled perithecia but recently was found to be polyphyletic and has been segregated into multiple genera. Not all cosmospora-like fungi have been treated systematically. Some of these species include *C. vilior* and many specimens often labeled as "*Cosmospora* sp." The objectives of this research were to establish the identity of *C. vilior* through epitypication using a recent collection that agrees with the type specimen in morphology, host and geography, and to determine its phylogenetic position within *Cosmospora* sensu lato and the Nectriaceae. A multilocus phylogeny was constructed based on six loci (ITS, LSU, MCM7, *rpb1*, *tef1*, and *tub*) to estimate a phylogeny. Results from the phylogenetic analyses indicated that *C. vilior* forms a monophyletic group with other *cosmopora*-like fungi that have an acremonium-like anamorph and that parasitize *Eutypa* and *Eutypella* (Ascomycota, Sordariomycetes, Xylariales, Diatrypaceae). The group is

phylogenetically distinct from other previously segregated genera. A new genus, *Pseudocosmospora* is described to accommodate the type species, *P. eutypellae*, and nine additional species in this clade.

INTRODUCTION

Cosmospora sensu Rossman (Nectriaceae, Hypocreales, Ascomycota; Gräfenhan et al. 2011) was erected to accommodate nectroid fungi with small, reddish, KOH+, smooth, thin-walled, laterally collapsing when dry, non- or weakly stromatic perithecia (Samuels et al. 1991, Rossman et al. 1999). These fungi have been reported throughout the world, but they are assumed to have greater diversity in warm temperate and tropical regions. In addition, they tend to have a higher diversity in recently disturbed stands (1–2 years old) compared to early successional stands (25–27 years old) and old-growth stands in tropical forests (Chaverri and Vílchez 2006). In that study, frequently collected species in recently disturbed stands, where newly killed woody substrates and herbaceous debris are prevalent, were members of *Chaetopsinectria* Lou & Zhuang and *Volutellonectria* Lou & Zhang, two genera segregated from *Cosmospora* sensu Rossman (Luo and Zhuang 2010, 2012). Many species of *Cosmospora* sensu Rossman are parasites of their fungal hosts (see Tsuneda 1982). Among genera segregated from *Cosmospora* sensu Rossman, some members of *Cosmospora* sensu stricto grow on basidiomycetes or xylariaceous hosts, species of *Dialonectria* (Sacc.) Cooke occur on *Diatrype* Fr. (Diatrypaceae), and *Microcera* Desm. parasitize scale insects (Gräfenhan et al. 2011).

The generic name *Cosmospora* has been a source of much taxonomic

confusion. Rabenhorst (1862) described this genus that was later reduced to a subgenus of *Nectria* (Fr.) Fr. by Saccardo (1883). Much later, it was synonymized with *Dialonectria* (Moravec 1954), which had been elevated from a subgenus of *Nectria* to generic rank by Cooke (1884). Rossman et al. (1999) resurrected the generic name *Cosmospora* based on priority. The group has also been referred to as *Nectria* subgenus *Dialonectria* or the '*Nectria episphaeria*-group' (Booth 1959, Samuels et al. 1991, Rossman et al. 1999). Early on, the group was presumed to be polyphyletic given its range of anamorphs and ecological niches (Samuels et al. 1991), and at some point, there were about 70 species classified under *Cosmospora* (www.indexfungorum.org). The polyphyly of *Cosmospora* was confirmed by recent phylogenetic studies (Zhuang and Zhuang 2006, Luo and Zhuang 2008, Samuels et al. 2009, Gräfenhan et al. 2011). Following the genus-for-genus concept, i.e. the delimitation of a genus based on the correlation of the teleomorph to its corresponding anamorph (Rossman 1993), *Cosmospora* was segregated into new or revived genera that correlate roughly with the anamorphs: *Chaetopsinectria*, *Cyanonectria* Samuels & P. Chaverri, *Nectricladiella* Crous & Schoch, *Fusicolla* Bonord., *Macroconia* (Wollenw.) Gräfenhan et al., *Microcera*, *Stylonectria* Höhn., and *Volutellonectria* (see Schoch et al. 2000, Samuels et al. 2009, Luo and Zhuang 2010, 2012, Gräfenhan et al. 2011). With the change to one scientific name for each species as directed in the International Code of Nomenclature for algae, fungi, and plants (ICN) (McNeill et al. 2012), *Chaetopsinectria* and *Volutellonectria* are considered synonyms of the older genera *Chaetopsina* Rambelli and *Volutella* Fr.

Cosmospora vilior was described as *Nectria vilior* by Starbäck (1899) with the

diagnosis “Peritheciis discretis, superficialibus, ovoideis, coccineis...Hab. in fungillo valsaceo.” Traditionally, the name has been applied to collections of cosmospora-like fungi having short, coarsely warted ascospores occurring on black stromata, particularly those of the Xylariales (Weese 1916, Samuels et al. 1990, Samuels et al. 1991). *Nectria vilior* has been reported to have a wide tropical and temperate distribution (Samuels et al. 1990). Re-examination of the type specimen of *C. vilior* revealed that its associated host is a species of *Eutypella* (Nitschke) Sacc. (Diatrypaceae). Our recent molecular analyses suggest that true *C. vilior* is unrelated to species of *Cosmospora* that occur on xylariaceous fungi, hereafter referred to as the *C. viliuscula* species complex. Species of the *C. vilior* complex occur only on species of *Eutypella* (Diatrypaceae) while *C. viliuscula* and related species are restricted to xylariaceous fungi.

The present paper deals with the phylogenetic and taxonomic reassessment of the *Cosmospora vilior* and similar taxa. The objectives of this research are: (i) to establish the identity of *C. vilior* and stabilize the name using epitypification, (ii) to elucidate the phylogenetic placement of *C. vilior* and related species within *Cosmospora* sensu Rossman and in the Nectriaceae, (iii) to describe a new genus, *Pseudocosmospora*, to accommodate *C. vilior* and related species, and (iv) to describe new species within *Pseudocosmospora* including the type *P. eutypellae*.

MATERIALS AND METHODS

Teleomorph and anamorph morphological characterization

Herbarium specimens were borrowed from the U.S. National Fungus Collections

(BPI), the William and Lynda Steere Herbarium, New York Botanical Garden (NY), and the Linnean Herbarium, Swedish Museum of Natural History (S). Fresh specimens were collected on trips to Argentina, Brazil, Costa Rica, France, and USA. For the characterization of the teleomorph, the following observations were made for perithecia: shape, size (length and width), color, ornamentation, and habit, e.g. perithecia being solitary or gregarious, immersed in substrata or superficial, stromatic or non-stromatic, and collapsing laterally or not when dry. Reaction to 3% w/v potassium hydroxide (KOH) and 100% lactic acid was observed for the perithecial wall. Sections of perithecia (ca. 11 μm in thickness) were made with the aid of a freezing microtome. Measurements of continuous characters (e.g. length and width) were made with Scion Image software beta 4.0.2 (Scion Corp., Frederick, Maryland) and summarized by descriptive statistics (e.g., minimum, maximum, mean and standard deviation).

Cultures were obtained from the culture collection at USDA, ARS, Systematic Mycology and Microbiology Laboratory (SMML). Additional cultures were obtained by isolating single ascospores from freshly collected samples with the aid of a micromanipulator and grown in cornmeal dextrose agar (CMD; Difco™ cornmeal agar + 2% w/v dextrose + antibiotics). Morphological observations of the colony were made by growing three pseudoreplicates of each isolate on CMD and Difco™ potato dextrose agar (PDA) in an incubator that alternates 12h/12h between fluorescent light and darkness at 25 C. Cultural morphology is described based on strains grown on PDA; cultures on CMD exhibit little variability. Colony color is described using the color terms in Rayner (1970). Culture growth was measured

weekly for two weeks. The anamorph was observed by cutting an agar block of a culture grown in synthetic nutrient-poor agar (SNA; Nirenberg 1976) under the same conditions mentioned above, covering it with a cover-slip, and examining it by light microscopy (Olympus BX50; Olympus, Tokyo, Japan). Measurements of continuous characters were made and analyzed as described above.

DNA Extraction, PCR, and Sequencing

Hirooka et al. (2010) described the DNA extraction protocol used here. Briefly, the isolates were grown in Difco™ potato dextrose broth (PDB), and the mycelial mat was harvested after a week of growth. DNA was extracted with PowerPlant® DNA Isolation Kit (MO BIO Laboratories Inc., Solana Beach, California).

Six partial loci were amplified. These loci are internal transcribed spacer (ITS; primers: ITS5 and ITS4; White et al. 1990), large subunit nuclear ribosomal DNA (LSU; primers: LROR and LR5; Vilgalys and Hester 1990), MCM7 (a DNA replication licensing factor; primers: Mcm7-709for & Mcm7-1348rev; Schmitt el al. 2009), RNA polymerase II subunit one (*rpb1*; primers: Crpb1a & rpb1c; Castlebury et al. 2004), translation elongation factor 1-α (*tef1*; primers: Tef1-728 and Tef1-986; Carbone and Kohn 1999), and β-tubulin (*tub*; O'Donnell and Cigelnik 1997). The PCR reaction mixture (25 µL total volume) consisted of 12.5 µL GoTaq®Green Master Mix 2X (Promega Corporation, Madison, Wisconsin), 1.25 µL for the forward and reverse primers each (10 mM), 1.0 µL of dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, Missouri), up to 5.0 µL genomic DNA template, and RNAse-free water to complete the total volume. PCR reactions were carried out in an Eppendorf

Mastercycler thermocycler (Eppendorf, Westbury, New York) under the cycle conditions listed in TABLE I. I. PCR products were cleaned with ExoSAP-IT® (USB Corp., Cleveland, Ohio). Clean PCR products were sequenced at the DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland) and McLAB DNA sequencing services (San Francisco, California). Sequences were assembled and edited with Sequencher 4.9 (Gene Codes, Madison, Wisconsin). Sequences were deposited in GenBank (SUPPLEMENTARY TABLE I. I.).

Phylogenetic Analyses

Two separate phylogenetic analyses were performed on two separate data sets as described below. The first data set contained a reduced number of isolates of *Cosmospora vilior* and related taxa as well as species of other cosmospora-like fungi to elucidate their phylogenetic placement in the Nectriaceae. The second data set contained all isolates of *Cosmospora vilior* and related taxa to determine their relationships.

ITS-LSU, MCM7, *rpb1*, *tef1*, and *tub* sequences were aligned with MAFFT 6 (Katoh 2008), and manually edited, if necessary, in Mesquite 2.75 (Maddison and Maddison 2011). Gaps (insertions/deletions) were treated as missing data. Alignments were deposited in TreeBASE (http://www.treebase.org; accession no. S14038). Maximum likelihood (ML) and Bayesian (BI) analyses were performed on each of the datasets of individual loci first and then on the concatenated dataset. CONCATEPILLAR 1.4 (Leigh et al. 2008) was used to determine whether loci could

be analyzed by concatenating the datasets or whether loci should be analyzed separately. Loci were concatenated if the p-value was greater than the default α-level of 0.05, which indicated that the null hypothesis (i.e., congruence of loci) could not be rejected.

For both ML and BI analyses, jModeltest (Guindon and Gascuel 2003, Posada 2008) was used to infer the models of nucleotide substitution for each locus. Default settings in jModeltest were used: 11 substitution schemes with equal or unequal base frequencies (+F) and invariable sites (+I) and/or rate variation among sites (+G). The base tree for likelihood calculations was ML optimized. Once likelihood scores were calculated, the models were selected according to the Akaike Information Criterion (AIC).

Maximum likelihood (ML) analyses were performed with GARLI v2.0 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl 2006) by submitting the job via the GARLI web service at http://www.molecularevolution.org (Bazinet and Cummings 2011), which uses a grid computing system (Cummings and Huskamp 2005) associated with The Lattice Project (Bazinet and Cummings 2008). Fifty independent search replicates were performed to search for the best tree. The starting tree was generated using a fast ML stepwise-addition algorithm. Two thousand bootstrap replicates were used for bootstrap analysis. Bayesian analyses were performed in MrBayes v3.2.1 (Ronquist et al. 2012). A majority rule consensus tree was generated by running four chains for 10,000,000 Markov Chain Monte Carlo generations, sampling trees every 100th generation, and discarding the first 25% of the sampled trees as burn-in. Tracer version 1.5 (Rambaut and Drummond 2007) was

used to confirm whether the negative log likelihoods had reached convergence.

RESULTS

Phylogenetic analyses: phylogenetic placement of C. vilior *and related species within* Cosmospora sensu *Rossman.*—The analysis performed in CONCATEPILLAR failed to reject the null hypothesis of congruence among loci (P =0.08). Therefore, all loci were concatenated. The concatenated matrix included 22 ingroup isolates that formed five major groups plus two outgroup taxa (*Corallomycetella repens* and *Pseudonectria pachysandricola*). It consisted of 3591 base pairs of which 970 were parsimony-informative, 318 were parsimony-uninformative, and 1858 were invariable sites. The topologies of the generated phylogenetic trees in both ML and BI were congruent. The negative log likelihoods for the phylogenetic trees were −16460.154 and −16513.115, respectively. The best tree (ML) is shown (FIG. 1.1).

Cosmospora vilior, *C. joca* and related species formed a highly supported clade (94% BP, 100% PP). This clade is related to *Dialonectria, Cosmospora* sensu stricto and an orphan group that includes *C. flavoviridis, C. obscura*, and *C. stegonsporii*. These clades of cosmospora-like fungi were highly supported as well (>70% BP, >95% PP), but the inner nodes connecting these clades were poorly supported. Basal to all of these groups is *Microcera*, another segregate genus of cosmospora-like fungi.

Phylogenetic analyses: relationship among C. vilior *and related species.*—The null hypothesis of congruence among loci (P =0.11) was not rejected in CONCATERPILLAR, and therefore, the five loci were concatenated to estimate a phylogeny. The concatenated matrix included 25 isolates belonging to the ingroup

and two outgroup taxa (*C. repens* and *M. larvarum*). The concatenated matrix consisted of 3353 bp of which 651 were parsimony-informative, 456 were parsimony-uninformative, and 1785 invariable sites. The tree topologies generated with ML and BI were congruent with one another. The log likelihoods for these two analyses were −23024.3191 and −23055.9286, respectively. The best tree generated with ML is shown (FIG. 1.2).

The combined analyses of *Cosmospora vilior* and related species revealed that there were as many as 16 independently evolving lineages (= putative species). Clade I is a complex of species whose hosts are *Eutypella* species. Three species are recognized within this clade, which include *C. vilior* and two species described below (*Pseudocosmospora eutypellae* and *P. rogersonii*). Sister to clade I is *P. eutypae* (described below), whose host is a species of *Eutypa* Tul. & C. Tul. Sister to clade II (*P. eutypae* + clade I) is clade III, which comprises two monotypic species, *C. joca* and *P. metajoca* (described below). *Cosmospora joca* is associated with a species of *Biscogniauxia* Kuntze (Xylariaceae), while *P. metajoca* is associated with a species of *Eutypa*. All clades corresponding to recognized species received maximum BP and PP support (with one exception).

TAXONOMY

Pseudocosmospora C. Herrera & P. Chaverri, **gen. nov**.

MycoBank MB 802432

Type species: *Pseudocosmospora eutypellae* C. Herrera & P. Chaverri

Etymology: "*Pseudo*" from Greek referring to the morphological similarity to both the teleomorphic and anamorphic states of *Cosmospora* sensu stricto.

Teleomorph: Stroma absent. Perithecia superficial or slightly immersed in fungal host stroma, scattered to gregarious, subglobose to obpyriform with a blunt papilla, generally less than 250 µm high, soft-textured, smooth-walled, scarlet, KOH+ blood-red, LA+ yellow, collapsing laterally when dry, uniloculate. Perithecial surface cells forming *textura angularis*. Perithecial wall generally 20–30 µm thick, of two regions; outer region of cells forming *textura globulsa* to *t. angularis*; inner region of cells forming *textura prismatica*. Asci unitunicate, cylindrical to narrowly clavate, increasing in size as ascospores mature, without a conspicuous apical ring, with eight spores arranged uniseriately. Ascospores ellipsoidal, 1-septate, slightly constricted at septum, yellow-brown, verrucose, sometimes appearing smooth at maturity.

Anamorph in culture: After 21 d at room temperature on PDA, colony surface crustose with no aerial mycelium or cottony with aerial mycelium, rosy-buff, pale-luteous, or salmon-pink. Sporulation on SNA usually abundant, arising directly from agar surface. Anamorphic state acremonium-like to verticillium-like; conidiophores generally simple, unbranched, sometimes verticillately branched, rarely densely aggregated. Phialides monophialidic, cylindrical, hyaline. Conidia ellipsoidal, ovoid, or reniform, smooth, sometimes guttulated, non-septate, hyaline.

Habitat: On stromata of diatrypaceous fungi, particularly species of *Eutypa* and *Eutypella*, rarely on species of *Biscogniauxia*.

Distribution: Asia, Africa, Europe, North America, Oceania, South America, possibly

cosmopolitan.

Notes: *Pseudocosmospora* is similar to *Cosmospora* sensu stricto in its cosmospora-like teleomorph and acremonium-like anamorph, although they differ in cultural characteristics and host preference. *Pseudocosmospora* is most common on diatrypaceous fungi except for *P. joca*, which occurs on a *Biscogniauxia* sp. The latter does not belong among the known hosts of *Cosmospora* sensu stricto, although these attack xylariaceous fungi as well as polypores. In general, *s*pecies of *Pseudocosmospora* have pinkish-colored colonies, while species of *Cosmospora* sensu stricto have olivaceous-green colonies on PDA. Phylogenetically, *Pseudocosmospora* appears to be closely related to *Dialonectria*. Both occur on diatrypaceous fungi, although they attack different genera. The genera also differ in their anamorphic state; *Dialonectria* has a fusarium-like anamorph.

***Key to species of* Pseudocosmospora**

1. On *Biscogniauxia* (Xylariaceae)…….*P. joca*
1. On *Eutypa* or *Eutypella* (Diatrypaceae)……...2

2. On *Eutypa*…….3
2. On *Eutypella*…….....4

3. Ascospores smooth, 6.3–8.7 µm long; colony rosy buff, 6–15 mm diam after 14 d at 25 C on PDA……..…….*P. eutypae*
3. Ascospores verrucose, 7.7–11.9 µm long; colony salmon-pink, 24–25 mm diam after 14 d at 25 C on PDA……...……..*P. metajoca*

4. Perithecia with a discoidal apex……...5

4. Perithecia with a blunt apex……..…...6

5. Fungal host on *Alnus* sp.; ascospores smooth, 9–10.4 µm……...……..*P. pithoides*

5. Fungal host on *Espeletia* sp.; ascospores verrucose, 11–14 µm long……..…….*P. pseudepisphaeria*

6. Conidiophores branching, becoming densely ramulose (fasciculate) on SNA……..…….....*P. triqua*

6. Conidiophores not branching or sparingly branched on SNA……..…...7

7. Colonies pale-luteous on PDA; conidia reniform, with two guttules at opposite ends, 3.4–7.4 µm long……..…..…..…..…..…*P. vilior*

7. Colonies white to salmon-pink on PDA……..…….....8

8. Colonies white on PDA; ascospores smooth, 10–15 µm long…….. *P. metepisphaeria*

8. Colonies salmon-pink on PDA……..……...9

9. Ascospores verrucose, 7.1–12.5 µm long; colonies 7.5–20 mm diam after 14 d at 25 C on PDA; conidia oblong to ellipsoidal, with two guttules at opposite ends, 3.1–6.2 µm long…….…...*P. eutypellae*

9. Ascospores smooth, 7.9–12.2 µm long; colonies 18–27.5 mm diam after 14 d at 25 C on PDA; conidia oblong to ellipsoidal, without guttules, 2.9–5.5 µm long……..……..…...*P. rogersonii*

Pseudocosmospora eutypae C. Herrera & P. Chaverri, **sp. nov**. FIG. 1.3.

Mycobank MB 802433

Holotype: FRANCE, Poitou-Charentes, Saint George de Rex (Marais Poitevin), on *Eutypa* sp., 26 Apr. 2011, *C. Herrera* (C.H. 11-01), BPI 884164, ex-holotype culture CBS 133961.

Etymology: In reference to its fungal host, *Eutypa.*

Teleomorph: Perithecia solitary, superficial, nonstromatic, subglobose with a discoidal apex, collapsing laterally when dry, scarlet, smooth, 171–200 × 150–183 µm (mean = 182 × 165; SD 16.1, 16.5; n = 3). Asci cylindrical to slightly clavate, with eight spores arranged uniseriately, 54–66 × 5.5–7 µm (mean = 59 × 6; SD 5.4, 0.7; n = 4). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, smooth, hyaline, 6.3–8.7 × 3.1–4.1µm (mean = 7.8 × 3.6; SD 0.7, 0.3; n = 30).

Anamorph: Colonies 6–15 mm diam (mean = 11.4; SD 4.1; n = 5) after 14 d. at 25 C on PDA, cottony with rosy-buff aerial mycelium, reverse concolorous. Sporulation on SNA usually abundant, arising directly from agar surface. Anamorphic state acremonium-like; conidiophores generally simple, unbranched. Phialides monophialidic, cylindrical, collarette not flared, hyaline, length 36–53 µm (mean = 41.3; SD 4.1; n = 13), width at base 1.5–2.0 µm (mean = 1.8; SD 0.2; n = 13), width at tip 1–1.3 µm (mean = 1.1; SD 0.1; n = 13). Conidia oblong, unicellular, smooth, hyaline, 4.6–6.7 × 1.2–2.1 µm (mean = 5.7 × 1.7; SD 0.6, 0.2; n = 30).

Habitat: On *Eutypa* cf. *lata* (Diatrypaceae) on bark.

Distribution: France, United Kingdom.

Additional isolates examined: UNITED KINGDOM, on *Crataegus* sp., 1958, *S. Francis*, culture IMI 73016.

Notes: *Pseudocosmospora eutypae* occurs on *Eutypa* cf. *lata* and has small and smooth ascospores. *Pseudocosmospora metajoca* is the only other species of *Pseudocosmospora* on *Eutypa*, but it has longer and verrucose ascospores.

Pseudocosmospora eutypellae C. Herrera & P. Chaverri, **sp. nov**. FIG. 1.4.

Mycobank MB 802434

Holotype: USA, Maryland, Beltsville, on *Eutypella* sp., on dead twigs of unidentified tree, 7 Oct. 2008, *Y. Hirooka* (Y.H. 08-17), BPI 884165, ex-holotype culture CBS 133966 = A.R. 4562.

Etymology: In reference to its fungal host, *Eutypella.*

Teleomorph: Perithecia gregarious, slightly immersed in host stromata, subglobose with a blunt apex to obpyriform, collapsing laterally, scarlet, smooth, 143–303 × 108–205 µm (mean = 202 × 150; SD 39, 22.7; n = 22). Asci cylindrical to slightly clavate, eight-spored, uniseriately arranged, 63–78.6 × 5.9–7.9 µm (mean = 71.6 × 6.7; SD 4.5, 0.6; n = 27). Ascospores ellipsoid to fusiform, equally two celled, slightly verrucose, yellow-brown, 7.1–12.5 × 3.6–5.6 µm (mean = 9.9 × 4.5; SD 0.9, 0.4; n = 136).

Anamorph: Colonies 7.5–20 mm diam (mean = 12.6, SD 3.4, n = 18) after 14 d. at 25

C on PDA, sometimes crustose, with or without aerial mycelium, buff, rosy-buff, or salmon-pink, reverse concolorous. Sporulation on SNA usually abundant, arising directly from agar surface, sometimes from lateral pegs. Anamorphic state acremonium-like to verticillium-like; conidiophores simple, unbranched, or branched, becoming densely branched. Phialides monophialidic, cylindrical, collarette not flared, hyaline, length (3.8–) 7.8–15.1 (–18.5) µm (mean = 10.2; SD 3.0; n = 29), width at base 0.9–2.3 µm (mean = 1.3; SD 0.3; n = 29), width at tip 0.7–1.2 µm (mean = 0.9; SD 0.14; n = 29). Conidia oblong to ellipsoidal, unicellular, with two guttules at opposite ends, smooth, hyaline, 3.1–6.2 × 1.0–2.4 µm (mean = 4.2 × 1.5; SD 0.6, 0.2; n =1 80).

Habitat: On *Eutypella* sp. (Diatrypaceae) on bark.

Distribution: France and U.S.A.

Additional specimens and isolates examined: FRANCE, Oloron, Forêt de Bugangue, on *Eutypella* sp., on bark of *Robinia pseudoacacia* (?), 17 May 1993, *F. Candoussau & J.D. Rogers* (F. 262), BPI 802567, culture CBS 128986 = G.J.S. 93-15; USA, Kentucky, Clermont, Bernheim Arboretum and Research Forest, on *Eutypella* sp., on dead branch of unidentified tree, 27 June 2010, *Y. Hirooka*, BPI 884169, culture CBS 133977 = G.J.S. 10-248; Maryland, Frederick County, Cunningham Falls State Park, on *Eutypella* sp., on *Rhus typhina*, 26 Aug. 2007, *L. Vasilyeva*, BPI 878454, culture CBS 129430 = A.R. 4453; Pennsylvania, Greensburg, on *Eutypella* sp., Aug. 2008, *J. Plitschke*, culture CBS 133965 = A.R. 4527; West Virginia, Grafton, on *Eutypella* sp., on bark of unidentified tree, 26 June 2010, *Y. Hirooka*, BPI 884168, culture CBS

133960 = C.H. 10-02.

Notes: *Pseudocosmospora eutypellae* is most closely related and similar to *P. rogersonii*, but can be distinguished from the latter by the ornamentation of its ascospores. *Pseudocosmospora eutypellae* has verrucose ascospores, while *P. rogersonii* has smooth ascospores.

Pseudocosmospora joca (Samuels) C. Herrera & P. Chaverri, **comb. nov**. FIG. 1.5.

Mycobank MB 802435

Basionym: *Nectria joca* Samuels, Mycol. Pap. 164: 21. 1991.

≡ *Cosmospora joca* (Samuels) Rossman & Samuels, Stud. Mycol. 42: 122. 1999.

Teleomorph: Perithecia gregarious, superficial, nonstromatic, subglobose with a minute papilla, collapsing laterally, scarlet at first, becoming blood red, darker at apex, smooth, 375–384 × 317–349 μm (mean = 380.2 × 336.3; SD 4.9, 16.9; n = 3). Asci cylindrical to clavate, eight-spored, uniseriately arranged, 90.7–112.6 × 8.8–11.1 μm (mean = 102.2 × 9.8; SD 8.5, 0.7; n = 9). Ascospores ellipsoid, equally two-celled, one-septate, constricted at septum, verrucose, yellow-brown, 10.9–14 × 6.4–7.7 μm (mean = 12.8 × 7; SD 0.8, 0.3; n = 30).

Anamorph: Colonies 4 mm diam (n = 3) after 14 d. at 25 C on PDA, crustose, salmon-pink to orange colony, reverse concolorous. Rarely sporulating on SNA. Anamorphic state acremonium-like; conidiophores generally simple, unbranched. Phialides monophialidic, cylindrical, hyaline, length 14.3–24.2 μm (mean = 18.8; SD

5.0; n = 3), width at base 1.6–2.4 μm (mean = 2.0; SD 0.4; n = 3), width at tip 1.1 (n = 3). Conidia oblong, unicellular, smooth, hyaline, 3.0–5.5 × 1.3–2.1 μm (mean = 4.1 × 1.6; SD 0.6, 0.2; n = 30).

Habitat: On *Biscogniauxia* sp. on bark.

Distribution: Argentina and Brazil.

Holotype: BRAZIL, Amazonas, Pico Rondon, Km 211 on Perimetral Norte, ca 3h walk from FUNAI post toward summit, 01°32'N, 02°48'W, on *Biscogniauxia* sp., 25 Mar. 1984, *G.J. Samuels* (1094), *Pipoly & Guedes*, INPA (not seen), ISOTYPES BPI 802606, NY 00671973 (not seen).

Epitype designated herein: ARGENTINA, Río Negro Province, San Carlos de Bariloche, Luma forest, on *Biscogniauxia* sp., on very rotten wood, 15 Apr. 2011, *A. Romero*, BPI 884175, ex-epitype culture CBS 133967 = A.R. 4779.

Notes: The application of the name is restricted here to species of *Pseudocosmospora* on *Biscogniauxia*. The isotype and the designated epitype both occur on species of *Biscogniauxia*. The colony and the anamorph are similar to the morphology described in the original description although the perithecia and ascospores of the epitype are larger than those reported in the literature.

Pseudocosmospora metajoca C. Herrera & P. Chaverri, **sp. nov**. FIG. 1.6.

Mycobank MB 802436

Holotype: NEW ZEALAND, North Island, Mt. Williams, on *Eutypa* sp., on dead

woody branch of *Beilschmiedia tawa*, 7 Mar. 2009, *A.Y. Rossman & P. Chaverri* (P.C. 952), BPI 879088, ex-holotype culture CBS 133968 = A.R. 4576.

Etymology: '*Meta*' from Greek meaning adjacent and '*joca*' in reference to the fact that it was originally classified as *C. joca*, and later found to be phylogenetically close to *C. joca*.

Teleomorph: Solitary or gregarious, superficial, nonstromatic, subglobose with a discoidal apex, some collapsing laterally, scarlet, smooth, 222–251 × 204–213 μm (mean = 236 × 208; n = 2). Asci clavate, eight-spored, uniseriately arranged, 62.2–69.2 × 5.9–7.2 μm (mean = 65.5 × 6.4; SD 2.5, 0.5; n = 5). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, slightly verrucose, yellow-brown, 7.7–11.9 × 3.3–5.3 μm (mean = 8.9 × 4.3; SD 0.9, 0.5; n = 29).

Anamorph: Colonies 24–25 mm diam (mean = 24.5; SD 0.5; n = 3) after 14 d. at 25 C on PDA, slightly cottony, pale salmon-pink, reverse concolorous. Sporulation on SNA usually abundant, arising directly from agar surface. Anamorphic state acremonium-like; conidiophores generally simple, unbranched. Phialides monophialidic, cylindrical, collarette not flared, hyaline, length 26–49 μm (mean = 37.3; SD 5.7; n = 9), width at base 2.1–2.8 μm (mean = 2.4; SD 0.2; n = 9), width at tip 1–1.3 μm (mean = 1.2; SD 0.1; n = 9). Conidia oblong to ellipsoidal, unicellular, guttulated, smooth, hyaline, 3.8–6.1 × 1.6–3.1 μm (mean = 4.8 × 2.1; SD 0.6, 0.3; n = 30).

Habitat: On *Eutypa* sp. (Diatrypaceae) on dead branch of *Beilschmiedia tawa*.

Distribution: New Zealand.

Notes: *Pseudocosmospora metajoca* was originally identified as *C. joca* based on its occurrence on what was thought to be a stroma of a *Biscogniauxia* species and its salmon-pink culture on PDA. On close examination of the specimen, the host was found to be a species of *Eutypa*. The colony of *P. metajoca* has a faster growth rate than that of *P. joca*. In addition, *P. metajoca* has much smaller perithecia and ascospores compared to *P. joca*. *Pseudocosmospora metajoca* differs from *P. eutypae*, the other *Pseudocosmospora* on *Eutypa*, by having verrucose ascospores.

Pseudocosmospora metepisphaeria (Samuels) C. Herrera & P. Chaverri, **comb. nov**.

Mycobank MB 802437

Basionym: *Nectria metepisphaeria* Samuels, Mycol. Pap. 164: 29. 1991.

≡ *Cosmospora metepisphaeria* (Samuels) Rossman & Samuels, Stud. Mycol. 42: 123. 1999.

Anamorph: Acremonium-like

Habitat: On *Eutypella* sp. (Diatrypaceae) on unidentified bark.

Distribution: Venezuela (known only from the type collection)

Holotype: VENEZUELA, Drt. Federale, vic. Macarao, on *Eutypella* sp., on unidentified bark, 21 Jun. 1971, *K.P. Dumont* (VE 335), *J.H. Haines, G. Morillo & E. Moreno*, VEN (not seen), ISOTYPE NY.

Notes: The isotype specimen was studied and determined to occur on a *Eutypella* sp.

Based on this host, it can be predicted that *C. metepisphaeria* would fall within the *Pseudocosmospora* clade. In addition to the host, the reported acremonium-like anamorphic state supports the placement of this species in *Pseudocosmospora*. Unique to this species is its smooth ascospores, (10–)11–14(–15) μm long and the white, crustose colony on PDA, reverse brown (Samuels et al. 1991). A culture no longer exists.

Pseudocosmospora pithoides (Ellis & Everh.) C. Herrera & P. Chaverri, **comb. nov**.

Mycobank MB 802438

Basionym: *Nectria pithoides* Ellis & Everh., Proc. Acad. Nat. Sci. Philad. 43: 247 (1891).

Anamorph: Unknown

Habitat: On an *Eutypella* sp. (Diatrypaceae) on bark of dead alder.

Distribution: British Columbia (known only from the type collection).

Holotype: CANADA, British Columbia, on bark of dead alder, May 1889, *J. Macoun* (122), NY 00927939.

Notes: The holotype specimen of *Nectria pithoides* was examined and determined to agree with the concept of *Pseudocosmospora* in regard to the host, which appears to be a *Eutypella* species. The perithecia have a prominent discoidal apex, which according to the description, gives an impression of being barrel-shaped (*pithos* from Greek = barrel). No asci were observed. The ascospores are ellipsoidal, one-septate, slightly constricted at the septum, smooth, 9–10.4 × 4.1–4.5 μm (mean = 9.7 × 4.4;

SD 0.5, 0.1; n = 8).

Pseudocosmospora pseudepisphaeria (Samuels) C. Herrera & P. Chaverri, **comb. nov**.

Mycobank MB 802439

Basionym: *Nectria pseudepisphaeria* Samuels, Mycol. Pap. 164: 34. 1991.

≡ C*osmospora pseudepisphaeria* (Samuels) Rossman & Samuels, Stud. Mycol. 42: 124. 1999.

Anamorph: Acremonium-like.

Habitat: On *Eutypella* sp. (Diatrypaceae) on branch of *Espeletia* sp.

Distribution: Venezuela (known only from the type collection).

Holotype: VENEZUELA, Merida, Parque Nacional Sierra Nevada, near Apartaderos, E. of Laguna Mucubaji, Laguna Negra, on *Eutypella* sp., on *Espeletia* sp., 18 Jul. 1971, *K.P. Dumont* (VE 2277), *J.H. Haines, G.J. Samuels & A. Revas,* NY 01013169.

Notes: Based on our examination of the holotype specimen, the fungal host of *C. pseudepisphaeria* is a *Eutypella* sp. The fungal host and the reported acremonium-like anamorphic state support the placement of *C. pseudepisphaeria* in the genus *Pseudocosmospora*. Unique to this species are the discoidal perithecial apices, its verrucose, (11–) 11.2–13(–14) μm long ascospores, and its white to pale salmon-colored colony (Samuels et al. 1991). A culture no longer exists.

Pseudocosmospora rogersonii C. Herrera & P. Chaverri, **sp. nov**. FIG. 1.7.

Mycobank MB 802440

Holotype: USA, New York, Dutchess County, Pawling, Pawling Nature Reserve, on *Eutypella* sp., 6–8 Oct. 1990, *G.J. Samuels & C.T. Rogerson*, BPI 1107121, ex-holotype culture CBS 133981 = G.J.S. 90-56.

Etymology: In honor of Clark T. Rogerson for his work on the Hypocreales that has guided all of us.

Teleomorph: Perithecia gregarious, slightly immersed in host stromata, subglobose with a blunt papilla, collapsing laterally, scarlet, smooth, 163–245 × 131–180 μm (mean = 193 × 152; SD 37, 21; n = 7). Asci broadly cylindrical to narrowly clavate, eight-spored, uniseriately arranged, 54-69 × 5.7-8.4 μm (mean = 63 × 6.7; SD 4.9, 0.7; n = 12). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, smooth, yellow-brown, 7.9–12.2 × 3.3–4.9 μm (mean = 9.6 × 4.1; SD 0.9, 0.3; n = 86).

Anamorph: Colonies 18–27.5 mm diam (mean = 22.4; SD 3.4; n = 8) after 14 d. at 25 C on PDA, crustose, rosy-buff to salmon-pink, reverse concolorous. Sporulation on SNA usually abundant, arising directly from agar surface. Anamorphic state acremonium-like; conidiophores generally simple, unbranched. Phialide cylindrical, smooth, straight, collarette not flared, hyaline, length 6.8–29.4 μm (mean = 12.3; SD 5; n = 30), width at base 1.0–2.3 μm (mean = 1.5; SD 0.3; n = 30), width at tip 0.7–1.2 μm (mean = 0.9; SD 0.13; n = 30). Conidia oblong to ellipsoidal, unicellular, smooth, hyaline, 2.9–5.5 × 1.1–2.6 μm (mean = 3.8 × 1.6; SD 0.6, 0.3; n = 89).

Habitat: On *Eutypella* sp. (Diatrypaceae) on bark.

Distribution: USA.

Additional specimens and isolates examined: USA, New York, Dutchess County, Pawling, Pawling Nature Reserve, on *Eutypella* sp., 6–8 Oct. 1990, *G.J. Samuels & C.T. Rogerson*, BPI 1107120; New York, Huguenot, YMCA Greenkill Retreat enter, on *Eutypella* sp., 26 Sept. 2009, *C. Herrera* (C.H. 09-02), BPI 884167, culture = G.J.S. 09-1384; New York, Painted Post, Watson Homestead Conference and Retreat Center, on *Eutypella* sp., on dead branch of *Fagus grandifolia*, 17 Sept. 2010, *C. Herrera* (C.H. 10-11), BPI 884166, culture CBS 133978 = G.J.S. 10-296; New York, Painted Post, Watson Homestead Conference and Retreat Center, on *Eutypella* sp., on dead branch of *Fagus grandifolia*, 17 Sept. 2010, *C. Herrera* (C.H. 10-12), BPI 884170, culture CBS 133979 = G.J.S. 10-297.

Notes: *Pseudocosmospora rogersonii* is closely related to *P. eutypellae*, but differs conspicuously in the ornamentation of its ascospores. *Pseudoscosmospora rogersonii* has smooth ascospores in contrast to *P. eutypellae*, which has verrucose ascospores.

Pseudocosmospora triqua (Samuels) C. Herrera & P. Chaverri, **comb. nov**.

Mycobank MB 802441

Basionym: *Nectria triqua* Samuels, Mycol. Pap. 164: 40. 1991.

≡ *Cosmospora triqua* (Samuels) Rossman & Samuels, Stud. Mycol. 42: 125. 1999.

Anamorph: Acremonium-like.

Habitat: On *Eutypella* sp. (Diatrypaceae) on unidentified bark.

Distribution: French Guiana (known only from the type collection).

Holotype of Nectria triqua: FRENCH GUIANA, Upper Marouini River, vic. roche Koutou, 02°55'N, 54°04'W, elev. 400 m., on *Eutypella* sp., on unidentified bark, 17 Aug. 1987, *G.J. Samuels* (5818), *J.-J. de Granville, L. Allorge, W. Hahn, M. Hoff,* NY 01013269.

Notes: Examination of the holotype revealed that the host is a *Eutypella* sp., which suggests that *C. triqua* should be placed in the genus *Pseudocosmospora*. Additionally, the reported anamorphic state is similar to that of *P. vilior* and *P. eutypellae* in having branching conidiophores branch that terminate with multiple phialides. Cultural morphology in PDA was not reported in the description of *Nectria triqua* (Samuels et al. 1991). The culture no longer exists. The ascospores are verrucose and (6.8–) 7.8–9.7 (–10.5) μm long.

Pseudocosmospora vilior (Starbäck) C. Herrera & P. Chaverri, **comb. nov**. FIG. 1.8.

Mycobank MB 802442

Basionym: *Nectria vilior* Starbäck, Bih. Kongl. Svenska Vet.-Acad. Handl. 25(3,1): 28. 1899.

≡ *Cosmospora vilior* (Starbäck) Rossman & Samuels, Stud. Mycol. 42: 126. 1999.

Teleomorph: Perithecia gregarious, slightly immersed in host stromata, subglobose with blunt apex, collapsing laterally, scarlet, smooth, 195–224 × 136–183 μm (mean = 213 × 164; SD 9.1, 14.7; n = 10). Asci cylindrical to clavate, eight-spored, uniseriately arranged, 59–81 × 5.3–11.0 μm (mean = 69 × 7.6; SD 6.2, 1.7; n = 18). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, slightly verrucose, yellow-brown, 8.3–13.0 × 4.1–6.4 μm (mean = 10.2 × 5.2; SD 1.0, 0.5; n = 90).

Anamorph: Colonies 23–65 mm diam (mean = 49; SD 16.9; n = 8) after 21 d. at 25 C on PDA, cottony with pale luteous aerial mycelium, reverse concolorous. Sporulation on SNA usually abundant, arising directly from agar surface; acremonium-like to verticillium-like; conidiophores simple and unbranched at first, becoming densely branched. Phialides cylindrical, smooth, straight, collarette not flared, hyaline, length 5.9–19.5 μm (mean = 12.9; SD 3.4; n = 9), width at base 1.1–1.8 μm (mean = 1.5; SD 0.2; n = 29), width at tip 0.7–1.3 μm (mean = 1.0; SD 0.2; n = 29). Conidia reniform, unicellular, smooth, with two guttules at opposite ends, hyaline, 3.4–7.4 × 1.1–2.3 μm (mean = 4.8 × 1.7; SD 0.7, 0.3; n = 90).

Habitat: On *Eutypella* sp. (Diatrypaceae) on bark.

Distribution: Argentina and Brazil.

Holotype: BRAZIL, Rio Grande do Sul, Santo Angelo pr. Cachoaira, on *Eutypella* sp., 12 Jan. 1893, *Gustav Malme* (114), S F46424.

Epitype designated herein: ARGENTINA, Misiones Province, Iguazú Biological

Station, on *Eutypella* sp., 25 Apr. 2011, *A.Y. Rossman, C. Salgado, A. Romero, R. Sanchez*, BPI 884176, ex-epitype culture CBS 133971 = A.R. 4810.

Additional specimens and isolates examined: ARGENTINA, Tucuman Province, Tucuman, on *Eutypella* sp., on standing dead branch of *Piper tucumanum*, 19 Apr. 2011, *A. Romero*, BPI 884174, culture CBS 133970 = A.R. 4771; BRAZIL, Bahia, Igrapiúna, on *Eutypella* sp., 12 Aug. 2010, *P. Chaverri* (P.C. 1246), *O. Liparini Pereira, D. Pinho, A. Luiz Firmino*, BPI 884172, culture CBS 133963.

Notes: An epitype was needed to establish an anamorph for *P. vilior* and to determine its phylogenetic placement. The epitype was selected based on the relatively close proximity to the collecting site of the holotype. The application of the name is restricted to species of *Pseudocosmospora* on *Eutypella* from South America that have pale-luteous colonies on PDA. However, it is recognized here that *P. vilior* consists of a species complex.

DISCUSSION

Genus Concept

The generic concept *Cosmospora* sensu stricto is based on its type *Cosmospora coccinea* Rabenh., which has *Verticillium olivaceum* W. Gams as its anamorph. Although the anamorph bears the name *Verticillium* Nees, the anamorphic state is acremonium-like (single phialide, unbranched) to verticillium-like (branching into multiple phialides). Accepted species in *Cosmospora* sensu stricto have an acremonium-like anamorph, and it is the character that circumscribes the genus (Gräfenhan et al. 2011). Conidiophore branching is not unique to the anamorph of *C.*

coccinea as this is also observed in some anamorphs in the *Cosmospora viliuscula* species complex.

Pseudocosmospora (described above) is recognized as a new genus based on the one-to-one genus concept suggested by Rossman (1993) to accommodate *C. vilior* and related species. The one-to-one genus concept has been used extensively in the Ascomycota to delimit genera (e.g. Gräfenhan et al. 2011, Luo and Zhang 2010, 2012). Briefly, this genus concept suggests that a genus should be circumscribed based on the correlation of its teleomorph to its unique anamorph state and vice versa. The groups circumscribed based on this concept are monophyletic and often supported by ecological traits (e.g. Gräfenhan et al. 2011, Luo and Zhang 2010, 2012). In Gräfenhan et al. (2011), the reported hosts for members of *Cosmospora* sensu stricto were basidiomycetes (e.g., *Fomitopsis* P. Karst., *Inonotus* P. Karst. and *Stereum* Hill ex Pers.) and xylariaceous fungi (e.g., *Hypoxylon* Bull.). *Microcera* and *Dialonectria* species have fusarium-like anamorphs, *Microcera* species are parasites of scale insects, and the lectotype species of *Dialonectria*, *D. episphaeria*, is reported on *Diatrype stigma* (Hoffm.) Fr. (Diatrypaceae; Booth 1959). The host of *Dialonectria ullevolea* Seifert & Gräfenhan has not been identified, but it is predicted here that the host will be a diatrypaceous fungus. The orphan clade consisting of *C. flavoviridis* (Fuckel) Rossman & Samuels, *C. stegonsporii* Rossman, Farr & Akulov, and *C. obscura* Lowen has not been taxonomically revised, and may require generic recognition. Species in this clade have a fusarium-like anamorphs, but little is known about their fungal hosts. Only the host of *C. stegonsporii*, *Stegonsporium pyriforme* (Hoffm.: Fr.) Corda (Diaporthales, Sordariomycetes), has been identified to species.

It is possible that all fungal hosts of species in this clade are members of the Diaporthales.

The one-to-one genus concept is ideal for the circumscription of genera in the Ascomycota because it forces the study of the holomorph and not only the teleomorph or anamorph. Such view is crucial in shifting to one name (Norvell 2011). Discarding information of either the teleomorph or anamorph in order to favor one generic hypothesis over the other may result in para- or polyphyletic groups. For example, a weak case could be made to group *Cosmospora*, *Dialonectria*, *Pseudocosmospora* and the orphan clade that consists of *C. flavoviridis*, *C. stegonsporii* and *C. obscura* into one genus because they have a cosmospora-like teleomorph and occur generally on Sordariomycetes. However, when the anamorphs are superimposed on the phylogeny, a paraphyletic group is formed with two groups having acremonium-like anamorphs and the remaining two groups fusarium-like anamorphs. It suggests that the teleomorp state is probably a symplesiomorphic character (or ancestral), while the anamorp represents a synapomorphic character (derived). Moreover, segregation of the discussed genera is supported by specialization to different host taxa.

The cosmospora-like teleomorphic state of *Pseudocosmospora* was correlated here to an acremonium-like anamorph. Our phylogeny (FIG. 1.2) demonstrates that *Pseudocosmospora* (BP 100%, PP 100%) is not congeneric with *Cosmospora s.str.*, the only other group of cosmospora-like fungi with an acremonium-like anamorph (Hirooka et al. 2010, Gräfenhan et al. 2011). The two groups differ primarily by their cultural characteristics. In general, *Pseudocosmosp*ora produces pinkish colonies,

while *Cosmospora* sensu stricto, produces olivaceous-green colonies on PDA.

Members of each genus considered in this study occur only on a particular group of host fungi. *Pseudocosmospora* is reported here to occur primarily on *Eutypa* and *Eutypella* species (Diatrypaceae) with the exeption of *Cosmospora joca* (Samuels) Rossman & Samuels, whose host is a species of *Biscogniauxia*. The genus *Dialonectria* also occurs on diatrypaceous fungi but has a fusarium-like anamorphic state as do species in the genus *Microcera* that occur primarily on insects.

Species Concept

The Genealogical Concordance Phylogenetic Species Recognition was used to delimit species boundaries (GCPSR; Taylor et al. 2000). According to this operational species concept, putative species are clades that are concordant across all single gene trees. The morphological species recognition was also used to support the species inferences made when applying GCPSR. Hence, inferred species may be associated with unique morphological features that set them apart from other closely related species.

Another species concept that could be useful in determining additional characters to delimit species is the ecological species concept. According to this species concept, ecological niches or adaptive zones can be used to delimit species (reviewed in de Queiroz 2007). Host, an ecological niche, could be a character specific to a particular *Pseudocosmospora* species. However, host identification of *Eutypa* and *Eutypella* to species was not possible based on morphology alone. Identification of the fungal host based on DNA sequences would resolve this

problem. Moreover, analyzing DNA sequences of cosmospora-like fungi and their associated fungal hosts would allow testing the hypothesis of cospeciation. Evidence for cospeciation would provide independent evidence for the delimitation of species in this genus.

A problem of GCPSR is that it requires multiple individuals per species. By definition a clade is formed by a minimum of two individuals per species (reviewed in Vinuesa 2010). In the case of this paper, only a single collection was made for many of the lineages, and it left us with a dilemma on how to deal with the many singletons present in the phylogeny (FIG. 1.2). It was decided to use the rule of rarity (reviewed in Lim et al. 2012) to recognize a singleton, *Pseudocosmospora metajoca* (described below), as a species. This species is morphologically and ecologically distinct from species recognized with GCPSR and other singletons. *Pseudocosmospora metajoca* occurs on an *Eutypa* sp. on *Beilschmiedia tawa* (A.Cunn.) Kirk (Lauraceae), which is a broadleaf tree native to New Zealand, and has verrucose ascospores. Also supporting the view that *P. metajoca* is a distinct species is the relatively long branch length, which indicates that there have been multiple substitutions per site since its segregation.

Cosmospora vilior represents a case where morphology is insufficient to distinguish closely related species. This species is characterized by its relatively fast growing, pale luteous colony on PDA. However, the clade probably represents a species complex given the highly supported subclade that consists of the strains AR 4771 and PC 1246. The species complex may consist of up to three species, but the selected epitype strain, AR 4810, is considered closer to the true *C. vilior* based on

geographical proximity to the original collecting site of the type specimen and its host.

Phylogenetic placement of Cosmospora joca*.*—The phylogenetic placement of *Cosmospora joca*, the host of which is a *Biscogniauxia sp.*, is puzzling considering that other members of *Pseudocosmospora* have an *Eutypa* or *Eutypella* species (Diatrypaceae) as their host. Two potential explanations for this observation are i) that the *Biscogniauxia* sp. represents the ancestral host for *Psedocosmospora* species, or ii) that the *Biscogniauxia* host of *C. joca* represents an independent host shift. Given that *Pseudocosmospora* species have diatrypaceous and xylariaceous hosts and assuming the first view, *Pseudocosmospora* could represent a link in the divergence from *Cosmospora* sensu stricto to *Dialonectria* (or vice-versa). Phylogenies of the fungal hosts have placed the Diatrypaceae as a sister clade to Xylariaceae (Moster et al. 2004, Tang et al. 2009), and these cosmospora-like fungi could have tracked their hosts faithfully as they diverged. Host specificity is not uncommon in the Hypocreales (e.g., species of *Cordyceps* sensu lato are known to be host specific to insect species; Sung et al. 2007). Coevolution/cospeciation analyses are needed to test these hypotheses.

TABLE I.I. Loci used in the phylogenetic analyses

Locus		ITS	LSU	Mcm7	Rpb1	Tef1	Tub	Combined
Cosmospora sensu lato dataset	Nucleotide substitution models	TIM2+I+2		TrN+I+G	TIM2+I+G	TrN+I+G	TPM3uf+I+G	
	Included sites	1384		570	692	365	580	3591
	Phylogenetically informative sites	94		201	235	236	204	970
	Uninformative polymorphic sites	77		29	80	42	90	318
	Invariable sites	1028		324	282	25	199	1858
Pseudocosmospora dataset	Nucleotide substitution models	TIMef+I+G		TrN+I+G	TIM2+I+G	HKY+G	TIM3+I+G	
	Included sites	1323		561	642	280	547	3353
	Phylogenetically informative sites	65		44	211	172	159	651
	Uninformative polymorphic sites	64		166	70	64	92	456
	Invariable sites	992		327	253	0	213	1785
Primers used (reference)		LR5, LROR (Vilgalys n.d.)	ITS5, ITS4 (White et al. 1990)	mcm7-709for, mcm7-1348rev (Schmitt et al. 2009)	crpb1a, rpb1c (Castlebury et al. 2004)	tef1-728, tef1-986 (Carbone & Kohn 1999)	Btub-T1, Btub-T2 (O'Donnell & Cigelnik 1997)	
PCR protocol: Annealing temp. & cycles		53 °C, 1 min, 40x		56 °C, 50 s, 38x	50 °C, 2 min, 40x	66 °C, 55 s, 9x 56 °C, 55 s, 35x	55 °C, 30 s, 35x	

SUPPLEMENTARY TABLE I.I. Isolates and accession numbers used in the phylogenetic analyses.

Species	Isolate No.	Herbarium No.	Substrate/Host	Country	GeneBank Accession No.					
					ITS	LSU	*mcm7*	*rpb1*	*tef1*	*tub*
"Cosmospora" flavoviridis	IMI 338173	-	Branch partially submerged in stream	United Kingdom	KC291747	KC291785	KC291821	KC291863	-	KC291902
"Cosmospora" obscura	MAFF 241484	-	Twig	Japan	KC291719	KC291788	KC291824	KC291864	KC291858	KC291903
"Cosmospora" stegonsporii	A.R. 4385, CBS 122305	BPI 878274	*Tilia cordata*	Ukraine	KC291718	KC291755	KC291792	KC291862	KC291828	KC291901
Corallomycetella repens	A.R. 4547, CBS 123826	BPI 881071	Bark	French Guiana	JF832594	JF832679	KC291795	JF832763	JF832517	JF832838
Cosmospora coccinea	A.R. 2741, CBS 114050	BPI 802729	*Inonotus nodulosus*	Germany	HM484537	GQ505990	-	GQ506020	HM484515	HM484589
Cosmospora viliuscula	G.J.S. 09-411	BPI 878994	*Kretzchmaria* sp.	Australia	JN995627	JN939826	JN993322	KC291866	KC291841	KC291905
Cosmospora viliuscula	G.J.S. 10-247	-	*Hypoxylon fragiforme*	USA	JN995629	JN939824	JN993326	KC291869	KC291843	KC291908
Cosmospora viliuscula	G.J.S. 83-197, CBS 124032	PDD	*Hypoxylon bovei*	New Zealand	KC291732	KC291777	KC291813	KC291868	KC291849	KC291907
Cosmospora viliuscula	G.J.S. 86-315	NY	*Xylaria* sp.	French Guiana	KC291748	KC291779	KC291815	KC291867	KC291851	KC291906
Cosmospora viridescens	CBS 102433	-	*Tilia* sp.	Czech Republic	KC291731	KC291765	KC291804	KC291865	KC291836	KC291904
Dialonectria episphaeria	G.J.S. 10-193, C.H. 10-01	-	*Diatrype stigma*	USA	KC291744	KC291771	-	KC291892	KC291842	KC291932

SUPPLEMENTARY TABLE I.I. Continued.

Species	Isolate No.	Herbarium No.	Substrate/Host	Country	GeneBank Accession No.					
					ITS	LSU	*mcm7*	*rpb1*	*tef1*	*tub*
***Dialonectria* sp.**	G.J.S. 10-298, C.H. 10-14	-	Diatrypaceae	USA	KC291743	KC291776	KC291812	KC291893	KC291848	KC291933
***Dialonectria* sp.**	C.H. 11-06	-	Diatrypaceae	USA	KC291741	KC291768	-	KC291890	-	KC291934
***Dialonectria* sp.**	G.J.S. 09-1197	-	*Diatrype bullata*	Belgium	KC291742	KC291769	KC291807	KC291891	KC291839	KC291931
Microcera coccophila	G.J.S. 83-198	-	scale insect	New Zealand	KC291753	KC291778	KC291814	KC291896	KC291850	-
Microcera coccophila	G.J.S. 98-50	BPI 748393	scale insect	Puerto Rico	KC291754	KC291784	KC291820	KC291897	KC291855	KC291937
Microcera coccophila	MAFF 241482	-	*Hemiberlesia lataniae* (Scale insect)	Japan	KC291752	KC291787	KC291823	KC291895	KC291857	KC291936
Microcera larvarum	A.R. 4580	-	scale insect	New Zealand	KC291751	KC291759	KC291798	KC291894	KC291832	KC291935
Pseudocosmospora eutypae	C.H. 11-01	BPI 884164	*Eutypa* sp.	France	KC291735	KC291766	KC291805	KC291884	KC291837	KC291925
Pseudocosmospora eutypae	IMI 73016	-	Unknown	Great Britain	KC291736	KC291786	KC291822	KC291885	KC291856	-
Pseudocosmospora eutypellae	A.R. 4453, CBS 129430	BPI 878454	*Eutypella* sp.	USA	JF832595	JF832680	KC291793	JF832764	JF832593	JF832839
Pseudocosmospora eutypellae	A.R. 4527	-	*Eutypella* sp.	USA	KC291720	KC291756	KC291794	KC291870	KC291829	KC291909
Pseudocosmospora eutypellae	A.R. 4562	BPI 884165	*Eutypella* sp.	USA	KC291721	KC291757	KC291796	KC291871	KC291830	KC291912

SUPPLEMENTARY TABLE I.I. Continued.

Species	Isolate No.	Herbarium No.	Substrate/Host	Country	GeneBank Accession No.					
					ITS	LSU	*mcm7*	*rpb1*	*tef1*	*tub*
Pseudocosmospora eutypellae	G.J.S. 10-294, C.H. 10-02	BPI 884168	*Eutypella* sp.	USA	KC291723	KC291773	KC291809	KC291873	KC291845	KC291910
Pseudocosmospora eutypellae	G.J.S. 10-248, C.H. 10-10	BPI 884169	*Eutypella* sp.	USA	KC291722	KC291772	KC291808	KC291872	KC291844	KC291911
Pseudocosmospora eutypellae	G.J.S. 93-15, CBS 128986	BPI 802567	*Eutypella* sp.	France	HM484856	GQ506006	KC291817	GQ506035	HM484849	HM484878
Pseudocosmospora joca	A.R. 4779	BPI 884175	*Biscogniauxia* sp.	Argentina	KC291746	KC291762	KC291801	KC291887	-	KC291924
Pseudocosmospora metajoca	A.R. 4576	BPI 879088	*Eutypa* sp.	New Zealand	KC291745	KC291758	KC291797	KC291886	KC291831	KC291923
Pseudocosmospora rogersonii	G.J.S. 10-296, C.H. 10-11	BPI 884166	*Eutypella* sp.	USA	KC291727	KC291774	KC291810	KC291876	KC291846	KC291917
Pseudocosmospora rogersonii	G.J.S. 10-297, C.H. 10-12	BPI 884170	*Eutypella* sp.	USA	KC291728	KC291775	KC291811	KC291877	KC291847	KC291916
Pseudocosmospora rogersonii	G.J.S. 09-1384	BPI 884167	*Eutypella* sp.	USA	KC291726	KC291770	-	KC291875	KC291840	KC291914
Pseudocosmospora rogersonii	G.J.S. 90-56	BPI 1107121	*Eutypella* sp.	USA	KC291729	KC291780	KC291816	KC291878	KC291852	KC291915
***Pseudocosmospora* sp.**	A.R. 4768	BPI 884173	*Eutypella* sp.	Argentina	KC291724	KC291760	KC291799	KC291881	-	KC291922
***Pseudocosmospora* sp.**	A.R. 4826	BPI 884177	*Eutypella* sp.	Argentina	KC291740	KC291764	KC291803	KC291888	KC291835	KC291929

SUPPLEMENTARY TABLE I.I. Continued.

Species	Isolate No.	Herbarium No.	Substrate/Host	Country	GeneBank Accession No.					
					ITS	LSU	*mcm7*	*rpb1*	*tef1*	*tub*
***Pseudocosmospora* sp.**	C.H. 11-02	BPI 884171	*Eutypella* sp.	France	KC291725	KC291767	KC291806	KC291882	KC291838	KC291919
***Pseudocosmospora* sp.**	G.J.S. 95-141	BPI 737706	*Eutypella* sp.	Uganda	KC291749	KC291781	KC291818	KC291883	KC291853	KC291921
***Pseudocosmospora* sp.**	G.J.S. 95-143	BPI 737708	*Eutypella* sp.	Uganda	KC291750	KC291782	-	KC291880	KC291854	KC291920
***Pseudocosmospora* sp.**	G.J.S. 96-216, CBS 133984	-	Unknown	USA	KC291733	KC291783	KC291819	KC291889	-	KC291930
***Pseudocosmospora* sp.**	MAFF 241499	-	Unknown	Japan	KC291739	KC291789	KC291825	KC291874	KC291859	KC291913
***Pseudocosmospora* sp.**	MAFF 241531	-	Unknown	Japan	KC291730	KC291790	KC291826	KC291879	KC291860	KC291918
Pseudocosmospora vilior	A.R. 4771	BPI 884174	*Eutypella* sp.	Argentina	KC291734	KC291761	KC291800	KC291898	KC291833	KC291926
Pseudocosmospora vilior	A.R. 4810	BPI 884176	*Eutypella* sp.	Argentina	KC291737	KC291763	KC291802	KC291900	KC291834	KC291928
Pseudocosmospora vilior	P.C. 1246	BPI 884172	*Eutypella* sp.	Brazil	KC291738	KC291791	KC291827	KC291899	KC291861	KC291927
Pseudonectria pachysandricola	A.R. 4592, CBS 128674	BPI 879936	*Pachysandra* sp.	USA	JF832658	JF832715	-	JF832791	JF832544	JF832909

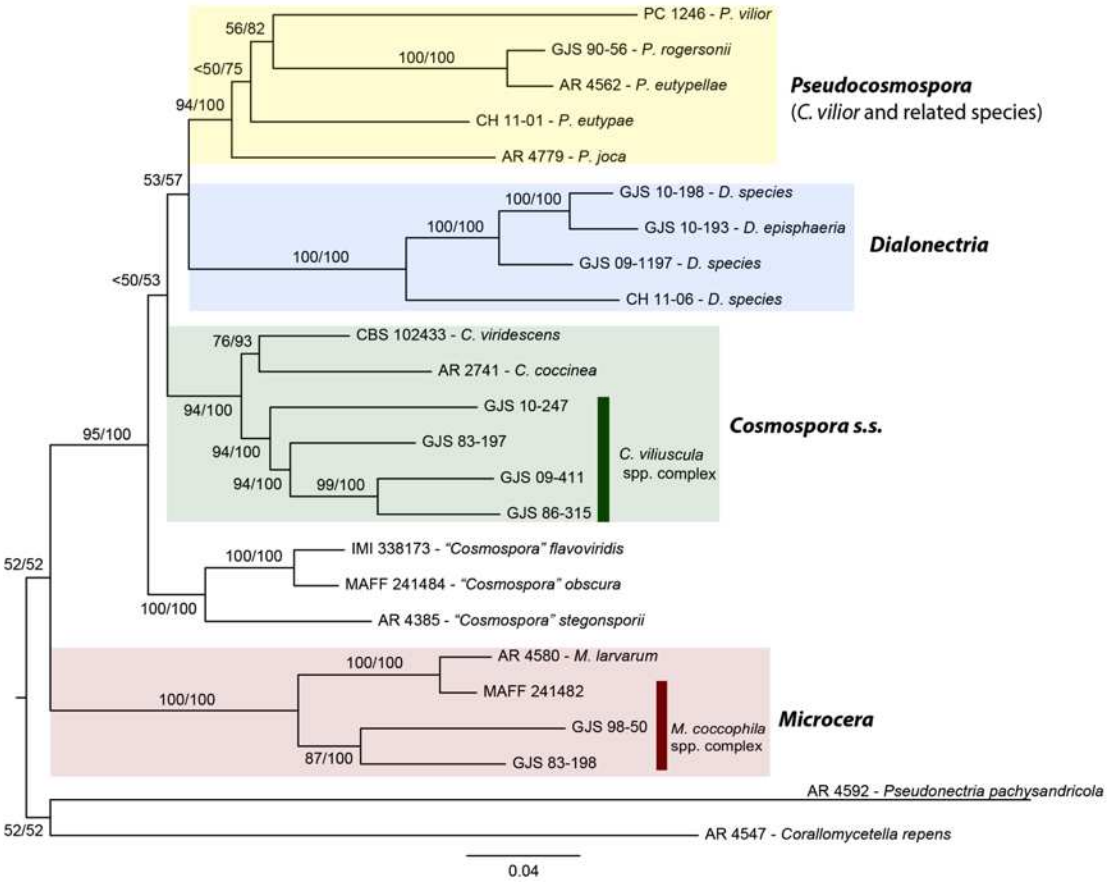

FIG. 1.1. Phylogenetic placement of *C. vilior* and related species within *Cosmospora* sensu Rossman based on a combined 5-loci (ITS-LSU, MCM7, *rpb1*, *tef1*, and *tub*) dataset. Best tree generated with ML analysis (−16460.154). Values at branches indicate Maximum Likelihood bootstrap (ML BP)/Bayesian posterior probabilities (BI PP).

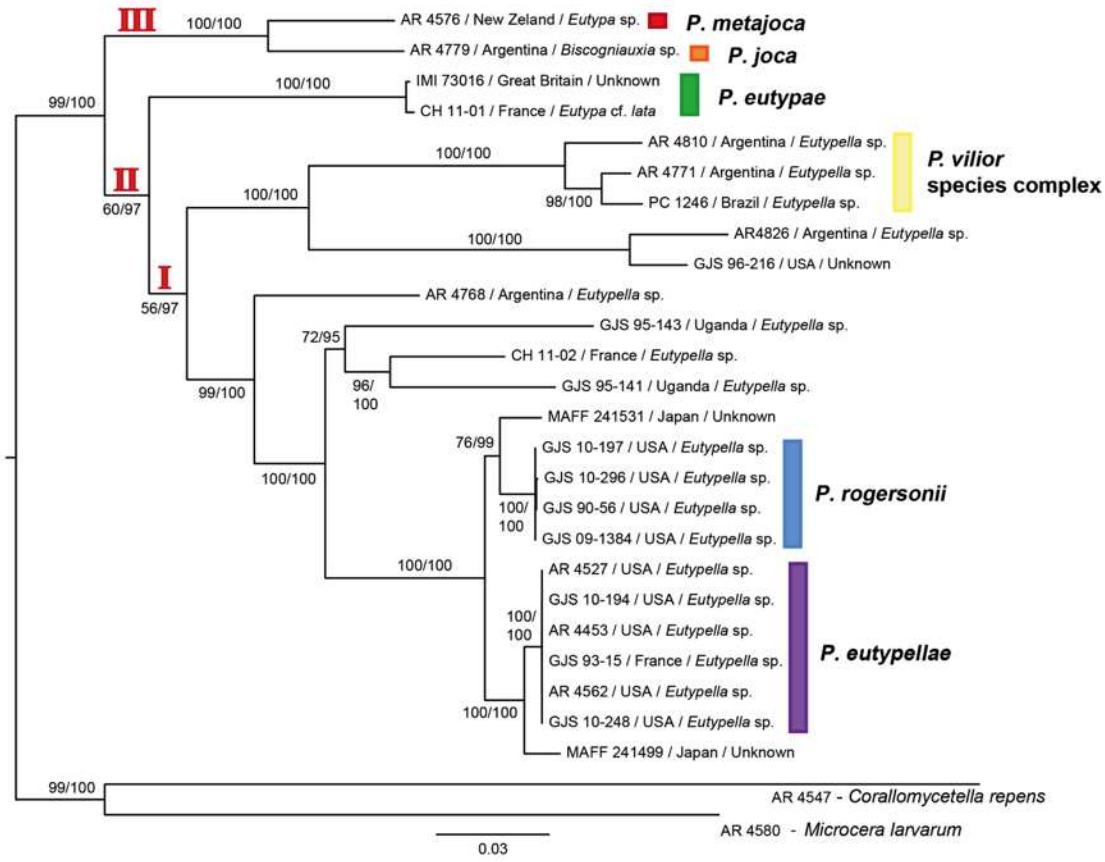

FIG. 1.2. Phylogenetic relationship of *C. vilior* and related species based on a combined 5-loci (ITS-LSU, MCM7, *rpb1*, *tef1*, and *tub*) dataset. Best tree generated with ML analysis (−23024.3191). Values at branches indicate Maximum Likelihood bootstrap (ML BP)/Bayesian posterior probabilities (BI PP).

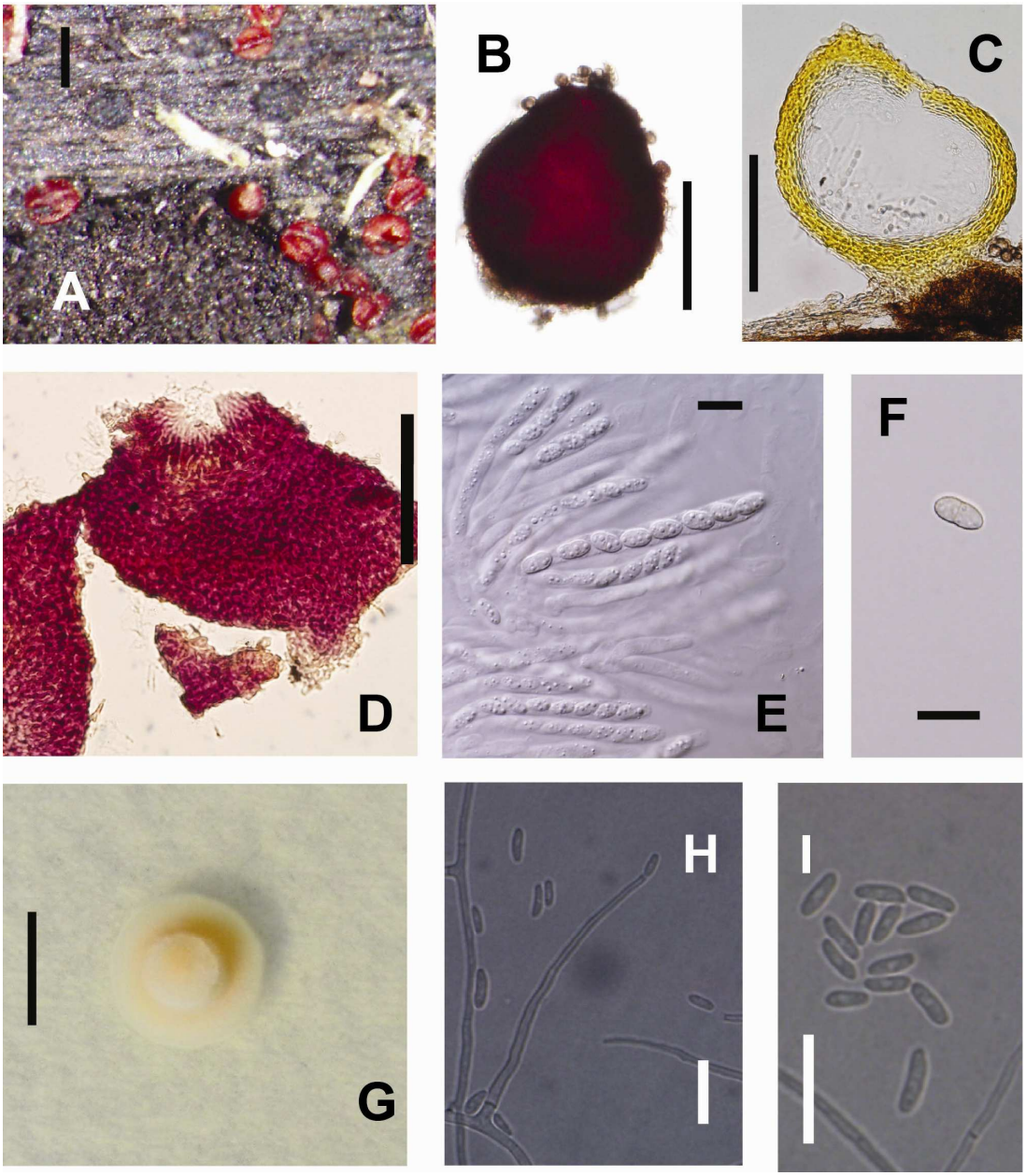

FIG. 1.3. *Pseudocosmospora eutypae*. (A) Perithecia on natural substrata. Scale bar = 200 µm. (B) Perithecium in 3% KOH. Scale bar = 100 µm. (C) Median section of perithecium. Scale bar = 100 µm. (D) Perithecial surface cells. Scale bar = 100 µm. (E) Asci. Scale bar = 10 µm. (F) Ascospore. Scale bar = 10 µm. (G) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (H) Phialide. Scale bar = 10 µm. (I) Conidia. Scale bar = 10 µm.

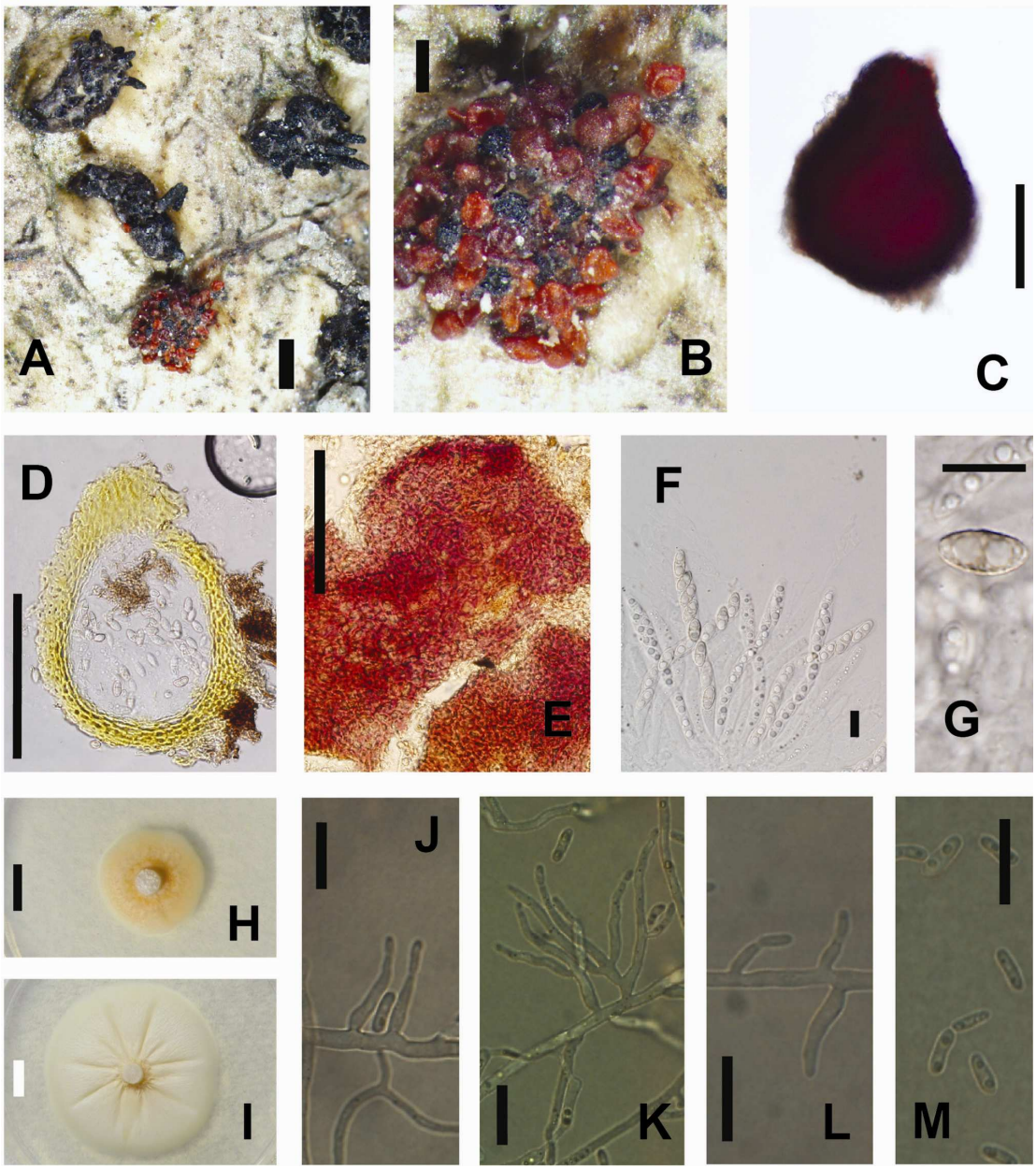
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FIG. 1.4. *Pseudocosmospora eutypellae*. (A, B) Perithecia on natural substrata. A. Scale bar = 600 µm. B. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Median section of perithecium. Scale bar =100 µm (E) Perithecial surface cells. Scale bar = 100 µm. (F) Asci. Scale bar = 10 µm. (G). Ascopore. Scale bar = 10 µm. (H, I) Cultures after 3 wks at 25 C on PDA. Scale bars = 10 mm. (J, K) Phialides. Scale bars = 10 µm. (L) Lateral phialidic pegs. Scale bar = 10 µm. (M) Conidia. Scale bar = 10 µm.

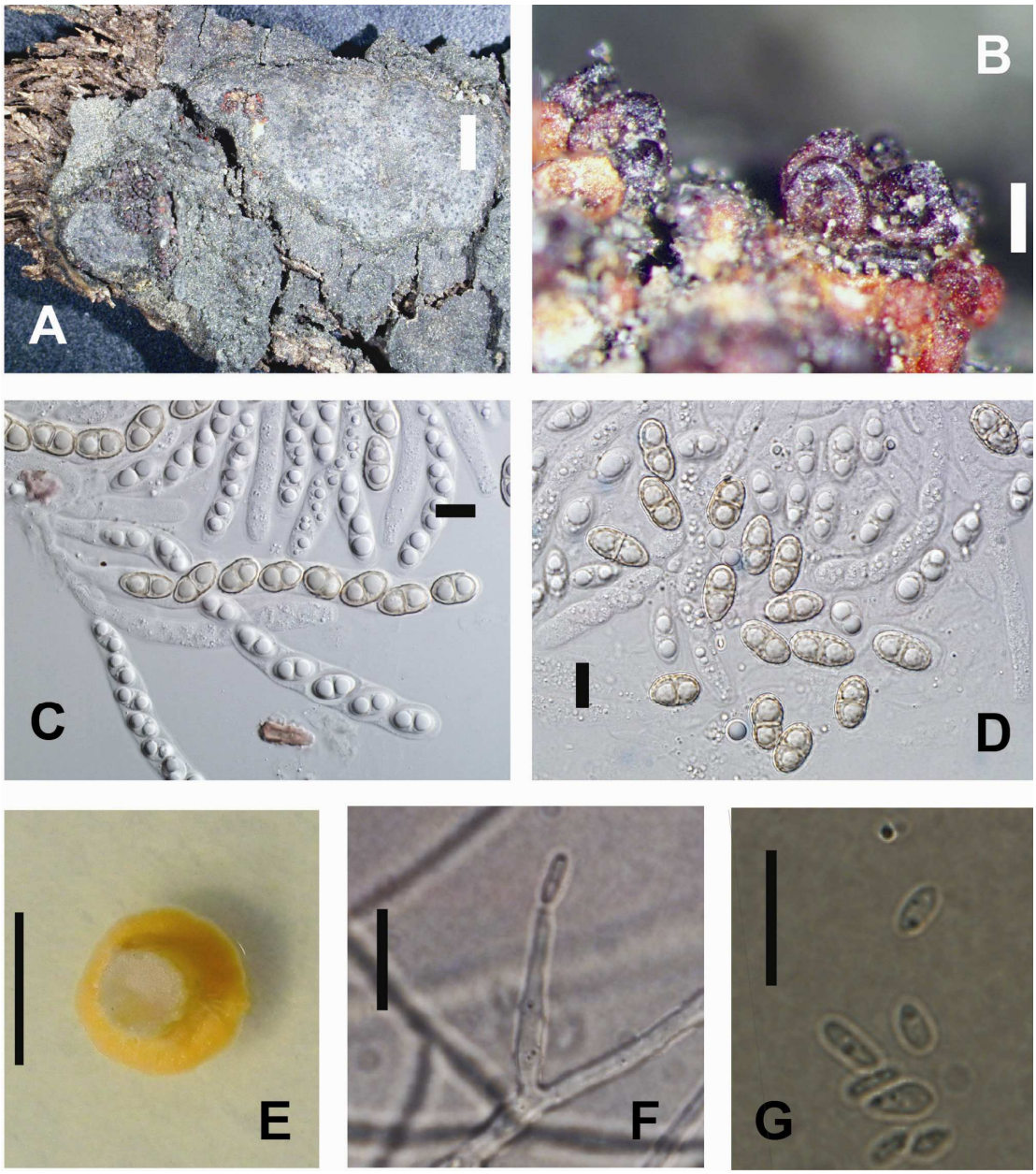

FIG. 1.5. *Pseudocosmospora joca*. (A, B) Perithecia on natural substrata. A. Scale bar = 2 mm. B Scale bar = 200 µm. (C) Asci. Scale bar = 10 µm. (D) Ascospores. Scale bar = 10 µm. (E) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (F) Phialide. Scale bar = 10 µm. (G). Conidia. Scale bar = 10 µm.

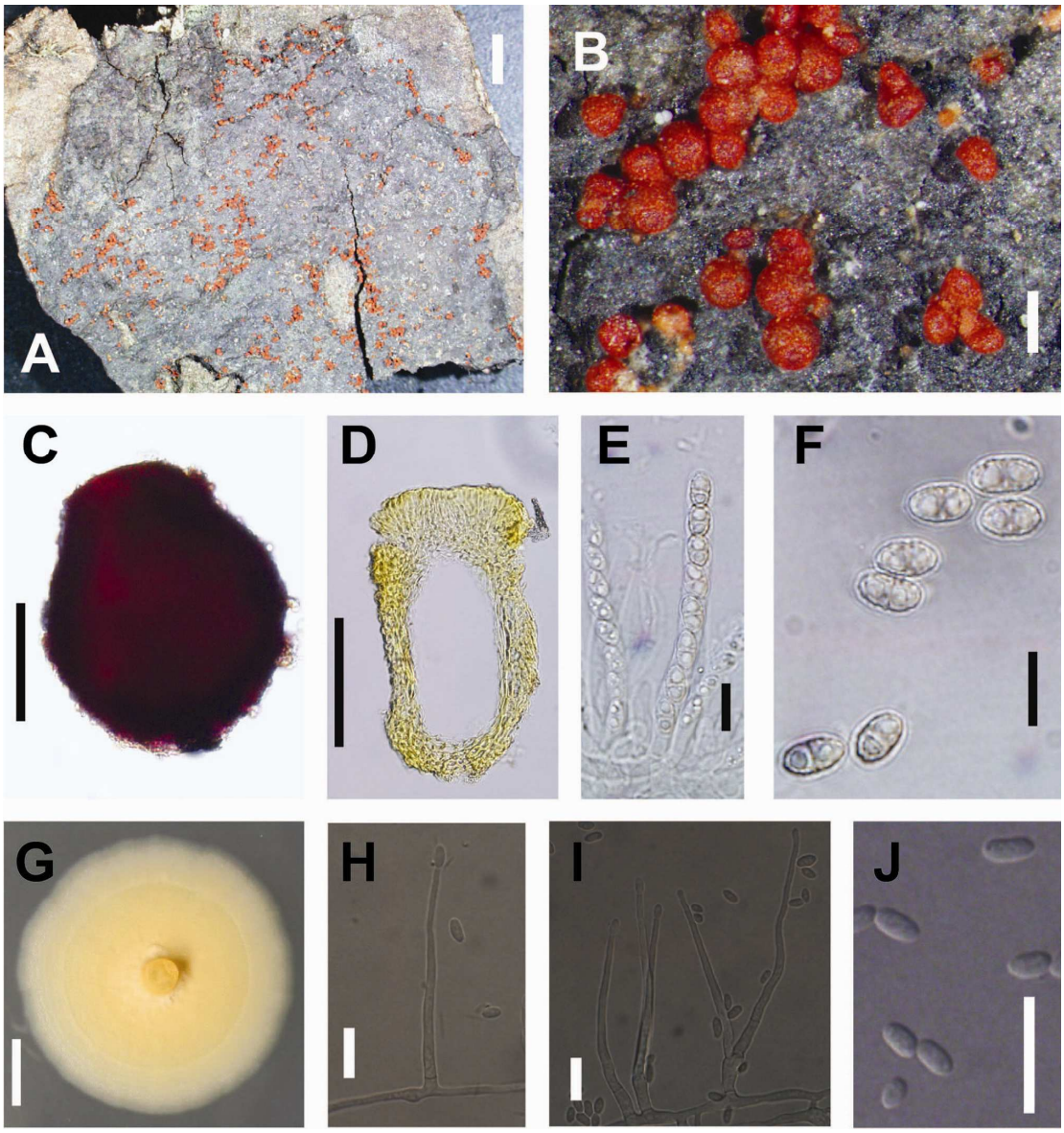

FIG. 1.6. *Pseudocosmospora metajoca*. (A, B) Perithecia on natural substrata. A. Scale bar = 2 mm. B. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Median section of perithecium. Scale bar = 100 µm. (E) Asci. Scale bar = 10 µm. (F) Ascospores. Scale bar = 10 µm. (G) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (H, I) Phialides. Scale bars = 10 µm. (J) Conidia. Scale bar = 10 µm.

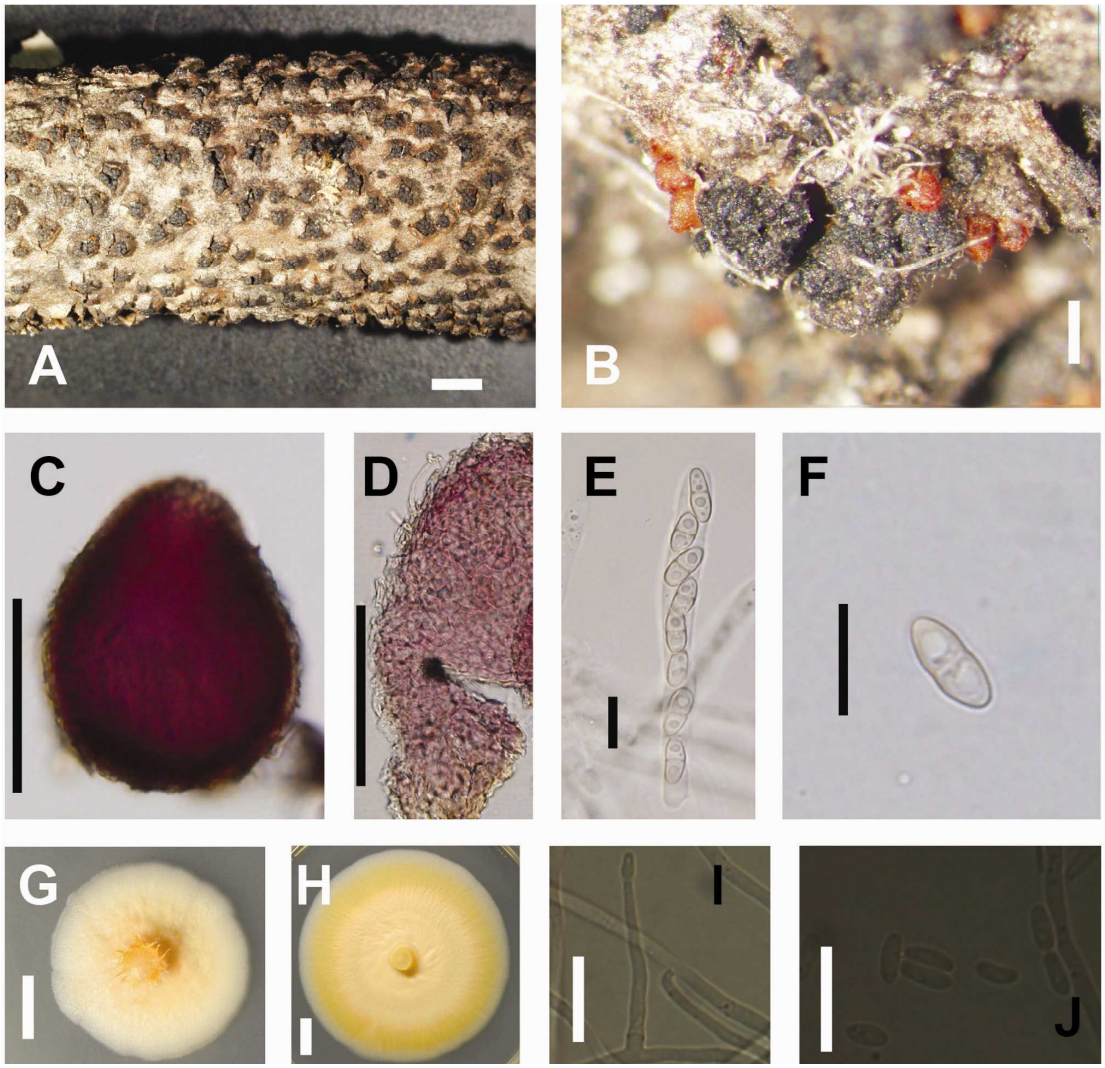

FIG. 1.7. *Pseudocosmospora rogersonii*. (A, B) Perithecia on natural substrata. A. Scale bar = 2 mm. B. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Perithecial surface cells. Scale bar = 100 µm. (E) Ascus. Scale bar = 10 µm. (F) Ascospores. Scale bar = 10 µm. (G, H) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (I) Phialide. Scale bar = 10 µm. (J) Conidia. Scale bar = 10 µm. Scale bars: (A) 2 mm, (B) 200 µm, (C, D) 100 µm, (E,F, I–J) 10 µm, (G, H) 10 mm.

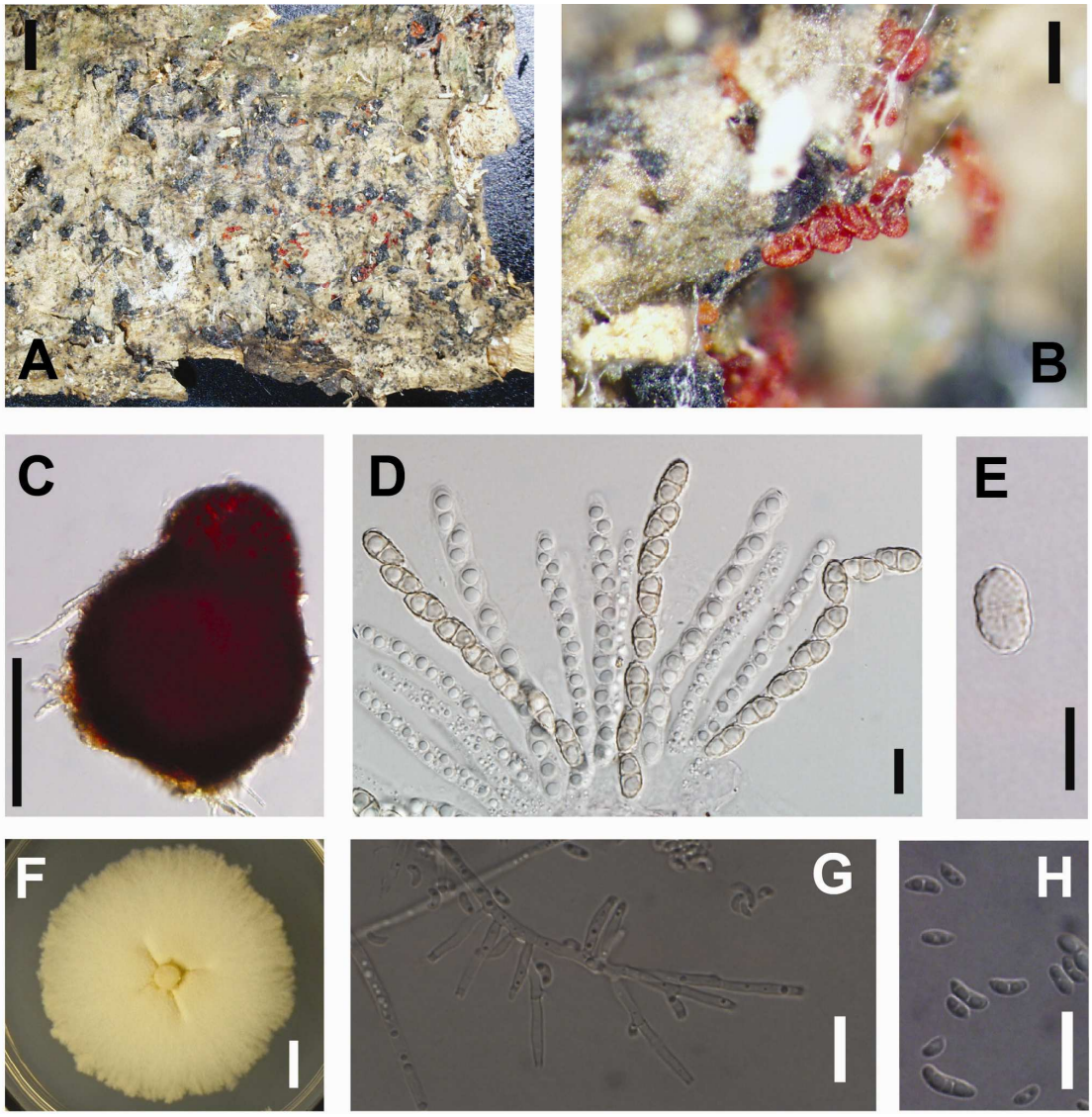

FIG. 1.8. *Pseudocosmospora vilior*. (A, B) Perithecia on natural substrata. A. Scale bar = 2 mm. B. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Asci. Scale bar = 10 µm. (E) Ascospore. Scale bar = 10 µm. (F) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (G) Phialides. Scale bar = 10 µm. (H) Conidia. Scale bar = 10 µm.

Chapter 2: Revision of the genus *Corallomycetella* with *Corallonectria* gen. nov. for *C. jatrophae* (Nectriaceae, Hypocreales)

C.S. Herrera, A.Y. Rossman, G.J. Samuels, C. Lechat and P. Chaverri. 2013. *Mycosystema* 32(3): 518–544. *Reprinted with permission of* Mycosystema.

ABSTRACT

The genus *Corallomycetella* (Ascomycota, Sordariomycetes, Hypocreales, Nectriaceae) has been defined to include red nectrioid fungi associated with rhizomorphs in nature and culture. With the recent collection of an unusual specimen having striated ascospores, the genus was re-examined using this and previously obtained cultures. A multilocus tree was constructed based on three loci (ITS, *mcm7*, β-tubulin) to determine phylogenetic relationships. Our results indicate that *Corallomycetella repens sensu lato* forms two clades associated with biogeography. *Corallomycetella repens sensu stricto* is restricted to specimens from Asia while *C. elegans* is resurrected for specimens from Africa and America. Minute striations in the ascospores are an overlooked character in species of *Corallomycetella*. *Corallomycetella jatrophae* is related to *Neonectria sensu lato* and unrelated to *C.*

repens and *C. elegans*; thus, a new genus, *Corallonectria*, is described to accommodate this species. *Corallonectria* is characterized by furfuraceous perithecia and synnematous fusarium-like anamorph.

INTRODUCTION

The genus *Corallomycetella* Henn. is recognized for two species having large, orange-red to red, smooth to scurfy ascomata arranged in caespitose clusters, and smooth to roughened ascospores (Rossman *et al.* 1999). These species occur primarily in tropical regions. Based on the reddish, KOH+ ascomata this genus is placed in the Nectriaceae, Hypocreales. One species, *C. repens* (Berk. & Broome) Rossman & Samuels, has a synnematal asexual state with red, rhizomorph-like strands at the base that has been referred to as *Rhizostilbella hibisci* (Pat.) Seifert. The reddish rhizomorph-like strands are also produced in culture as well as ellipsoid, non-septate conidia each with a truncate base. This species causes a number of diseases, specifically 'violet root rot' of *Theobroma cacao* L., root rot of *Carica papaya* L., and 'stinking root disease' of several tropical woody plants (Booth & Holliday 1973). *Corallomycetella jatrophae* (A. Møller) Rossman & Samuels has a similar looking ascomatal state with a reddish synnematal asexual state that produces large, fusiform, multi-septate conidia.

A specimen collected in French Guiana has striate ascospores although otherwise is similar to *C. repens*. As part of a study to determine if this unusual specimen is a distinct species, the phylogenetic placement of *Corallomycetella* within the Nectriaceae was investigated. Previous studies had suggested that it was basal to the

genus *Cosmospora* Rabenh., which has recently been shown to be polyphyletic (Gräfenhan *et al.* 2011).

MATERIALS AND METHODS

Herbarium specimens and cultures

Fresh specimens of *Corallomycetella* sensu Rossman *et al.* (1999) were collected on trips to Brazil, Costa Rica, French Guiana, and Gabon (Kadri Põldmaa). Cultures were obtained by isolating single asci or ascospores and grown in cornmeal dextrose agar (CMD; Difco™ cornmeal agar + 2% w/v dextrose + antibiotics). Dried specimens were deposited at the U.S. National Fungus Collections (BPI), Beltsville, Maryland, USA. Cultures were deposited at Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands (Table 1) from where additional fungal strains were obtained.

Herbarium specimens of *Corallomycetella* were borrowed from the U.S. National Fungus Collections (BPI), Farlow Reference Library and Herbarium of Cryptogamic Botany (FH), Royal Botanic Gardens Kew (K), and William and Lynda Steere Herbarium, New York Botanical Garden (NY).

Morphological characterization

The macro-morphology of the teleomorph was observed using a stereoscope (Olympus SZX12 Olympus, Tokyo, Japan). The color, shape, size, ornamentation, and habit of the perithecia were characterized. To observe their internal structures, the perithecia were rehydrated in 3% KOH and the centrum isolated on a glass slide and

covered with a coverslip. Microscopic characters, e.g. asci and ascospores, were observed with a compound microscope (Olympus BX50; Olympus, Tokyo, Japan). The color reaction of the perithecial wall was observed using 3% KOH and 100% lactic acid (LA). Sections of perithecia (ca. 10μm in width) were made with the aid of a freezing microtome.

To observe colony morphology strains were grown on Difco™ potato dextrose agar (PDA) in an incubator that alternates between fluorescent light and darkness (12h/12h) at 25°C. Two replicates with two pseudoreplicates were grown for each isolate. Culture growth was measured weekly for two weeks. Colony color is described using the terms in Rayner (1970). To observe the mononematous anamorph isolates were grown in synthetic nutrient-poor agar (SNA; Nirenberg 1976) under the conditions described above. A block of agar was cut, placed on a microscope slide, covered with a coverslip, and examined by light microscopy (Olympus BX50; Olympus, Tokyo, Japan).

Measurements of continuous characters, e.g. length and width, were made with Scion Image software beta 4.0.2 (Scion Corp., Frederick, Maryland), and summarized by descriptive statistics, e.g., minimum, maximum, mean and standard deviation.

DNA extraction, PCR, and sequencing

The detailed DNA extraction protocol is described in Hirooka *et al.* (2010). Briefly, the strains were grown in Difco™ potato dextrose broth (PDB) for one week, and the mycelial mat harvested for DNA extraction. DNA was extracted with PowerPlant®

DNA Isolation Kit (MO BIO Laboratories Inc., Solana Beach, California). DNA of *Corallomycetella jatrophae* (P.C. 1300) was amplified directly from the several centra of the perithecia because the isolate did not survive –80°C storage. The centra were isolated in antibiotics, transferred to a microcentrifuge tube with 10µm RNAse-free water, incubated for 10min at 65°C, and homogenized using a micropestle. The sample was centrifuged, and 5µm of the supernatant was transferred to a new tube. DNA was amplified with Illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare Bio-Sciences Corp., Piscataway, New Jersey) following the manufacturer's instructions.

Four partial loci were amplified. These loci include ITS ribosomal DNA (ITS; White *et al.* 1990) and three protein coding regions: α-actin (*act*; Samuels *et al.* 2006), *mcm7* (a DNA replication licensing factor; Schmitt *et al.* 2009), and β-tubulin (*tub*; O'Donnell and Cigelnik 1997). The PCR reaction mixture (25µL total volume) consisted of 12.5µL GoTaq® Green Master Mix 2X (Promega Corporation, Madison, Wisconsin), 1.25µL 10mmol/L forward primer, 1.25µL 10mmol/L reverse primer, 1.0µL of dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, Missouri), 2.0µL genomic DNA template, and 7µL of sterile RNAse-free water. PCR amplifications were carried out in an Eppendorf Mastercycler thermocycler (Eppendorf, Westbury, New York) under the cycle conditions listed in Table 2. PCR products were cleaned with ExoSAP-IT® (USB Corp., Cleveland, Ohio). Clean PCR products were sequenced at the DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland) and McLAB DNA sequencing

services (San Francisco, California). Sequences were assembled and edited with Sequencher 4.9 (Gene Codes, Madison, Wisconsin). Sequences were deposited in GenBank (Table 2.1).

Phylogenetic analyses

A multiple sequence alignment for each locus was performed in the MAFFT v.6 web service (http://mafft.cbrc.jp/alignment/server/; Katoh 2008) with the E-INS-i alignment strategy. Alignments were manually edited in in Mesquite 2.75 (Maddison & Maddison 2011).

CONCATEPILLAR 1.4 (Leigh et al. 2008) was used to determine which loci could be concatenated and analyzed to generate a phylogeny. Loci were concatenated if the p-value was greater than the default α-level of 0.05, which indicated that the null hypothesis, i.e. congruence of loci, could not be rejected.

JModeltest (Guindon and Gascuel 2003; Posada 2008) was used to infer the model of nucleotide substitution for each locus. Default settings in jModeltest were used: 11 substitution schemes with equal or unequal base frequencies (+F), and with/without invariable sites (+I) and/or rate variation among sites (+G). The base tree for likelihood calculations was ML optimized. Once likelihood scores were calculated, the models were selected according to the Akaike Information Criterion (AIC).

Maximum likelihood (ML) analyses were performed with GARLI v2.0 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl 2006) in the GARLI web service (http://www.molecularevolution.org; Bazinet and Cummings 2011), which uses a

grid computing system associated with The Lattice Project (Cummings and Huskamp 2005; Bazinet and Cummings 2008). Fifty independent search replicates were performed to generate the starting tree and search for the best tree with a fast ML stepwise-addition algorithm. Two thousand bootstrap replicates were used in the bootstrap analysis. Bayesian analyses were performed in MrBayes v3.2.1 (Ronquist *et al.* 2012). A majority rule consensus tree was generated by running four chains for 10,000,000 Markov Chain Montecarlo generations sampling trees every 100th generation, and discarding the first 25% of the sampled trees as burn-in. Tracer version 1.5 (Rambaut and Drummond 2007) was used to confirm whether the negative log likelihoods had reached convergence.

Species recognition in *Corallomycetella* sensu stricto was based on genealogical concordance phylogenetic species recognition (GCPSR; Taylor *et al.* 2000), 95% connection limit in statistical parsimony networks (Posada & Crandall 2001; Templeton 2001), and genealogical sorting index (*gsi*; Cummings et al. 2008). Haplotype networks based on statistical parsimony were generated for each locus and the combined multilocus dataset in TCS v1.21 (Clement *et al.* 2000). Haplotypes are joined by an edge only if the edge has a probability of parsimony greater than or equal to 95% (default settings in TCS). The *gsi* is a statistic that measures genealogical exclusivity of a group of individuals/species on a rooted tree with 0=polyphyly and 1=monophyly. The *gsi* was implemented in the *gsi* web interface (http://www.genealogicalsorting.org/) with single locus trees generated with GARLI (as described above) and 10,000 permutations to test statistical significance of the *gsi*

value (P<0.05). The ensemble statistic (gis_T) was estimated from a multi-tree file containing all single locus trees.

RESULTS

The analysis performed in CONCATEPILLAR rejected the null hypothesis of congruence among all loci (P-value < 0.001), and for this reason *act* was analyzed separately (Fig. 2.1). The analysis determined that only ITS, *mcm7*, and *tub* were congruent (P-value=0.3), and therefore these loci could be concatenated. The concatenated matrix consisted of 2,304 base pairs of which 799 were parsimony-informative, 236 were parsimony-uninformative, and 345 were invariable (Table 2.2). The topologies of the generated phylogenetic trees in both ML and BI were congruent. The negative log likelihoods for the phylogenetic trees were −17491.8148 and −17588.5333, respectively. The best tree (ML) is shown in Figure 2.2.

The combined analyses of species in *Corallomycetella* sensu Rossman *et al.* (1999) revealed that these taxa consisted of three clades, although distantly related, i.e. the genus is polyphyletic. One clade is composed of *Corallomycetella repens* sensu Rossman *et al.* (1999) (100% BP, 100% PP), and allied with *Cosmospora* sensu stricto, *Dialonectria* (Sacc.) Cooke, *Fusicolla* Bonord., *Macroconia* (Wollenw.) Gräfenhan *et al.*, and *Microcera* Desm. (94.5% PP).

Corallomycetella repens sensu Rossman *et al.* (1999) clustered into two well-supported subclades that correlated with geographic origin (Fig. 2.2). One subclade is known only from Asia (99.6% BP, 100% PP), and represented in Fig. 2.2 by isolates from India, Java, and Sri Lanka. In single gene trees, this clade was present in *act*,

ITS, and *mcm7*, but only *act* and *mcm7* strongly supported this clade (Fig. 2.3). This subclade is recognized below as *C. repens* sensu stricto.

The second subclade of *Corallomycetella repens* sensu Rossman *et al.* (1999) is known from tropical regions in the western hemisphere and Africa. It is well supported (87.9% BP, 100% PP; Fig. 2.2) and referred to as *C. elegans* (see Taxonomy section). It is present and strongly supported in *act*, ITS, and *tub* single gene trees (Fig. 2.3). Within the *C. elegans* clade, ITS was the only locus supporting the monophyly of specimens from America, while *tub* was the only locus strongly supporting the monophyly of specimens from Africa (Fig. 2.3).

TCS analyses resolved two segregate haplotype networks of *Corallomycetella repens* sensu Rossman *et al.* (1999; Fig. 2.4). The smaller haplotype network comprised two haplotypes from Asia, and the larger one included four haplotypes from tropical America and Africa. Within the larger haplotype network, eight polymorphic sites separated two small subgroups that showed geographic congruence. In single gene haplotype networks, only the analysis of *tub* reconstructed two separate haplotype networks as seen in the combined haplotype network, although ITS and *mcm7* had a relatively high number of mutational differences between the "American" and "African" subgroups (five and six, respectively; Fig. 2.5).

Gsi analyses did not support the monophyly of *Corallomycetella repens* sensu Rossman *et al.* (1999). The ensemble gsi_T value was 1, but not significant (p-value = 0.075). Complete sorting was not observed in any of the subclades. The ensemble gsi_T values for the *C. repens* sensu stricto and *C. elegans* subclades were 0.928 (p-value =

<0.001) and 0.735 (p-value = 0.001). The ensemble gsi_T values for the "American" and "African" populations of *C. elegans* were 0.928 (p-value = <0.001) and 0.735 (p-value = 0.001), respectively. *Gsi* values for each locus are reported in Table 2.3.

The second species included in *Corallomycetella* sensu Rossman *et al.* (1999), *C. jatrophae*, formed a well-supported clade distinct from that genus (100% BP, 100% PP, Fig. 2.2). This species is closely allied with *Ilyonectria* P. Chaverri & Salgado, *Neonectria* Wollenw., and *Viridispora* Samuels & Rossman, although this inner node is not well supported in the combined analysis (87% PP, Fig. 2.2). *Act* strongly supports a clade comprising *C. jatrophae*, *Ilyonectria*, and *Neonectria* (100% PP, Fig. 1). Given the distinctive morphology of *C. jatrophae* and the lack of affinity with any known genus, this species is placed in a new genus, *Corallonectria* (described below).

Three isolates identified as "*Nectria" mauritiicola* (Henn.) Seifert & Samuels, a name considered a synonym of *Corallomycetella repens* (Rossman *et al.* 1999), were distinct from all isolates referred to as *Corallomycetella*. The ITS sequence of these isolates are identical (Max. Ident.=100% in Blast®; blast.ncbi.nlm.nih.gov/) to the sequence of CBS 122.29, ex-type culture of *Sarocladium kiliense* (Grütz) Summerb. (76% BP, 82% PP; Fig. 2.2), and related to other species of *Sarocladium* W. Gams & D. Hawksw. (100% BP, 100% PP; Fig. 2.2).

DISCUSSION

Genus concepts

In the prior taxonomic revision by Rossman *et al.* (1999), *Corallomycetella* was based on *C. repens* (neotype of *C. heinsenii*, designated by Rossman *et al.* 1999). The genus included nectrioid fungi with scurfy to furfuraceous perithecia with rhizomorphs, roughened or smooth ascospores, and synnematous, fusarium-like or *Rhizostilbella* anamorphs. The circumscription of *Corallomycetella* was revised based on the genus-for-genus concept (Rossman 1993) in which a teleomorphic fungal genus correlates with its unique anamorph. Using this concept teleomorphic and anamorphic genera are observed to be monophyletic (e.g., Chaverri *et al.* 2008, 2011; Gräfenhan *et al.* 2011; Luo and Zhuang 2010, 2012).

Our phylogenetic analyses of molecular sequence data reveal that *Corallomycetella* sensu Rossman *et al.* (1999) consists of two distantly related major clades (Fig. 2.2). The clade that comprises *Corallomycetella* sensu stricto is related to *Cosmopora* sensu Rossman (94.5% PP) as previously shown by Hirooka *et al.* (2012). The anamorph of *Corallomycetella* sensu stricto is a synnematous *Rhizostilbella*. The genus is also characterized by scurfy perithecia that develop from rhizomorphs or at the base of synnemata, and produce finely striated ascospores that appear roughened in median section because the outer wall is often sinuous.

Corallomycetella jatrophae forms a second clade of *Corallomycetella* sensu Rossman *et al.* (1999), but it is most closely related to neonectria-like fungi (92% PP; Fig. 2.2). Although the exact relationship is not well resolved (low node support), the topology

of this subclade is similar to that reported by Chaverri *et al.* (2011) in which the inner nodes are well supported due to the higher number of concordant loci used in the combined analyses. *Corallomycetella jatrophae* is characterized by its synnematous fusarium-like anamorph, which is unique among neonectria-like fungi. Synnematous fusarium-like anamorphs are also observed in *Atractium* Link and *Microcera* (Gräfenhan *et al.* 2011), but these genera appear to be unrelated to *C. jatrophae*. Representatives of *Atractium* were not included in our phylogeny, but Gräfenhan *et al.* (2011) showed that the type, *A. stilbaster* Link and a second species of *Atractium* form a distinct genus allied with *Pseudonectria* and *Volutella*. The genus *Atractium* is not included the major clade of neonectria-like fungi. *Microcera* and allies (*Cosmospora* sensu Rossman *et al.* 1999) are closely related to *Corallomycetella* (94.5% PP; Fig. 2.2). This suggests that the synnematous fusarium-like anamorph has been independently derived three separate times. Based on these results, the clade previously regarded as *Corallomycetella jatrophae* is segregated into a new monotypic genus *Corallonectria* (described below).

Species concepts

The species previously referred to as *Corallomycetella repens* sensu Rossman *et al.* (1999) is recognized in this study to comprise two species, namely *C. repens* sensu stricto and *C. elegans*, based on our multi-method approach (GCPSR, connection of haplotypes with ≥95% parsimony probability, and *gsi*). In addition to these criteria the two species are supported by morphological and biogeographical differences. *Corallomycetella repens* is restricted to isolates from South and Southeast Asia (Fig. 2.2), while isolates from Africa and America are recognized as *C. elegans*.

Corallomycetella repens is circumscribed in the strict sense based on our phylogenetic results. The clade is well supported in the combined analyses (99.6% BP, 100% PP; Fig. 2.2). The ensemble *gsi* value obtained for this clade (0.902, p-value = 0.004; Table 2.3) suggested that it is approximating monophyly (i.e., complete sorting). The clade is present in all single locus trees except *tub*, but only supported in *act* and ITS (Fig. 2.3). Under GCSPR (Taylor *et al.* 2000) the clade could not be recognized probably because complete lineage sorting has not occurred. This criterion seems too conserved, and we decided to follow the criterion used by Pringle *et al.* (2005), which recognizes a species if the clade is well supported in the majority of single gene trees. The segregate network of *C. repens* haplotypes also suggests that *C. repens* is not conspecific with *C. elegans*. A segregate network occurs when the observed mutational differences exceed the maximum number of mutational connections, i.e. the 95% parsimony probability cutoff, between haplotypes. Segregate parsimony networks have been associated to correspond to species boundaries (reviewed in Hart & Sunday 2007).

The case for *Corallomycetella elegans* is slightly more complicated, but also conforms to the species recognition used in this study. The clade is well supported (87.9% BP, 100% PP; Fig. 2.2), and exhibits a moderate genealogical exclusivity across single gene trees (gsi_T=0.838, p-value=0.001; Table 2.3). Monophyly of the clade was supported by ITS and *tub* single gene trees. Conflicting with the monophyly of this clade is the monophyly of the "American" isolates supported by the ITS locus, while *tub* supported the monophyly of these isolates for ones from Africa (Fig. 2.3). Under the applied criterion of GCPSR (Pringle *et al.* 2005), these

populations cannot be segregated into species because only a single gene supports its monophyly. Under the 95% parsimony probability, these subgroups were not separated into their own haplotype networks. Thus, this method also supports the hypothesis that isolates from Africa and America are conspecific.

As mentioned above, the recognized species of *Corallomycetella* are correlated to geographic origin. It is possible that the observed distribution of *Corallomycetella* species may be the result of vicariance. Supporting this hypothesis are the plant hosts of *Corallomycetella*, some of which are native to the same geographic region. For example, *C. elegans* parasitizes *Hevea brasiliensis* Müll. Arg. (Table 2.1), which is native to the Americas. It could explain why haplotypes from Africa are conspecific with those in America (Fig. 2.3). African haplotypes could be the result of anthropogenic introductions from America into plantations of *H. brasiliensis* in Africa. Vicariance has been used to explain the geographic distribution of species of the biotrophic fungus *Cyttaria* Berk. and their hosts in the plant genus *Nothofagus* Blume (Peterson *et al.* 2010).

The incorrect application of a name: "Nectria mauritiicola"

In our revision of the genus *Corallomycetella*, an isolate labeled CBS 400.52, initially identified as *Nectria mauritiicola*, a synonym of *C. repens*, was determined to be unrelated to the genus *Corallomycetella* sensu Rossman *et al.* (1999). ITS sequences labeled *Nectria mauritiicola* and retrieved from GenBank were identical to those for CBS 400.52. The source of the GenBank sequences was listed as human blood, which cast doubt on the identity of these fungi because specimens of *Corallomycetella* are

reported to be plant pathogens (reviewed in Booth and Holliday, 1973). Additionally, the observed anamorph of CBS 400.52 was acremonim-like, unlike the anamorph of species of *Corallomycetella* (described below).

The herbarium specimen of CBS 400.52 listed as IMI 44310 was not observed, and we could not determine whether the name was correctly applied to the original specimen/isolate. Examination of the type specimen of *Nectria mauritiicola* revealed that this name is correctly considered a synonym of *Corallomycetella elegans* (see Taxonomy section). Thus, the name *Nectria mauritiicola* was incorrectly applied to CBS 400.52 and the other sequences in GenBank.

The sequences of CBS 400.52 and others retrieved from GenBank were found to be identical to that of CBS 122.29, ex-type culture of *Sarocladium kiliense* (≡ *Acremonium kiliense*). The acremonium-like anamorph produced by CBS 400.52 also suggests this species. Therefore, the sequences labelled "*Nectria mauritiicola*" in GenBank are determined to be conspecific with *S. kiliense* (76% BP; Fig. 2.2). They are incorrectly annotated creating confusion in the scientific community especially those who depend on the database for identification. Novicki *et al.* (2003) and Jang *et al.* (2012) are examples of clinical literature where the incorrect identification was assigned, although Novicki *et al.* (2003) questioned the validity of the GenBank name because their samples did not cluster with CBS 313.72 (a true *Corallomycetella repens* included in our study). This example calls for a method to change annotations in GenBank and highlights the importance of taxonomic studies to correct these crucial errors.

TAXONOMY

Corallomycetella Henn., Hedwigia 43: 245. 1904.

Generic type: *Corallomycetella heinsenii* [as *heinesii*] (Henn.) Henn. (≡ *Corallomyces heinsenii* Henn., Bot. Jahrb. Syst. 23: 358. 1897).

[= *Corallomyces* Berk & M.A. Curtis, J. Acad. Nat. Sci. Philadelphia, Ser. 2, 2: 289. 1853, non Fr. 1849. — Type: *C. elegans* Berk. & M.A. Curtis]

= *Rhizostilbella* Wolk, Mycol. Zentbl. 4: 237. 1914.

Generic type: *R. hibisci* (Pat.) Seifert (≡ *Stilbum hibsci* Pat., = *R. rubra* Wolk, Mycol. Zentbl. 4: 237. 1914 fide Seifert, 1985).

Perithecia solitary to gregarious, associated with reddish rhizomorphs or synnemata, obpyriform, scarlet to blood-red, KOH+ blood-red, LA+ yellow, slightly scurfy, uniloculate. Perithecial surface cells forming *textura angularis*. Perithecial wall of one region of cells forming *textura angularis*, becoming narrow, compressed towards the centrum, 50–70µm thick. Asci narrowly clavate, apex with a ring, with eight-ascospores arranged uniseriately. Ascospores ellipsoid, smooth, one-septate, constricted at septum, thick-walled, outer wall sometimes sinuous, appearing rough (optical section), finely striated (surface view), yellow-brown.

Anamorph synnematous *Rhizostilbella*. Synnemata on natural substrata solitary or gregarious, 2–5 caespitose, arising laterally or as terminal extension of the rhizomorphs or directly from the substratum, cylindric-capitate, subulate-capitate, cylindrical, slender to robust, straight, curved or sinuous, unbranched or once or twice

branched, hirsute, pale luteous to luteous, KOH+ livid red to purple, LA+ yellow. Marginal hyphae echinulate to verrucose, pale luteous, KOH+ livid red, with clavate terminal cells, covering entire surface of stipe. Conidiophores unbranched, or once simple monochasial or monoverticillate. Phialides cylindrical, terminal, lateral and terminal, collarettes not flared, periclinal thickening conspicuous. Conidial mass white to yellow, subglobse. Conidia ellipsoidal, ovoidal with a truncate base, non-septate, smooth-walled, hyaline.

Habitat: On bark and roots of decaying or living (diseased) tropical trees, and also isolated from soil.

Distribution: Africa, Asia, America (pantropical).

Notes: Species of *Corallomycetella* are unique nectriaceous fungi in that they have a synnematous *Rhizostilbella* anamorph. *Corallomycetella* is similar to *Corallonectria* in that species in these genera produce rhizomorphs in PDA, but *Corallonectria* has a synnematous, fusarium-like anamorph.

Corallomycetella elegans (Berk. & M.A. Curtis) C. Herrera & P. Chaverri, **comb. nov**. Fig. 2.6

MycoBank No. MB803107

Basionym: [*Corallomyces elegans* Berk. & M.A. Curtis, J. Acad. Nat. Sci. Philadelphia, Ser. 2, 2: 239. 1853, genus illeg., Art. 53].

= *Stilbum hibsci* Pat., J. Bot. Paris 1891: 320 fide Seifert, 1985.

≡ *Rhizostilbella hibisci* (Pat.) Seifert, Stud. Mycol. 27: 162. 1985.

[= *Corallomyces heinsenii* Henn., Bot. Jahrb. Syst. 23: 538. 1897, genus illeg., Art. 53].

≡ *Corallomycetella heinsenii* (Henn.) Henn., Hedwigia 43: 245. 1904.

[= *Corallomyces elegans* var. *camerunensis* Henn., Bot. Jahrb. Syst. 22: 76. 1897, genus illeg., Art. 53].

[≡ *Corallomyces camerunensis* (Henn.) Henn., Bot. Jahrb. Syst. 23: 538. 1897, genus illeg., Art. 53].

[= *Corallomyces berolinensis* Henn., Verh. Bot. Vereins Prov. Brandenburg 40: 153. 1898, genus illeg., Art. 53].

= *Nectria coccinea* (Pers : Fr.) Fr. var. *platyspora* Rehm, Ann. Mycol. 7: 137. 1900.

≡ *Nectria platyspora* (Rehm) Weese, in Höhn. & Weese, Ann. Mycol. 8: 464. 1910.

[= *Corallomyces mauritiicola* Henn., Hedwigia 43: 244. 1904, genus illeg., Art. 53].

≡ *Nectria mauritiicola* (Henn.) Seifert & Samuels, Stud. Mycol. 27: 161. 1985.

= *Rhizostilbella rubra* Wolk, Mycol. Zentbl. 4: 237. 1914 fide Seifert, 1985.

Anamorph: synnematous *Rhizostilbella*.

Teleomorph: Perithecia solitary to gregarious, associated with reddish rhizomorphs and/or synnemata, obpyriform, scarlet, KOH+ blood-red, LA+ yellow, with concolorous scurf, 512–940(–1073) × 309–634 μm (mean = 711 × 486; SD 127, 70; n

= 30). Asci narrowly clavate, apex with a ring, with eight-ascospores arranged uniseriately, (123–)145–211 × 8.6–14.3µm (mean=162×11; SD 21, 1.6; n=24). Ascospores ellipsoid, smooth, one-septate, constricted at septum, thick walled, outer wall sometimes sinuous, appearing rough (optical section), finely striated (surface view), 13.7–22.1×5.8–9.2µm (mean=16.8×7.7; SD 1.5, 0.7; n = 110).

Culture and anamorph: Colonies 42–69 mm diam (mean=54; SD 7; n=34) after 14d. at 25°C on PDA. Colony surface with synnemata forming near the inoculum or scattered, aerial mycelium white to pale-luteous, cottony to velvety; below aerial mycelium greenish olivaceous; sometimes agar discoloring yellow-green; reverse isabelline at center becoming buff toward colony edge, with isabelline dichotomously branching rhizomorphs immersed in agar. Synnemata cylindrical-capitate, cylindrical, slender to robust, straight, hirsute, orange to luteous, KOH+ livid red, 876–2,536×248–621µm (mean=1,394×424; SD 545, 108; n=19). Marginal hyphae of synnemata, septate, echinulate, covering entire surface of stipe, with clavate terminal cells, 15–29×7.6–9.9 (mean=21×8.5; SD 5.1, 0.8; n=7). Conidiophores unbranched or once simple monochasial or monoverticillate. Phialides cylindrical, slightly tapering towards tip, hyaline. Conidial mass buff-colored. On SNA, conidiophores simple, unbranched, acremonium-like. Phialides cylindrical, slightly tapering towards tip, collarettes not flared, periclinal thickening conspicuous, hyaline, length 27–81µm (mean=55; SD 11.8; n=81), width at base 2.2–4.2µm (mean=3.3; SD 0.4; n=81), width at tip 1.5–2.8µm (mean=2.1; SD 0.3; n=81). Conidia ellipsoidal, ovoidal with a truncate base, non-septate, smooth-walled, hyaline, 13–26(–28.4) × 7–13 µm (mean=19×10; SD 2.5, 1.1; n=270).

Habitat: On bark and roots of decaying or living (diseased) tropical trees, and also isolated from soil.

Distribution: Brazil, Colombia, Costa Rica, French Guiana, Nicaragua, Panama, Venezuela (Samuels 1973; Samuels & Dumont, 1982; Rossman *et al.*, 1999), DR Congo, Gabon, Guadeloupe, Ivory Coast, Jamaica, Liberia.

Holotype of *Corallomyces elegans*: **Suriname**, on bark, **Holotype** ex herb. Schw. in herb. Berkeley (K; **Neotype** of *Corallomycetella heinsenii* designated in Rossman *et al.* 1999).

Epitype of Corallomyces elegans *designated herein*: **French Guiana**, Région de Saül, Layon des Eaux Claires, on bark of unidentified tree, 2 May 2008, C. Lechat (CLL8064), **Epitype** BPI 881071, ex-epitype culture CBS 123826 = AR 4547.

Additional type specimens examined: **Brazil**, Rio Grande do Sul, Saô Leopoldo, on bark, Oct. 1907, S.J. Rick (exsiccati no. 1813; NY, **Isotype** of *Nectria coccinea* var. *platyspora*); Estado de Amazonas, Rio Jurua, Miry, on *Mauritia flexuosa*, July 1901 (E. Ule Herbarium Brasiliense no. 2837, **Holotype** of *Corallomyces mauritiicola*); **Cameroon**, on bark, J.R. Jungner (FH, **Holotype** of *Corallomyces elegans* var. *camerunensis*).

Additional specimens and isolates examined: **Brazil**, Bahia, Igrapiúna, on dead bark, 12 Sep. 2010, P. Chaverri (PC 1261), O. Liparini Pereira, D. Pinho, A. Luiz Firmino, BPI 884207, culture CBS 134440; Estado do Pará, Belterra, Maguari, Floresta Nacional do Tapajós, elev. 28 m., S2°46'59.6", W55°01'38.9", on dead tree of *Hevea*, 7 May 2011, O. Liparini Pereira & P. Chaverri (PC 1307), BPI 884208, culture CBS

134441. **Colombia**, Puerto Japon, Rio Peneya, Caqueta, on bark, 25 Jul. 1973–28 Jul. 1973, Y. Doi, BPI 842181 = TNS-F-224942 = TNS.D-1720. **Costa Rica**, isolated from rhizosphere of *Musa sapientum*, R.D. Goos (No. 1127), culture CBS 276.60 = ATCC 14043 = IMI 84360; Heredia Province, La Selva Biological Station, succession plots 2-3 years old, N10° 25' 23.5" W84° 00' 04.8", on dying base of Musaceae, 16 Mar. 2010, P. Chaverri (PC 1123), BPI 881540; Limón, Santa Rita de Pococí, on canker of *Tectona grandis*, M. Arguedas, BPI 748185; Heredia Province, La Selva Biological Station, Sendero Tres Rios and Camino Experimental Norte, N10°26’7.3” W84°00’31.4”, alt. 64 m., on unidentified *Liana*, 17 Mar. 2010, P. Chaverri (PC 1166), BPI 881547, culture CBS 131288 = G.J.S. 10-133; Heredia Province, La Selva Biological Station, Sendero Tres Rios and Camino Experimental Norte, N10°26’7.3” W84°00’31.4”, alt. 64 m., on unidentified palm, 17 Mar. 2010, P. Chaverri (PC 1169), BPI 882353, culture CBS 131289 = G.J.S. 10-134. **DR Congo**, stem base, INEAC, Afd. Phyto, No. 1191, culture CBS 275.60. **French Guiana**, Cayenne. Montagne Cacao, Piste Coralie, 4°32'0"N 52°25'0"W, on bark, 14 Feb. 1988, A. Rossman & C. Feuillet (3142), BPI 1107213. **Gabon**, Crystal Mountains National Park, on dead bark, 30 Apr. 2009, K. Põldmaa (GAB 18), TU 107818 = BPI 879302, culture CBS 125531 = A.R. 4659. **Guadeloupe**, Sentier de la Cascade, Vauchelet, Gorges de la Riviere, alt. 600 m., on bark, 6 Jan. 1996, J. Vivant, BPI 744460. **Ivory Coast**, IRCA Plantation, near Abidjan, *Hevea brasiliensis*, 1968, J.J. Guillaumin, culture CBS 119.84 = IMI 135503. **Jamaica**, Surrey, St. Andrew Parish, on decaying seed pod, 12 Jan. 1971, RP Korf (CUP-MJ824), JR Dixon, DP Dumont, RW Erb, DH Pfister, DR Reynolds, AY Rossman, GJ Samuels, NY. **Liberia**,

Cavalla, Harbel, *Hevea brasiliensis*, 25 Nov. 1963, J. Schreurs (No. 72), culture CBS 379.64. **Venezuela**, Territorio Federal Amazonas, Neblina Base Camp on Rio Baria (= Rio Mawarinuma), left bank, downstream from camp, alt. 140 m., on undetermined substrate, 18 Feb. 1985, A. Rossman (A.R. 2175), BPI 552587; Edo. Barinas, Caimital Forest, La Montana. Ca. 5 km NE of Caimital Village and ca. 10 km NE of intersection with Barinas-Opisbo Rd., dry secondary forest, alt. 800 m., 8°35'N 70°15'W, on bark, 25 Nov. 1990, G.J. Samuels, B. Hein, S. M. Huhndorf (7501), BPI 744870.

Notes: *Corallomycetella elegans* and *C. repens* are indistinguishable morphologically, except for the synnemata produced in PDA. The synnemata of *C. elegans* can attain a height of up to 2,500 μm as described by Seifert (1985). The synnemata of *C. repens* only reaches 600 μm and are cushion-shaped. *Corallomycetella elegans* is apparently restricted to the tropical Western Hemisphere and Africa.

Corallomycetella repens (Berk. & Broome) Rossman & Samuels, Stud. Mycol. 42: 113. 1999. Fig. 2.7

Basionym: *Sphaerostilbe repens* Berk. & Broome, J. Linn. Soc., Bot. 14: 114. 1875.

= *Stilbum hibisci* Pat., J. Bot., Paris 1891: 320. 1891.

≡ *Rhizostilbella hibisci* (Pat.) Seifert, Stud. Myco. 27: 162. 1985.

= *Rhizostilbella rubra* van der Wolk, Mycol. Centralbl. 4: 237. 1914.

= *Stilbum incarnatum* Wakker, Ziekten van het Suikerriet op Java, Leiden, p. 197.

1898.

= *Stilbum incarnatum* var. *dioscoreae* Sacc., Boll. Orto Bot. Regia Univ. Napoli 6: 63. 1918.

= *Cephalosporium kashiense* R.Y. Roy & G.N. Singh, Curr. Sci. 37: 535. 1968.

≡ *Acremonium kashiense* (R.Y. Roy & G.N. Singh) W. Gams, *Cephalosporium*-artige Schimmelpilze (Hyphomycetes) p. 138. 1971.

Anamorph: synnematous *Rhizostilbella*.

Teleomorph: Perithecia solitary to gregarious, associated with reddish rhizomorphs and/or synnemata, obpyriform, scarlet, KOH+ blood-red, LA+ yellow, covered with scurfs, 386–659×264–367µm (mean= 548×318; SD 112, 34; n=6). Asci narrowly clavate, apex with a ring, with eight-ascospores arranged uniseriately, 194–228×16–20µm (mean =213×18; SD 12.9, 1.4; n=10). Ascospores ellipsoid, smooth, one-septate, constricted at septum, thick walled, outer wall sometimes sinuous, appearing rough (optical section), finely striated (surface view), yellow-brown, 15–21.5×6.7–8.8µm (mean=18 ×7.8; SD 2.2, 0.7; n=20).

Culture and anamorph: Colonies 42–61mm diam (mean=52; SD 6.5; n=12) after 14d. at 25°C on PDA. Colony surface with white aerial mycelium, cottony to velvety; conidial masses buff colored, slimy, pionnotal produced by synnemata near the inoculum; agar discoloring yellow-green; reverse isabelline with conspicuous isabelline dichotomously branching rhizomorphs immersed in agar. Surface of sterile colonies with white aerial mycelium, cottony, reverse saffron. In culture, synnemata

cushion-shaped, orange to salmon, KOH+ livid red, 403–523×520–594µm (mean=450×552; SD 64, 38; n=3). Hyphae of synnemata, septate, echinulate. Conidiophores unbranched or once simple monochasial or monoverticillate. Phialides monophialidic, cylindrical, hyaline. On SNA, conidiophores simple, unbranched, acremonium-like. Phialides cylindrical, collarettes not flared, periclinal thickening conspicuous, hyaline, length 39–79µm (mean=53; SD 12.9; n=10), width at base 2.5–3.8µm (mean=3.1; SD 0.5; n=10), width at tip 1.8–2.7µm (mean=2.3; SD 0.2; n=10). Conidia ellipsoidal to ovoidal with a truncate base, nonseptate, smooth-walled, hyaline, 13–19 × 7–11µm (mean=16×9; SD 1.5, 0.9; n=30).

Habitat: On bark and roots of decaying or living (diseased) tropical trees, and also isolated from soil.

Distribution: China, India, Indonesia, and Sri Lanka.

Holotype of *Sphaerostibe repens*: **Sri Lanka** (Ceylon), Peradeniya, on decaying wood of *Artocarpus integrifolia*, August, **Holotype** Herb. Berkeley (K), no. 1005.

Additional specimens and isolates examined: **China**, alt. 650m., on bark, 01 OCT. 1993, Y. Doi (93-10), BPI 802510. **India**, Uttar Pradesh, Varanasi, isolated from rhizosphere of *Linum usitatissimum*, Jan. 1967, G.N. Singh, culture ITCC 1330=CBS 313.72=IMI 132119 [ex-holotype of *Cephalosporium kashiense*]. **Indonesia**, Java, Bogor, on bark of *Carica papaya*, 16 May 1955, A. Kurnadi, BPI 631169; Java, on root of *Carica papaya*, Jun. 1948, K.B. Boedijn & J. Reitsma (No. B.R. 29/48), culture CBS 358.49. **Sri Lanka**, isolated from soil, 1963, O.S. Peries, culture IMI 101072=CBS 118.84.

Notes: *Corallomycetella repens* is only known from South to Southeast Asia. Morphologically, it is nearly indistinguishable from *C. elegans*, except for its short, cushion-shaped, synnemata in PDA. The observed synnema are from a single culture (CBS 118.84), which was isolated in 1963. One could question whether conidiomata development has changed from a stipitate synnemata to the cushion-shaped synnemata over the years. Fresh collections from Asia are needed to select an epitype in order to stabilize the name and determine the extent of morphological variation.

Corallonectria C. Herrera & P. Chaverri, **gen. nov**.

MycoBank No. MB803108

Generic type: *Corallonectria jatrophae* (A. Møller) C. Herrera & P. Chaverri

Etymology: From Greek *korallion*=coral. Referring to the short, red stalk on which the perithecia develop and making reference to the genus *Corallomycetella.*

Perithecia seated on a short red stalk, in caespitose clusters of 2 to several, ovoid to obpyriform, collapsing laterally or not collapsing when dry, scarlet, KOH+ blood-red, LA+ yellow, with a white to yellow furfuraceous coating of hyphae below apex; furfuraceous coating missing in age; apex acute, smooth, uniloculate. Perithecial surface cells forming *textura angularis*. Perithecial wall of one region of cells forming *textura angularis*, becoming narrow, compressed towards the centrum, 30–40µm thick. Asci clavate, apex simple, with eight ascospores arranged biseriately. Ascospores fusiform-ellipsoid, sometimes reniform, 1-septate, often constricted slightly at septum, pale brown when discharged, smooth-walled. Anamorph synnematous, fusarium-like.

Habitat: On bark of decaying or living (diseased) tropical trees.

Distribution: Tropical America and Greater Antilles.

Notes: *Corallonectria* is similar to *Corallomycetella* in that it produces rhizomorphs on PDA. However, the anamorphic states are different. *Corallomycetella* has a synnematous *Rhizostilbella* anamorph, while *Corallonectria* has a synnematous fusarium-like anamorph. *Corallonectria* is also characterized by a white to yellow furfuraceous coating below the apex of the perithecia, and relatively large, pale-brown, and smooth ascospores.

Corallonectria jatrophae (A. Møller) C. Herrera & P. Chaverri, **comb. nov**. Fig. 2.8

MycoBank No. MB803109

Basionym: [*Corallomyces jatrophae* A. Møller, Bot. Mitt. Tropen 9: 295. 1901, genus illeg., Art. 53].

≡ *Nectria jatrophae* (A. Møller) Wollenw., Z. Parasitenk. (Berlin) 3: 498. 1931

≡ *Corallomycetella jatrophae* (A. Møller) Rossman & Samuels, Stud. Mycol. 42: 114. 1999.

= *Nectria madeirensis* Henn., Hedwigia 43: 244. 1904.

[= *Corallomyces caricae* Henn., Hedwigia 43: 245. 1904, genus illeg., Art. 53].

= *Macbridella amazonensis* Bat., J.L. Bezerra & C.R. Almeida, An. XIV congr. Nac. Soc. Bot. Brasil, 1963: 118. 1964.

≡ *Nectria amazonensis* (Bat., J.L. Bezerra & C.R. Almeida) Samuels, Canad. J. Bot. 51: 1278. 1973.

Anamorph: synnematous, fusarium-like.

Teleomorph: Perithecia seated on a short red stalk, in caespitose clusters of 2 to several, ovoid to obpyriform, 729–1308×447–748µm (mean=932×575; SD 171, 83; n=17), not collapsing or collapsing by lateral pinching, orange-red to scarlet, with a white to yellow furfuraceous coating below apex; apex acute, smooth, scarlet. Asci clavate, apex simple, with eight ascospores arranged biseriately, 93–128×10.3–19.8µm (mean=108×15; SD 12.1, 3.1; n=9). Ascospores fusiform-ellipsoid, sometimes reniform, 1-septate, often constricted slightly at septum, pale brown when discharged, smooth, (26–)27–41.7×6.8–12.2 (mean=32×9; SD 2.9, 0.9; n=139).

Culture and anamorph: Colonies 42–57mm diam (mean=48; SD 9.7; n=4) after 14d. at 25°C on PDA. Colony surface with white aerial mycelium, cottony to velvety; agar discoloring amber; synnemata produced near inoculum; agar discoloring amber; reverse saffron, with saffron dichotomously branching rhizomorphs immersed in agar. Synnemata cylindrical, slender to robust, straight or curved, rarely branching, appearing furfuraceous with loose, white hyphae, with a terminal cupulate capitulum (several with age), pale-luteous, KOH+ apricot to scarlet, 1,178–2,464×225–359µm (mean=1,698×286; SD 425, 43; n=10; taller with age). Conidiophores unbranched or once simple monochasial or monoverticillate. Phialides cylindrical, hyaline, length 9–34µm (mean=22; SD 10.5; n=7), width at base 2.9–3.9µm (mean=3.3; SD 0.3; n=7), width at tip 1.9–2.7µm (mean=2.2; SD 0.3; n=7). Conidial mass forming inside

cupulate capitula, flame-shaped, luteous. Conidia fusarium-like, long-fusiform, slightly curving at the apical and basal ends, apical cell acute, basal cell pedicellate, hyaline, forming on PDA, not observed on SNA, 3–4(–5)-septate: 3-septate (68–)71–84× 5.2–7.7µm (mean=77×6.5; SD 3.8, 0.6; n=30), 4-septate 73–90 × 5.0–7.5µm (mean = 80×6.6; SD 3.9, 0.6; n=30), 5-septate (75–)92–100×5.0–7.2µm (mean = 91 × 6.0; SD 8.4, 0.7; n=6).

Habitat: On bark of decaying or living (diseased) tropical trees.

Distribution: South and Central America (Belize, Brazil, Colombia, Costa Rica, French Guiana, Nicaragua, Panama, Puerto Rico, Venezuela; Rossman et al., 1999; Samuels, 1973; Samuels & Dumont, 1982).

Lectotype of Corallomyces jatrophae *designated herein*: A plate of illustrations in the original paper of *Corallomyces jatrophae*, **Lectotype** (BPI-Stevenson Library) Møller 1901, Taf. I Figs. 21–28.

Epitype of Corallomyces jatrophae *designated herein*: **Puerto Rico**, Cordillera Central, Guavate Picnic Area, off Rte. 184, alt. 500m., base of living tree, 25 Feb. 1996, G.J. Samuels (8120), H.-J. Schroers, D.J. Lodge, **Epitype** BPI 745232, ex-epitype culture CBS 913.96 = G.J.S. 96-18.

Additional type specimens examined*:* **Brazil**, Manaus, on bark of unidentified plant, Batista, 20 Feb. 1961 (URM 22, **Holotype** of *Macbridella amazonensis*); Rio Jurua, Cacoeria, on dead stems of *Carica* sp., May 1901, Ule 2822 (FH, **Isotype** of *Corallomyces caricae*). Marmellos, Rio Madeira, on decaying bark, Mar. 1902, Ule 3115, Mycotheca Brasiliensis no. 69 (The Botanical Museum, University of

Copenhagen, **Isotype** of *Nectria madeirensis*).

Additional specimens examined: **Belize**, Cayo Distr., Blue Hole National Park, Hermons trail, 19 Nov. 2001, L. Ryvarden (44387), BPI 843763. **Brazil**, Estado do Pará, Belterra, Maguari, Floresta Nacional do Tapajós, elev. 28m., S2°46'59.6", W55°01'38.9", on bark, 7 May 2011, O. Liparini Pereira & P. Chaverri (PC 1300), BPI 884209. **French Guiana**, Route de Belizon, track to Montage Tortue, 15km. from road N2, 52°20', 4°25', on bark of newly killed tree, 18 Feb. 1988, A.Y. Rossman & C. Feuillet (3222), BPI 1107291; ibid., A.Y. Rossman & C. Feuillet (3230B), BPI 1107295. **Martinique,** Precheur, Anse Couleuvre, on bark, 18 Aug. 2011, C. Lechat CLL MAR11044C (BPI 829340). **Puerto Rico**, Luquillo Mountains, Bisley Watershed in Valley left of Walkup Tower, below Landslide, Luquillo Experimental Forest, on tree trunk, 14 Mar. 1990, D.J. Lodge (PR 700), BPI 1109351. **Venezuela**, Edo. Bolivar, along Rio Caroni near Rapids Just below Uriman, elevation 393m., on bark, 11 Jan. 1955, J.A. Steyermark & J.J.Wurdack (80), BPI 552420; Chimanta Massif, Torono-Tepui, Estado Bolivar, rainforest Slopes above Base Camp, on bark, 24 Jan. 1955, J.A. Steyermark & J.J. Wurdack, BPI 1107269; Amazonas, Cerro de La Neblina, Valley at N. Base of Pico Phelps, Cloud Forest, alt. 1,000–1,250m, 00°49'N, 66°00'W, on bark, 12 Apr. 1984–13 Apr. 1984, G.J. Samuels (1297), BPI 1107268; Edo. Miranda, Parque Nacional Guatopo, trail between Agua Blanca and La Cruceta, alt. 500-600m., 10°3'N 66°26'W, on bark, 27 Nov. 1990–30 Nov. 1990, G.J. Samuels, B. Hein, S.M. Huhndorf (7570), BPI 744831.

Illustrations. —Samuels (1973, Figs 10-13, as *N. amazonensis*); Wollenweber (1930, No. 684, as *C. jatrophae*).

Notes: *Corallonectria jatrophae* is the only species in the genus *Corallonectria*. It can be easily identified by furfuraceous perithecia on a short red stalk. On PDA, this species produces a luteous colony with rhizomorphs. Ideally an epitype from the same collecting region as the type would be designated, which it would make our isolate from Brazil (PC 1300) ideal. However, the isolate did not survive -80□C storage. Our phylogeny demonstrated that isolates from Puerto Rico (CBS 913.96) and Brazil (PC 1300) are conspecific, and suggesting a broad biogeographic range. Based on the teleomorph, they are indistinguishable. Thus, the specimen from Puerto Rico is designated as epitype.

Table 2.1 Isolates and accession numbers used in the phylogenetic analyses

Species	Isolate No.	Herbarium No.	Substrate/ Host	Country of origin	GeneBank Accession No.			
					ITS	*act*	*mcm7*	*tub*
Calonectria acicola	CBS 114813		-	-	GQ280546	-	-	DQ190591
Calonectria densa	CBS 125261, CMW 31182		-	-	GQ280647	-	-	GQ267232
Campylocarpon fasciculare	CBS 112613		*Vitis vinifera*	South Africa	AY677301	HM352881	-	AY677221
Campylocarpon pseudofasciculare	CBS 112679		-	-	AY677306	HM352882	-	AY677214
Corallomycetella elegans	CBS 276.60		*Musa sapientum*	Costa Rica	KC479748	KC479741	KC479769	KC479780
Corallomycetella elegans	G.J.S. 10-133, CBS 131288	BPI 881547	*Liana* sp.	Costa Rica	KC479746	KC479734	KC479761	KC479781
Corallomycetella elegans	G.J.S. 10-134, CBS 131289	BPI 882353	Decaying palm	Costa Rica	KC479747	KC479735	KC479762	KC479782
Corallomycetella elegans	A.R. 4547, CBS 123826	BPI 881071	Bark	French Guiana	JF832594	JF832440	KC291795	JF832838
Corallomycetella elegans	P.C. 1307, CBS 134441	BPI 884208	*Hevea brasiliensis*	Brazil	KC479749	KC479736	KC479763	KC479783
Corallomycetella elegans	CBS 119.84		*Hevea brasiliensis*	Ivory Coast	KC479752	KC479733	KC479768	KC479775
Corallomycetella elegans	A.R. 4659, CBS 125531	BPI 879302	Bark	Gabon	KC479750	KC479730	KC479764	KC479776
Corallomycetella elegans	CBS 379.64		*Hevea brasiliensis*	Liberia	KC479754	KC479732	KC479766	KC479778
Corallomycetella elegans	P.C. 1261, CBS 134440	BPI 884207	Bark	Brazil	KC479751	KC479731	KC479767	KC479779
Corallomycetella elegans	CBS 275.60		Stem base	Zaire	KC479753	KC479737	KC479765	KC479777
Corallomycetella jatrophae	P.C. 1300	BPI 884209	Bark	Brazil	KC479759	KC479745	-	KC479788
Corallomycetella jatrophae	G.J.S. 96-18, CBS 913.96	BPI 745232	Base of living tree	Puerto Rico	KC479758	KC479744	-	KC479787
Corallomycetella repens	CBS 118.84		Soil	Sri Lanka	KC479755	KC479738	KC479770	KC479784

Table 2.1. Continued

Species	Isolate No.	Herbarium No.	Substrate/ Host	Country of origin	GeneBank Accession No.			
					ITS	*act*	*mcm7*	*tub*
Corallomycetella repens	CBS 358.49		*Carica papaya*	Indonesia	KC479756	KC479740	KC479771	KC479785
Corallomycetella repens	CBS 313.72		Soil	India	KC479757	KC479739	KC479772	KC479786
Cosmospora coccinea	AR 2741, CBS 114050	BPI 802729	*Inonotus nodulosus*	Germany	HM484537	GQ505967	-	HM484589
Cosmospora viridescens	CBS 102433		*Tilia*	Czech Republic	KC291731	KC479742	KC291804	KC291904
Cyanonectria buxi	CBS 125554		-	-	HM626660	-	-	-
Cyanonectria cyanostoma	G.J.S. 98-127, CBS 101734	BPI 748307	Buxaceae	France	HM484558	GQ505961	-	HM484611
Dialonectria episphaeria	G.J.S. 10-193, C.H. 10-01		*Diatrype stigma*	USA	KC291744	-	KC479773	KC291932
Dialonectria episphaeria	CBS 125494		-	-	HQ897811	-	-	-
Fusicolla acetilerea	F-223,908		-	Comoros	EU860058	-	-	EU860031
Fusicolla violacea	F-167,589		-	France	EU860060	-	-	EU860032
Ilyonectria liriodendri	CBS 112602		-	-	HM364302	-	-	AY677242
Ilyonectria radicicola	A.R. 2553, ATCC 208837		-	-	HM364290	HM352871	-	HM352856
Lanatonectria flavolanata	strain 5622		-	-	-	-	-	HM054109
Lanatonectria flocculenta	GJS 01-66, CBS 126441		-	Ecuador	JF832656	JF832481	-	JF832913
Macroconia leptosphaeriae	CBS 100001		-	-	HQ897810	-	-	-
Macroconia papilionacearum	CBS 125495		-	-	HQ897826	-	-	-
Microcera larvarum	A.R. 4580, CBS 133964	-	scale insect	New Zealand	KC291751	-	KC291798	KC291935

Table 2.1. Continued

Species	Isolate No.	Herbarium No.	Substrate/ Host	Country of origin	GeneBank Accession No.			
					ITS	*act*	*mcm7*	*tub*
Microcera rubra	F-267,623		-	USA	EU860074	-	-	EU860020
Nectria cinnabarina	A.R. 4477, CBS 125165	BPI 879981	Dead twigs of *Aesculus* sp.	France	HM484548	HM484503	JN993335	HM484606
Nectria pseudotrichia	G.J.S. 09-1329	BPI 881041	dead bark of tree	Venezuela	JF832647	JF832506	JN993321	JF832902
Neonectria fuckeliana	A.R. 3103, CBS 125133		-	-	HM364291	HM352872	-	HM352857
Neonectria ramulariae	ATCC 16237		-	-	HM364297	HM352879	-	HM352863
Ophionectria trichospora	G.J.S. 01-206, CBS 109876	BPI 863854	*liana*	Cameroon	HM484867	-	-	HM484886
Pleonectria cucurbitula	A.R. 2778, CBS 125130	BPI 746348	*Pinus sylvestris*	Austria	JF832603	JF832464	-	JF832855
Pleonectria lamyi	A.R. 2779, CBS 115034	BPI 746349	*Berberis vulgaris*	Austria	HM484544	HM484507	-	HM484593
Rugonectria rugulosa	TPPH 32		*Myrica rubra*	Japan	AB233176	-	-	AB237526
Rugonectria rugulosa	Y.H. 10-01, CBS 129158	BPI 881070	-	USA	JF832661	JF832515	-	JF832911
Sarocladium attenuatum	CBS 399.73		-	-	AY566995	-	-	-
Sarocladium kiliense	CBS 122.29		skin infection of man	Germany	AJ621775	-	-	-
Sarocladium kiliense as *Nectria mauritiicola*	NRRL 20420	-	-	USA	AJ557830	-	-	-
Sarocladium kiliense as *Nectria mauritiicola*	NHRC-FC042	-	human blood	Russia	AJ558114	-	-	-
Sarocladium kiliense as *Nectria mauritiicola*	CBS 400.52		*Ficus carica*	England	KC479760	KC479743	KC479774	KC479789
Sarocladium strictum	CBS 346.70		-	-	AY214439	-	-	-
Stylonectria applanata	CBS 125489		-	-	HQ897805	-	-	-

Table 2.1. Continued

Species	Isolate No.	Herbarium No.	Substrate/ Host	Country of origin	GeneBank Accession No.			
					ITS	*act*	*mcm7*	*tub*
Stylonectria purtonii	DAOM 235818		-	-	HQ897831	-	-	-
Thelonectria discophora	A.R. 4324, CBS 125153		-	-	HM364294	HM352875	-	HM352860
Thelonectria westlandica	G.J.S. 83-156, CBS 112464		*Dacrydium cupressinum*	New Zealand	HM484559	GQ505959	-	HM484610
Viridispora alata	A.R. 1770, CBS 125123		-	Portugal	JF832678	GQ505985	-	JF832912
Viridispora diparietispora	CBS 102797		-	-	JN049838	-	-	-

* KC479730–KC479789 sequences were produced in this study.

Table 2.2. Loci used in the phylogenetic analyses. Information on the primers, including base pairs, PCR protocols, and models of nucleotide substitution are indicated.

Locus	ITS	*act*	*mcm7*	*tub*	Combined
Included sites	561	584	576	583	2304
Phylogenetically informative sites	248	102	167	282	799
Uninformative polymorphic sites	48	38	73	77	236
Invariable sites	0	154	0	192	345
Nucleotide substitution models (coded as)	TVM+G (nst = 6, rates = gamma)	TIM1+I+G (nst = 6, rates = invgamma)	TIM1+G (nst = 6, rates = gamma)	HKY+I+G (nst = 2, rates = invgamma)	
Primers used (reference)	ITS5, ITS4 (White *et al.* 1990)	Tact1, Tact2 (Samuels *et al.* 2006)	mcm7-709for, mcm7-1348rev (Schmitt et al. 2009)	Btub-TI, Btub-T2 (O'Donnell & Cigelnik 1997)	
PCR protocol: Annealing temp. & cycles	53 °C, 1 min, 40x	65 °C, 30 s, 15x 48 °C, 30 s, 30x	56 °C, 50 s, 38x	55 °C, 30 s, 35x	

Table 2.3. Genealogical Sorting Index (*gsi*) and probability values for single gene trees and the ensemble of gene trees.

Gene	*C. repens* sensu Rossman *et al.* (1999)		*C. repens* s. str. (Asian clade)		*C. elegans* (African + American clades)		*C. elegans* (African Clade)		*C. elegans* (American Clade)	
	gsi	P	*gsi*	P	*gsi*	P	*gsi*	P	*gsi*	P
act	1	0.071	1	0.003	0.675	0.001	0.711	0.001	0.711	<0.001
ITS	1	0.069	1	0.003	1	0.001	0.711	0.001	1	<0.001
mcm7	1	0.072	1	0.002	0.675	0.001	0.519	0.005	1	<0.001
tub	1	0.075	0.6061	0.014	1	0.001	1	<0.001	1	0.001
ALL (gsi_T)	1	0.075	0.902	0.004	0.838	0.001	0.735	0.001	0.902	0.003

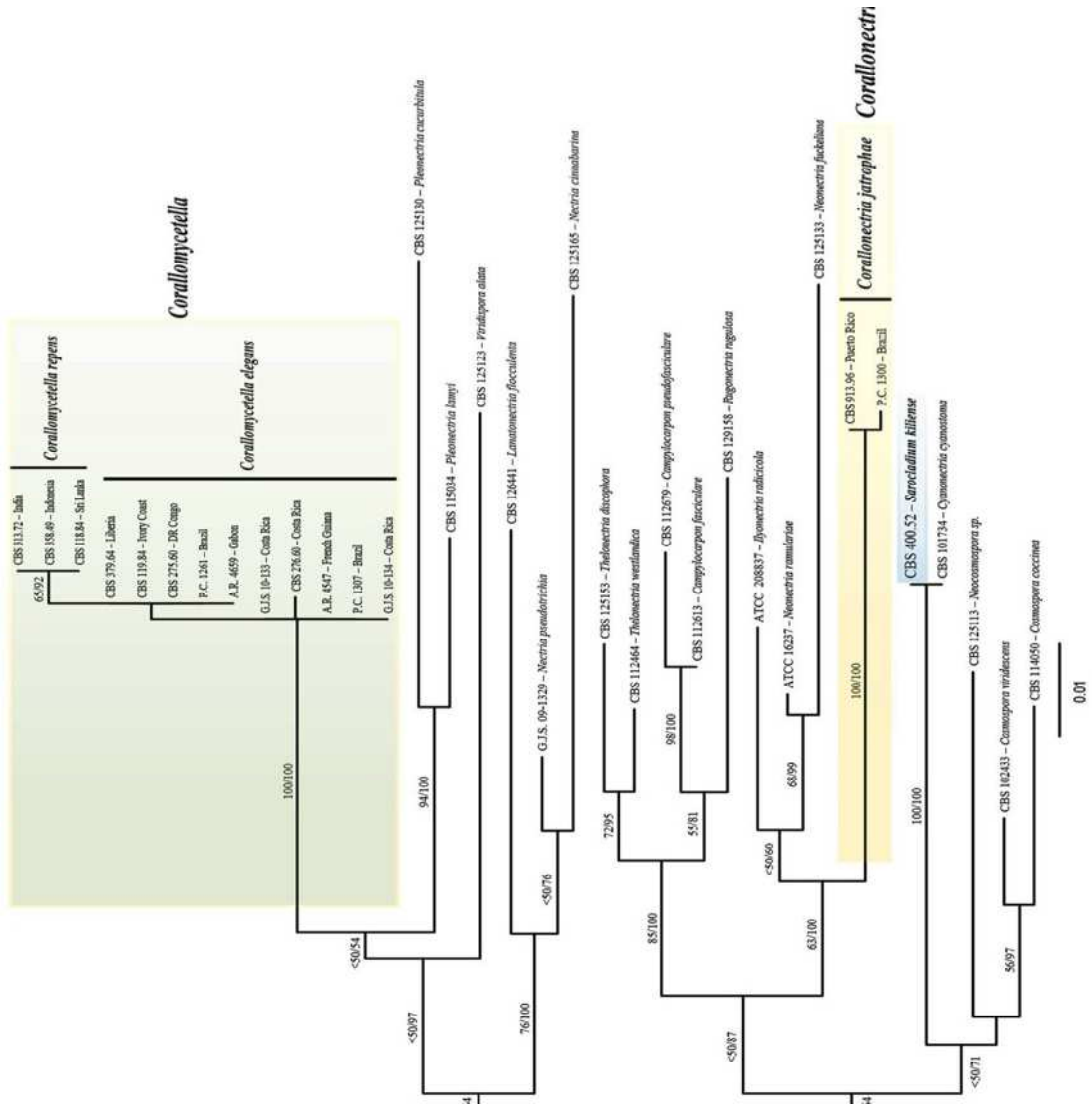

Fig. 2.1. Phylogenetic placement of *Corallomycetella* sensu Rossman et al. (1999) inferred from *act*. Best tree generated with ML analysis (−Ln = 2514.7837). Values at branches indicate Maximum Likelihood bootstrap (ML BP)/Bayesian posterior probabilities (BI PP).

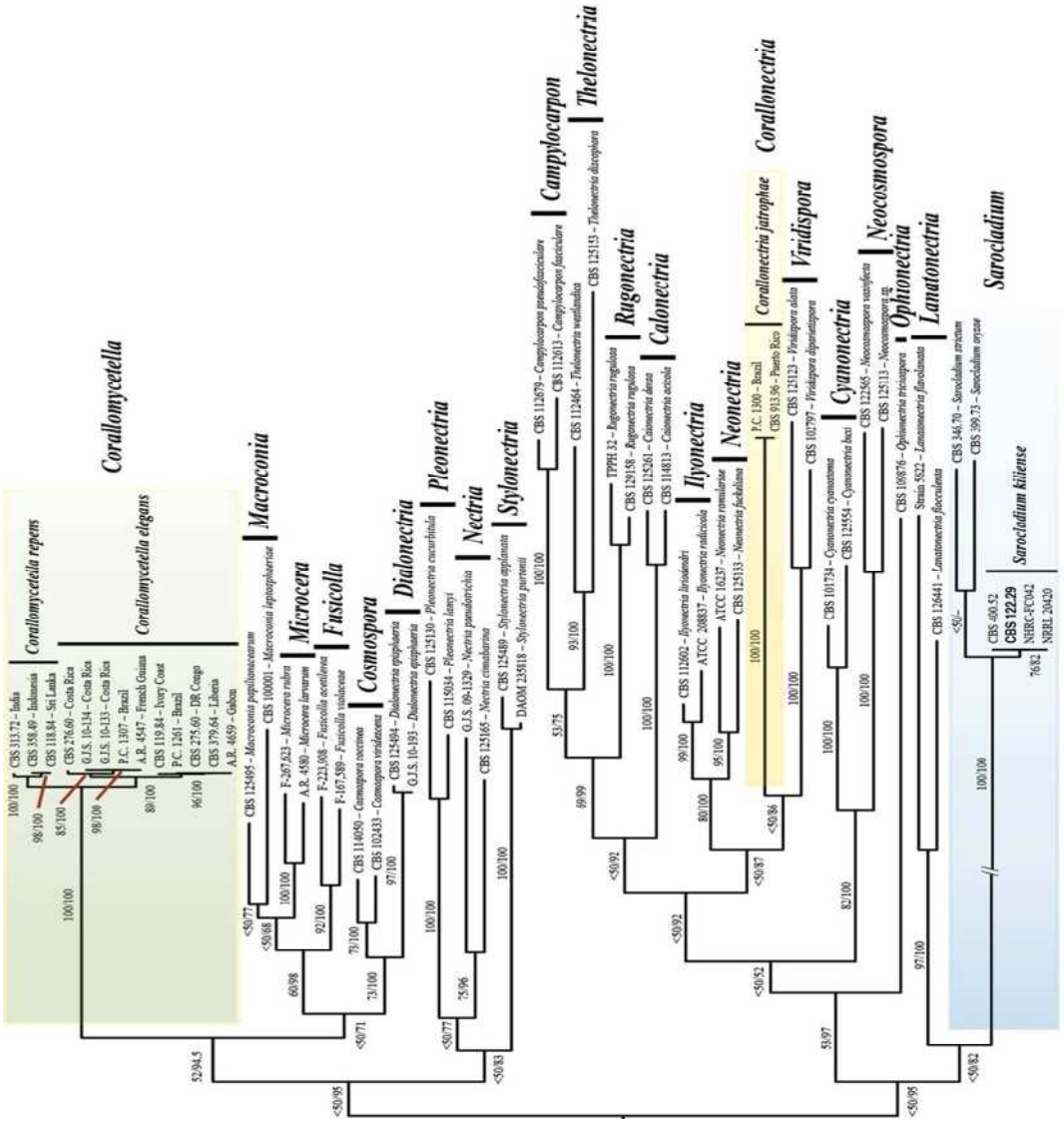

Fig. 2.2 Phylogenetic placement of Corallomycetella sensu Rossman et al. (1999) based on a combined 3-loci (ITS, mcm7, and tub) dataset. Best tree generated with ML analysis (−Ln = 17491.8148). Values at branches indicate Maximum Likelihood bootstrap (ML BP)/Bayesian posterior probabilities (BI PP).

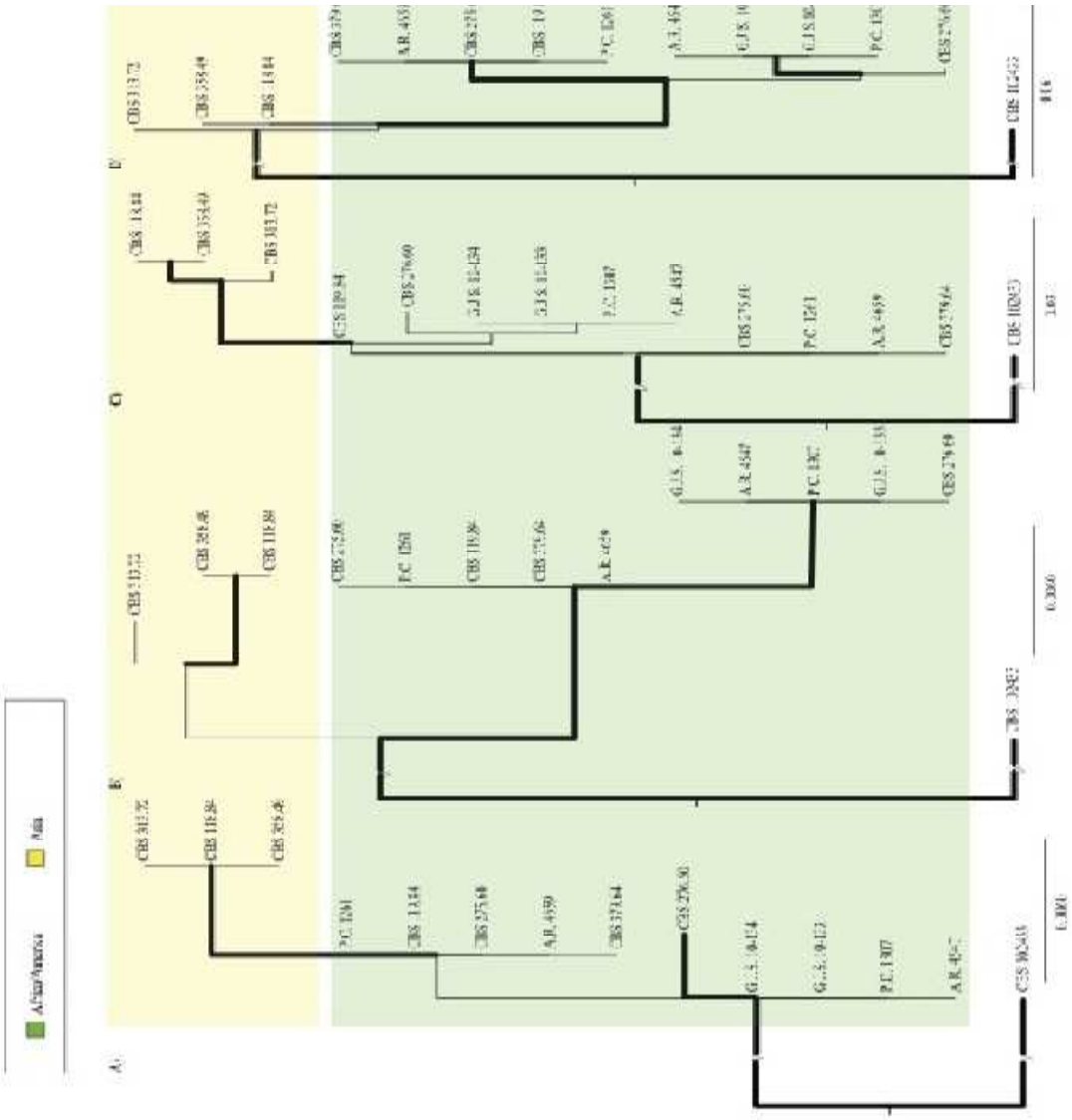

Fig. 2.3. Phylogenetic relationship of *Corallomycetella repens* sensu Rossman *et al.* (1999). Trees with the best log likelihood are presented for (A) *act*, (B) ITS, (C) *mcm7*, and (D) *tub*. Thicker lines indicate well-supported branches (>70% ML BP).

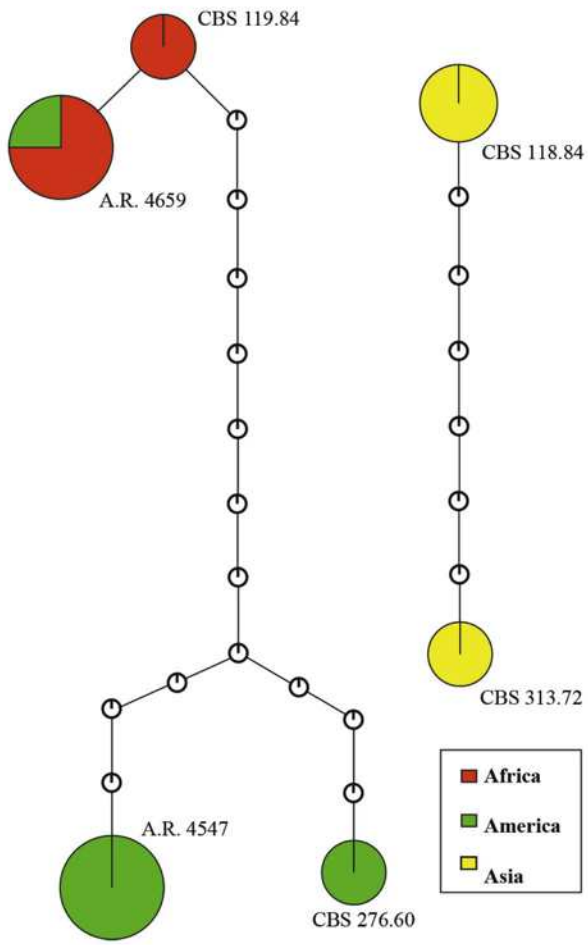

Fig. 2.4. Multilocus haplotype network for *Corallomycetella repens* sensu Rossman *et al.* (1999). The network was constructed in TCS v1.21. Each colored circle represents a haplotype; size of circle is proportional to haplotype frequency. Within each haplotype circle, geographic origins of isolates are proportionally represented as a pie chart. Empty circles represent intermediate haplotypes inferred by TCS. Each line segment represents a single mutation.

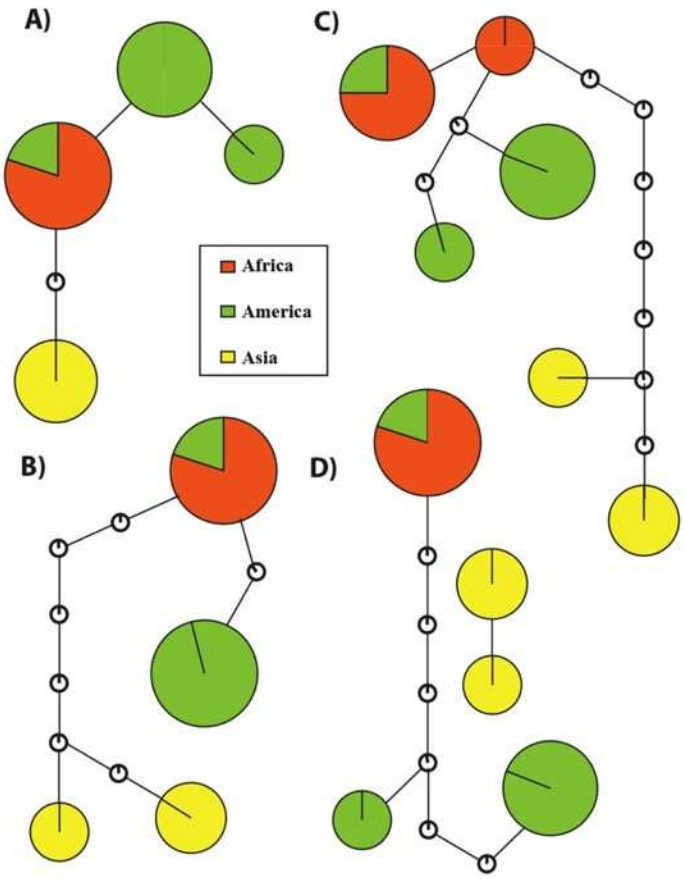

Fig. 2.5. Haplotype networks of *act*, ITS, *mcm7*, and *tub*. The network was constructed in TCS v1.21. **A)** *act*; **B)** ITS; **C)** *mcm7*; **D)** *tub*. Each colored circle represents a haplotype; size of circle is proportional to haplotype frequency. Within each haplotype circle, geographic origins of isolates are proportionally represented as a pie chart. Empty circles represent intermediate haplotypes inferred by TCS. Each line segment represents a single mutation.

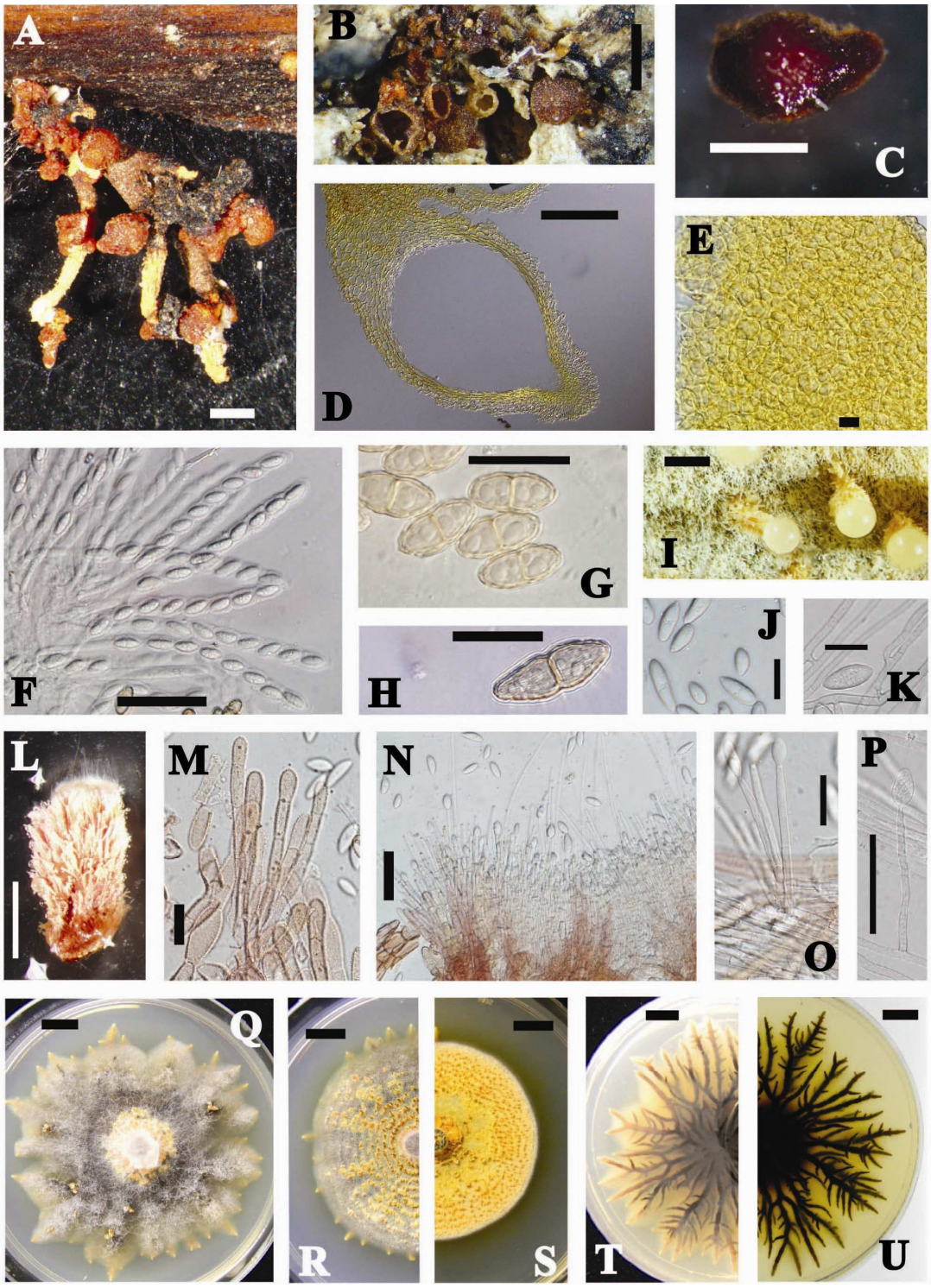
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Fig. 2.6. ***Corallomycetella elegans.*** A, B: Perithecia on natural substrata; C: Perithecium in 3% KOH; D: Median section of perithecium; E: Perithecial surface cells; F: Asci; G: Ascospores (optical section); H: Ascospore (surface view); I: Synnemata on PDA; J: Conidia produced by synnemata; K: Conidium produced by mononematous anamorph; L: Synnema in 3% KOH; M: Marginal hyphae of synnema; N–O: Conidiophores/phialides of synnema; P: Mononematous anamorph; Q–S: Colonies after 2 wks at 25□ on PDA. (T–U) Colony reverse after 2 wks at 25□ on PDA. Scale bars: A–C, I=500µm; D=200µm; E, G, H, J, K, O=20µm; F, M, N, P=50µm; R–U=10mm.

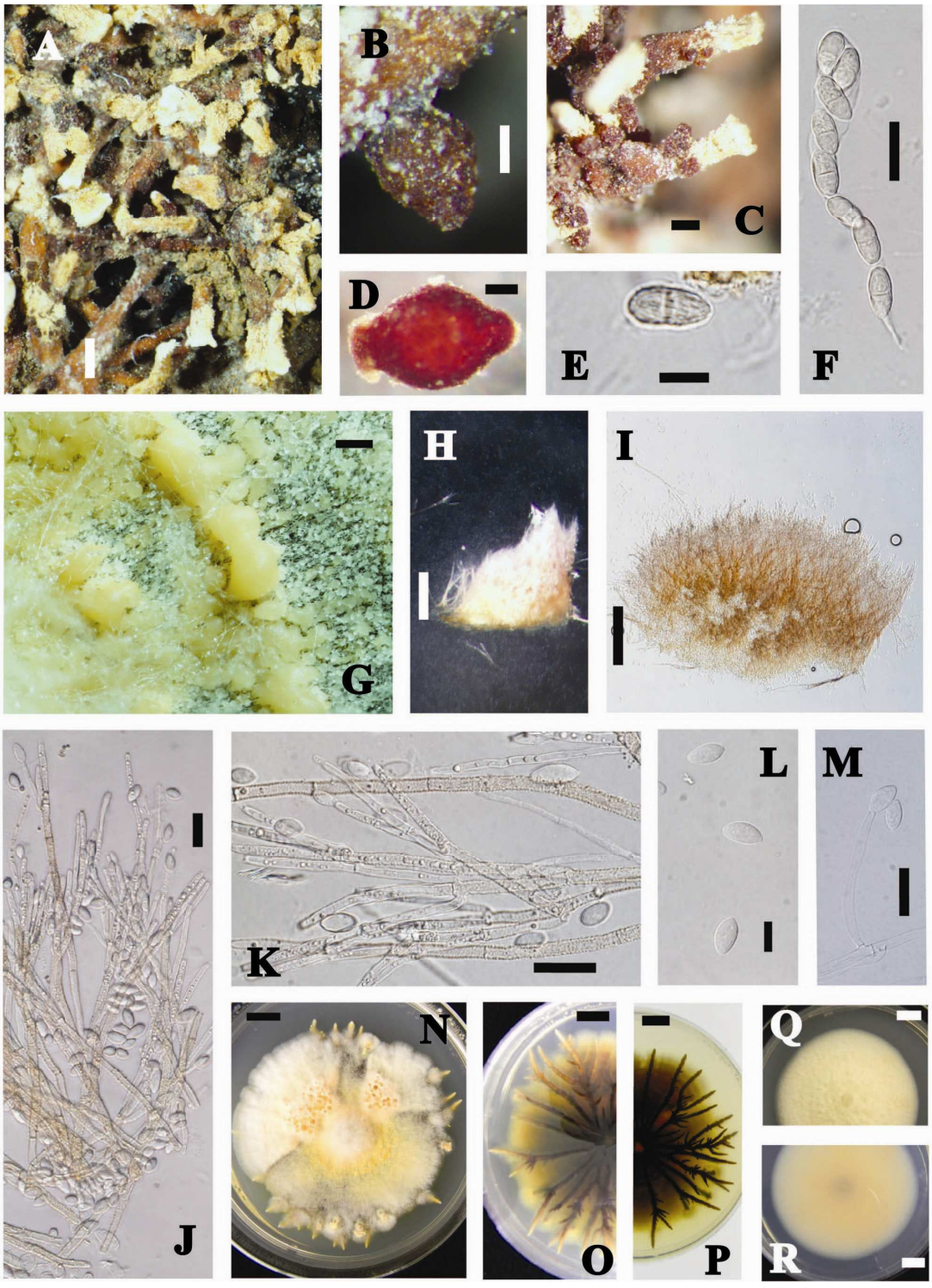
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Fig. 2.7. ***Corallomycetella repens.*** A: Perithecia/synnemata on natural substrata; B: Perithecia on natural substrata; C: Synnemata on natural substrata; D: Perithecium in 3% KOH; E: Ascospore (surface view); F: Ascus; G: Conidial masses; H, I: Synnema in 3% KOH; J–K: Conidiophores/phialides of synnema; L: Conidia produced by synnema; M: Mononematous anamorph; N: Colony after 2 wks at 25□ on PDA; O, P: Colony reverse; Q: Colony of sterile isolate after 2 wks at 25□ on PDA; R: Colony reverse of sterile isolate. Scale bars: A, G: 500µm; B, D: 100µm; C, H, I: 200µm; E, L: 10µm; F, J, K: 20µm; N–R: 10mm.

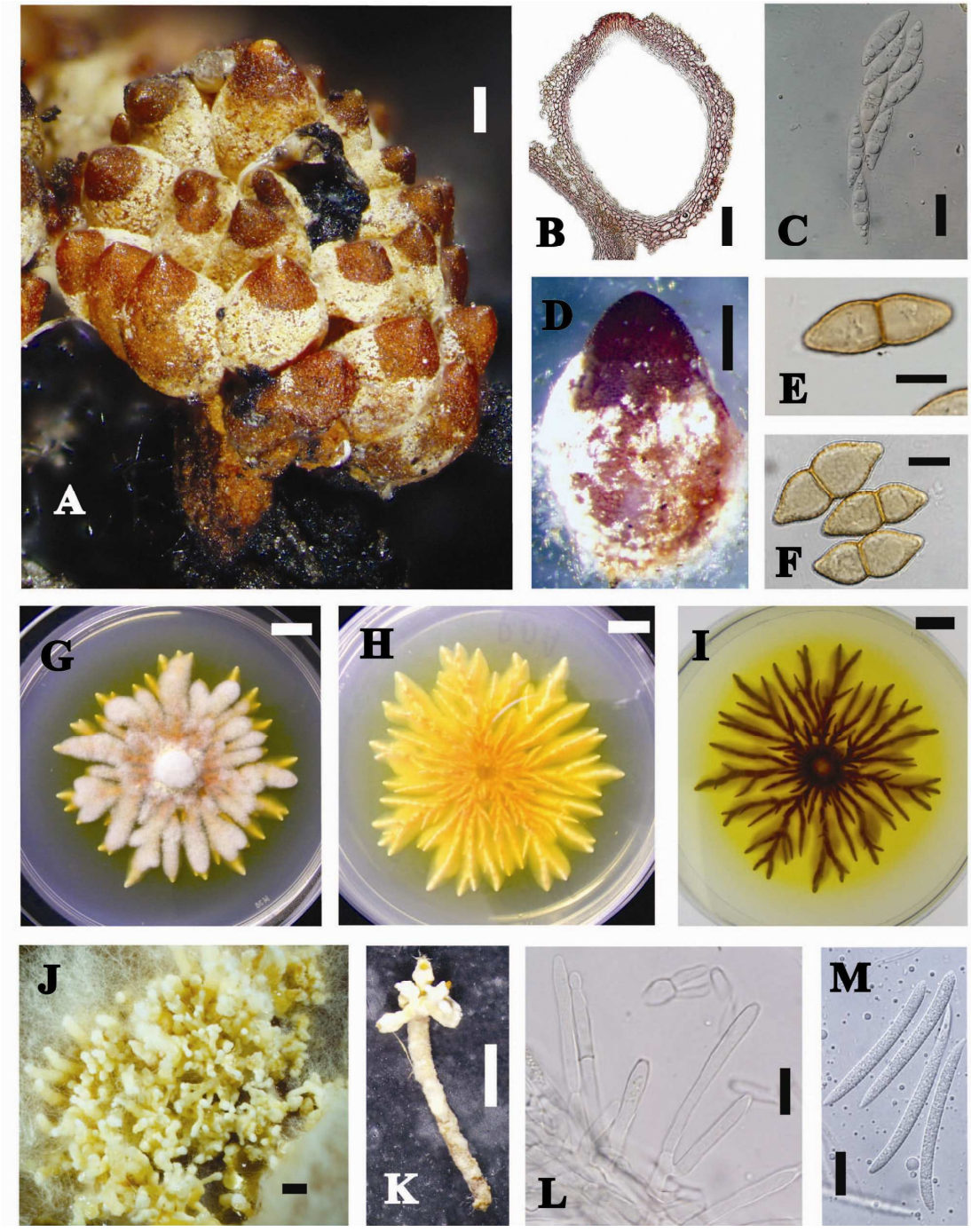
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Fig. 2.8. ***Corallonectria jatrophae.*** A: Perithecia on natural substrata; B: Median section of perithecium; C: Ascus; D: Perithecium in 3% KOH; E: Ascospores (optical section); F: Ascospore (surface view); G: Colony after 2 wks at 25□ on PDA; H, I: Colony reverse; J: Synnemata after 2 wks at 25□ on PDA; K: Synnema after 8 wks at 25□ on PDA; L: Phialides of synnema; M: Conidia produced by synnema. Scale bars: A, D: 200μm; B: 100μm; C: 20μm; E, F, L, M: 10μm; G–I: 10mm; J: 700μm; K: 1,000μm.

Chapter 3: Phylogenetic and taxonomic revision of the *Cosmospora viliuscula* species complex: *Cosmospora* species that grow on xylariaceous fungi.

ABSTRACT

The genus *Cosmospora* includes nectroid fungi that grow on polypores and xylariaceous fungi. The species growing on xylariaceous fungi have been known previously as '*Cosmospora vilior*,' although recently these fungi have been referred to as *Cosmospora viliuscula.* In this paper, the phylogenetic relationships and taxonomy of *C. viliuscula* are revised. A phylogeny was generated with maximum likelihood and Bayesian inference methods based on a three-partition dataset (ITS, LSU, and *mcm7-rpb1-tub2*). We demonstrate that *Cosmospora viliuscula* represents a species complex of which each well-supported clade is regarded as species. A few rare species were also described, namely *C. fomiticola*, *C. novazelandica*, and *C. stilbohypoxyli*. For the first time the sexual morphs of *C. arxii* and *C. khandalensis* are described.

INTRODUCTION

The generic concept of *Cosmospora* Rabenh. (Nectriaceae, Hypocreales, Sordariomycetes, Ascomycota) was based on sexual state morphology (Rossman et al. 1999; also referred to as *Cosmospora* sensu Rossman). It included nectria-like species with small, reddish, KOH+, smooth, thin-walled, laterally collapsing when

dry, non- or weakly stromatic perithecia (Samuels et al. 1991, Rossman et al. 1999). Asexual states of *Cosmospora* sensu Rossman included fusarium-like, acremonium-like, *Chaetopsina* Rambelli, *Stilbella* Lindau, and *Volutella* Fr. (Rossman et al. 1999). *Cosmospora* species exhibit greater diversity in warm temperate and tropical regions, and have a fungicolous, insecticolous, rarely lignicolous, corticolous, and herbicolous habits (Samuels et al. 1991, Rossman et al. 1999). Given its range of asexual states and ecological habits, the genus was considered polyphyletic, and has been split into monophyletic groups based on asexual characteristics: *Chaetopsina* Rambelli, *Nectricladiella* Crous & Schoch, *Fusicolla* Bonord., *Macroconia* (Wollenw.) Gräfenhan et al., *Microcera* Desm., *Pseudocosmospora* C. Herrera & P. Chaverri,, *Stylonectria* Höhn. and *Volutella* Fr. (Schoch et al. 2000, Gräfenhan et al. 2011, Luo and Zhang 2010, 2012, Herrera et al. 2013). Herrera et al. (2013) presented a detail history of *Cosmospora* sensu Rossman.

The name *Cosmospora vilior* (Starbäck) Rossman & Samuels (≡ *Nectria vilior*), a common tropical species of nectria-like fungi, was applied to collections growing on stromata of Xylariaceae (Xylariales, Sordariomycetes, Ascomycota). However, it was recognized early on that the type specimen of that name did not occur on a xylariaceous fungus (e.g. Samuels et al. 1990). It has since been demonstrated that the name "*C. vilior"* was misapplied to *Cosmospora* occurring on xylariaceous fungi (see Herrera et al. 2013). Herrera et al. (2013) referred to the group of fungi occurring on xylariaceous fungi as the *Cosmospora viliuscula* (Samuels, Yoshim. Doi & Rogerson) Rossman & Samuels species complex because its type specimen grew on a xylariaceous fungus (*Kretzschmaria* cf. *deusta* (Hoffm.) P.M.D. Martin). In addition,

Samuels et al. (1990) diagnosed *Cosmospora viliuscula* (as *N. viliuscula*) as being morphologically similar to '*Nectria*' *vilior* but having slightly smaller ascospores. *Cosmospora vilior* has been transferred to the newly described genus *Pseudocosmospora* C. Herrera & P. Chaverri, whose species occur mainly on *Eutypa* Tul. & C. Tul. and *Eutypella* (Nitschke) Sacc. species (Diatrypaceae, Xylariales, Sordariomycetes, Ascomycota; Herrera et al. 2013).

The present taxonomic treatment deals with the *Cosmospora viliuscula* species complex, which up to now, had been thought to be a single species. We describe many seven new species in this complex based on multi locus phylogenetic analyses, and report an apparent host specificity by the recognized species within this complex. Results from this study show once again the prevalence of morphologically similar species complexes in the Hypocreales and the utility of molecular methods for defining cryptic species.

MATERIALS AND METHODS

Herbarium specimens and cultures

Fresh specimens of *Cosmospora* spp. were collected on trips to Argentina, Brazil, Costa Rica, Peru, and USA. Pure cultures were obtained by isolating single ascospores from the freshly collected samples and grown in cornmeal dextrose agar (CMD; Difco™ cornmeal agar + 2% w/v dextrose + antibiotics). Dried specimens were deposited at the U.S. National Fungus Collections (BPI), Beltsville, Maryland, USA, and the cultures derived from the fresh specimens were deposited at

Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. Accession numbers are listed in Table 3.1.

Herbarium specimens of *Cosmospora* spp. were borrowed from the U.S. National Fungus Collections (BPI); Royal Botanic Gardens Kew (K); William and Lynda Steere Herbarium, New York Botanical Garden (NY); New Zealand National Fungal Herbarium (PDD); and Herbarium of the Botany Department, Swedish Museum of National History (S). Extant cultures corresponding to these herbarium specimens were obtained from the culture collection at USDA, ARS, Systematic Mycology and Microbiology Laboratory (SMML) and CABI Bioscience Fungus Collection (formerly International Mycological Institute, IMI). Additional cultures were obtained from CBS and Ministry of Agriculture, Forestry and Fisheries (MAFF) culture collection (see Table 3.1).

Morphological characterization

Sexual state morphology was observed using a stereoscope (Olympus SZX12 Olympus, Tokyo, Japan). The features of the perithecia were characterized (e.g. color, shape, size, ornamentation, and habit). To observe their internal structures, the perithecia were rehydrated in 3% KOH, and the centrum isolated on a glass slide and covered with a coverslip. Microscopic characters such as asci and ascospores were observed under a compound microscope (Olympus BX50; Olympus, Tokyo, Japan).

Colony morphology was characterized in two different growing media: cornmeal dextrose agar (CMD; Difco™ cornmeal agar + 2% w/v dextrose) and Difco™ potato dextrose agar (PDA). Two replicates with two pseudoreplicates were grown for each

isolate in each growing medium. Cultures were grown in an incubator that alternates between fluorescent light and darkness (12h/12h) at 25°C. Colony growth rate was measured weekly for two weeks. Colony color is described using the terminology in Rayner (1970).

Asexual state morphology (in culture) was characterized in synthetic nutrient-poor agar (SNA; Nirenberg 1976). Isolates were grown under the conditions described above. A block of agar was cut, placed on a microscope slide, covered with a coverslip, and examined by light microscopy (Olympus BX50; Olympus, Tokyo, Japan).

Measurements of continuous characters, such as length and width, were made with Scion Image software beta 4.0.2 (Scion Corp., Frederick, Maryland), and were summarized by descriptive statistics, e.g., minimum, maximum, mean and standard deviation.

DNA extraction, PCR, and sequencing

DNA extraction was performed as described in Hirooka et al. (2010). Briefly, *Cosmospora* isolates were grown in Difco™ potato dextrose broth, and the mycelial mat was harvested after one week of growth for DNA extraction. DNA was extracted with PowerPlant® DNA Isolation Kit (MO BIO Laboratories Inc., Solana Beach, California). Six partial loci were amplified and sequenced. These loci comprised two ribosomal DNA regions and four protein-coding regions. These loci and the primers used to amplify them are listed in Table 3.2. PCR reaction mixtures were prepared as described in Herrera et al. (2013). PCR amplifications were carried out in an

Eppendorf Mastercycler thermocycler (Eppendorf, Westbury, New York) under the cycle conditions listed in Table 3.2. PCR products were cleaned with ExoSAP-IT® (USB Corp., Cleveland, Ohio) and the cleaned products were sequenced at the DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland) or McLAB DNA sequencing services (San Francisco, California). Sequences were assembled and edited with Sequencher 4.9 (Gene Codes, Madison, Wisconsin). Sequences were deposited in GenBank (accession numbers are listed in Table 3.1).

Phylogenetic analyses

Multiple sequence alignment for each locus was performed via the MAFFT v.6 web service (http://mafft.cbrc.jp/alignment/server/; Katoh et al. 2002, 2013) with the E-INS-i alignment strategy and the 1PAM / κ=2 scoring matrix for nucleotide sequences. Alignments were manually edited in in Mesquite 2.75 (Maddison and Maddison 2011). Alignments were deposited in TreeBase under accession number XXXXX.

PartitionFinder (Lanfear et al. 2012) was used with default settings to find the best-fit partitioning scheme among the sequenced loci. CONCATEPILLAR 1.4 (Leigh et al. 2008) was used to determine which partitions could be concatenated and analyzed to estimate a phylogeny. Partitions were concatenated if the p-value was greater than the estimated α-level correction of 0.025, which indicated that the null hypothesis, i.e., congruence of loci, could not be rejected.

JModeltest (Guindon and Gascuel 2003; Darriba et al. 2012) was used to infer the model of nucleotide substitution for each locus with default settings (11 substitution schemes, +F, +I, +G). The base tree for likelihood calculations was ML optimized. Once likelihood scores were calculated, the best model for each partition was selected according to the Akaike Information Criterion (AIC).

The phylogeny for *Cosmospora* was estimated with maximum likelihood and Bayesian methods. Maximum likelihood (ML) analyses were performed with GARLI v2.0 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl 2006) via the GARLI web service (http://www.molecularevolution.org; Bazinet and Cummings 2011), which uses a grid computing system associated with The Lattice Project (Cummings and Huskamp 2005; Bazinet and Cummings 2008). Fifty independent search replicates were performed to find the best tree with a fast ML stepwise-addition algorithm. Two thousand bootstrap replicates were used in the bootstrap analysis. Bayesian analyses were performed in MrBayes v3.2.2 (Ronquist et al. 2012). A majority rule consensus tree was generated by running four chains for 10,000,000 Markov Chain Montecarlo generations sampling trees every 100th generation, and discarding the first 25% of the sampled trees as burn-in. Tracer version 1.5 (Rambaut and Drummond 2007) was used to confirm whether the negative log likelihoods had reached convergence.

Species Recognition

Species were recognized in *Cosmospora* based on the modified criterion used by Pringle et al. (2005) of the genealogical concordance phylogenetic species

recognition (GCPSR; Taylor et al. 2000). According to the criterion used by Pringle et al. (2005) of the GCPSR, a putative species is recognized if a clade is well supported in the majority of single gene trees. Single gene trees were generated with MrBayes v3.2.2 as described above, except that only 5,000,000 Markov Chain Montecarlo generations were run.

Morphological species recognition and ecological species recognition were used to support the species inferences made when applying GCPSR. Thus, a clade, or putative species, may be associated with unique morphological and/or ecological traits that set it apart from other closely related species.

RESULTS

Phylogenetic analyses

PartitionFinder determined four partitions: ITS, LSU, *mcm7+rpb1+tub2*, and *tef1*. The null hypothesis that these partitions were congruent was tested in CONCATEPILLAR, which rejected this null hypothesis ($P < 0.025$). In a separate analysis, the partitions ITS, LSU, and *mcm7+rpb1+tub2* were found to be congruent ($P = 0.043$; i.e., we failed to reject the null hypothesis that these loci were congruent), and were concatenated. *Tef1* was analyzed separately (Supplemental Figure 3.5). The ITS, LSU, and *mcm7+rpb1+tub2* concatenated matrix comprised of 3327 total characters; 681 characters were parsimony informative; 254 characters were parsimony uninformative; and 1991 characters were invariable. Table 3.2 lists information for each individual locus.

The topologies of the generated phylogenetic trees for ML and BI were not congruent (Figures 3.1, 3.2). The negative log likelihoods for the phylogenetic trees were −18580.293 and −18297.8348, respectively. The topologies differed primarily in deep node relationships of the genus *Cosmospora* (node labeled "A" in Figures 3.1 and 3.2; 74% ML BP; 100% BI PP). Basal relationships were unresolved in the Bayesian tree with a polytomy that includes the following lineages: *Cosmospora fomiticola* (described below), *C. viridescens*, and the *Cosmospora viliuscula* species complex (node labeled "B" in Figures 3.1 and 3.2). In addition, basal nodes of the Bayesian tree were unsupported (<70% BI PP). In the ML tree, the topology was resolved, although basal nodes were poorly supported (<70% ML BP). Basal species such as *C. coccinea* and *C. fomiticola* occur on polypores (Polyporaceae, Basidiomycota), while C. viridescens occurs on various ascomycetous and other substrates.

All lineages within the *Cosmospora viliuscula* species complex corresponding to recognized species received high ML BP and BI PP support (with one exception; Figures 3.1 and 3.2; Table 3.3). At least two genes supported the recognized species (Supplemental Figures S3.1–S3.6; Table 3.3). Clades within this complex correlate with specific fungal hosts belonging to the Xylariaceae (Ascomycota).

Morphological studies

Little morphological variation exists among species of the *Cosmospora viliuscula* complex. The confidence intervals of character means (e.g. ascospore length, width, ratio of length and width; conidia length, width, ratio of length and width; colony growth) generally overlapped among species in the complex with some exceptions.

The ascospores of *Cosmospora novazelandica* (described below) were longer and wider compared to other species in this complex. The conidia length of *Cosmospora arxii* and *C. stilbohypoxyli* (described below) were not different, but had longer conidia compared to other species in the complex. *Cosmospora stilbohypoxyli* had wider conidia. Full pairwise comparisons are presented in Supplemental Table 3.1.

DISCUSSION

Cosmospora Rabenh.

The generic concept of *Cosmospora* is based on its type *Cosmospora coccinea* Rabenh., which was lectotypified by Rossman et al. (1999). Its asexual state was described as *Verticillium olivaceum* W. Gams, although it is unrelated to *Verticillium* Nees in the strict sense. Morphologically the asexual state of *C. coccinea* varies from acremonium-like (single phialide, unbranched) to verticillium-like (branching into 1–3 phialides). The asexual state of some species of *Cosmospora* produce secondary branches, ultimately terminating in 1–3 phialides. The asexual state is the character that unites species in *Cosmospora* sensu stricto (Gräfenhan et al. 2011). Herrera et al. (2013) described *Pseudocosmospora* for a cosmospora-like clade that has an acremonium-like asexual state, but is unrelated to *Cosmospora* species. *Pseudocosmospora* species have different hosts, which belong to the Diatrypaceae (Xylariales, Ascomycota). The reported fungal hosts of *Cosmospora* species belong to two unrelated fungal families: Xylariaceae (Ascomycota) and Polyporaceae (Basidiomycota).

The genus *Cosmospora* sensu stricto is monophyletic and well supported (node

labeled A in Figs. 3.1 and 3.2; 74% ML BP; 100% BI PP). However, basal relationships are poorly resolved. In the ML tree, basal nodes have low support values (<70% ML BP), and in the Bayesian tree, a basal polytomy is present. *Cosmospora* is likely to have undergone a putative early fast radiation given this basal phylogenetic structure. Similar deep node relationships have been observed in other organisms that have undergone a fast radiation (e.g., birds, Pons et al. 2005; fishes, Chen et al. 2003; plants, Fishben and Soltis 2004; snakes, Kelly et al. 2009; among others). Reconstructing basal relationships in systems undergoing early radiations is difficult because phylogenetic signal is often masked by multiple hits, which increase with time (reviewed in Donoghue and Sanderson 1992). Phylogenetic resolution can be accomplished by increasing the number of characters and/or by doing a more exhaustive sampling of taxa (reviewed in Hillis and Wiens 2000).

Species recognition

Species were recognized as described in Herrera et al. (2013), except that we follow the GCPSR criterion used by Pringle et al. (2005). All clades with high support (>95% BI PP) in the majority of single gene trees were recognized as species (Supplemental Figures S3.1–S3.6). Further support for recognizing these clades as species is their apparent host specificity, and in some instances, these recognized species exhibit unique morphological characters that differentiate them. Species such as *Cosmospora viliuscula* are well supported (100% ML BP; 100% BI PP), produce a penicillate asexual state, and have *Kretzschmaria* cf. *deusta* as its host. This host differs from *Kretzschmaria deusta* in that it occurs in tropical regions, while the native range of *K. deusta* is in temperate regions (Rogers & Ju 1998). One species of

Cosmospora, *C. ustilinae*, occurs on *K. deusta* in temperate regions, and is highly supported as well (100% ML BP; 100% BI PP). *Cosmospora scruposae* produces long phialides, occurs on *Xylaria scruposa*, and is highly supported (100% ML BP; 100% BI PP). Similarly, *C. arxii*, *C. clavi*, and *C. micropedis* occur on diverse hosts (*Hypoxylon fragiforme*/*H. howeanum*, *Kretzschmaria clavus*/*K. sp*., and *K. micropus*/*H. cyclopicum*, respectively), and have maximum support. Species on each host set are probably closely related, but have not been treated phylogenetically. Identification of the hosts for *C. khandalensis* could not be determined to species rank. A more holistic approach, i.e. studying both parasite and host together, is needed as suggested by Herrera et al. (2013).

We also recognize some singletons as species based on the rule of rarity (reviewed in Lim et al. 2012) given the apparent high fidelity of *Cosmospora* species to their hosts. *Cosmospora fomiticola*, *C. novazelandica*, and *C. stilbohypoxyli* occur on *Fomes fomentarius* (Polyporaceae, Basidiomycota), *Annulohypoxylon bovei* (Xylariaceae, Ascomycota), and *Stilbohypoxylon quisquiliarum* (Xylariaceae, Ascomycota), respectively. With extensive searching in herbaria, we were able to identify additional specimens with the same hosts and similar morphology as these *Cosmospora* species. However, no extant cultures are associated with these specimens, and thus, DNA could not be extracted. These species have been poorly sampled due to lack of experts in their regions of origin. Apart from the unique ecological roles of these fungi, these species bear morphological differences that set them apart from other recognized species. For example, *C. novazelandica* has cells that protrude from the apex of the perithecia, which results in a roughened appearance, and *C. stilbohypoxyli* has

extremely slow growing colonies in PDA. Additionally, in the phylogenetic tree these species have relatively long branches, which suggest that there have been multiple substitutions per site since their speciation.

TAXONOMY

Cosmospora Rabenh., Hedwigia 2: 59. 1862.

Generic type: *Cosmospora coccinea* Rabenh., Fungi europ. exsicc.: no. 459. 1862.

Habitat: On polypores (e.g., *Fomes* (Fr.) Fr., *Fomitopsis* P. Karst., *Inonotus* P. Karst., and *Stereum* Hill ex Pers.), xylariaceous fungi (e.g., *Annulohypoxylon* Y.M. Ju, J.D. Rogers & H.M. Hsieh, *Hypoxylon* Bull., *Kretzschmaria* Fr., Stilbohypoxylon Henn., and *Xylaria* Hill ex Schrank); often isolated from soil.

Distribution: Cosmopolitan.

Descriptions and illustrations: Gräfenhan et al. (2011); Rossman et al. (1999)

Notes: The present paper follows the generic concept of *Cosmospora* by Gräfenhan et al. (2011). In Gräfenhan et al. (2011), the genus was restricted to cosmospora-like fungi tending to occur on other fungi, particularly on polypores and xylariaceous fungi, and have an acremonium-like to verticillium-like asexual state. The perithecia are nonstromatic, collapsing laterally, scarlet, and generally less than 300 µm. The ascospores are ellipsoid, minutely verrucose, verrucose, or tuberculate, yellow-brown at maturity, and <10 µm. On PDA, colonies are velvety, slightly flocculose, olivaceous, and on CMD, colonies are dark-green, flat.

Key to species of Cosmospora *sensu stricto*

1. On bone and *Ruzenia spermoides* (Lasiosphaeriaceae)…….....…..*C. viridescens*

1. On Polyporaceae and Xylariaceae (excluding *Biscogniauxia* spp.) …….2

2. On Polyporaceae……3

2. On Xylariaceae……...4

3. On *Inonotus* spp.; ascospores tuberculate…….*C. coccinea*

3. On *Fomes fomentarius*; ascospores verrucose……*C. fomiticola*

4. On *Hypoxylon fragiforme* and *H. howeanum*……..*C. arxii*

4. On other xylariaceous fungi……...5

5. On *Annulohypoxylon* spp……...6

5. On other xylariaceous fungi……...8

6. On *Annulohypoxylon bovei*; ascospore length mean =10 μm……*C. novazelandica*

6. On *Annulohypoxylon* spp.; ascospore length mean <10 μm……..7

7. On *Annulohypoxylon cohaerens* and *A. multiforme*; ascospore length mean =7.5 μm; on CMD, colony amber……..…….*C. annulohypoxyli*

7. On an *Annulohypoxylon* sp.; ascospore length mean = 6.8 μm; on CMD, colony dark-green……...*C. khandalensis*

8. On *Kretzschmaria* spp……...9

8. On other xylariaceous fungi…….13

9. On ustulinoid fungi……..10

9. On kretzschmariod fungi……..11

10. On *Kretzschmaria deusta* (temperate forest); asexual state simple; ascospore length mean =7.0 µm; conidia length mean =4.7 µm……...*C. ustulinae*

10. On *Kretzschmaria* cf. *deusta* (tropical forest); asexual state penicillate; ascospore length mean =7.8 µm; conidia length mean =4.0 µm.…….*C. viliuscula*

11. On *Kretzschmaria cetrarioides*; ascospore length mean >10 µm……..……..*C. rickii*

11. On other kretzschmariod fungi with ascospore length mean < 10 µm……..……...…12

12. On *Kretzschmaria clavus* and *K.* cf. *pavimentosa*; ascospores 7.5 × 3.9 µm (mean); conidia 4.4 × 2.1 µm (mean).…….*C. clavi*

12. On *Kretzschmaria micropus* and *Hypoxylon cyclopicum*; ascospores 6.9 × 3.7 µm (mean); conidia 3.7 × 1.8 µm (mean).……...*C. micropedis*

13. On *Stilbohypoxylon quisquiliarum*; conidia 5.9 × 2.8 µm (mean); on CMD, 9.5 mm diam (mean)……..…….*C. stilbohypoxyli*

13. On *Xylaria scruposa*; conidia 4.8 × 2.0 µm (mean); on CMD, 49.5 mm diam (mean)…….*C. scruposae*

Cosmospora annulohypoxyli C. Herrera & P. Chaverri, **sp. nov**. Fig. 3.3.

Mycobank MB XXXXXX

Holotype: **USA**, Louisiana, East Baton Rouge Parish, Port Hudson, Port Hudson State Commemorative Area, on *Annulohypoxylon* cf. *cohaerens*, on bark of unidentified tree, 19 Aug. 1996, *G.J. Samuels*, *M. Blackwell* & *M. Camara*, BPI 744521, ex-

holotype culture G.J.S. 96-186 = CBS XXXXXX.

Etymology: In reference to the fungal host, *Annulohypoxylon* species.

Sexual state: Perithecia solitary, sometimes in groups of a few (< five), superficial, subglobose with a blunt apex, collapsing laterally, scarlet, smooth, 207–274 × 181–232 μm (mean = 232.6 × 198.4; SD 21.2, 15.2; n = 10). Asci cylindrical, eight-spored, uniseriately arranged, 50–68 × 4.0–5.7 μm (mean = 59.0 × 5.0; SD 4.9, 0.6; n = 20). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, minutely verrucose, yellow-brown, 6.5–8.9 × 3.2–4.2 μm (mean = 7.5 × 3.6; SD 0.5, 0.2; n = 60).

Culture and asexual state: On PDA colonies 30–39 mm diam (mean = 35.1; SD 2.8; n = 7) after 14 d at 25°C, velvety, slightly floccose, olivaceous, reverse amber. On CMD colonies 40–46 mm diam (mean = 42.7; SD 2.4; n = 7) after 14 d at 25°C, flat, amber, with aerial mycelium sparsely spread, reverse concolorous. Sporulation on SNA abundant, arising directly from agar surface. Asexual state acremonium-like; conidiophores simple, unbranched, or dichotomously branched. Phialides monophialidic, cylindrical, collarette flared, hyaline, length (26–)36–68 μm (mean = 50.4; SD 8.5; n = 20), width at base 1.8–2.6 μm (mean = 2.2; SD 0.3; n = 20), width at tip 0.8–1.4 μm (mean = 1.1; SD 0.2; n = 20). Conidia ovoid to ellipsoidal, unicellular, smooth, hyaline, 3.0–5.4 × 1.6–2.7 μm (mean = 4.0 × 2.0; SD 0.5, 0.2; n = 60).

Habitat: Fungicolous on *Annulohypoxylon cohaerens* and *A. multiforme*

(Xylariaceae), on unidentified bark.

Distribution: United States (Indiana, Louisiana).

Additional specimens and isolates examined: **USA**, Indiana, Yellow Wood State Forest, Brown Co., alt. 200 m., 39°09’ N, 86°06’ W, Jackson Creek Management Trail, on *Annulohypoxylon* cf. *multiforme,* on bark of unidentified tree, 30 Sep. 1995, *G.J. Samuels*, BPI 737773, culture G.J.S. 95-199 = CBS XXXXXX.

Notes: This species is only known to grow on *Annulohypoxylon cohaerens and A. multiforme*. *Cosmospora novazelandica* and *C. khandalensis* also occur on *Annulohypoxylon* species, but have different ascospore lengths. *Cosmospora novazelandica* has longer ascospores (mean = 10 µm) than C. annulohypoxyli (mean = 7.5 µm), and *C. khandalensis* have slightly shorter ascospores (mean = 6.8 µm).

Cosmospora arxii (W. Gams) Gräfenhan & Schroers, Studies in Mycology 68: 95. 2011. Fig. 3.4.

Basionym: *Acremonium arxii* W. Gams, Cephalosporium-artige Schimmelpilze (Stuttgart) p. 123. 1971.

Asexual state: acremonium-like.

Habitat: Fungicolous on *Hypoxylon fragiforme* (Pers.) J. Kickx f. and *H. howeanum* Peck (Xylariaceae).

Distribution: France, Germany, and USA (KY, NY).

Sexual state: Perithecia solitary, superficial, globose with a blunt apex, collapsing

laterally, scarlet, smooth, 172–226 × 162–209 µm (mean = 206.2 × 186.8; SD 19.4, 18.7; n = 8). Asci cylindrical, eight-spored, uniseriately arranged 55.7–68.0 × 4.5–5.9 µm (mean = 63.1 × 5.2; SD 3.7, 0.4; n = 17). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, minutely verrucose, yellow-brown, 6.4–8.6(–9.9) × 3.0–4.2 µm (mean = 7.6 × 3.5; SD 0.7, 0.3; n = 40).

Culture and asexual state: On PDA colonies 16–23 mm diam (mean = 20.1; SD 2.1; n = 8) after 14 d at 25°C, velvety, slightly floccose, pale salmon-pink, reverse concolorous. On CMD colonies 23–31 mm diam (mean = 27.1; SD 3.0; n = 8) after 14 d at 25°C, flat, hyaline, with no aerial mycelium, reverse concolorous. Sporulation on SNA abundant, arising directly from agar surface. Asexual state acremonium-like; conidiophores simple, unbranched, or dichotomously branched. Phialides monophialidic, cylindrical, collarette flared, hyaline, length 42–68 µm (mean = 52.1; SD 7.7; n = 20), width at base 1.6–2.3 µm (mean = 2.0; SD 0.2; n = 20), width at tip 0.9–1.3 µm (mean = 1.2; SD 0.1; n = 20). Conidia ellipsoidal, unicellular, smooth, hyaline, 4.0–8.4 × 1.5–2.4 µm (mean = 5.4 × 2.0; SD 1.0, 0.2; n = 60).

Specimens and isolates examined: **France**, Foret de I'Hermitage, on *Hypoxylon howeanum* (originally identified as *H. fuscum*), on *Prunus spinosa*, 17 Apr. 2008, *A. Gminder*, BPI 879925, culture A.R. 4521 = CBS XXXXXX. **USA**, Kentucky, Clermont, Bernhein Arbor & Research Forest, Jackson-Yoe Loop, 37º54.3225'N, 85º38.4079'W, on *Hypoxylon fragiforme*, on *Fagus grandifolia*, 27 Jun. 2010, C. Herrera (C.H. 10-05), BPI XXXXXX, culture G.J.S. 10-247 = CBS XXXXXX.

Additional descriptions and illustrations: Gams (1971), Gräfenhan et al. (2011).

Notes: Identity of these isolates was confirmed by sequencing RNA polymerase II 2nd largest subunit (RPB2; A.R. 4521: JQ014128; G.J.S. 10-247: JQ014118) and comparing the sequences against the sequence of the ex-type culture, CBS 748.69 (HQ897725 retrieved from GenBank). CBS 748.69 was 99% similar to G.J.S. 10-247 and 97% similar to A.R. 4521. Unique to this species is its fungal hosts (*Hypoxylon fragiforme* or *H. howeanum*) and its salmon-pink colony on PDA.

Cosmospora clavi C. Herrera & P. Chaverri, **sp. nov**. Fig. 3.5.

Mycobank MB XXXXXX

Holotype: Costa Rica, Heredia, Puerto Viejo de Sarapiquí, La Selva Biological Station, Sendero Tres Rios and Camino Experimental Norte, N 10°26'7.3", W 84°00'31.4", elev. 600 m, on *Kretzschmaria clavus*, 17 Mar. 2010, *P. Chaverri* (P.C. 1167), *G.J. Samuels*, *A.Y. Rossman*, *C. Salgado* & *C. Herrera*, **holotype** BPI XXXXXX, ex-holotype culture G.J.S. 10-112 = CBS XXXXXX.

Etymology: Referring to the fungal host of the type specimen, *Kretzschmaria clavus.*

Sexual state: Perithecia solitary, superficial, globose with blunt apex to obpyriform, collapsing laterally, scarlet, smooth 181– 258 × 135–245μm (mean = 214.3 × 179.1; SD 27.8, 30.5; n = 15). Asci cylindrical, eight-spored, uniseriately arranged, 49–67 × 5–6 μm (mean = 56.3 × 5.2; SD 3.9, 0.4; n = 30). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, verrucose, yellow-brown, 6.1–9.1 × 3.2–4.7 μm (mean = 7.5 × 3.9; SD 0.6, 0.3; n = 90).

Culture and asexual state: On PDA colonies (32–)37–53 mm diam (mean = 43.7; SD 6.5; n = 15) after 14 d at 25°C, velvety, floccose, greenish-olivaceous or pale-yellow,

with a sienna pigment diffusing in medium, reverse umber. On CMD colonies 50–61 mm diam (mean = 54.4; SD 3.4; n = 16) after 14 d at 25°C, flat, dark-green, becoming greenish-yellow at edge, reverse concolorous. Sporulation on SNA abundant, arising directly from agar surface. Asexual state acremonium-like; conidiophores simple, unbranched, or dichotomously branched, rarely with three phialides. Phialides monophialidic, cylindrical, collarette flared, hyaline, length 21–68 µm (mean = 47; SD 11.2; n = 49), width at base 1.5–2.7 µm (mean = 2.1; SD 0.3; n = 49), width at tip 0.9–1.4 µm (mean = 1.1; SD 0.1; n = 39). Conidia rarely ovoid, ellipsoidal to reniform, unicellular, smooth, hyaline, 3.5–6.6 × 1.5–2.6 µm (mean = 4.4 × 2.1; SD 0.5, 0.2; n = 150).

Habitat: Fungicolous on *Kretzschmaria clavus* and *K.* cf. *pavimentosa* (Xylariaceae), on unidentified bark.

Distribution: Possibly pan Neotropical, known from Brazil, Costa Rica, Puerto Rico and Venezuela.

Additional specimens and isolates examined: **Brazil**, State of Amazonas, Pico Rondon, Perimetral Norte Road, km 211, vine forest ca. 3 hr. walk from FUNAI, 01º31'N, 62º48'W, on *Kretzschmaria clavus*, on bark of unidentified tree, 24–26 Mar. 1984, *G.J. Samuels* (1076), *J. Pipoly* & *W. Rodrigues*, NY, culture G.J.S. 84-290 = CBS XXXXXX. **Puerto Rico**, Caribbean Nat. Forest, Luquillo Mts., El Verde Research Area, along La Prieta Creek, elev. 350–400 m., on *Kretzschmaria* cf. *pavimentosa*, 20 Feb. 1996, *G.J. Samuels* (8047) & *H.-J. Schroers*, BPI 744678, culture G.J.S. 96-7 = CBS XXXXXX. Cordillera Central, Chario Azul, off Rte.184,

elev. 550 m, on *Kretzschmaria* cf. *pavimentosa*, 25 Feb. 1996, *G.J. Samuels* (8106), *H.-J. Schroers & D.J. Lodge*, BPI 745249, culture G.J.S. 96-48 = CBS 448.96. **Venezuela**, Edo. Bolivar, on road between El Dorado and Sta. Elena, ca 118 km S of El Dorado, trail up N-facing slope of Uei-Tepui, from old military camp 'Ciento Veinticinco', on *Kretzschmaria clavus*, on bark of unidentified tree, 5 Aug 1972, *K.P. Dumont, R.F. Cain, G.J.Samuels & C. Blanco,* Dumont-VE 6918 (NY). Distrito Federal, on the road between El Portachuelo and Chichiriviche, ca 7 km south of Chichiriviche, on *Kretzschmaria clavus*, on bark of unidentified tree, 30 July 1972, *K.P. Dumont, R.F. Cain, G.J. Samuels, B. Manara*, Dumont-VE 6706 (NY).

Notes: This *Cosmospora* species grows on *Kretzschmaria clavus* and on a *Kretzschmaria* species with an ustulinoid shape.

Cosmospora coccinea Rabenh., Fungi europ. exsicc.: no. 459. 1862. Fig. 3.6.

= *Nectria cosmariospora* Ces. & De Not., Comm. Soc. Crittog. Ital. 1(4): 195. 1863.

≡ *Dialonectria cosmariospora* (Ces. & De Not.) Cooke, Grevillea 12(no. 64): 110. 1884.

= *Verticillium olivaceum* W. Gams, Cephalosporium-artige Schimmelpilze (Stuttgart) p. 123. 1971.

Asexual state: Acremonium-like.

Habitat: Fungicolous on *Inonotus* spp. on *Fagus* and *Alnus*.

Distribution: Europe.

Specimens and isolates examined: **Germany**, Bavaria, Burghausen-Unterhadermark, on dead crusts of *Inonotus nodulosus*, on fallen branch of *Fagus sylvatica*, 02 Oct.

1993, *R. Boesmiller*, BPI 802729, culture A.R. 2741 = CBS 114050, also A.R. 2743.

Additional descriptions and illustrations: Gams (1971; as *V. olivaceum*), Samuels et al. (1991; as *N. cosmariospora*), Rossman et. al (1999), Gräfenhan et al. (2011).

Notes: *Cosmospora coccinea* was lectotypified by Rossman et al. (1999), and reported to grow on pores of *Inonotus* spp. Its ascospores are tuberculate. *Cosmospora cymosa* is also known to occur on *Inonotus* spp., and is presumed to be sister species with *C. coccinea* (Gräfenhan et al. 2011). However, the sexual state of *C. cymosa* is not known. It is possible that *C. cymosa* is a morphological variation of *C. coccinea*.

Cosmospora fomiticola C. Herrera & P. Chaverri, **sp. nov**. Fig. 3.7.

Mycobank MB XXXXXX

Holotype: **New Zealand**, Westland, Mt Aspiring National Park, Haast Pass, on *Fomes fomentarius*, 12 Apr. 1983, *G.J. Samuels*, *R.E. Beever* & *R.H. Petersen*, PDD 46398, ex-holotype culture G.J.S. 83-194 = CBS XXXXXX.

Etymology: From *fomitis* (latin) = tinder and *-cola* (latin) = growing on; referring to the host of this species, the tinder fungus, a common name for *Fomes fomentarius.*

Sexual state: Perithecia solitary, rarely in groups of a few, superficial, globose to ovoid with an acute apex, collapsing laterally, orange, smooth, 212–304 × 176–272 µm (mean = 240.4 × 201.3; SD 33.2, 35.4; n = 6). Asci cylindrical, eight-spored, uniseriately arranged, 59–85 × 5–8 µm (mean = 73.9 × 6.0; SD 7.7, 1.1; n = 10). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum,

minutely verrucose, yellow-brown, 6.9–9.7 × 2.8–4.1 μm (mean = 7.9 × 3.6; SD 0.6, 0.3; n = 50).

Culture and asexual state: On PDA colonies 24–27.5 mm diam (mean = 25.8; n = 2) after 14 d at 25°C, velvety, slightly floccose, white, with a sienna pigment diffusing in medium, reverse umber. On CMD colonies 12–17 mm diam (mean = 14.5; n = 2) after 14 d at 25°C, flat, olivaceous, with white aerial mycelium towards center, with an olivaceous pigment diffusing into medium, reverse concolorous. Sporulation on SNA abundant, arising directly from agar surface. Asexual state acremonium-like; conidiophores branched with 3–4 phialides. Phialides monophialidic, cylindrical, collarette flared, hyaline, length 19–42 μm (mean = 35; SD 6.3; n = 10), width at base 1.3–2.8 μm (mean = 1.9; SD 0.4; n = 10), width at tip 0.9–1.3 μm (mean = 1.1; SD 0.1; n = 10). Conidia ovoid to ellipsoidal, unicellular, smooth, hyaline, 2.8–4.8 × 1.6–2.4 μm (mean = 3.7 × 2.0; SD 0.5, 0.2; n = 30).

Habitat: Fungicolous on *Fomes fomentarius* (Polyporaceae, Basidiomycota).

Distribution: New Zealand.

Additional specimens examined: **New Zealand**, North Island, Gisborne District, Track to Lake Ruapani (Site 74), on *Fomes* sp., 31 May 1983, *A.Y. Rossman, P. Matsushima, P. Johnston* & *G.J. Samuels*, BPI 745118.

Notes: This monotypic species is the only species known to occur on *Fomes fomentarius*. The shape of the perithecia resembles the shape a lemon with a minute acute apex. Also, the colonies produced by *C. fomiticola* on PDA and CMD are very

distinct. On PDA, the colony is floccose and white, and on CMD, the colony is olivaceous with sporulation occurring towards the center.

Cosmospora khandalensis (Thirum. & Sukapure) Gräfenhan & Seifert, Stud. Mycol. 68: 96. 2011. Fig. 3.8.

Basionym: *Cephalosporium khandalense* Thirum. & Sukapure, Mycologia 58: 359. 1966.

Habitat: Fungicolous on *Annulohypoxyon* sp. (Xylariaceae); asexual state on decaying stem and stump of *Bambusa*.

Distribution: Argentina, Brazil, India, and Japan.

Sexual state: Perithecia solitary, superficial, globose with a blunt apex, collapsing laterally, scarlet, smooth, 163–228 × 143–208 μm (mean = 188.9 × 164.9; SD 20.3, 19.5; n = 11). Asci cylindrical, eight-spored, uniseriately arranged, 44–61 × 4–7 μm (mean = 52.7 × 5.2; SD 4.5, 0.7; n = 15). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, verrucose, yellow-brown, 5.5–8.0 × 2.6–4.2 μm (mean = 6.8 × 3.3; SD 0.5, 0.3; n = 80).

Culture and asexual state: On PDA colonies (23–)31.5–43 mm diam (mean = 36.5; SD 5.8; n = 12) after 14 d at 25°C, velvety, floccose, citrine, with a sienna pigment diffusing in medium, reverse sienna. On CMD colonies 40–51 mm diam (mean = 45.6; SD 3.4; n = 11) after 14 d at 25°C, flat, dark-green, becoming pale-luteous at edge, with white aerial mycelium sparsely spread, reverse concolorous. Sporulation on SNA abundant, arising directly from agar surface. Asexual state acremonium-like;

conidiophores simple, unbranched, or dichotomously branched. Phialides monophialidic, cylindrical, collarette flared, hyaline, length 34–64 µm (mean = 50.5; SD 9.2; n = 30), width at base 1.5–2.6 µm (mean = 2.0; SD 0.2; n = 30), width at tip 0.8–1.3 µm (mean = 1.0; SD 0.1; n = 30). Conidia ovoid to ellipsoidal, unicellular, smooth, hyaline, 3.0–5.2 × 1.5–2.4 µm (mean = 3.8 × 2.0; SD 0.4, 0.2; n = 90).

Holotype of Cosmospora khandalensis: **India**, Maharashtra, Khandala, on decaying stem and stump of *Bambusa*, Aug. 1964., *M. J. Thirumalachar*, **holotype** HACC 148. (not seen), **isotypes** CBS H-15076 (not seen) = K(M) 169348, **ex-type** cultures ATCC 16091 (not seen) = CBS 356.65 (not seen) = IMI 112790 = MUCL 7974 (not seen).

Specimens and isolates examined: **Argentina**, Tucuman Province, Tucuman, San Javier trail just past Yerba Buena, on *Annulohypoxylon* sp., 21 Apr. 2011, *C. Salgado*, BPI XXXXXX, Culture A.R. 4798 = CBS XXXXX; Tucuman Province, Tucuman, San Javier trail just past Yerba Buena, on *Annulohypoxylon* sp., 21 Apr. 2011, *A. Romero*, BPI XXXXXX, Culture A.R. 4799 = CBS XXXXX. **Brazil**, Estado do Pará, Belterra, Maguari, Floresta Nacional do Tapajós, S2°46'59.6", W55°01'38.9", elev. 28 m., on *Annulohypoxylon* sp., on decaying tree, 7 May 2011, *O. Liparini Pereira* & *P. Chaverri* (P.C. 1306), BPI XXXXXX, culture CBS XXXXXX. **Japan**, Chinen-son, Shimajiri-gun, Okinawa Pref., Okinawa Islands, on unidentified pyrenomycete, 19 Jan. 2003, *Y. Hirooka* (h81), TFM FPH-7823 (not seen), culture MAFF 251500.

Additioinal descriptions and illustrations: Sukapure & Thirumalachar (1966), Hirooka et al. (2008; as *Cosmospora triqua*), Gräfenhan et al. (2011).

Notes: The sexual state of *Cosmospora khandalensis* is reported for the first time. Isolates derived from single ascospores (e.g. A.R. 4798, A.R. 4799, and P.C. 1306) formed a well-supported clade with the ex-type culture of *C. khandalensis* (IMI 112790). This result resolved the identity of these isolates, and suggested a wide distribution of this species.

Cosmospora micropedis C. Herrera & P. Chaverri, **sp. nov**. Fig. 3.9.

Mycobank MB XXXXXX

Holotype: **French Guiana**, Saul, ca. 20 km SW of Saul (03º60'N, 53º20'W) toward Mt. Galbao (03º50'N, 53º20'W), elev. 650 m, on *Kretzschmaria micropus*, 22 Jan. 1986, *G.J. Samuels* (3182) & *J.R. Boise*, NY, ex-holotype culture G.J.S. 86-108 = CBS XXXXXX.

Etymology: Referring to the fungal host of the type specimen, *Kretzschmaria micropus*.

Sexual state: Perithecia solitary, superficial, subglobose with a blunt apex, collapsing laterally, scarlet, smooth, 186–227 × 167–233 µm (mean = 208.3 × 196.8; SD 14.7, 23.1; n = 6). Asci cylindrical, eight-spored, uniseriately arranged, 50–61 × 4–7 µm (mean = 55.5 × 5.3; SD 3.5, 0.6; n = 9). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, verrucose, yellow-brown, 5.2–8.7 × 2.8–4.4 µm (mean = 6.9 × 3.7; SD 0.6, 0.3; n = 84).

Culture and asexual state: On PDA colonies 41–54 mm diam (mean = 47.6; SD 4.3; n = 12) after 14 d at 25°C, velvety, slightly floccose, olivaceous, with an amber

pigment diffusing into medium, reverse amber. On CMD colonies 48–63 mm diam (mean = 57.6; SD 5.2; n = 12) after 14 d at 25°C, flat, dark-green, becoming pale-luteous towards edge, with white aerial mycelium sparsely spread, reverse concolorous. Sporulation on SNA abundant, arising directly from agar surface. Asexual state acremonium-like; conidiophores simple, unbranched. Phialides monophialidic, cylindrical, collarette flared, hyaline, length 22–53 µm (mean = 41.2; SD 7.9; n = 29), width at base 1.6–2.4 µm (mean = 2.1; SD 0.2; n = 29), width at tip 0.9–1.4 µm (mean = 1.1; SD 0.1; n = 29). Conidia ovoid to ellipsoidal, unicellular, smooth, hyaline, 2.9–5.8 × 1.2–2.4 µm (mean = 3.7 × 1.8; SD 0.5, 0.2; n = 90).

Habitat: Fungicolous on *Kretzschmaria micropus* and *Hypoxylon cyclopicum* (Xylariaceae).

Distribution: Possibly pan Neotropical, known from Brazil, Costa Rica, French Guiana and Guadeloupe.

Additional specimens and isolates examined: **Brazil**, Estado do Pará, Município de Rurópolis, Comunidade Novo Horizonte, S4°06'49.7", W55°00'24.9", elev. 69 m., on *Hypoxylon cyclopicum*, on rotten wood, 02 May 2011, *G. Barata*, *O. Liparini Pereira, D.N. Skaltsas* & *P. Chaverri* (P.C. 1285), BPI XXXXXX, culture CBS XXXXXX; 0–3 km S. of Central Portion of Serra Araca & 0–8 km E. of Rio Javari, elev. 60 m, 00º49'N, 63º19'W, on *Hypoxylon cyclopicum*, 1–5 & 12–13 Mar. 1984, *G.J. Samuels* (661), NY; Serra Araca, elev. 60 m, on *Hypoxylon cyclopicum*, 10–13 Mar. 1984, *G.J. Samuels* (805), NY. **Costa Rica**, Heredia, Puerto Viejo de Sarapiquí, La Selva Biological Station, Sendero Tres Rios and Camino Experimental Norte, N

10°26’7.3”, W 84°00’31.4”, elev. 800 m, on *Hypoxylon cyclopicum*, 17 Mar. 2010, *P. Chaverri* (P.C. 1164), *G.J. Samuels*, *A.Y. Rossman*, *C. Salgado* & *C. Herrera*, BPI XXXXXX, culture G.J.S. 10-113 = CBS XXXXXX; Puerto Viejo de Sarapiquí, La Selva Biological Station, Sendero Tres Rios and Camino Experimental Norte, N 10°26’7.3”, W 84°00’31.4”, elev. 800 m, on *Hypoxylon cyclopicum*, 17 Mar. 2010, *P. Chaverri* (P.C. 1158), *G.J. Samuels*, *A.Y. Rossman*, *C. Salgado* & *C. Herrera*, BPI XXXXXX, culture G.J.S. 10-121 = CBS XXXXXX. **Guadeloupe**, Trace Delgrès, Basse Terre, on *Kretzschmaria micropus*, Jan. 1997, *J. Vivant*, BPI 744480.

Notes: This *Cosmospora* species grows on *Kretzschmaria micropus* and *Hypoxylon cyclopicum*. Experts in the Xylariaceae consider these fungal hosts to be morphological variants of the same species (Rogers & Ju 1998). The conidia of *C. micropedis* are generally smaller compared to those of other *Cosmospora* species growing on *Kretzschmaria* species.

Cosmospora novazelandica C. Herrera & P. Chaverri, **sp. nov**. Fig. 3.10.

Mycobank MB XXXXXX

Holotype: **New Zealand**, Buller, 21 Km S. of Murchison, on *Annulohypoxylon bovei*, 17 Apr. 1983, *G.J. Samuels*, *P.R. Johnson*, *R.E. Beever* & *R.H. Petersen*, PDD 46401, ex-holotype culture G.J.S. 83-197 = CBS XXXXXX.

Etymology: In reference to the geographical origin of this species, New Zealand.

Sexual state: Perithecia solitary, superficial, obpyriform, collapsing laterally, scarlet, with cells protruding around the apex (appearing roughened), 292– 330 × 211– 330

μm (mean = 312.1 × 289.9; SD 19.1, 54.1; n = 4). Asci cylindrical to clavate, eight-spored, uniseriately arranged, 70–90 × (5–)6–8 μm (mean = 80.5 × 7.0; SD 5.8, 0.8; n = 18). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, minutely verrucose, yellow-brown, 8.0–11.39 × 4.0–5.8 μm (mean = 10.0 × 4.8; SD 0.6, 0.4; n = 60).

Culture and asexual state: On PDA colonies 16–20 mm diam (mean = 18.3; SD 1.7; n = 4) after 14 d at 25°C, velvety, slightly floccose, citrine to olivaceous, with a citrine pigment slightly diffusing into medium, reverse olivaceous. On CMD colonies 18–19 mm diam (mean = 18.5; SD 0.6; n = 4) after 14 d at 25°C, flat, citrine, with an citrine pigment diffusing in medium, reverse greyish yellow-green to citrine green. Sporulation on SNA abundant, arising directly from agar surface. Asexual state acremonium-like; conidiophores simple, unbranched. Phialides monophialidic, cylindrical, collarette flared, hyaline, length 38–51 μm (mean = 42.7; SD 4.2; n = 10), width at base 1.9–3.0 μm (mean = 2.2; SD 0.4; n = 10), width at tip 1.0–1.2 μm (mean = 1.1; SD 0.1; n = 10). Conidia ovoid to ellipsoidal, unicellular, smooth, hyaline, 2.6–5.3 × 1.4–2.4 μm (mean = 3.8 × 1.8; SD 0.5, 0.2; n = 50).

Habitat: Fungicolous on *Annulohypoxylon bovei* (Xylariaceae) on unidentified bark.

Distribution: New Zealand.

Additional specimens examined: **New Zealand**, South Island, Boyle River Lodge, 172º23’E, 42º31’S, on *Annulohypoxylon bovei*, on unidentified bark, 13 Sep. 1981, *L. Brako* (No. 3991A), NY.

Notes: *Cosmospora novazelandica* is host specific to *Annulohypoxylon bovei*. Also, this species is unusual in respect to the ornamentation at the apex in which cells are protruding, thus giving the apex the appearance of being roughened. Perithecial ornamentation is generally absent in *Cosmospora*. Additionally, this species have the largest ascospores among species of the *Cosmospora viliuscula* species complex.

Cosmospora rickii (Rehm) Rossman & Samuels, Stud. Mycol. 42: 124. 1999.

Basionym: *Nectria rickii* Rehm, Hedwigia 44: 2. 1905.

= *Nectria episphaeria* var. *kretzschmariae* Henn., Bot. Jb. 14(4): 364. 1891.

≡ *Nectria kretzschmariae* (Henn.) Weese, Akad. Wiss. Wien Sitzungsber., Math.-Naturwiss. Kl., Abt. 1, 125: 506. 1916.

= *Nectria stigme* Rehm, Hedwigia 44: 2. 1905.

Anamorph: not known.

Habitat: Fungicolous on *Kretzschmaria cetrarioides*.

Distribution: Brazil and Republic of the Congo.

Holotype of Nectria rickii: **Brazil**, Sao Leopoldo, on disintegrated stroma of *Kretzschmaria cetrarioides* (as *K. lichenoides*), 1903, *S.J. Rick*, F10125. (holotype of *N. rickii*).

Additional specimens examined: **Brazil**, S. Sao Leopoldo, on *Kretzschmaria cetrarioides* (as *K. lichenoides*), 1903, *S.J. Rick*, F10189 (S - holotype of *N. stigme*). **Republic of the Congo** (as Loango), on *Kretzschmaria cetrarioides* (as *K. pechuelii*),

1876, *Pechuel-Lösche*, B (holotype of *Nectria episphaeria* var. *kretzschmariae* not seen; destroyed by the war-dependent fire of 1943).

Notes: Weese (1916) was the last researcher to have examined all three holotype specimens. He concluded that *N. episphaeria* var. *kretzschmariae*, *N. rickii*, and *N. stigme* represented the same species. He raised *N. episphaeria* var. *kretzschmariae* to species rank in 1916. The principle of priority applies only to names within the same rank, thus *N. rickii* or *N. stigme*, published in the same article in 1905 and thus having equal priority, provide older names at the species rank. Rossman et al. (1999) already selected *N. rickii* to represent this species and transferred the name to *Cosmospora*. The holotype of *N. rickii* consists of the mature form (ascospores ellipsoid, minutely verrucose, yellow-brown, 14.1–18.3 × 6.1–8.9 µm, mean = 16.0 × 7.5 µm) while the holotype of *N. stigme* represents an immature form of the same species (ascospores ellipsoid, minutely verrucose, yellow-brown, 7.1–9.6(–13.2) × 3.8–4.8(–7.4) µm, mean = 8.0 × 4.1 µm). The holotype of *Nectria episphaeria* var. *kretzschmariae* has been destroyed. All three type specimens are reported to occur on the same host, *Kretzschmaria cetrarioides*, which supports the idea that these specimens each represent the same single species. A living culture from a specimen is needed to determine the phylogenetic placement of this species.

Cosmospora scruposae C. Herrera & P. Chaverri, **sp. nov**. Fig. 3.11.

Mycobank MB XXXXXX

Holotype: **French Guiana**, Montagne de Kaw, Route de l'est, km 50, on *Xylaria scruposa*, 25 Mar. 1986, *G.J. Samuels* & *C. Feuillet*, GJS 4487 (NY), ex-holotype

culture G.J.S. 86-331 = CBS XXXXXX.

Etymology: In reference to its fungal host, *Xylaria scruposa.*

Sexual state: Perithecia solitary, superficial, subglobose with an acute apex, collapsing laterally, orange, or scarlet, smooth, 177–239 × 159–224 μm (mean = 212.6 × 189.2; SD 17.0, 18.0; n = 18). Asci cylindrical, eight-spored, uniseriately arranged, 45–71 × 4–6 μm (mean = 56.4 × 4.8; SD 6.4, 0.5; n = 20). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, verrucose, yellow-brown, 6.0–9.0 × 2.9–4.8 μm (mean = 7.3 × 3.7; SD 0.6, 0.3; n = 130).

Culture and asexual state: On PDA colonies 28–41.5 mm diam (mean = 33.3; SD 3.8; n = 16) after 14 d at 25°C, velvety, floccose, olivaceous buff, with a sienna to umber pigment diffusing into medium, sometimes with white aerial mycelium densely spread, reverse sienna to umber. On CMD colonies 33–55 mm diam (mean = 49.5; SD 6.6; n = 17) after 14 d at 25°C, flat, white, sometimes with an amber pigment, reverse concolorous. Sporulation on SNA abundant, arising directly from agar surface. Asexual state acremonium-like; conidiophores simple, unbranched, or dichotomously branched, rarely terminating in three phialides. Phialides monophialidic, cylindrical, collarette flared, hyaline, length 36–66 μm (mean = 59.6; SD 9.0; n = 38), width at base 1.4–2.8 μm (mean = 2.1; SD 0.4; n = 38), width at tip 0.8–1.3 μm (mean = 1.1; SD 0.2; n = 38). Conidia ovoid to ellipsoidal, unicellular, smooth, hyaline, 3.5–6.7 × 1.4–2.8 μm (mean = 4.8 × 2.0; SD 0.8, 0.3; n = 90).

Habitat: Fungicolous on *Xylaria* cf. *scruposa* (Xylariaceae).

Distribution: Possibly pan Neotopical, known from French Guiana, Guyana, Puerto Rico and Venezuela.

Additional specimens and isolates examined: **French Guiana**, Piste de Saint-Elie, km 16 on road between Sinnamary and St. Elie, ECEREX, ORSTOM research area, 05°20' N, 53°W, on stromata of *Xylaria* cf. *scruposa* Feb–Mar 1986, *G.J. Samuels*, G.J.S. 3899 (NY); Paul Isnard Area, ca. 150 km S of St. Laurent du Moroni, Citron, Mt. Lucifer, 04º70'N, 53º90'W, on stroma of *Xylaria* cf. *scruposa*, Mar. 1986, *G.J. Samuels* & *P. Searwar*, GJS 4129 (NY), culture G.J.S. 86-278 = CBS XXXXXX; Paul Isnard Area, ca. 150 km S of St. Laurent du Moroni, Citron, small ridge at SW edge of the village, 04º70'N, 53º90'W, on *Xylaria* cf. *scruposa*, 16–17 Mar. 1986, *G.J. Samuels* & *P. Searwar*, G.J.S. 4326 (NY), culture G.J.S. 86-315 = CBS XXXXXX; Montagne de Kaw, Route de l'est, km 27, 04º60'N, 52º40'W, on *Xylaria* cf. *scruposa*, 21 Mar. 1986, *G.J. Samuels* & *C. Feuillet*, G.J.S. 4393 (NY), culture G.J.S. 86-320 = CBS XXXXXX. **Guyana**, Upper Demerara-Berbice Region, Upper Demerara subregion, Mabura Hill, along Waraputa Creek, elev. 0–50 m, 05º14'N, 58º47'W, on stroma of *Xylaria* cf. *scruposa*, 28 Mar. 1987, *G.J. Samuels* & *L. Wong Kam*, G.J.S. 5303 (NY); Demerara-Mahaica Region, Mahaica subregion, Linden Highway between Georgetown & Yarowcabra, Yarowcabra Forestry Station, elev. 50–100 m, 06º30'N, 58º15'W, on stromata of *Xylaria* cf. *scruposa*, 26–27 Apr. 1987, *G.J. Samuels* & *J. Pipoly*, G.J.S. 5563 (NY); Cuyuni-Mazarun Region, Mazarun Subregion, along Koatze River, ca. 2 km E of Pong River, ca. 5 hr walk of Chinoweing Village, elev. 600–650 m, 05º28'N, 60º04'W, on stroma of *Xylaria* cf. *scruposa*, Feb–Mar 1990, *G.J. Samuels*, *J. Pipoly*, G. Gharbarran, J. Chin & R.

Edwards, G.J.S. 5002 (NY). **Puerto Rico**, Caribbean National Forest, Luquillo Mts., La Prieta Creek, elev. 350–400 m, on stroma of *Xylaria* cf. *scruposa*, 20 Feb. 1996, *G.J. Samuels* (8039) & *H.-J. Schroers*, BPI 744671, culture G.J.S. 96-6 = CBS 455.96. **Venezuela**, Edo. Aragua, Henry Pittier National Park, Rancho Grande Biological Station, Toma Trail to water source, elev. 1200–1300 m, 10°21'N, 67°41'W, on stroma of *Xylaria* cf. *scruposa*, 03 Dec. 1990, *G.J. Samuels* (7853), *B. Hein* & *S.M. Huhndorf*, BPI 745150, culture G.J.S. 90-224 = CBS XXXXXX; Edo. Aragua, Henry Pittier National Park, Rancho Grande Biological Station, Trail to Guacamayo, elev. 1250–1400 m, 10°21'N, 67°41'W, on stroma of *Xylaria* cf. *scruposa*, 04 Dec. 1990, *G.J. Samuels* (7891), *B. Hein* & *S.M. Huhndorf*, BPI 744778, culture G.J.S. 90-217= CBS XXXXXX.

Notes: *Cosmospora* species growing on *Xylaria* tend to have perithecia with an acute apex. Although fresh cultures were apparently dark-green as indicated by the dried culture specimens in herbarium packets, regrown isolates of *C. scruposae* produced a white colony on CMD, thus the white colony cannot be considered to an unique character of this species. It suggests that the stored isolates have degraded in storage such that they no longer produce the pigmentation.

Cosmospora stilbohypoxyli C. Herrera & P. Chaverri, **sp. nov**. Fig. 3.12.

Mycobank MB XXXXXX

Holotype: **Argentina**, Tucuman Province, San Javier, on *Stilbohypoxylon quisquiliarum*, on decorticated wood, 20 Apr. 2011, *C. Salgado*, BPI XXXXXX, ex-holotype culture A.R. 4783 = CBS XXXXXX.

Etymology: In reference to its fungal host, *Stilbohypoxylon quisquiliarum.*

Sexual state: Perithecia solitary, superficial, subglobose with blunt apex, sometimes appearing acute, collapsing laterally, scarlet, smooth, 152–188 × 145–185 µm (mean = 169 × 158; SD 11.2, 12.8; n = 8). Asci narrowly clavate, eight-spored, uniseriately arranged, 45–61 × 4– 6 µm (mean = 51.6 × 4.9; SD 4.5, 0.5; n = 10). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, minutely verrucose, yellow-brown, 6.5–7.9(–10.1) × 3.0–4.0 µm (mean = 6.9 × 3.5; SD 0.4, 0.2; n = 40).

Culture and asexual state: On PDA colonies 5–6 mm diam (mean = 5.3; SD 0.6; n = 4) after 14 d at 25°C, velvety, slightly floccose, herbage-green, with a slight sulphur-yellow pigment diffusing into medium, reverse concolorous. On CMD colonies 8–11.5 mm diam (mean = 9.5; SD 1.5; n = 4) after 14 d at 25°C, flat, greenish-yellow at center, becoming white towards edge, reverse concolorous. Sporulation on SNA abundant, arising directly from agar surface. Asexual state acremonium-like; conidiophores simple, unbranched. Phialides monophialidic, cylindrical, collarette flared, hyaline, length 34–44 µm (mean = 40.1; SD 3.2; n = 10), width at base 2.0–3.0 µm (mean = 2.3; SD 0.3; n = 10), width at tip 1.1–1.6 µm (mean = 1.3; SD 0.2; n = 10). Conidia ovoid to ellipsoidal, unicellular, smooth, hyaline, 4.6–7.5 × 2.2–3.7 µm (mean = 5.9 × 2.8; SD 0.6, 0.3; n = 30).

Habitat: Fungicolous on *Stilbohypoxylon quisquiliarum* (Xylariaceae) on unidentified bark.

Distribution: Argentina and Venezuela.

Additional specimens and isolates examined: **Venezuela**, Distrito Federal, South of Los Caracas, along Rio Los Caracas, on *Stilbohypoxylon quisquiliarum*, on bark of unidentified tree, 22 July 72, *K.P. Dumont* (Dumont-VE 5665), *R.F. Cain*, *G.J. Samuels* & *B. Manara*, NY.

Notes: *Cosmospora stilbohypoxyli* is only known to grow on *Stilbohypoxylon quisquiliarum*. This species is extremely slow growing on PDA and CMD compared to other species.

Cosmospora ustulinae (Teng) C. Herrera & P. Chaverri, **comb. nov**. Fig. 3.13.

Mycobank MB XXXXXX

Basionym: *Nectria ustulinae* Teng, Sinensia, Shanghai 4: 275 (1934).

Habitat: Fungicolous on *Kretzschmaria deusta* (Xylariaceae).

Distribution: China, Japan, Portugal, USA (AL, FL, MI, NY, TN).

Holotype of *Nectria ustulinae*: **China**, Kiangsu, Pao-hua Shan, on stroma of *Kretzschmaria deusta* (as *Hypoxylon ustulatum*), *S.C. Teng* (2027), holotype BPI 553261.

Sexual state: Perithecia solitary, in clusters (<10), rarely densely aggregated, superficial, subglobose with a blunt apex, collapsing laterally, scarlet, smooth, 185–225 × 146–195 µm (mean = 205.6 × 169.9; SD 15.9, 17.7; n = 6). Asci narrowly clavate, eight-spored, uniseriately arranged, 41–61 × 4–5 µm (mean = 50.3 × 4.8; SD

6.5, 0.4; n = 12). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, verrucose, yellow-brown, 5.8–8.3 × 2.7–4.8 μm (mean = 7.0 × 3.6; SD 0.6, 0.4; n = 62).

Culture and asexual state: On PDA colonies 35–51 mm diam (mean = 42.9; SD 5.3; n = 14) after 14 d at 25°C, velvety, slightly floccose, olivaceous to citrine, sometimes becoming greenish-yellow at edge, with a slight greenish-yellow or sienna pigment diffusing in medium, sometimes black droplets forming at center of colony, reverse concolorous. On CMD colonies 44–52 mm diam (mean = 48.4; SD 2.8; n = 14) after 14 d at 25°C, flat, dark-green, or greenish-yellow at the center, becoming hyaline towards edge of colony, reverse concolorous. Sporulation on SNA abundant, arising directly from agar surface. Asexual state acremonium-like; conidiophores simple, unbranched, or dichotomously branched. Phialides monophialidic, cylindrical, collarette flared, hyaline, length 35–57 μm (mean = 45.9; SD 5.8; n = 28), width at base 1.7–3.1 μm (mean = 2.3; SD 0.3; n = 28), width at tip 0.9–1.3 μm (mean = 1.1; SD 0.1; n = 28). Conidia ovoid to ellipsoidal, unicellular, smooth, hyaline, 3.3–6.5 × 1.6–3.1 μm (mean = 4.7 × 2.2; SD 0.7, 0.3; n = 120).

Epitype designated herein: **USA**, Tennessee, Blount Co., Great Smoky Mts. National Park, 25 mi W Gatlinburg, Cades Cove, Gum Swamp, elev. 1800 ft, 35°35’13.7”N, 83°50’19.2”W, on stroma of *Kretzschmaria deusta*, 06 Sep. 2005, *S. Huhndorf*, BPI 871089, ex-epitype culture A.R. 4215 = CBS XXXXXX.

Additional specimens and isolates examined: **Japan**, Kumamoto Prefecture, Kikushi City, Kikuchi valley, on remnants of old stroma of *Kretzschmaria* cf. *deusta*, 31 May

2004, *Y. Hirooka* (h278), BPI XXXXXX, culture MAFF 241532**. USA**, Alabama, Talledega Nat. Forest, Cheaha State Park, Clay Co., Creek Trail, on stroma of *Kretzschmaria deusta*, 23 Sep. 1992, *G.J. Samuels, C.T. Rogerson, S.M. Huhndorf*, BPI 802840, culture G.J.S. 92-95 = CBS XXXXXX. **Portugal**, isolated from cut treated stump of *Clethra arborea*, 2000, culture IMI 389101.

Additional description: Teng (1934; no illustrations).

Notes: This species grows on *Kretzschmaria deusta* in temperate forests. In contrast to the asexual state of *C. viliuscula*, which is highly branched, i.e. penicillate, the asexual state of *C. ustulinae* is simple, unbranched or branching into two phialides.

Cosmospora viliuscula (Samuels) Rossman & Samuels, Stud. Mycol. 42: 126 (1999). Fig. 3.14.

Basionym: *Nectria viliuscula* Samuels, Mem. New York Bot. Gard. 59: 44 (1990).

Habitat: Fungicolous on *Kretzschmaria* cf. *deusta* (Xylariaceae).

Distribution: Australia, Costa Rica, Indonesia and New Zealand.

Holotype of *Nectria viliuscula*: **Indonesia**, North Sulawsi, Eastern Dumoga-Bone National Park, Gn. Muajat, Danau Alia, elev. 1400 m, 00°45’N, 124°25’E, on *Kretzschmaria* cf. *deusta* (as *Hypoxylon* cf. *deustum*), 26 Oct. 1985, G.J. Samuels 2385, Holotype BO (Not Seen), Isotype NY 01013285.

Sexual state: Perithecia solitary, or in clusters (<10), superficial, subglobose with a blunt apex, collapsing laterally, scarlet, smooth, 198–255 × 159–236 µm (mean =

224.9 × 236; SD 20.7, 29.4; n = 10). Asci cylindrical, eight-spored, uniseriately arranged, 46–63 × 4–6(–7) µm (mean = 54.2 × 5.1; SD 4.9, 0.7; n = 12). Ascospores ellipsoid, equally two-celled, one-septate, slightly constricted at septum, verrucose, yellow-brown, 6.2–9.8 × 2.2–5.1 µm (mean = 7.8 × 3.8; SD 0.8, 0.4; n = 54).

Culture and asexual state: On PDA colonies 39–56 mm diam (mean = 46.4; SD 6.7; n = 11) after 14 d at 25°C, velvety, slightly floccose, amber, or olivaceous, sometimes sienna pigment diffusing in medium, sometimes with white aerial mycelium sparsely or densely spread, reverse sienna. On CMD colonies 27–55 mm diam (mean = 45.8; SD 10.2; n = 12) after 14 d at 25°C, flat, white, reverse concolorous. Sporulation on SNA abundant, arising directly from agar surface. Asexual state acremonium-like to penicillate; conidiophores simple, unbranched, or dichotomously branched; forming primary, secondary, and rarely tertiary branches, each terminal branch producing 2–4 phialides. Phialides monophialidic, cylindrical, collarette flared, hyaline, length 28–57 µm (mean = 43.6; SD 6.3; n = 30), width at base 1.4–2.5 µm (mean = 1.8; SD 0.3; n = 30), width at tip 0.8–1.2 µm (mean = 1.0; SD 0.1; n = 30). Conidia ovoid to ellipsoidal, unicellular, smooth, hyaline, 2.2–5.7 × 1.3–2.9 µm (mean = 4.0 × 2.1; SD 0.6, 0.3; n = 90).

Epitype designated herein: **Australia**, Queensland, Lake Barrine, elev. 750 m, 17.0°14.0’43.0”S, 145°38.0’, 21.0”E, on stroma of *Kretzschmaria* cf. *deusta* (as *Ustulina deusta*), 20 Feb. 2009, *P. Chaverri* (P.C. 858) & *A.Y. Rossman*, BPI 878994, ex-epitype culture G.J.S. 09-411 = CBS XXXXXX.

Additional specimens and isolates examined: **Costa Rica**, Heredia, Puerto Viejo de

Sarapiquí, La Selva Biological Station, Sendero Tres Rios and Camino Experimental Norte, N 10°26’7.3”, W 84°00’31.4”, elev. 100–300 m, on stroma of *Kretzschmaria* cf. *deusta*, 17 Mar. 2010, *P. Chaverri* (P.C. 1150), *G.J. Samuels*, *A.Y. Rossman*, *C. Salgado* & *C. Herrera*, BPI XXXXXX, culture G.J.S. 10-114 = CBS XXXXXX. **New Zealand**, Auckland Prov. Bay of Islands Co., Puketi Forest, on stroma of *Kretzschmaria* cf. *deusta* (as *Ustulina* sp.), 02 Mar. 1973, *J.M. Dingley*, PDD 30869, culture G.J.S. 73-2 = CBS XXXXXX.

Additional descriptions and illustrations: Samuels, Doi, & Rogerson (1990), Samuels et al. (1991), Rossman et al. (1999).

Notes: This species grows on *Kretzschmaria* cf. *deusta* in tropical forests. In addition this species is unique in producing a penicillate asexual state.

Cosmospora viridescens (C. Booth) Gräfenhan & Seifert, Stud. Mycol. 68: 96. 2011.

Basionym: *Nectria viridescens* C. Booth, Mycol. Pap. 73: 89. 1959.

Habitat: On bone and fungicolous on *Ruzenia spermoides* (Lasiosphaeriaceae).

Distribution: Czech Republic, Denmark and United Kingdom.

Holotype of *Nectria viridescens*: **UK**, England, Yorkshire, Sawley Woods, on black pyrenomycete on branches of *Salix*, 22 Apr. 1954, C. Booth, holotype IMI 56376 (not seen), isotype BPI 553304 = DAOM 83074 (not seen); Wales, Llanrwst, Gwydyr Forest, on *Ruzenia spermoides*, on *Betula*, May 1958, *C. Booth*, paratype K(M) 169349, ex-paratype culture IMI 73377a.

Additional isolates examined: **Czech Republic**, Sumava Mts. National Park, vicinity

of Breznik, Mt. Studna hora, on bark of dead standing trunk, 11 Aug. 1999, *M. Reblova*, culture CBS 102430; Sumava Mts. National Park, vicinity of Breznik, Pytlacky, on dead tree, 14 Aug. 1999, *M. Reblova*, culture CBS 102433. **Denmark**, on bone, 24 Jul. 1998, *T. Laessoe*, culture A.R. 2783 = CBS XXXXXX.

Descriptions and illustrations: Booth (1959), Gräfenhan et al. (2011).

Notes: The isolate IMI 73377a represents the only living culture derived from specimens cited by Booth (1959) in his original description of *Nectria viridescens*. By definition, K(M) 169349 represents a paratype of *C. viridescens*, and phylogenetic placement of this species in the strict sense was possible with this isolate. It clustered with other isolates previously considered to be *C. viridescens* (see Gräfenhan et al. 2011), but the clade may consist of a species complex. The ascospores are ellipsoid, slightly constricted at septum, minutely verrucose, yellow-brown, 7.6–12.3 × 3.7–4.7 μm (mean = 9.4 × 4.3 μm).

Additional accepted species not treated in this paper

***Cosmospora berkeleyana* (P. Karst.) Gräfenhan, Seifert & Schroers**, *Stud. Mycol.* **68**: 95. 2011.

Basionym: *Verticillium berkeleyanum* P. Karst., Meddeland. Soc. Fauna Fl. Fenn. 18: 64. 1891.

≡ *Acremonium berkeleyanum* (P. Karst.) W. Gams, Netherlands J. Pl. Pathol. 88: 76. 1982.

Asexual state: Acremonium-like.

Habitat: On *Inonotus radiatus* and *Stereum hirsutum*.

Distribution: Canada, Finland, Germany, and Netherlands.

Descriptions and illustrations: Karsten (1891), Gams (1971), and Gams & Zaayen (1982).

Notes: The sexual state of this species is unknown. The identity of the isolate CBS 258.70 reported to be growing on *Inonotus radiatus* is questionable. Given the host, the isolate could be a morphological variant of *Cosmospora coccinea*. This isolate has not been sequenced.

***Cosmospora butyri* (J.F.H. Beyma) Gräfenhan, Seifert & Schroers**, *Stud. Mycol.* **68**: 96. 2011.

Basionym: *Tilachlidium butyri* J.F.H. Beyma, Zentralbl. Bakteriol., 2 Abt. 99: 388. 1938.

≡ *Acremonium butyri* (J.F.H. Beyma) W. Gams, Cephalosporium-artige Schimmelpilze (Stuttgart) p. 126. 1971.

Asexual state: Acremonium-like.

Habitat: Isolated from butter.

Distribution: Denmark (only known from the type).

Descriptions and illustrations: Beyma (1938) and Gams (1971).

Notes: The sexual state of this species is unknown. Summerbell et al. (2011) noted

that the ex-type culture might comprise a mixed culture. We suspect that *C. butyri* has a fungal host, but it has not been found. The reported substrate suggests that this fungus might have a secondary nutrition mode (e.g., saprophytic).

***Cosmospora cymosa* (W. Gams) Gräfenhan, Seifert & Schroers**, *Stud. Mycol.* **68**: 96. 2011.

Basionym: *Acremonium cymosum* W. Gams, Cephalosporium-artige Schimmelpilze (Stuttgart) p. 131. 1971.

Asexual state: Acremonium-like.

Habitat: On *Inonotus radiatus*.

Distribution: Germany.

Descriptions and illustrations: Gams (1971).

Notes: The sexual state of this species is unknown. The phylogeny in *Gräfenhan* et al. (2011) includes *C. coccinea* and *C. cymosa* as sister taxa. It is possible that *C. cymosa* is a morphological variant of *C. coccinea* given that they are 97% similar based on ITS sequences. However, protein-coding loci showed them to be more divergent. Differences are found primarily at third codon position sites of the protein-coding genes.

***Cosmospora lavitskiae* (Zhdanova) Gräfenhan, Seifert & Schroers**, *Stud. Mycol.* **68**: 96. 2011.

Basionym: *Gliomastix lavitskiae* Zhdanova, Mikrobiol. Zhurn. 28: 37. 1966.

Asexual state: Acremonium-like.

Habitat: Isolated from soil rhizosphere of *Zea mays*.

Distribution: Ukraine.

Descriptions and illustrations: Zhdanova (1966) and Gams (1971).

Notes: The sexual state of this species is unknown. We suspect that *C. lavitskiae* has a fungal host, but it has not been found. This fungus could have a secondary nutrition mode (e.g., saprophytic).

Table 3.1. Isolates and accession numbers used in the phylogenetic analyses.

Species	Isolate No.	Herbarium No.	Substrate/Host	Geographic Origin	GeneBank Accession No.					
					ITS	LSU	*mcm7*	*rpb1*	*tef1*	*tub2*
Corallomycetella elegans	A.R. 4547, CBS 123826	BPI 881071	Bark	French Guiana	JF832594	JF832679	KC291795	JF832763	JF832517	JF832838
Cosmospora annulohypoxyli	G.J.S. 95-199, CBS XXXXXX	BPI 737773	*Annulohypoxylon* cf. *multiforme*	USA	JN995634	JN939817	JN993307	JQ031066	KJ676365	KJ676284
Cosmospora annulohypoxyli	G.J.S. 96-186, CBS XXXXXX	BPI 744521	*Annulohypoxylon* cf. *cohaerens*	USA	JN995635	JN939816	JN993308	JQ031065	KJ676366	KJ676285
Cosmospora arxii	A.R. 4521, CBS XXXXXX	BPI 879925	*Hypoxylon cf. howeanum*	France	JN995621	JN939839	JN993336	JQ031086	KJ676338	KJ676255
Cosmospora arxii	G.J.S. 10-247, CBS XXXXXX	BPI XXXXXX	*Hypoxylon fragiforme*	USA	JN995629	JN939824	JN993326	KC291869	KC291843	KC291908
***Cosmospora* sp.**	G.J.S. 96-251, CBS 123961	-	unidentified black pyrenomycete	USA	KJ676150	KJ676187	KJ676306	KJ676224	KJ676345	KJ676263
***Cosmospora* sp.**	IMI 133984	-	on undescribed plant substrate	Ukraine	KJ676167	KJ676204	KJ676325	KJ676241	KJ676369	KJ676288
***Cosmospora* sp.**	KAS 1105	-	on woodpile	Canada	KJ676172	KJ676209	KJ676330	KJ676246	-	KJ676293
***Cosmospora* sp.**	KAS 5072	BPI XXXXXX	*Hypoxylon* sp.	Canada	XXXXXX	XXXXXX	-	-	-	-
Cosmospora clavi	G.J.S. 87-4, CBS 123941	-	*Kretzchmaria* sp.	Brazil	KJ676149	KJ676186	-	KJ676223	KJ676344	KJ676262
Cosmospora clavi	CBS 251.78	-	*Kretzchmaria* sp.	Brazil	KJ676151	KJ676188	KJ676307	KJ676225	KJ676346	KJ676264
Cosmospora clavi	G.J.S. 96-48, CBS 448.96	BPI 745249	*Kretzchmaria* sp.	Puerto Rico	JN995625	JN939834	JN993319	JQ031081	KJ676347	KJ676265

Table 3.1. Continued.

Species	Isolate No.	Herbarium No.	Substrate/Host	Geographic Origin	GeneBank Accession No.					
					ITS	LSU	*mcm7*	*rpb1*	*tef1*	*tub2*
Cosmospora clavi	G.J.S. 84-290, CBS XXXXXX	G.J.S. 1076 (NY)	*Kretzschmaria clavus*	Brazil	KJ676159	KJ676196	KJ676316	KJ676233	KJ676356	KJ676275
Cosmospora clavi	G.J.S. 96-7, CBS XXXXXX	BPI 744678	*Kretzchmaria* sp.	Puerto Rico	KJ676165	KJ676202	-	KJ676239	KJ676367	KJ676286
Cosmospora coccinea	A.R. 2741, CBS 114050	BPI 802729	*Inonotus nodulosus*	Germany	HM484537	GQ505990	-	GQ506020	HM484515	HM484589
Cosmospora coccinea	A.R. 2743, CBS XXXXXX	-	*Inonotus nodulosus*	Germany	KJ676141	KJ676178	-	KJ676215	KJ676335	KJ676252
Cosmospora fomiticola	G.J.S. 83-194 , CBS XXXXXX	PDD 46398	*Fomes fomentarius*	New Zealand	KJ676158	KJ676195	KJ676314	KJ676232	KJ676355	KJ676274
Cosmospora khandalensis	A.R. 4770, CBS XXXXX	BPI XXXXXX	*Annulohypoxylon* sp.	Argentina	KJ676143	KJ676180	KJ676300	KJ676217	KJ676339	KJ676256
Cosmospora khandalensis	A.R. 4798, CBS XXXXX	BPI XXXXXX	*Annulohypoxylon* sp.	Argentina	KJ676145	KJ676182	KJ676302	KJ676219	KJ676340	KJ676258
Cosmospora khandalensis	A.R. 4799, CBS XXXXX	BPI XXXXXX	*Annulohypoxylon* sp.	Argentina	KJ676146	KJ676183	KJ676303	KJ676220	KJ676341	KJ676259
Cosmospora khandalensis	IMI 112790	IMI 112790	on decaying stem and stump of *Bambusa*	India	KJ676166	KJ676203	KJ676324	KJ676240	KJ676368	KJ676287
Cosmospora khandalensis	MAFF 241500	-	on unidentified pyronomycete	Japan	KJ676174	KJ676211	KJ676331	KJ676248	KJ676375	KJ676295

Table 3.1. Continued.

Species	Isolate No.	Herbarium No.	Substrate/Host	Geographic Origin	GeneBank Accession No.					
					ITS	LSU	*mcm7*	*rpb1*	*tef1*	*tub2*
Cosmospora micropedis	G.J.S. 10-113, CBS XXXXXX	BPI XXXXXX	*Hypoxylon cyclopicum*	Costa Rica	KJ676154	KJ676191	KJ676310	KJ676228	KJ676351	KJ676269
Cosmospora micropedis	G.J.S. 10-121, CBS XXXXXX	BPI XXXXXX	*Hypoxylon cyclopicum*	Costa Rica	KJ676156	KJ676193	KJ676312	KJ676230	KJ676352	KJ676271
Cosmospora micropedis	G.J.S. 86-108, CBS XXXXXX	GJS 3182 (NY)	*Kretzschmaria micropus*	French Guiana	KJ676161	KJ676198	KJ676318	KJ676235	KJ676358	KJ676277
Cosmospora micropedis	P.C. 1285, CBS XXXXXX	BPI XXXXXX	*Hypoxylon* cf. *cyclopicum*	Brazil	KJ676176	KJ676213	KJ676333	KJ676250	KJ676377	KJ676297
Cosmospora novazelandica	G.J.S. 83-197, CBS 124032	PDD 46401	*Annulohypoxylon bovei*	New Zealand	KC291732	KC291777	KJ676315	KC291868	KC291849	KC291907
Cosmospora scruposae	G.J.S. 86-315, CBS XXXXXX	GJS 4326 (NY)	*Xylaria scruposa*	French Guiana	KC291748	KC291779	KJ676320	KC291867	KC291851	KC291906
Cosmospora scruposae	G.J.S. 90-217, CBS XXXXXX	BPI 744778	*Xylaria scruposa*	Venezuela	JF832596	JF832681	-	JF832765	JF832518	JF832840
Cosmospora scruposae	G.J.S. 96-6, CBS 455.96	BPI 744671	*Xylaria scruposa*	Puerto Rico	HM484855	GQ506003	KJ676323	GQ506032	HM484851	HM484876
Cosmospora scruposae	C.T.R. 71-62, CBS XXXXXX	CUP-MJ 858 (NY)	*Xylaria scruposa*	Jamaica	KJ676152	KJ676189	KJ676308	KJ676226	KJ676348	KJ676266
Cosmospora scruposae	G.J.S. 86-278, CBS XXXXXX	GJS 4129 (NY)	*Xylaria scruposa*	French Guiana	KJ676162	KJ676199	KJ676319	KJ676236	KJ676359	KJ676278
Cosmospora scruposae	G.J.S, 86-320, CBS XXXXXX	GJS 4393 (NY)	*Xylaria scruposa*	French Guiana	KJ676163	KJ676200	KJ676320	KJ676237	KJ676360	KJ676279

Table 3.1. Continued.

Species	Isolate No.	Herbarium No.	Substrate/Host	Geographic Origin	GeneBank Accession No.					
					ITS	LSU	*mcm*7	*rpb*1	*tef*1	*tub*2
Cosmospora scruposae	G.J.S. 86-331, CBS XXXXXX	GJS 4487 (NY)	*Xylaria scruposa*	French Guiana	JN995631	JN939822	-	JQ031070	KJ676361	KJ676280
Cosmospora scruposae	G.J.S. 90-224, CBS XXXXXX	BPI 745150	*Xylaria scruposa*	Venezuela	JN995632	JN939820	-	JQ031068	KJ676362	KJ676281
***Cosmospora* sp.**	G.J.S. 01-301, CBS XXXXXX	BPI 871586	*Xylaria* sp.	Thailand	KJ676153	KJ676190	KJ676309	KJ676227	KJ676349	KJ676267
***Cosmospora* sp.**	G.J.S. 82-275, CBS XXXXXX	PDD 44440	*Annulohypoxylon* sp.	New Zealand	KJ676157	KJ676194	KJ676313	KJ676231	KJ676354	KJ676273
***Cosmospora* sp.**	G.J.S. 85-200, CBS XXXXXX	GJS 2384 (NY)	*Xylaria* sp.	Indonesia	KJ676160	KJ676197	KJ676317	KJ676234	KJ676357	KJ676276
***Cosmospora* sp.**	G.J.S. 95-142, CBS XXXXXX	BPI 737707	*Xylaria* sp.	Uganda	KJ676164	KJ676201	KJ676322	KJ676238	KJ676364	KJ676283
***Cosmospora* sp.**	IMI 318025	K(S) 169350	*Xylaria* cf. *polymorpha*	England	KJ676168	KJ676205	KJ676326	KJ676242	KJ676370	KJ676289
***Cosmospora* sp.**	IMI 362240	-	stem tissue of *Vitis*	-	KJ676169	KJ676206	KJ676327	KJ676243	KJ676371	KJ676290
***Cosmospora* sp.**	KAS 3751	-	*Xylaria* cf. *polymorpha*	Canada	KJ676173	KJ676210	-	KJ676247	KJ676374	KJ676294
Cosmospora stilbohypoxyli	A.R. 4783, CBS XXXXXX	BPI XXXXXX	*Stilbohypoxylon quisquiliarum*	Argentina	KJ676144	KJ676181	KJ676301	KJ676218	-	KJ676257
Cosmospora ustulinae	A.R. 4215, CBS XXXXXX	BPI 871089	*Kretzschmaria deusta*	USA	JN995619	JN939841	-	JQ031088	KJ676337	KJ676254
Cosmospora ustulinae	G.J.S. 92-95, CBS XXXXXX	BPI 802840	Kretzschmaria deusta	USA	JN995633	JN939818	-	JQ031067	KJ676363	KJ676282

Table 3.1. Continued.

Species	Isolate No.	Herbarium No.	Substrate/Host	Geographic Origin	GeneBank Accession No.					
					ITS	LSU	*mcm*7	*rpb*1	*tef*1	*tub*2
Cosmospora ustulinae	IMI 389101	-	stump of *Clethra arborea*	Portugal	KJ676170	KJ676207	KJ676329	KJ676245	KJ676372	KJ676291
Cosmospora ustulinae	MAFF 241532	BPI XXXXXX	*Kretzschmaria* cf. *deusta*	Japan	KJ676175	KJ676212	KJ676332	KJ676249	KJ676376	KJ676296
Cosmospora viliuscula	G.J.S. 09-411, CBS XXXXXX	BPI 878994	*Kretzschmaria* cf. *deusta*	Australia	JN995627	JN939826	-	KC291866	KC291841	KC291905
Cosmospora viliuscula	G.J.S. 10-114, CBS XXXXXX	BPI XXXXXX	*Kretzschmaria* cf. *deusta*	Costa Rica	KJ676155	KJ676192	KJ676311	KJ676229	-	KJ676270
Cosmospora viliuscula	G.J.S. 73-2, CBS XXXXXX	PDD 30869	Kretzschmaria cf. deusta	New Zealand	JN995630	JN939823	-	JQ031093	KJ676353	KJ676272
Cosmospora viridescens	A.R. 2783	?	Bone	Denmark	KJ676142	KJ676179	KJ676299	KJ676216	KJ676336	KJ676253
Cosmospora viridescens	CBS 102430	-	standing trunk	Czech Republic	KJ676147	KJ676184	KJ676304	KJ676221	KJ676342	KJ676260
Cosmospora viridescens	CBS 102433	-	dead tree	Czech Republic	KJ676148	KJ676185	KJ676305	KJ676222	KJ676343	KJ676261
Cosmospora viridescens	IMI 73377a	K(S) 169349	*Ruzenia spermoides*	Wales	KJ676171	KJ676208	KJ676328	KJ676244	KJ676373	KJ676292
Dialonectria episphaeria	G.J.S. 10-193, C.H. 10-01	-	*Diatrype stigma*	USA	KC291744	KC291771	KC479773	KC291892	KC291842	KC291932
Microcera larvarum	A.R. 4580	-	scale insect	New Zealand	KC291751	KC291759	KC291798	KC291894	KC291832	KC291935
Pseudocosmospora eutypellae	A.R. 4562	BPI 884165	*Eutypella* sp.	USA	KC291721	KC291757	KC291796	KC291871	KC291830	KC291912

Table 3.2. Loci used in the phylogenetic analyses. Information on the primers, including base pairs, PCR protocols, and models of nucleotide substitution are indicated.

Locus	**ITS**	**LSU**	***Mcm7***	***Rpb1***	***Tub2***	**Combined**	***Tef1***
Nucleotide substitution models	SYM+I+G	HKY+I	GTR+I+G			—	HKY+G
Included sites	574	783	1970			3327	352
Phylogenetically informative sites	28	29	624			681	201
Uninformative polymorphic sites	46	20	188			254	80
Invariable sites	352	723	916			1991	0
Primers used (reference)	ITS5, ITS4 (White et al. 1990)	LR5, LROR (Vilgalys & Hester 1990)	mcm7-709for, mcm7-1348rev (Schmitt et al. 2009)	crpb1a, rpb1c (Castlebury et al. 2004)	Btub-TI, Btub-T2 (O'Donnell & Cigelnik 1997)	—	tef1-728, tef1-986 (Carbone & Kohn 1999)
PCR protocol: Annealing temp. & cycles	53 °C, 1 min, 40×		56 °C, 50 s, 38×	50 °C, 2 min, 40×	55 °C, 30 s, 35×	—	66 °C, 55 s, 9x 56 °C, 55 s, 35×

Table 3.3. Support received by each recognized species.

	ITS (BI PP)	**LSU (BI PP)**	***mcm*7 (BI PP)**	***rpb*1 (BI PP)**	***tef*1 (BI PP)**	***tub*2 (BI PP)**	**Combined (BI PP/ML BP)**
Cosmospora annulohypoxili	-	94%	100%	100%	94%	100%	100%/100%
Cosmospora arxii	100%	100%	100%	100%	98%	100%	100%/100%
Cosmospora sp.	-	-	100%	100%	-	100%	100%/73%
Cosmospora clavi	-	-	98%	100%	-	-	100%/97%
Cosmospora coccinea	100%	100%	N/A	100%	99%	100%	100%/100%
Cosmospora khandalensis	98%	-	100%	97%	94%	100%	100%/100%
Cosmospora micropodis	-	-	100%	100%	-	100%	100%/100%
Cosmospora scruposae	-	-	-	100%	-	100%	100%/100%
Cosmospora ustulinae	-	-	99%	100%	97%	100%	100%/100%
Cosmospora viliuscula	100%	-	100%	100%	93%	100%	100%/100%
Cosmospora viridescens	91%	-	100%	94%	-	100%	100%/99%

Supplemental Table 3.1. Morphological differences in the *Cosmospora viliuscula* species complex (K-W test, P < 0.05). A) Ascospore Length. B) Ascospore Width. C) Ascospore Qratio. D) Conidia Length. E) Conidia Width. F) Conidia Qratio. G) Growith in CMD. H) Growith in PDA.

	C. viliuscula	*C. ustulinae*	*C. stilbohypoxiii*	*C. scruposae*	*C. novaezelandica*	*C. micropodis*	*C. khandalensis*	*C. fomiticola***	*C. clavi*	*C. arxii*	*C. annulohypoxili*
C. annulohypoxili		ADEF	ADE	DF	AB	ACEGH	AB		BCDG	DF	
C. arxii	DFH	ACDEFH	AEF	CG	ABDF	ACDFGH	ADF	DF	BCDFGH		
C. clavi	CDF	AB	ABDEGH	BEFH	ABCDEGH	ABDE	ABDFG	BCDF			
*C. fomiticola***		ACDF	ACDE	ACDF	AB	AC	ABC				
C. khandalensis	AB	BDEF	DE	ABDF	AB	BCEG					
C. micropodis	ACE	DE	DEGH	ACDFH	ABCGH						
C. novaezelandica	ABEH	ABDEH	ABDE	ABDFG							
C. scruposae	ADFH	EF	DEFG								
C. stilbohypoxili	ABDEH	DEH									
C. ustulinae	ADEF										
C. viliuscula											

** = *C. fomiticola* excluded in G & H comparisons

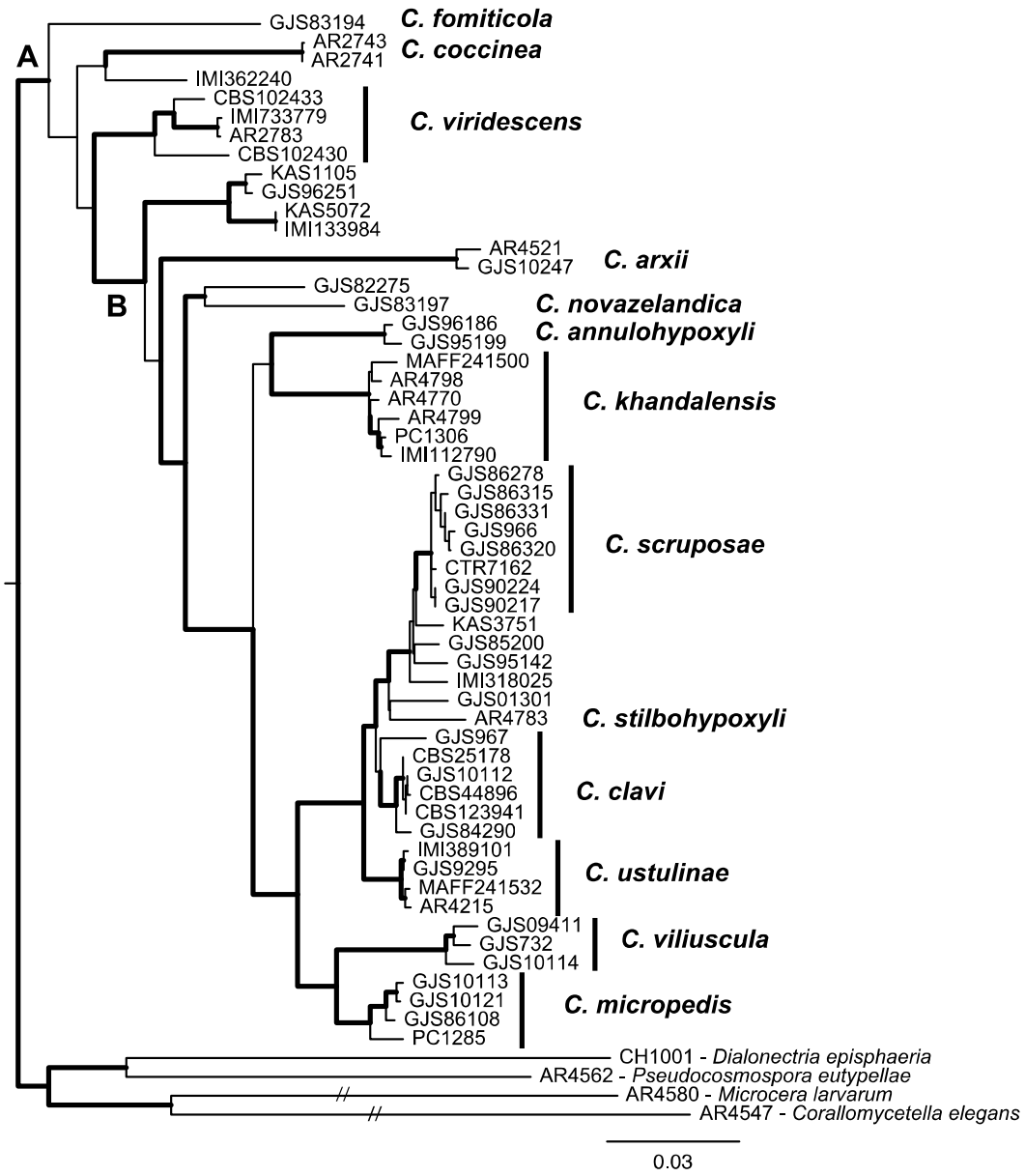


Fig. 3.1 ML phylogeny (best tree; LnL = −18580.293) of *C. viliuscula* species complex based on three partition (ITS, LSU, *mcm7-rpb1- tub2*) dataset. Label A, *Cosmospora* node; label B, *Cosmospora viliuscula* species complex. Black bold branches indicate >70% ML BP.

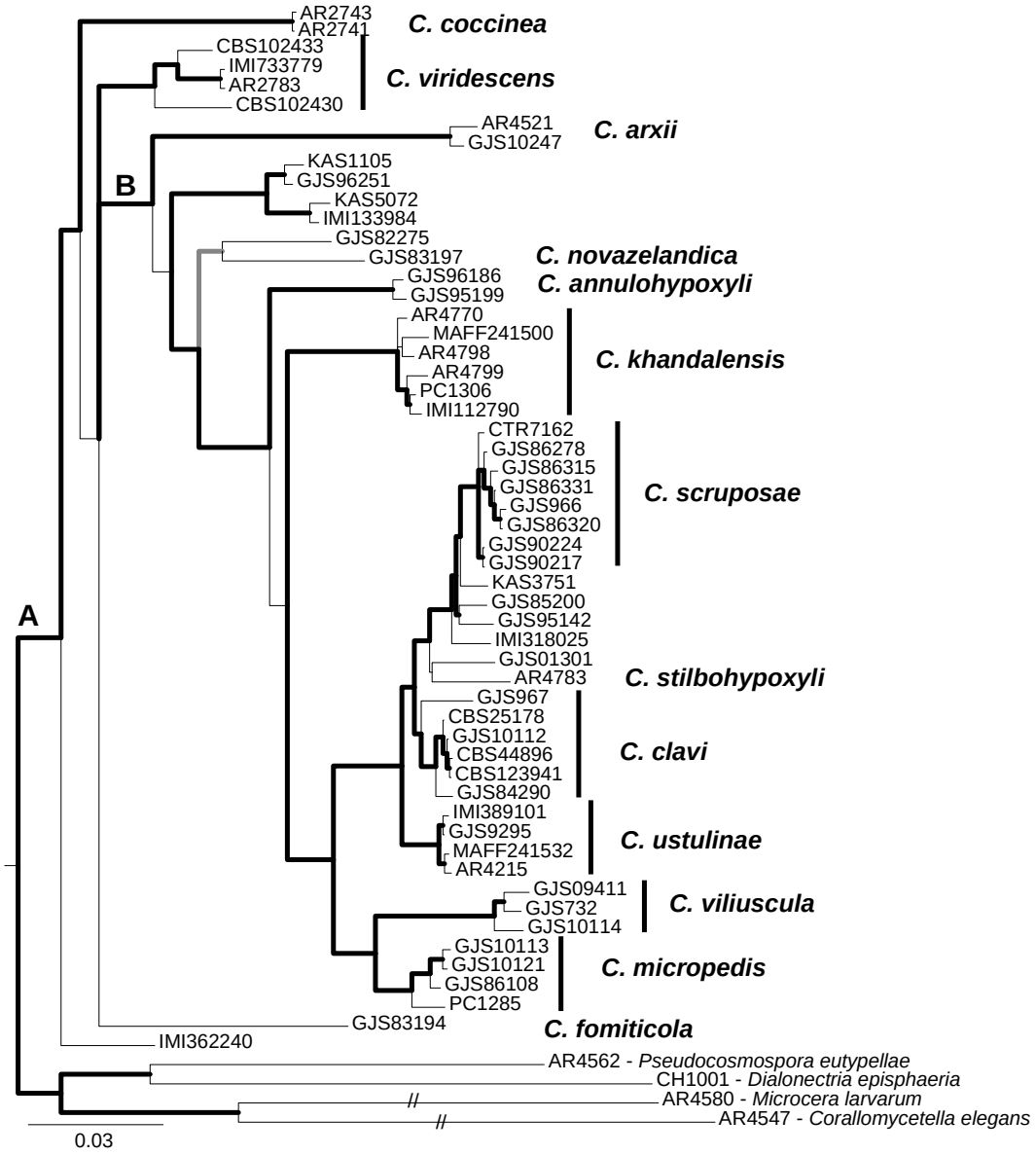


Fig. 3.2 BI phylogeny (consensus tree; LnL = −18297.835) of *C. viliuscula* species complex based on three partition (ITS, LSU, *mcm7-rpb1- tub2*) dataset. Label A, *Cosmospora* node; label B, *Cosmospora viliuscula* species complex. Gray bold branches indicate >90% BI PP; black bold branches indicate >95% BI PP.

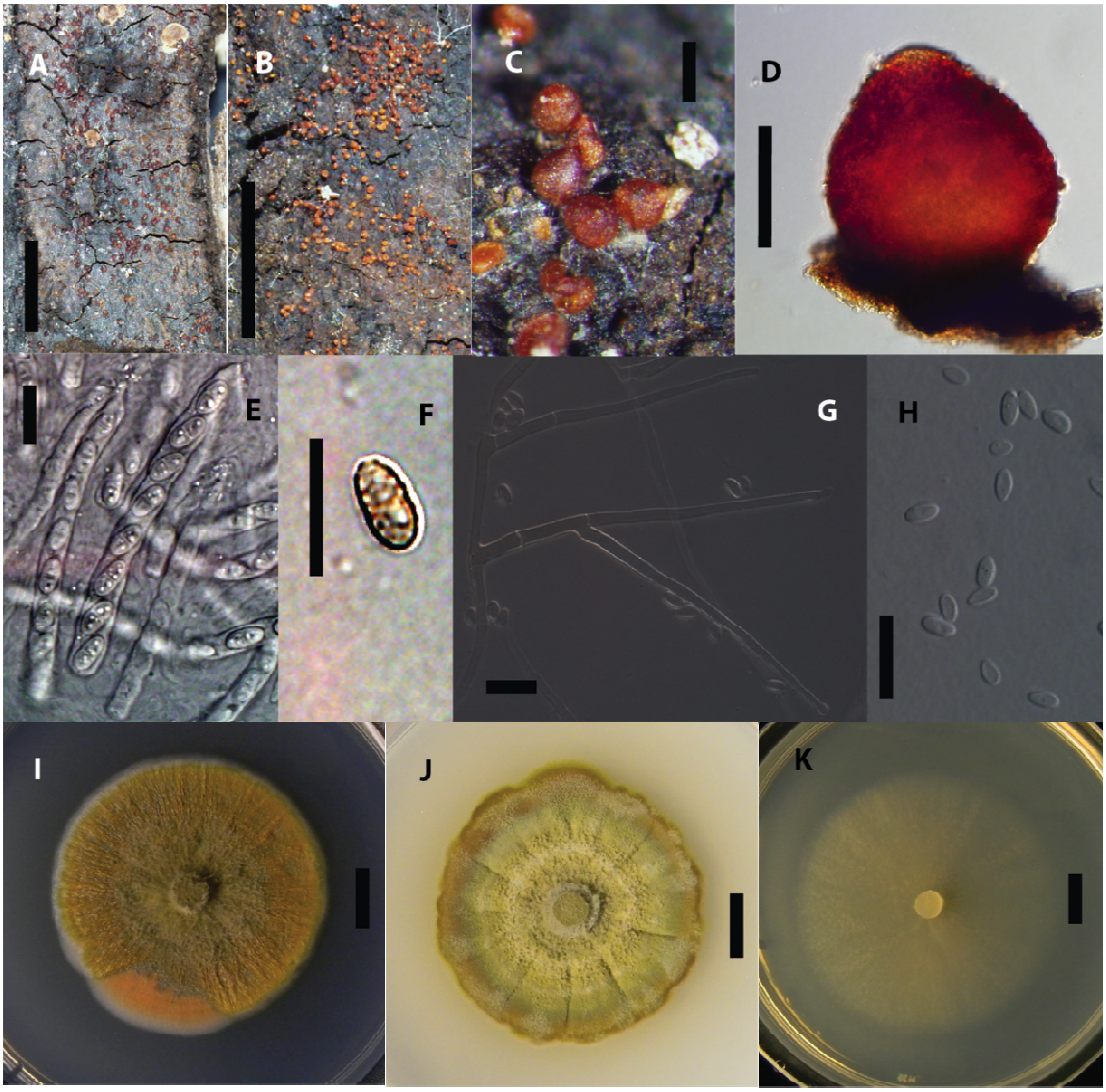


Fig. 3.3 *Cosmospora annulohypoxyli*. (A) Habit. Scale bar = 3 mm. (B) Habit. Scale bar = 4 mm. (C) Perithecia on natural substrata. Scale bar = 200 µm. (D) Perithecium in 3% KOH. Scale bar = 100 µm. (E) Asci. Scale bar = 10 µm. (F) Ascospore. Scale bar = 10 µm. (G) Phialide. Scale bar = 10 µm. (H) Conidia. Scale bar = 10 µm. (I–J) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (K) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm

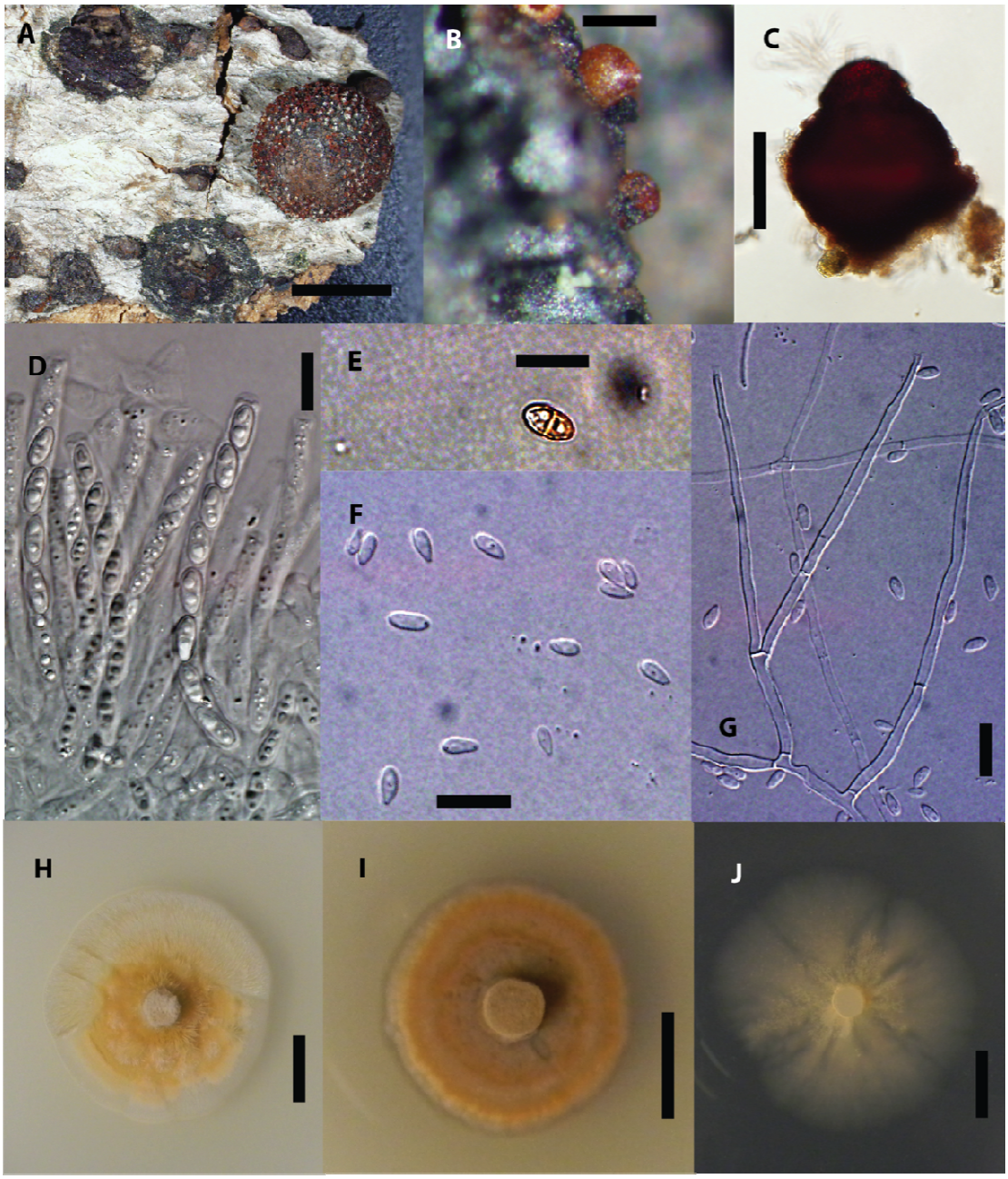


Fig. 3.4 *Cosmospora arxii*. (A) Habit. Scale bar = 4 mm. (B) Perithecia on natural substrata. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Asci. Scale bar = 10 µm. (E) Ascospore. Scale bar = 10 µm. (F) Conidia. Scale bar = 10 µm. (G) Phialide. Scale bar = 10 µm. (H−I) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (J) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm

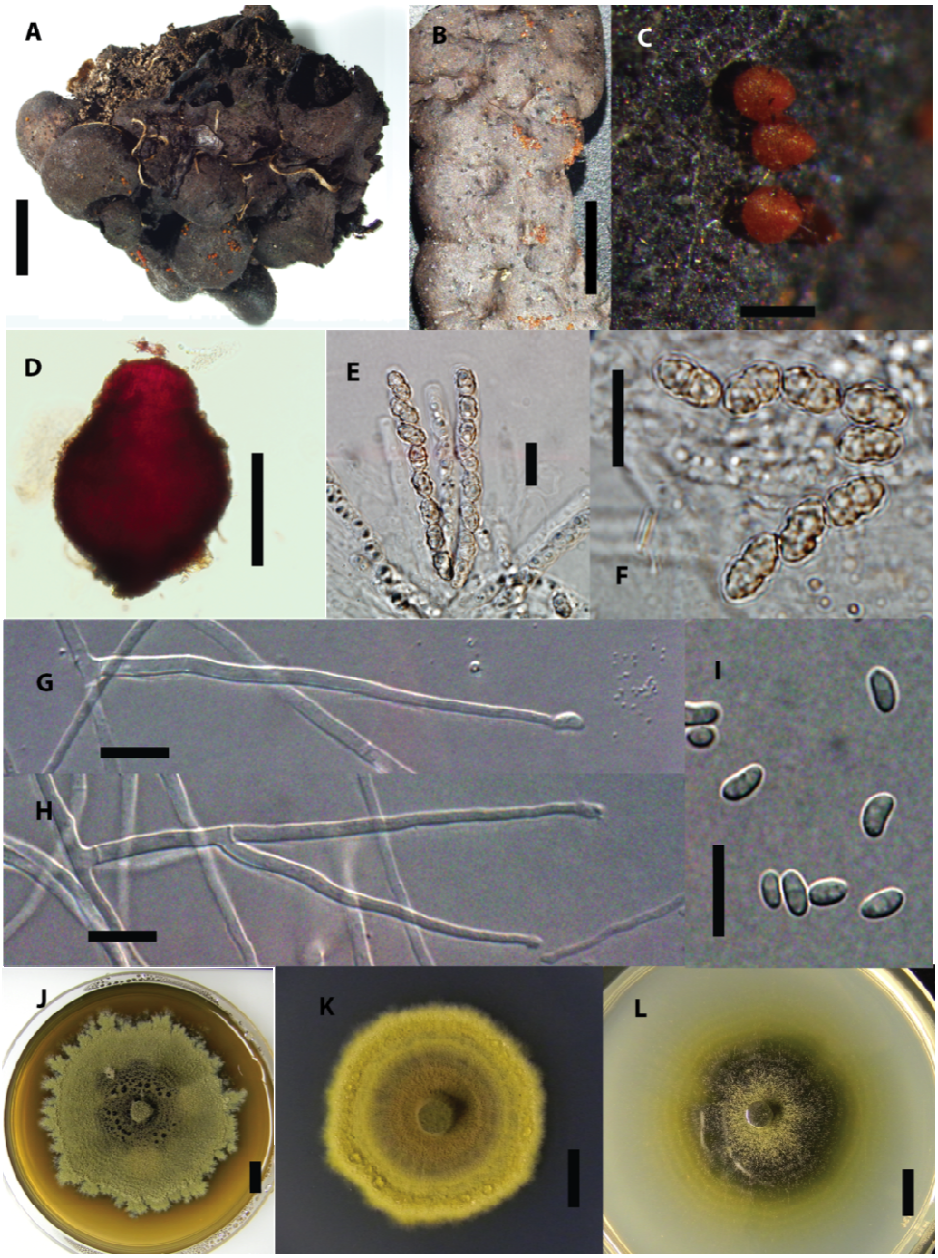


Fig. 3.5 *Cosmospora clavi*. (A−B) Habit. Scale bar = 4 mm. (C) Perithecia on natural substrata. Scale bar = 200 µm. (D) Perithecium in 3% KOH. Scale bar = 100 µm. (E) Asci. Scale bar = 10 µm. (F) Ascospore. Scale bar = 10 µm. (G−H) Phialide. Scale bar = 10 µm. (I) Conidia. Scale bar = 10 µm. (J−K) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (L) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm

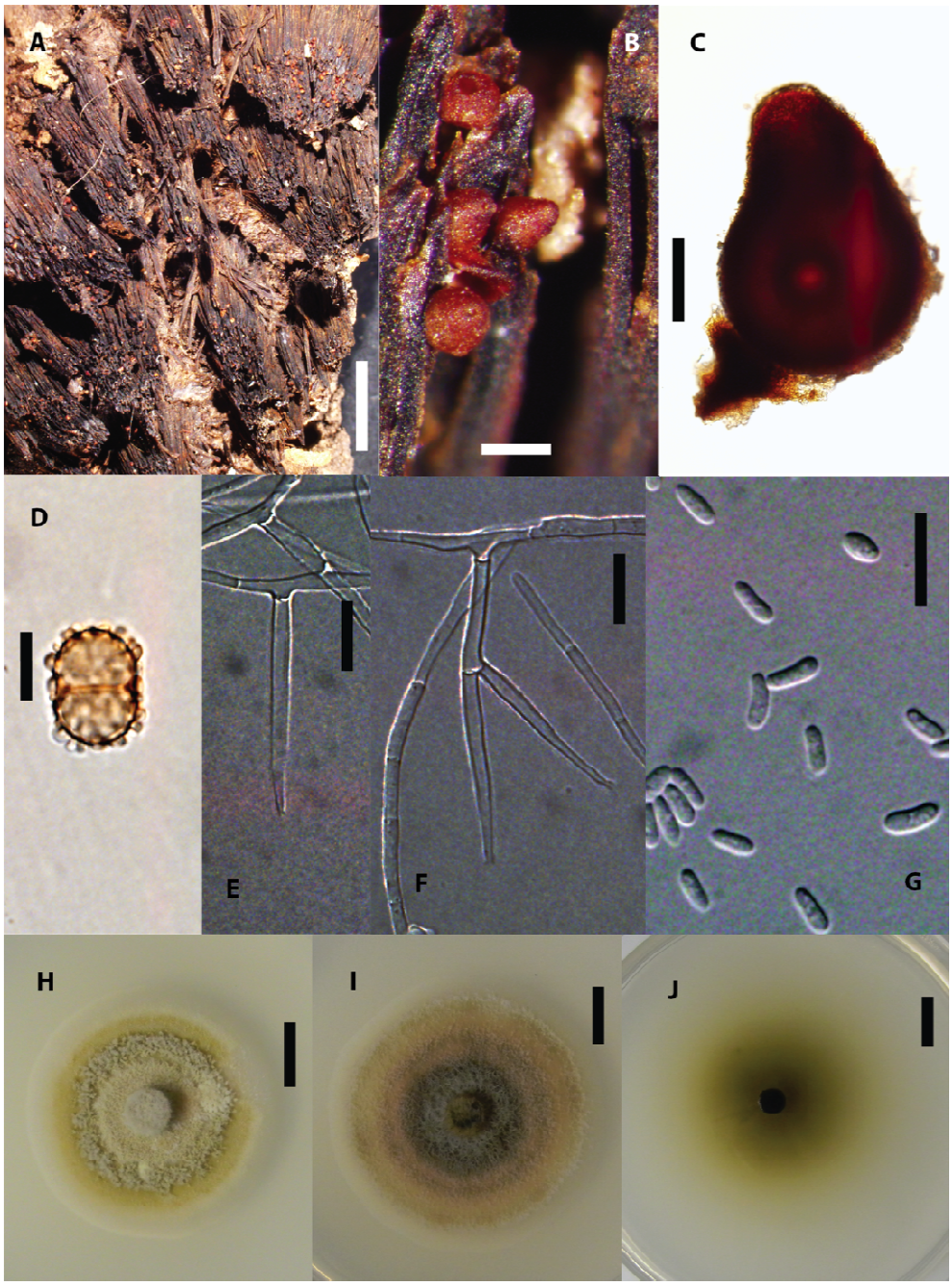


Fig. 3.6 *Cosmospora coccinea*. (A) Habit. Scale bar = 4 mm. (B) Perithecia on natural substrata. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Ascospore. Scale bar = 10 µm. (E−F) Phialide. Scale bar = 10 µm. (G) Conidia. Scale bar = 10 µm. (H−I) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (J) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm

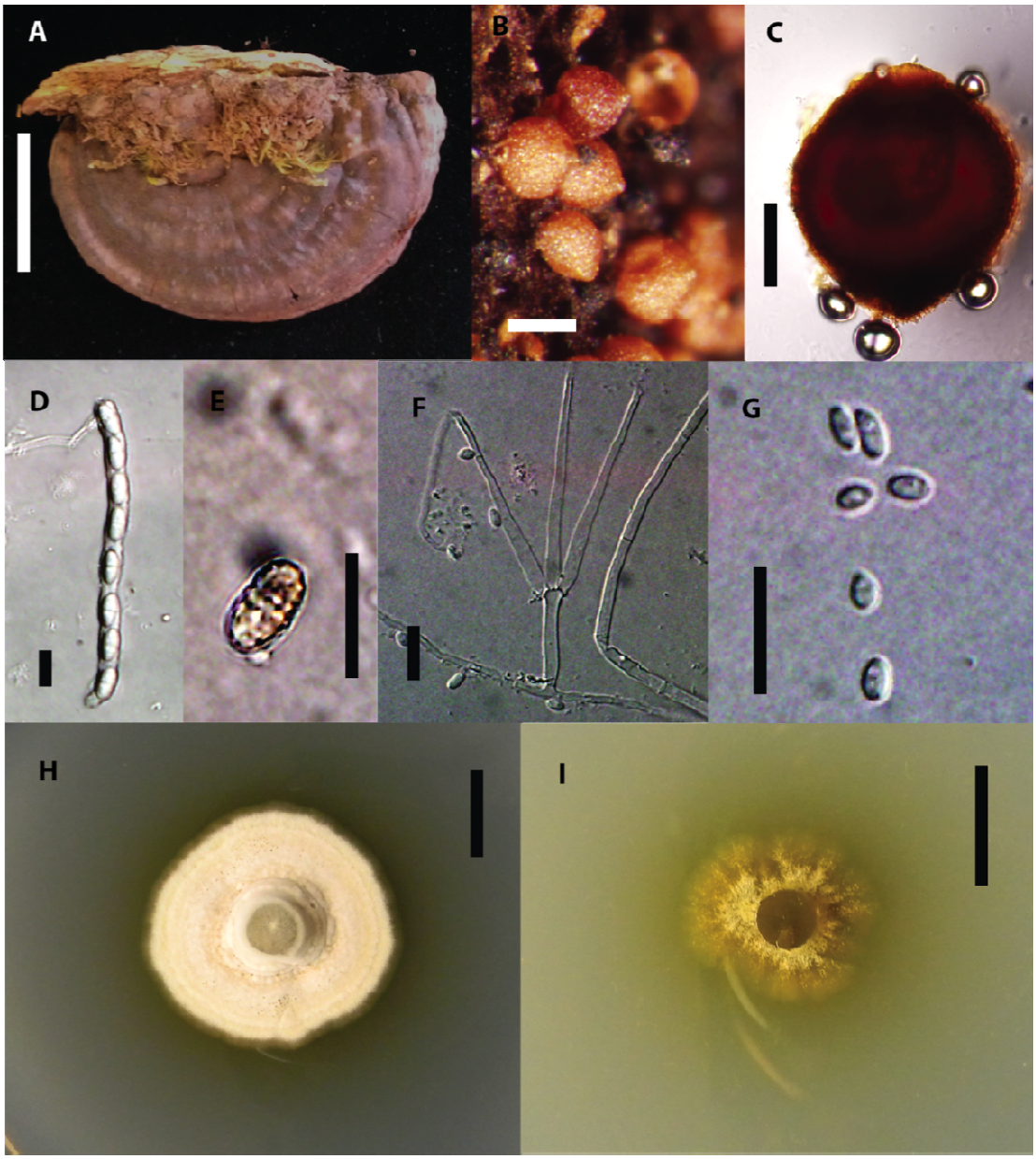


Fig. 3.7 *Cosmospora fomiticola*. (A) Habit. Scale bar = 3 mm. (B) Perithecia on natural substrata. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Asci. Scale bar = 10 µm. (E) Ascospore. Scale bar = 10 µm. (F) Phialide. Scale bar = 10 µm. (G) Conidia. Scale bar = 10 µm. (H) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (I) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm

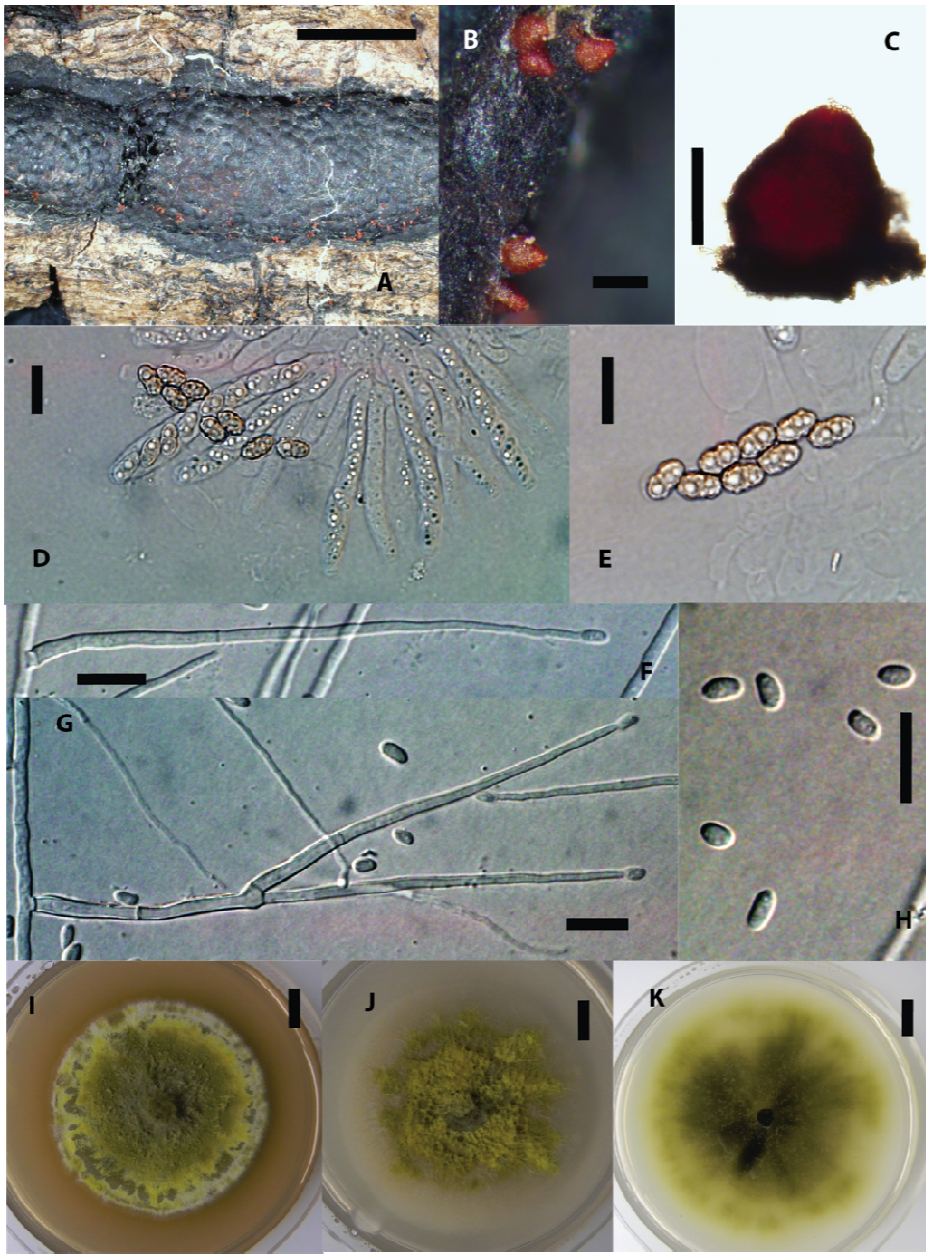


Fig. 3.8 *Cosmospora khandalensis*. (A) Habit. Scale bar = 5 mm. (B) Perithecia on natural substrata. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Asci. Scale bar = 10 µm. (E) Ascospores. Scale bar = 10 µm. (F−G) Phialide. Scale bar = 10 µm. (H) Conidia. Scale bar = 10 µm. (I−J) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (K) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm

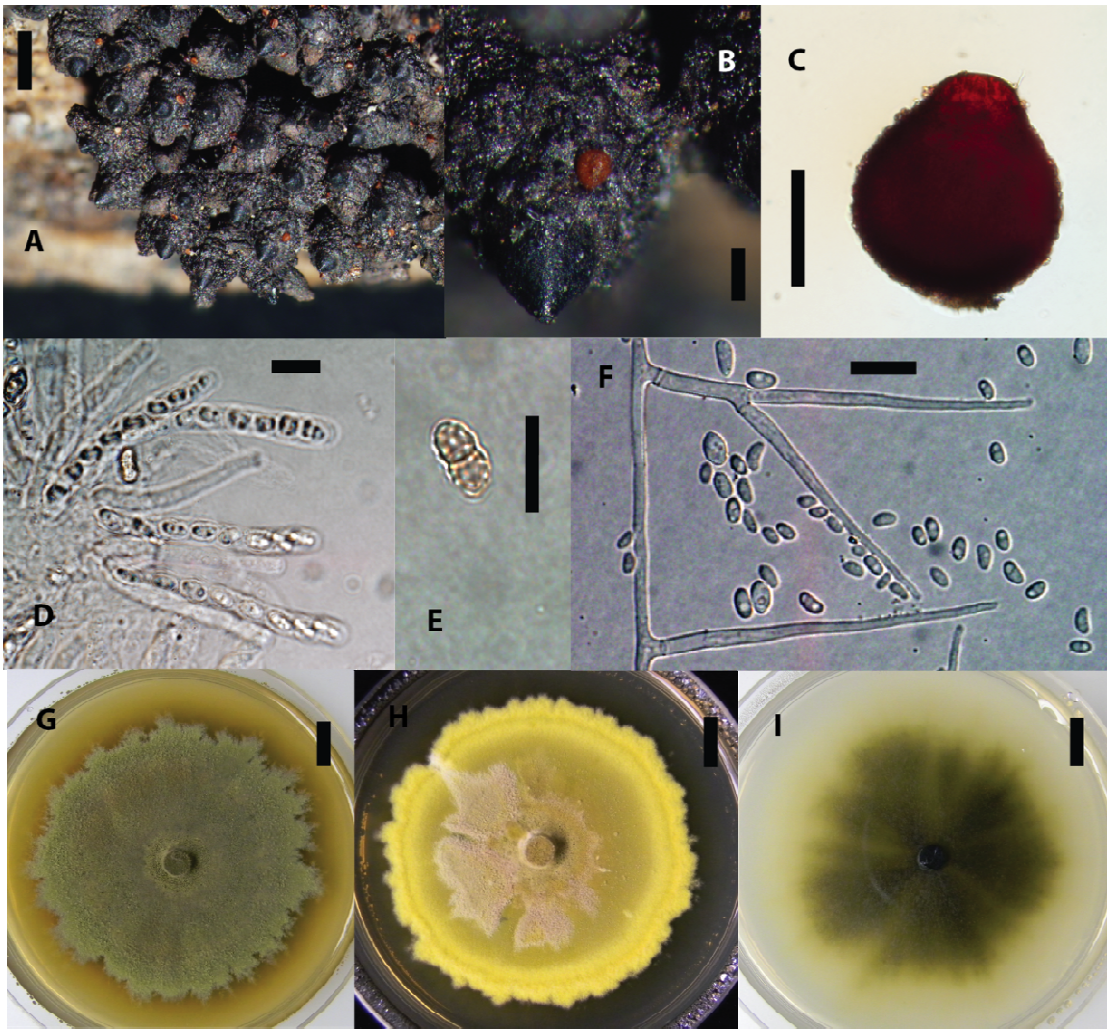


Fig. 3.9 *Cosmospora micropedis*. (A) Habit. Scale bar = 1 mm. (B) Perithecia on natural substrata. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Asci. Scale bar = 10 µm. (E) Ascospores. Scale bar = 10 µm. (F) Phialides and conidia. Scale bar = 10 µm. (G−H) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (I) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm

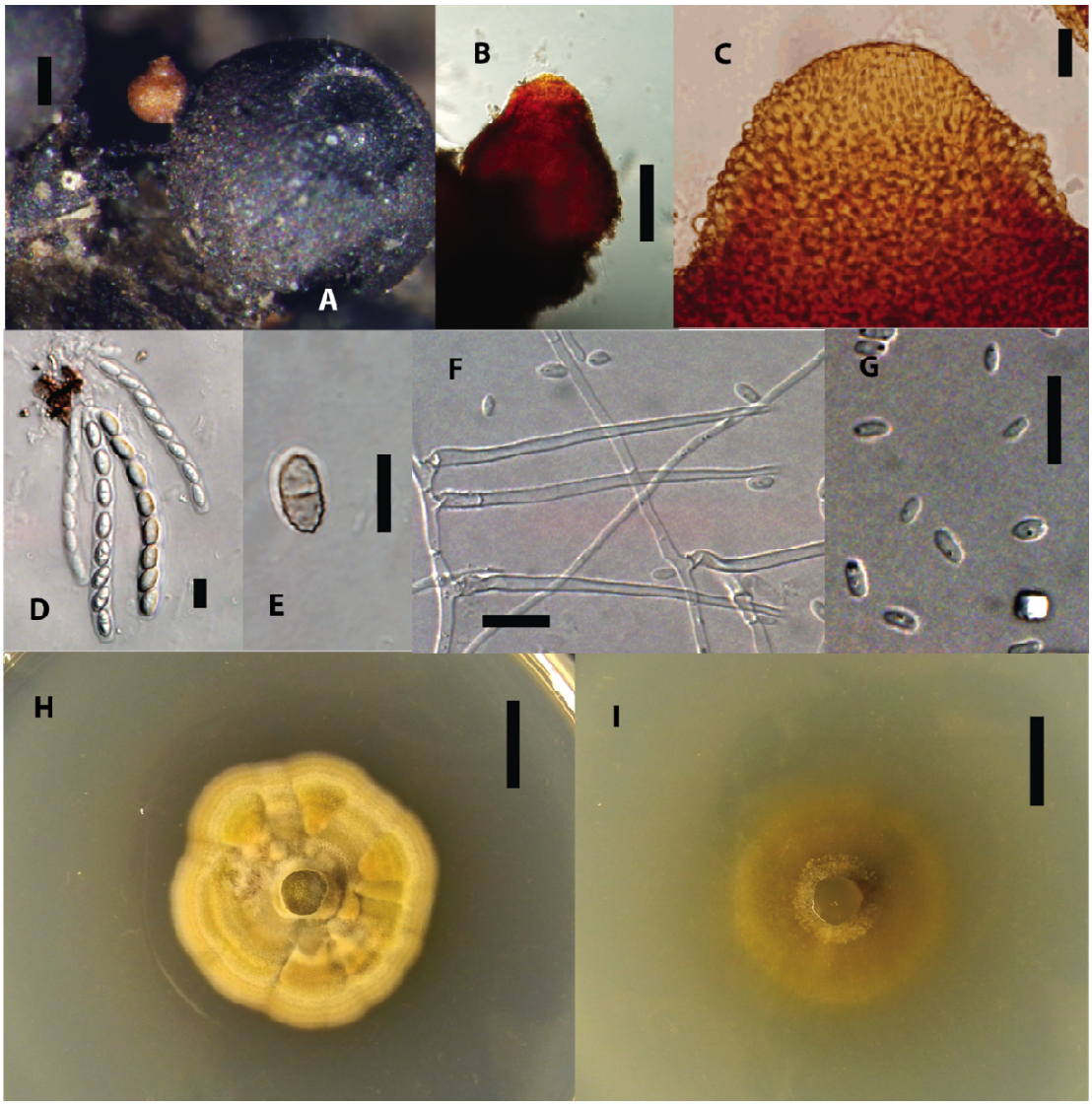


Fig. 3.10 *Cosmospora novazelandica*. (A) Perithecia on natural substrata. Scale bar = 200 μm. (B) Perithecium in 3% KOH. Scale bar = 100 μm. (C) Cells protruding around the perithecial apex. Scale bar = 10 μm. (D) Asci. Scale bar = 10 μm. (E) Ascospores. Scale bar = 10 μm. (F) Phialides and conidia. Scale bar = 10 μm. (G) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (H) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm

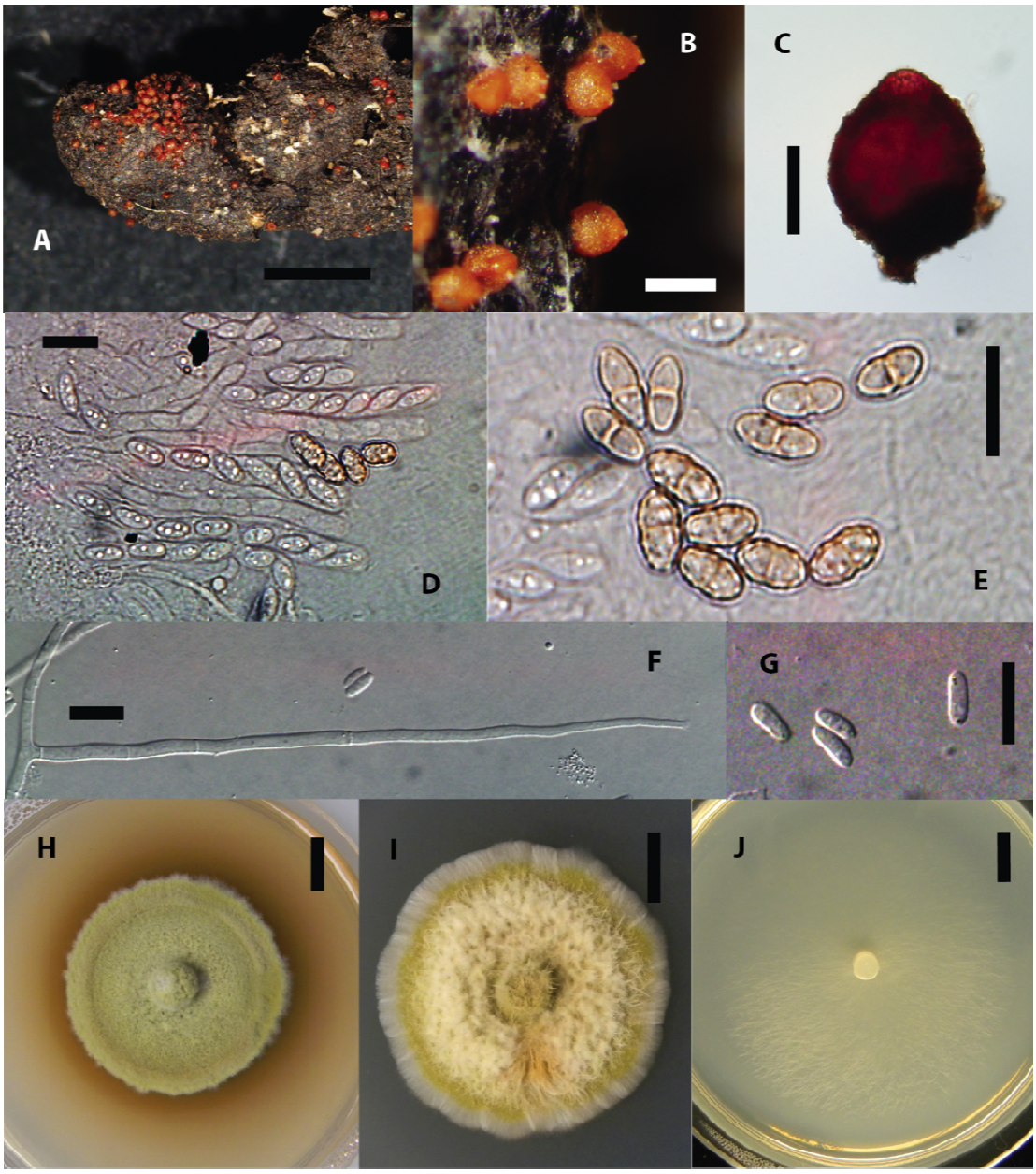


Fig. 3.11 *Cosmospora scruposae*. (A) Habit. Scale bar = 2 mm. (B) Perithecia on natural substrata. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Asci. Scale bar = 10 µm. (E) Ascospores. Scale bar = 10 µm. (F) Phialides. Scale bar = 10 µm. (G) Conidia. Scale bar = 10 µm. (H−I) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (J) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm

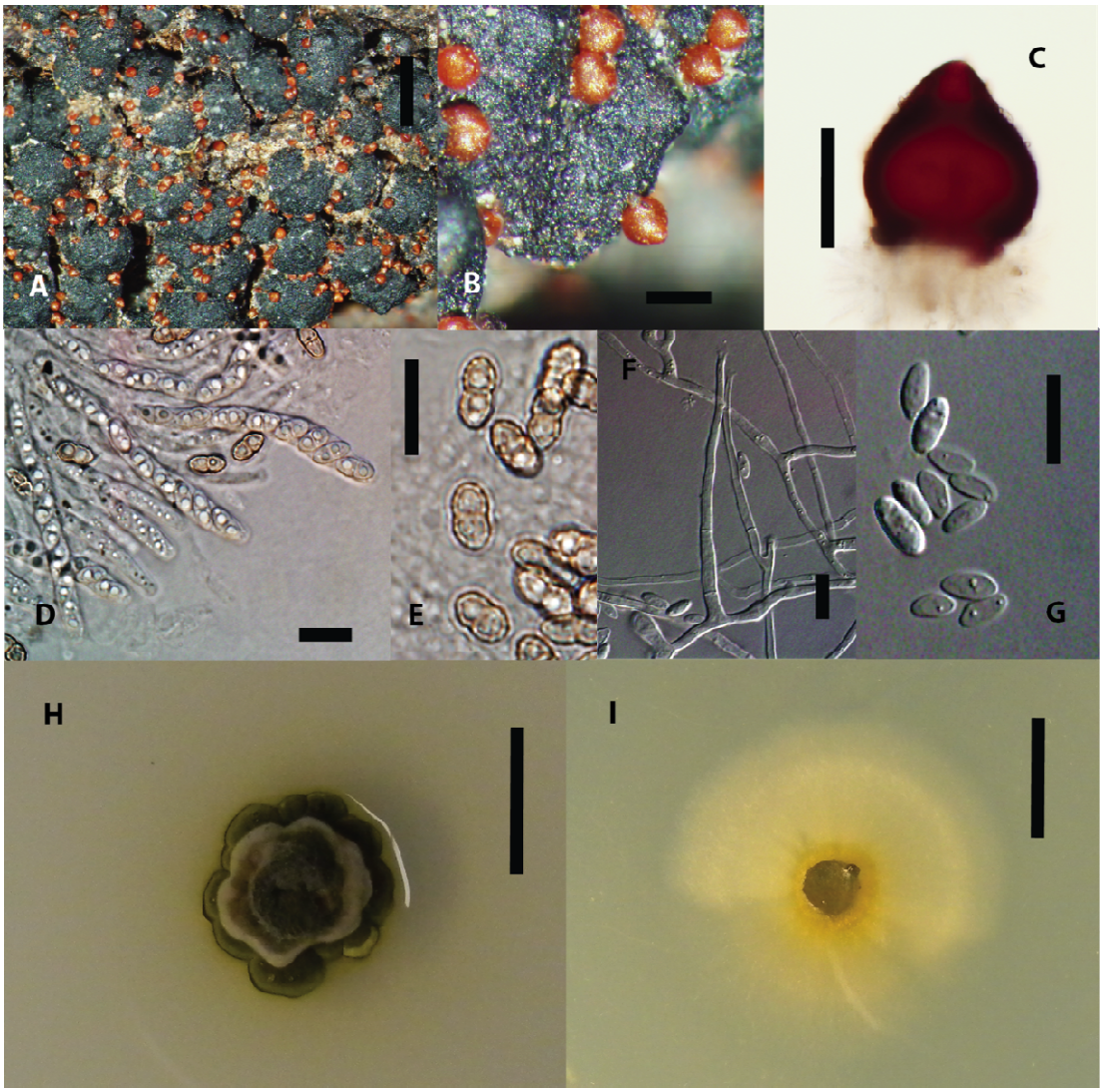


Fig. 3.12 *Cosmospora stilbohypoxyli*. (A) Habit. Scale bar = 1 mm. (B) Perithecia on natural substrata. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Asci. Scale bar = 10 µm. (E) Ascospores. Scale bar = 10 µm. (F) Phialides. Scale bar = 10 µm. (G) Conidia. Scale bar = 10 µm. (H) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (I) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm

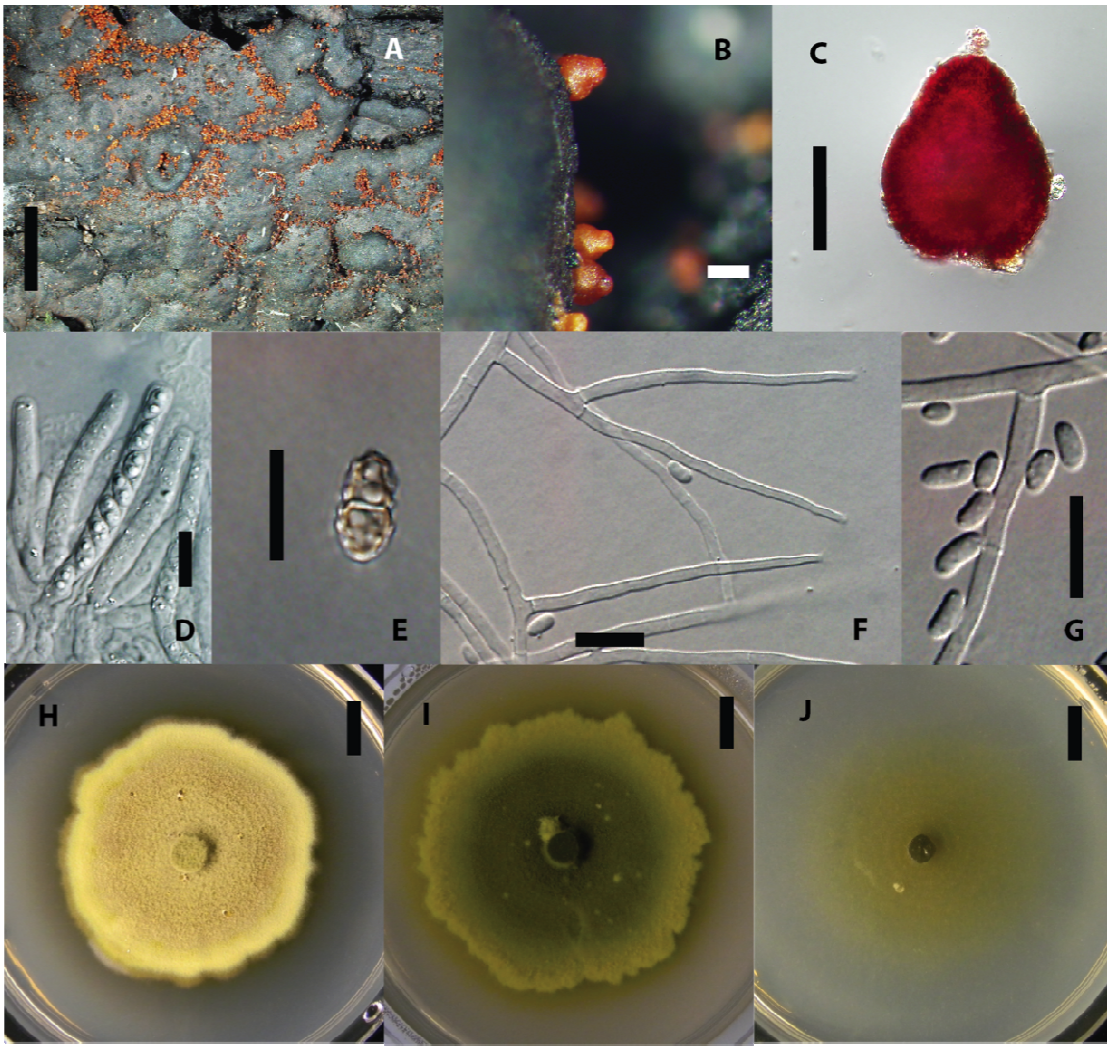


Fig. 3.13 *Cosmospora ustulinae*. (A) Habit. Scale bar = 4 mm. (B) Perithecia on natural substrata. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Asci. Scale bar = 10 µm. (E) Ascospores. Scale bar = 10 µm. (F) Phialides. Scale bar = 10 µm. (G) Conidia. Scale bar = 10 µm. (H−I) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (J) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm

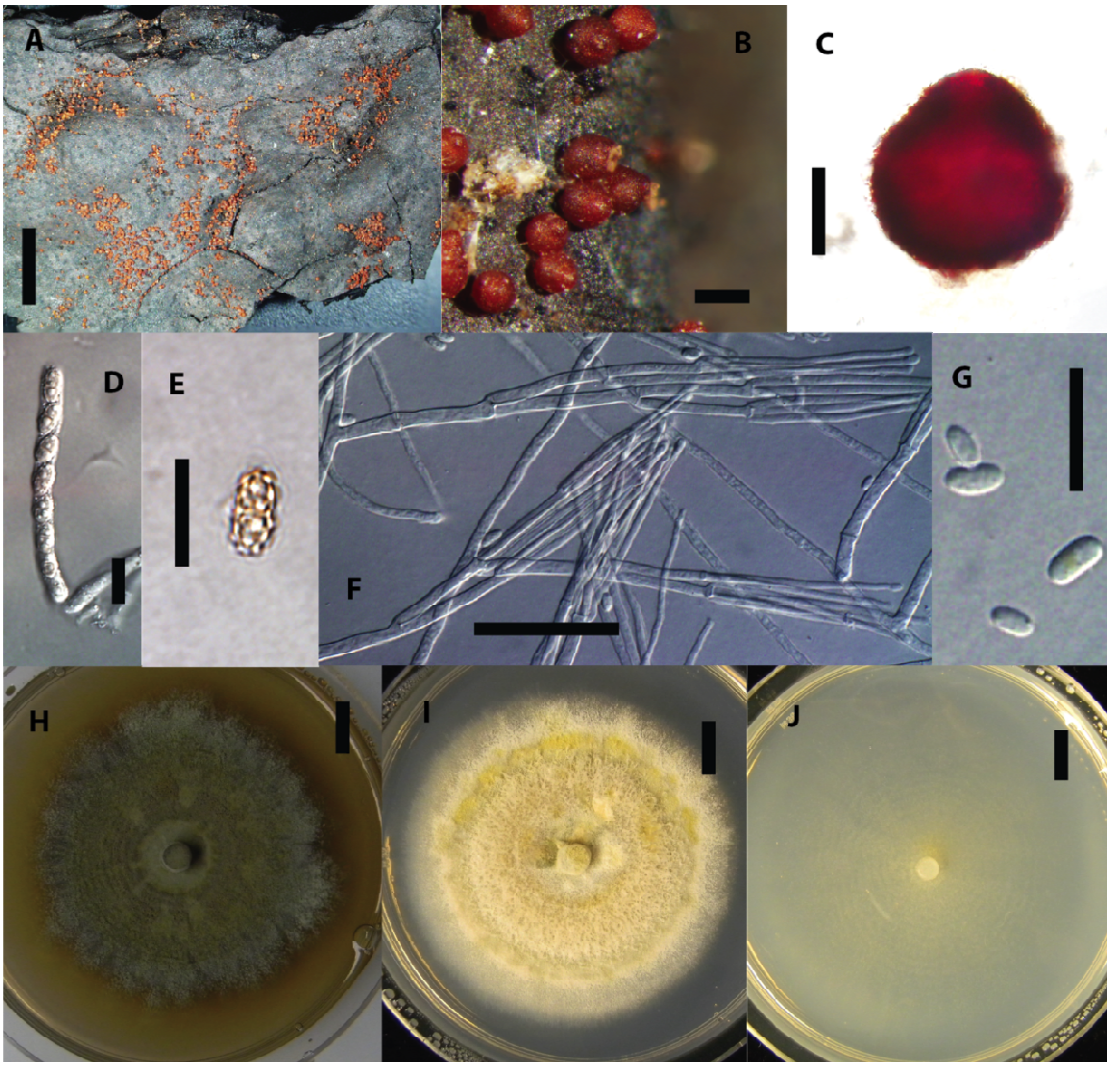
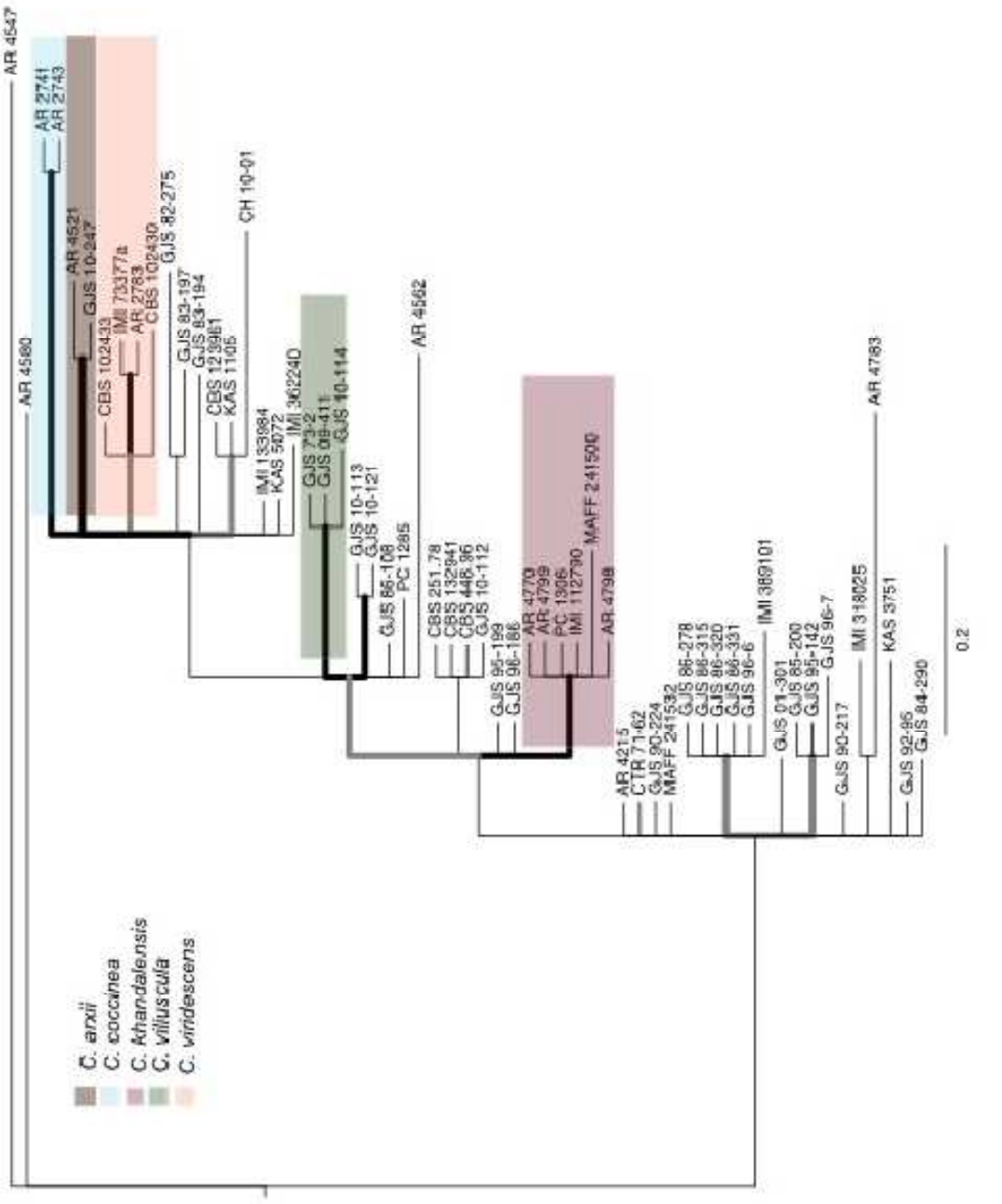


Fig. 3.14 *Cosmospora viliuscula*. (A) Habit. Scale bar = 4 mm. (B) Perithecia on natural substrata. Scale bar = 200 µm. (C) Perithecium in 3% KOH. Scale bar = 100 µm. (D) Asci. Scale bar = 10 µm. (E) Ascospores. Scale bar = 10 µm. (F) Phialides. Scale bar = 10 µm. (G) Conidia. Scale bar = 10 µm. (H−I) Cultures after 3 wks at 25 C on PDA. Scale bar = 10 mm. (J) Cultures after 3 wks at 25 C on CMD. Scale bar = 10 mm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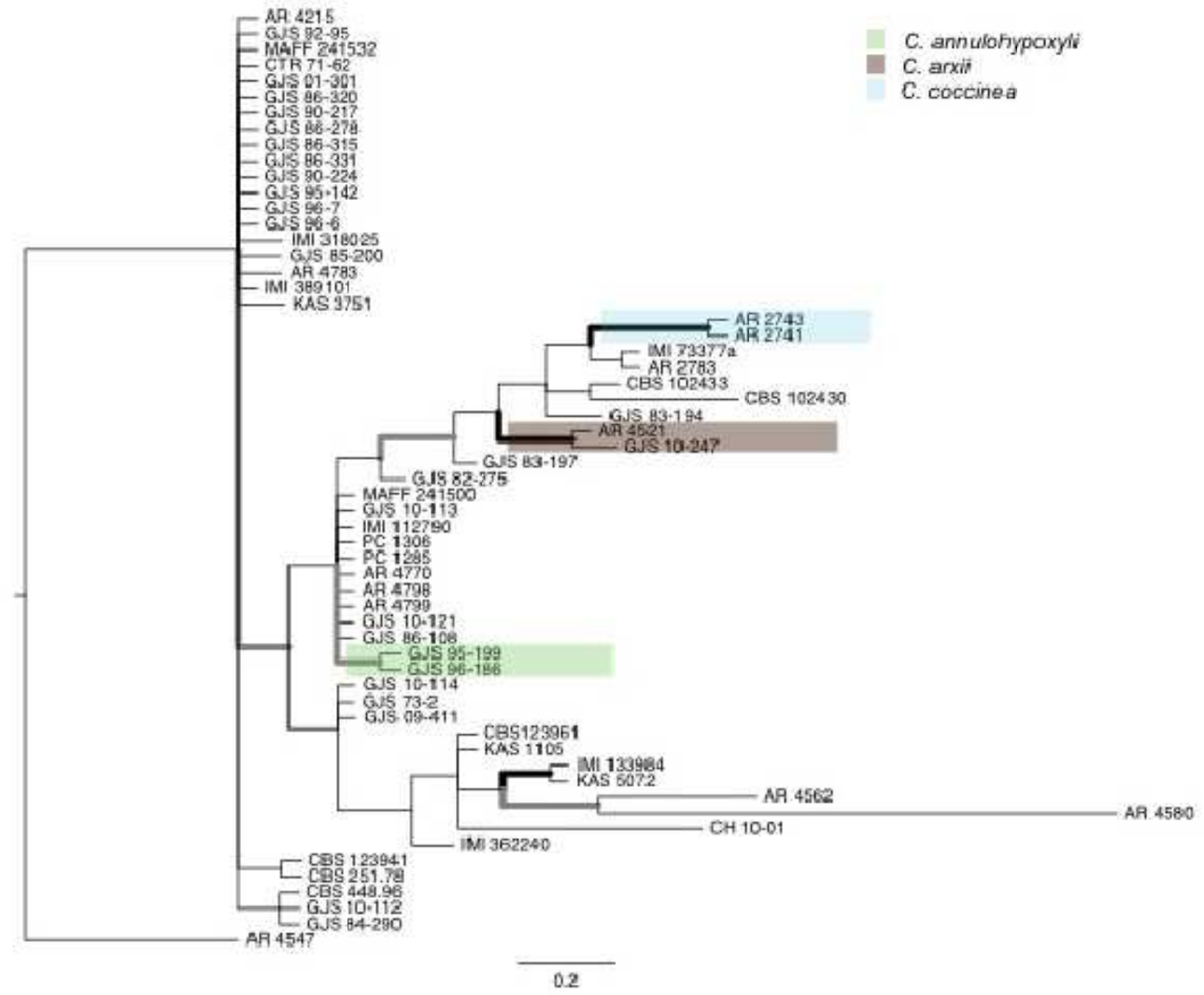
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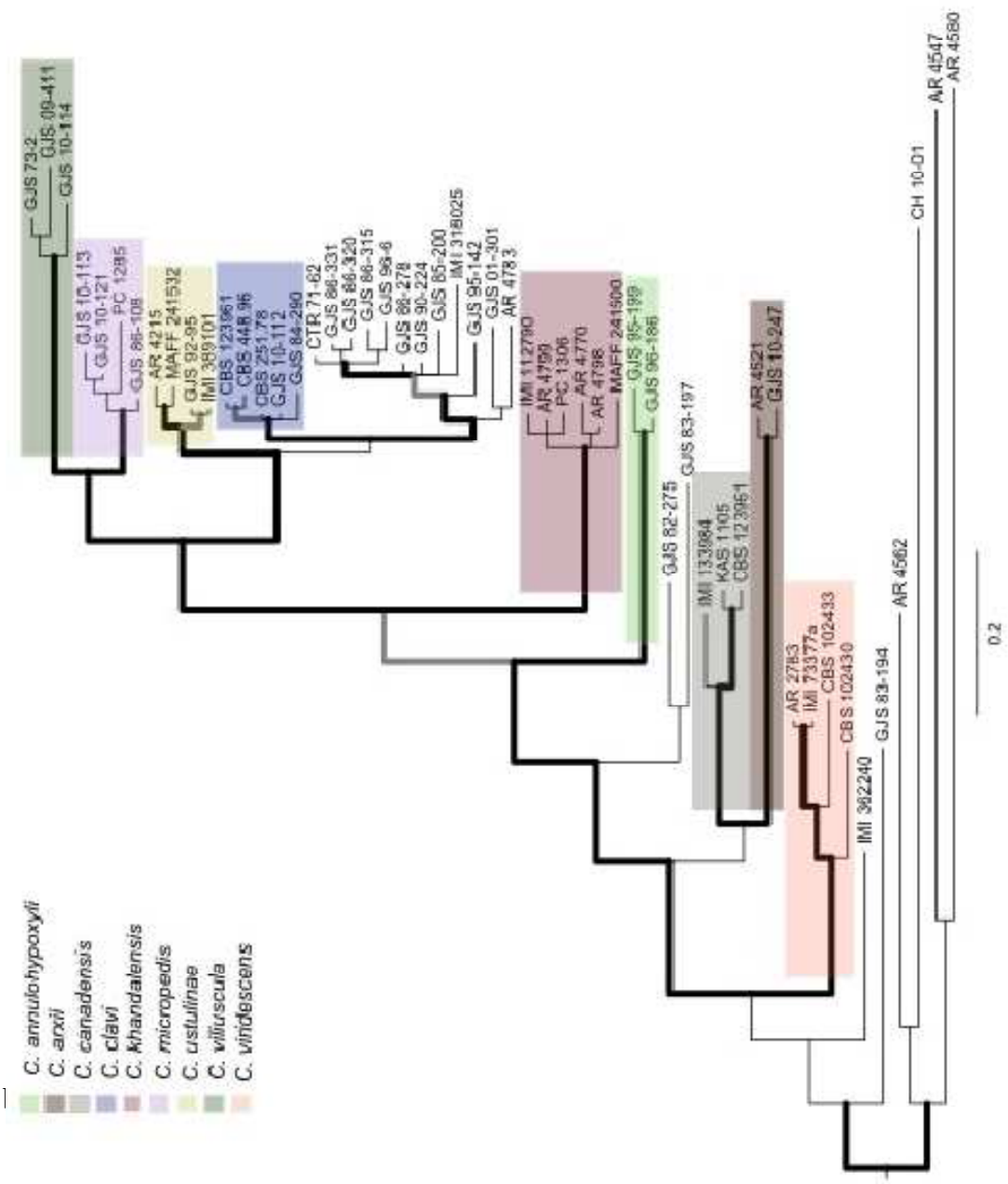
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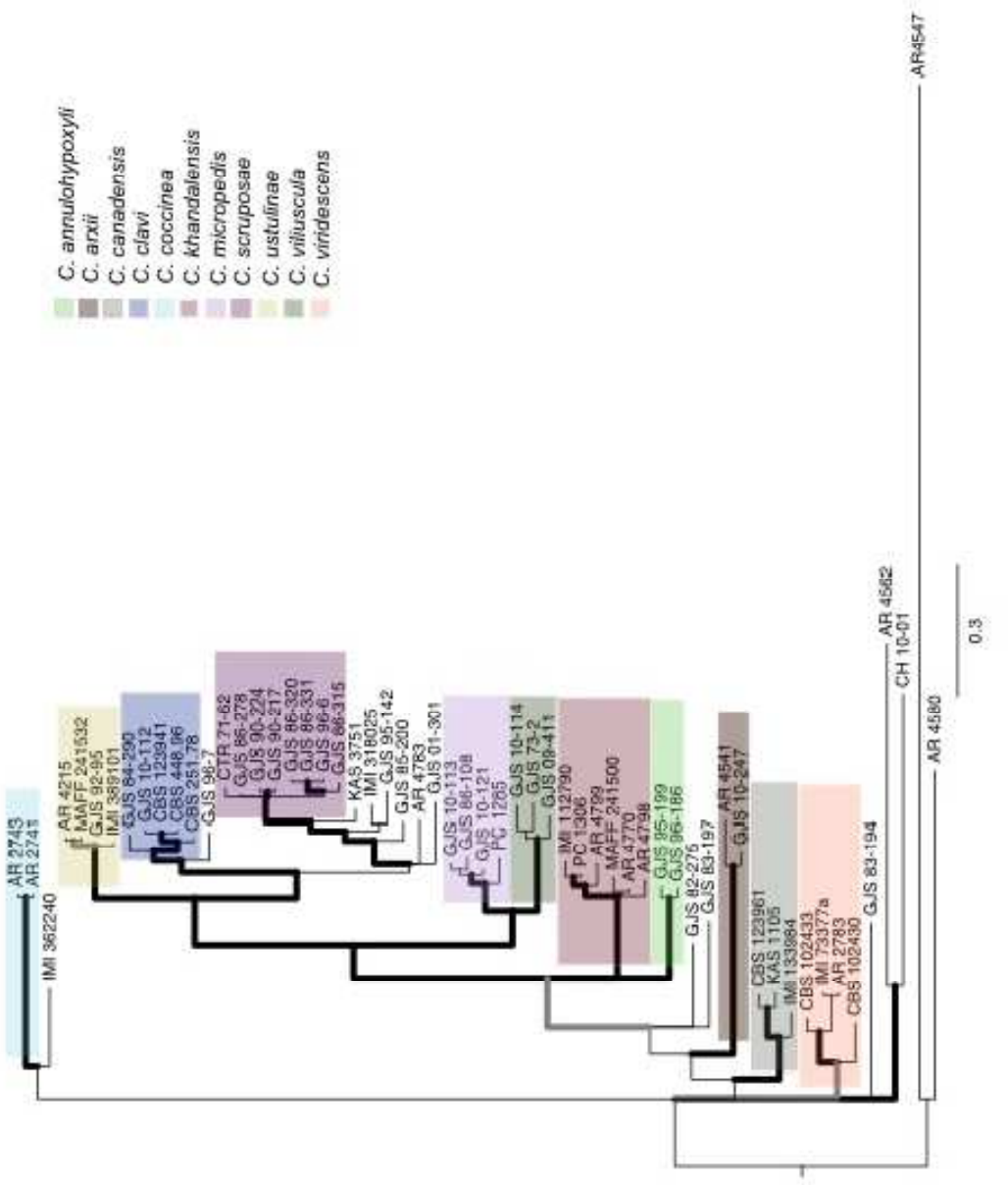
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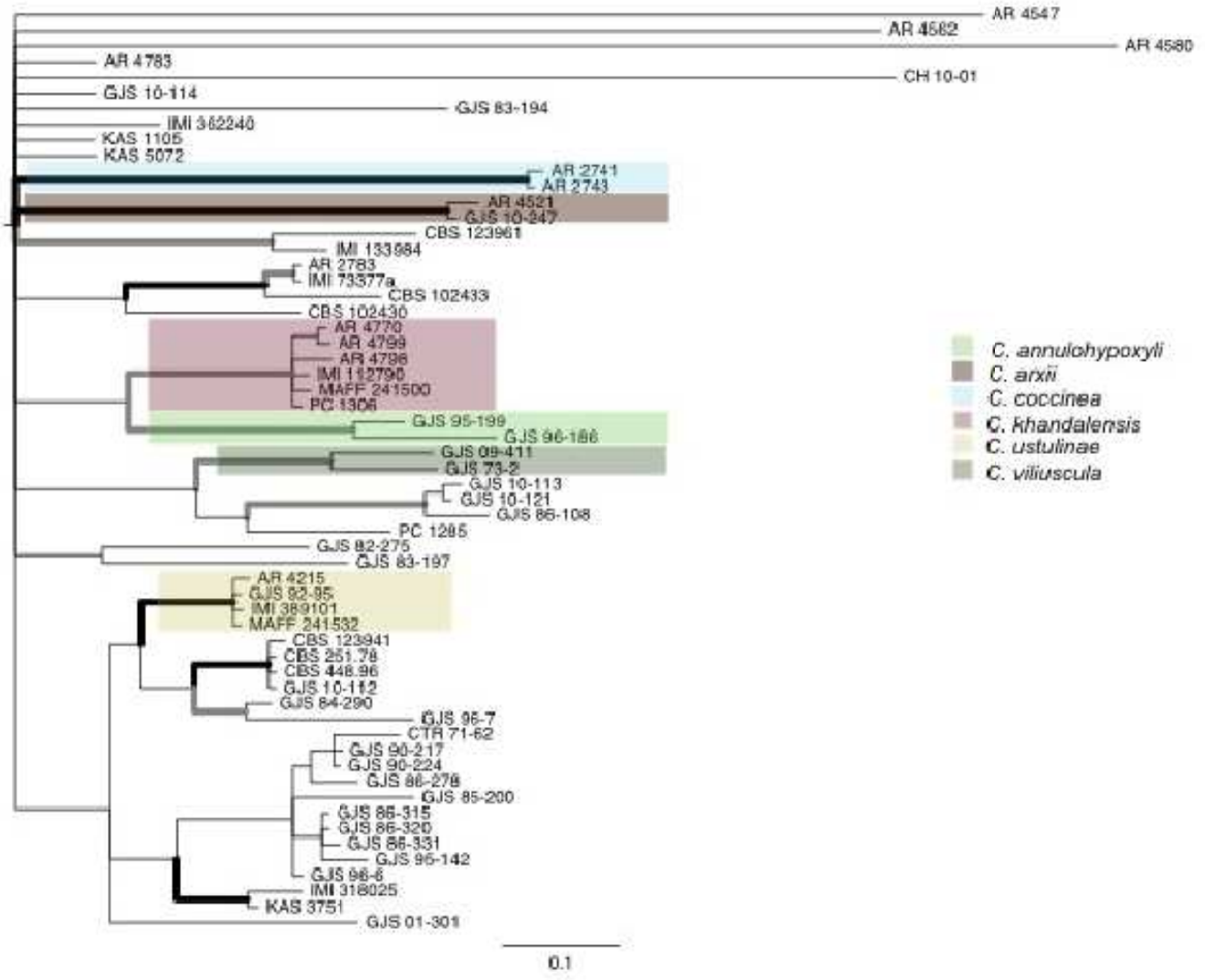
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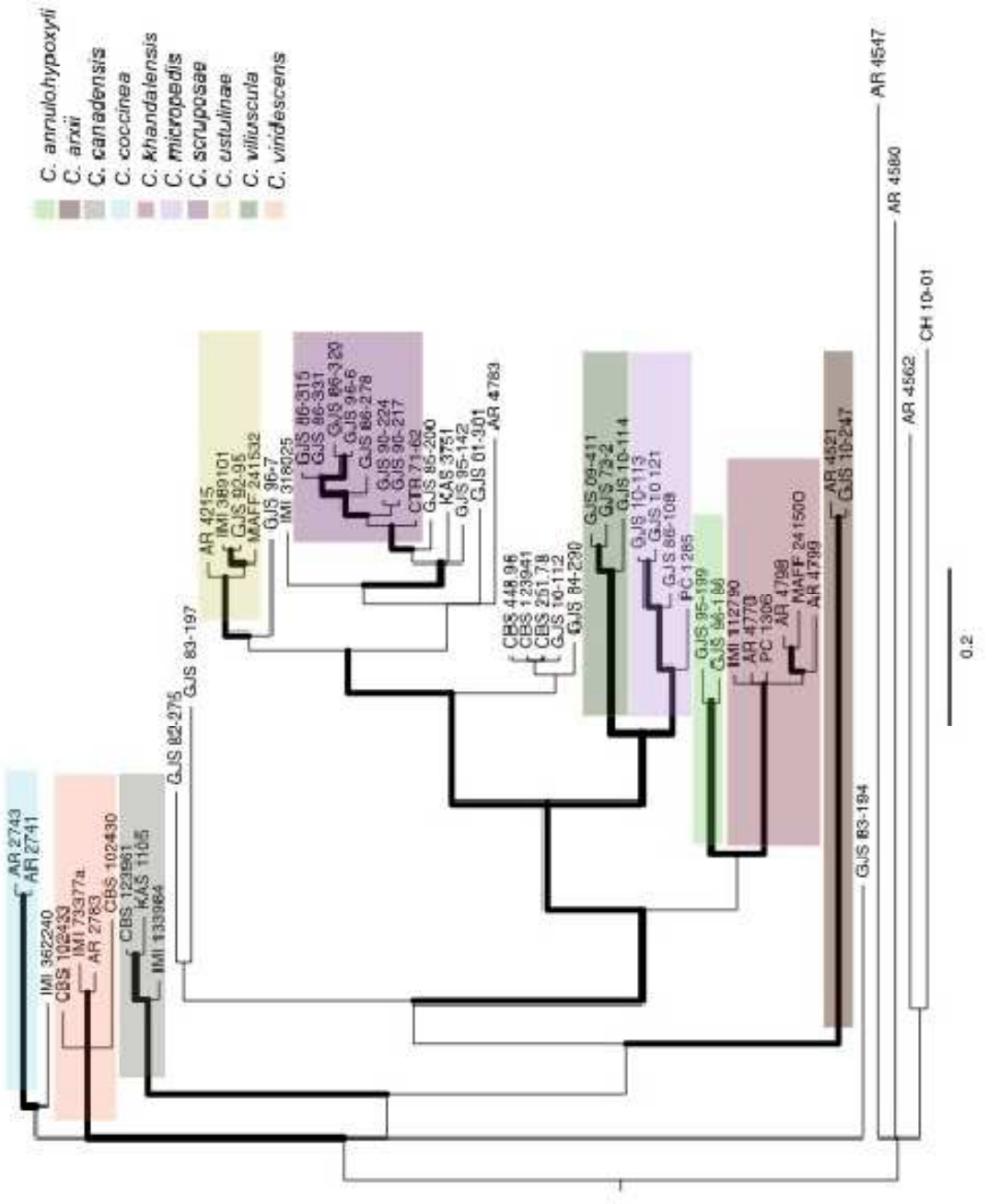
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Chapter 4: Pseudocospeciation of the mycoparasite *Cosmospora* with their associated fungal hosts.

ABSTRACT

Species of *Cosmospora* are parasites of other fungi (mycoparasites), including species belonging to the Xylariales. Based on prior taxonomic work, these fungi were determined to be highly host specific. We suspected that the association of *Cosmospora* and their hosts could not be a result of random chance, and tested the cospeciation of *Cosmospora* and the their hosts with contemporary methods (e.g. Parafit, PACo, and Jane). The cophylogeny of *Cosmospora* and their hosts was found to be congruent, but only host-parasite links in more recent evolutionary lineages of the host were determined as coevolutionary. Reconciliation reconstructions determined at least five host switch events early in the evolution of *Cosmospora*. This pattern is more likely to be explained by pseudocospeciation (i.e., host switches followed by cospeciation), which also produce congruent cophylogenies.

INTRODUCTION

Evolutionary relationships of fungus-fungus systems have been rarely studied. Millanes et al. (2014) studied the *Biatoropsis* Räsänen-*Usnea* Dill. ex Adans. system (a fungal parasite-fungal host association), and demonstrated that host-switch events played a more prevalent role than cospeciation events in their reconciliation

reconstructions of *Biatoropsis* and *Usnea* phylogenies. Also, the fungal cultivars of the fungus-growing ants (fungi belonging to the Agaricaceae and Tricholomataceae) and their associated fungal parasites, *Escovopsis* J.J. Muchovej & Della Lucia, have been shown to have highly congruent phylogenies (Currie et al. 2003). In other non-fungal systems, host-parasite relationships have also produced congruent cophylogenies (e.g. Clayton and Johnson 2003; Banks et al. 2006; Hosokawa et al. 2006; Marussich and Machado 2007; Hughes et al. 2007; Noda et al. 2007; Jackson et al. 2008; Lanterbecq et al. 2010; Göker et al. 2011), which have been taken as evidence of cospeciation between hosts and parasites. However, congruent cophylogenies can also result from other evolutionary mechanisms besides cospeciation such as coevolution and sequential evolution. Coevolution is the evolution in two or more species that leads to reciprocal evolutionary changes, and in sequential evolution, changes in one taxon leads to changes in the other taxon, but the change is not reciprocal (reviewed in Ridley 2004). Cospeciation involves the joint speciation of two or more species that are ecologically associated (e.g. host-parasites; Page 2003). There are also evolutionary events that would lead to incongruent cophylogenies: 1) duplication (independent speciation), 2) host-switching, and 3) lineage sorting (e.g. extinction and "missing the boat"; reviewed in Page 2003; reviewed in Paterson and Banks 2001).

In the present study, we studied the association between species of *Cosmospora* Rabenh. (sensu lato; a mycoparasite—a fungus that parasitizes other fungi) and their associated fungal hosts. *Cosmospora* (Ascomycota, Hypocreales, Nectriaceae) is a fungal genus that was determined to be artificial, and segregated into many

monophyletic genera (Schoch and Crous 2000; Lou and Zhuang 2010, 2012; Gräfenhan et al. 2011; Herrera et al. 2013). The sexual fruiting bodies (perithecia) in *Cosmospora* sensu lato are highly conserved to the degree of being indistinguishable. Briefly, the perithecia are reddish, small-sized (<300 microns), and pear-shaped (Fig. 4.1A). The sexual spores (ascospores) are ellipsoid to ellipsoid-fusiform, one-septate, yellow-brown and warted at maturity (Samuels et al. 1991; Rossman et al. 1999). The perithecia usually grow in-clusters on other fungi, scale insects, rarely on wood and herbaceous substrata (Rossman 1983; Samuels et al. 1991; Rossman et al. 1999). Cosmospora-like fungi are reported to be most common in recently disturbed forest stands (Chaverri and Vilchez 2006), and to have much greater diversity in warm temperate and tropical regions (Rossman et al. 1999). However, they are not infrequent outside those regions.

Most cosmospora-like fungi are mycoparasites of fungi in the families Xylariaceae and Diatrypaceae (Xylariales, Ascomycota; Gräfenhan et al. 2011). Tsuneda (1982) first described the attack by these mycoparasites. Briefly, the fruiting bodies of the fungal host are penetrated by the *Cosmospora* species, and the fleshy insides of the fungal host are slowly attacked and consumed by the *Cosmospora*'s vegetative hyphae. It is thought that the slow attack ensures an extended period of nutrient uptake. The fungal host is able to mature but not to release ascospores. Ultimately, the host's fleshy insides are replaced by vegetative hyphae of the *Cosmospora*. The mycoparasitic attack ends with the formation of its own perithecia directly on the surface of the host's fruiting bodies (Fig. 4.1B), while simultaneously consuming its own vegetative hyphae for the production of perithecia (Tsuneda 1982).

Cosmospora sensu stricto include species that grow on xylariaceous fungi (fungi belonging to the Xylariaceae, Xylariales, Ascomycota). During the taxonomic revision of these fungi (see Herrera et al. 2014), it was observed that these species demonstrated a high degree of host-specificity (i.e., their association was not random). Host-specificity is a trait that often characterizes the intimate relationship of a host and its associated parasite. Given this host-specific trait, we hypothesized that species of *Cosmospora* have cospeciated with their xylariaceous fungal hosts following Fahrenholz' rule (i.e., host and associated parasites form cophylogenies; reviewed in Ridley 2004). In this paper, we investigated associations between *Cosmospora* species and their associated xylariaceous hosts.

METHODS

Cosmospora phylogeny

Thirteen species were selected based on the availability of host data (see below). Sequences were generated in prior taxonomic work (Herrera et al. 2013; Herrera et al. 2014). Briefly, DNA was extracted from mycelium grown for one week in Difco™ potato dextrose broth with PowerPlant® DNA Isolation Kit (MO BIO Laboratories Inc., Solana Beach, California). Internal transcribed spacer (ITS), large subunit nuclear ribosomal DNA (LSU), DNA replication licensing factor (*mcm7*), RNA polymerase II Subunit one (*rpb1*), and β-tubulin (*tub2*) were amplified in an Eppendorf Mastercycler thermocycler (Eppendorf, Westbury, New York) and sequenced at the DNA Sequencing Facility (Center for Agricultural Biotechnology,

University of Maryland, College Park, Maryland). The selected species and the associated sequences are listed in Table 4.1.

Sequences were aligned via the MAFFT v.6 web service (http://mafft.cbrc.jp/alignment/server/; Katoh et al. 2002, 2013) implementing the E-INS-i alignment strategy and the 1PAM / κ=2 scoring matrix for nucleotide sequences. Alignments were manually edited in Mesquite 2.75 (Maddison and Maddison 2011). Ambiguous regions were excluded. The best-fit partitioning scheme among the sequenced loci and the model of nucleotide substitution for each partition were determined with PartitionFinder v1.1.1 (Lanfear et al. 2012) using the default settings.

Phylogenetic analysis was performed using GARLI v2.01 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl 2006) via the GARLI web service (http://www.molecularevolution.org; Bazinet and Cummings 2011), which uses a grid computing system associated with The Lattice Project (Cummings and Huskamp 2005; Bazinet and Cummings 2008). Fifty independent search replicates were performed to find the best tree with a fast ML stepwise-addition algorithm. One thousand bootstrap replicates were used in the bootstrap analysis.

Host phylogeny

Effort was made to extract DNA directly from the fruiting bodies of the host. However, in most cases, we obtained sequences of the associated *Cosmospora* species suggesting that the mycoparasite had already attacked the host. We were able to obtain ITS sequences from the hosts of *Cosmospora khandalensis* and

Pseudocosmospora joca by amplifying DNA with Illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare Bio-Sciences Corp., Piscataway, New Jersey) following the manufacturer's instructions. The identity of the host for *P. joca* was determined to be *Biscogniauxia capnodes* (Xylariaceae), while the host for *C. khandalensis* could only be identified to genus rank as *Annulohypoxylon* (Xylariaceae). In our previous taxonomic revision of the *Cosmospora viliuscula* species complex (Herrera et al., *Chapter 3*), we conservatively identified the hosts in the complex based on morphological characters. We extracted sequences (ITS, *act*A, *rpb*2, and tub2) from GenBank for these species, and these sequences are listed in Table 4.2. Phylogenetic analysis was performed as described for the mycoparasite (above).

Cophylogenetic analyses

Thirteen host species and thirteen *Cosmospora* species were included in the cophylogenetic analyses. We performed two distance-based methods: PACo (Balbuena et al. 2013) and ParaFit (Legendre et al. 2002). Additionally, two tree-reconciliation methods were performed: Jane v.4 (Conow et al. 2010) and CoRe-PA v0.5.1 (Merkle et al. 2010). A tanglegram between *Cosmospora* species and their associated host was generated with TreeMap v3.0β (Charleston 2011).

Distance-based methods were implemented in R (*R Core Team* 2013) with the APE package (Paradis et al. 2004). Host and parasite phylogenies were transformed into matrices of patristic distances, and transformed again into principle coordinates to describe the phylogenies. The host principle coordinates, parasite principle

coordinates, and host-parasite association matrices were used to test the degree of congruence between the host and parasite phylogenies with a global host-parasite statistic, and the significance of the statistic was determined using a permutation test. 100000 permutations were run for PACo, whereas 999 permutations were run for ParaFit. PACo and ParaFit algorithms test the null hypothesis that the host and parasite phylogenies are independent (or randomly associated).

In an evaluation of tree reconciliation methods (CoRe-PA, Jane and TreeMap), CoRe-PA was determined to be the most precise tool available in predicting the associations between hosts and parasites, although it does not produce an optimal estimate of the number of cospeciation and switching events. Jane was determined to yield the correct estimate of cospeciation events (Keller-Schmidt et al. 2011). Because they are based on the optimality criterion of Maximum Parsimony, these methods seek to find the cophylogeny with the minimum cost. CoRe-PA and Jane assign costs to four evolutionary events: cospeciation, duplication, host switch and sorting. Additionally, Jane assigns a cost to failure to diverge. We used the default cost settings in CoRe-PA and Jane.

RESULTS

Phylogenetic analyses

PartitionFinder determined three partitions in the *Cosmospora* supermatrix, which included 3217 total characters (ITS: 570; LSU: 782; *mcm7*: 615; *rpb1*: 690; and *tub2*: 560). These partitions were ITS, LSU, and *mcm7+rpb1+tub2*. The best model of nucleotide substitution was TIMef+I, K80+I, and TrNef+G, for each partition

respectively. The negative log likelihood for the best tree was −10885.2025. *Cosmospora* lineages were well supported with some exceptions (Fig. 4.2).

Three partitions were determined for the host supermatrix that comprised 3159 total characters (ITS: 466; *act*A: 301; *rpb2*: 1199; and *tub2*: 1193). These partitions were ITS, *act*A+*rpb*2, and *tub2*. TIMef+G, K80+I, and TrN+G were selected as the best models for each partition, respectively. The negative log likelihood for the best tree was −12636.6736. Lineages of xylariaceous fungi were well supported with some exceptions (Fig. 4.2).

Distance-based analyses

A procrustean superimposition plot of axes one and two, corresponding to patristic distances of *Cosmospora* and their fungal hosts, suggested three groups of host-parasite associations (Fig. 4.3). One group is composed of *Cosmospora* species associated with *Annulohypoxylon* and *Hypoxylon*. Another group is composed of *Cosmospora* species associated with *Kretzschmaria*, *Stilbohypoxylon* and *Xylaria*. A third group is composed of *Dialonectria episphaeria*, *Pseudocosmospora* and their associated hosts.

Distance-based methods supported an overall congruence between the phylogenies of *Cosmospora* and their associated hosts. The PACo analysis produced a residual sum of squares (m^2_{XY}) of 0.4193 with an associated permutational $P = 0.00001$. Similarly, the ParaFit global fit statistic was 0.0275 ($P = 0.005$). The contribution of each host-parasite to the global fit was assessed with a jackknife procedure applied in PACo, which estimated the squared residual and its 95% confidence interval of each

individual link (Fig. 4.4). Most links associated with *Kretzschmaria*, *Stilbohypoxylon* and *Xylaria* hosts contributed relatively little to the residual sum of squares. The *Eutypa lata-Pseudocosmospora eutypae* and *Eutypella scoparia-Pseudocosmospora eutypellae* links were also determined to contribute relatively little to the residual sum of squares. ParaFitLink1 analysis also considered these links + *Kretzschmaria deusta-Cosmospora ustulinae* as coevolutionary at 0.05 significance level.

Tree reconciliation analyses

The Tanglegram between *Cosmospora* and host phylogenies showed some internal congruence (Fig. 4.2). The reconciliation of the *Cosmospora* tree with the host tree revealed a maximum of seven cospeciation events might have happened in their evolution (Fig. 4.5). This reconciliation also contained five host-switches and three sorting events. The total cost for this reconciliation was 18 in CoRe-Pa and 13 in Jane. Jane generated another equally parsimonious reconciliation between *Cosmospora* and host trees (Fig. 4.6). This reconciliation had six cospeciations, six host-switches, and one sorting event. In only one instance out of 100 did a better random sample solution produced a reconciliation cost below 13 ($P = 0.01$).

DISCUSSION

Distance based methods confirmed that the phylogenies of *Cosmospora* and the fungal host were more congruent than expected by chance (PACo, $P = 0.00001$; ParaFit, $P = 0.005$). The global congruence between host and parasite phylogenies have been interpreted as a result of cospeciation in many studies prior to this one (e.g. ants and plants, Itino et al. 2001; fungi and plants, Jackson 2004; penguins and their

lice, Banks et al. 2006; mycoviruses and their fungal hosts, Göker et al. 2011; among others). However, not all individual host-parasite links were found to be coevolutionary (Fig. 4.4). Most host-parasite links considered coevolutionary included *Cosmospora* associated with *Kretzschmaria*, *Stilbohypoxylon*, and *Xylaria* hosts. These host genera represent recent evolutionary lineages of the Xylariaceae (Tang et al. 2009; Hsieh et al. 2010). Charleston and Robertson (2002) observed a similar global congruency of host-parasite cophylogenies and codivergences occurring at the tip of the host phylogeny. Given that there was a large difference in evolutionary rates between host and parasites, Charleston and Robertson (2002) determined that the observed evolutionary pattern could not be explained by cospeciation events alone, and suggested that this pattern was a result of host-switches followed by cospeciation events. Cospeciation is expected to have congruent phylogenies but also to have similar divergence times (reviewed in Page 2003). Similar congruent topologies as seen in cospeciation could arise as a result of host-switches followed by cospeciation events (or pseudocospeciation) but not have similar divergence times (Hafner and Nadler 1988; reviewed in Page 2003; de Vienne et al. 2007, 2013).

Tree reconciliation based methods also supported the idea that the cophylogeny between *Cosmospora* and their fungal hosts could not be interpreted from strict cospeciation events (Fig. 4.5 and 4.6). The reconciled trees contained five–six host switch events (Fig. 4.5 and 4.6), which occurred early in the host phylogeny. Cospeciation events were more prevalent towards the tip of the host phylogeny. Divergence time estimates could not be determined in the current study due the lack

of fossil records for fungi in general (Taylor and Berbee 2006). Calibration points are needed within the in-group of study to obtain more accurate estimates of divergence times. Therefore, we cannot determine whether or not *Cosmospora* and the host have similar divergent times or not. Strict speciation cannot be ruled out, but strict cospeciation is probably unlikely given the relatively high number of suspected host switch events in the reconciliation reconstructions. Pseudocospeciation represents a better hypothesis to explain the apparent congruency between *Cosmospora* and the host phylogenies.

Pseudocospeciation is often confused in the literature as cospeciation given the significant global congruency between host and parasite phylogenies, even though the parasites have been shown to diverge more recently than the host (e.g. Reed et al. 2007; Light and Hafner 2008). The lack of congruency in divergence times (or temporal congruency) between host and parasites should have refuted the hypothesis of cospeciation (e.g. Charleston and Robertson 2002; Sorenson et al. 2004; Huyse and Volckaert 2005). De Vienne et al. (2013) reviewed cospeciation literature, and determined than only seven percent of the literature represented convincing cases of cospeciation. These cases involved symbionts that were transmitted vertically, which does seem to be the case for *Cosmospora*. In contrast, Hafner and Nadler (1988) posited that pseudocospeciation resulted from host switches by the symbiont onto closely related hosts of the original host (horizontal transmission) followed by speciation on the new host. The resulting phylogenies of the host and the symbiont resemble the phylogenetic signature of cospeciation (i.e. cophylogenies) as result of the conserved host switching of the symbionts (Hafner and Nadler 1988; Charleston

and Robertson 2002; Sorenson et al. 2004; Huyse and Volckaert 2005). Host switching consists of a two-step process (reviewed in Norton and Carpenter 1998). Firstly, the acquisition of a new host by the parasite requires that the new host is found within the parasite's range and is related to the old host (i.e. phylogenetically similar; e.g. Davies and Pedersen 2008), or has a similar ecological habitat to the old host (i.e. ecologically similar; e.g. Nikoh and Fukatsu 2000). Secondly, the parasite has to adapt to the new host in a way that diminishes gene flow between populations on the old host and populations on the new host. Ultimately, the parasite on the new host will speciate as a result of limited gene flow over time. Host switching involves an initial decrease in host specificity during the colonization of a new host, and an increase in host specificity as speciation on the new host occurs (Norton and Carpenter 1998).

This study represents a preliminary account of the evolutionary relationships between cosmospora-like fungi and their associated hosts, and further study of this group of fungi is likely to yield intriguing and complex results. Some species of *Cosmospora* sensu stricto are associated with basidiomycetes (Basidiomycota, Mycota; Herrera et al. 2014; Gräfenhan et al. 2011), which could represent a putative inter-phylum host-switch early in the evolution of *Cosmospora*. In other fungi, rapid speciation was observed after host switches, particularly those exploiting new adaptive zones (Zaffarano et al. 2008; Chaverri and Samuels 2013). Additionally, species of *Microcera* Desm. (Nectriaceae, Hypocreales, Ascomycota), a former group of fungi of *Cosmospora* sensu lato, are parasites of scale insects (Coccoidea, Hemiptera,

Insecta; Gräfenhan et al. 2011) and lichens (unpublished data). This lineage of cosmospora-like fungi could represent a putative interkingdom host switch.

Table 4.1. Isolates and accession numbers used in the phylogenetic analyses										
Species	**ID code**	**Isolate No.**	**Herbarium No.**	**Host**	**Geographic origin**	**GeneBank Accession No.**				
						ITS	**LSU**	***mcm7***	***rpb1***	***tub2***
Cosmospora annulohypoxili	Can	G.J.S. 96-186, CBS XXXXXX	BPI 744521	Annulohypoxylon cf. cohaerens	USA	JN995635	JN939816	JN993308	JQ031065	KJ676285
Cosmospora arxii	Car	G.J.S. 10-247, CBS XXXXXX	BPI XXXXXX	Hypoxylon fragiforme	USA	JN995629	JN939824	JN993326	KC291869	KC291908
Cosmospora clavi	Ccl	G.J.S. 84-290, CBS XXXXXX	G.J.S. 1076 (NY)	Kretzschmaria clavus	Brazil	KJ676159	KJ676196	KJ676316	KJ676233	KJ676275
Cosmospora khandalensis	Ckh	A.R. 4799, CBS XXXXX	BPI XXXXXX	Annulohypoxylon sp.	Argentina	KJ676146	KJ676183	KJ676303	KJ676220	KJ676259
Cosmospora novaezelandica	Cno	G.J.S. 83-197, CBS 124032	PDD 46401	Annulohypoxylon bovei	New Zealand	KC291732	KC291777	KJ676315	KC291868	KC291907
Cosmospora scruposae	Csc	G.J.S. 86-331, CBS XXXXXX	GJS 4487 (NY)	Xylaria scruposa	French Guiana	JN995631	JN939822	-	JQ031070	KJ676280
Cosmospora sp.	Csp	KAS 3751	-	Xylaria cf. polymorpha	Canada	KJ676173	KJ676210	-	KJ676247	KJ676294
Cosmospora stilbohypoxili	Cst	A.R. 4783, CBS XXXXXX	BPI XXXXXX	Stilbohypoxylon quisquiliarum	Argentina	KJ676144	KJ676181	KJ676301	KJ676218	KJ676257
Cosmospora ustulinae	Cus	A.R. 4215, CBS XXXXXX	BPI 871089	Kretzschmaria deusta	USA	JN995619	JN939841	-	JQ031088	KJ676254

Table 4.1. Continued

Species	ID code	Isolate No.	Herbarium No.	Host	Geographic origin	GeneBank Accession No.				
						ITS	LSU	*mcm7*	*rpb1*	*tub2*
Dialonectria episphaeria	Dep	G.J.S. 10-193, C.H. 10-01	-	Diatrype stigma	USA	KC291744	KC291771	KC479773	KC291892	KC291932
Pseudocosmospora eutypae	Pe1	C.H. 11-01	BPI 884164	Eutypa sp.	France	KC291735	KC291766	KC291805	KC291884	KC291925
Pseudocosmospora eutypellae	Pe2	A.R. 4562	BPI 884165	Eutypella sp.	USA	KC291721	KC291757	KC291796	KC291871	KC291912
Pseudocosmospora joca	Pjo	A.R. 4779	BPI 884175	Biscogniauxia sp.	Argentina	KC291746	KC291762	KC291801	KC291887	KC291924

Table 4.2. Isolates and accession numbers used in the phylogenetic analyses

Species	ID code	Isolate/ specimen no.	Geographic origin	Associated with:	GeneBank Accession No.			
					ITS	*act*	*tub2*	*rpb2*
Stilbohypoxylon quisquiliarum	Squ	172 (JDR)	French Guiana	*Cosmospora stilbohypoxili*	EF026119	EF025590	EF025605	GQ853020
Kretzschmaria clavus	Kcl	114 (JDR)	French Guiana	*Cosmospora clavi*	EF026126	EF025596	EF025611	GQ844789
Kretzschmaria deusta	Kde	JF05154	France	*Cosmospora ustulinae*	-	-	DQ840092	-
Annulohypoxylon bovei	Abo	YMJ 90081914	Taiwan	*Cosmospora novaezelandica*	EF026141	AY951765	AY951654	-
Hypoxylon fragiforme	Hfr	YMJ 387	France	*Cosmospora arxii*	JN979419	AY951831	AY951719	-
Biscogniauxia capnodes	Bca	YMJ 138	Taiwan	*Pseudocosmospora joca*	EF026131	AY951787	AY951675	JX507779
Xylaria polymorpha	Xpo	1012 (JDR)	USA	*Cosmospora sp.*	GU322460	GQ452364	GQ495954	GQ848343
Xylaria scruposa	Xsc	CLL5025	Martinique	*Cosmospora scruposae*	GU322458	GQ452362	GQ495952	GQ848341
Annulohypoxylon cohaerens	Aco	YMJ 310	France	*Cosmospora annulohypoxili*	EF026140	AY951766	AY951655	GQ844766
Annulohypoxylon sp.	Asp	AR4799b	Argentina	*Cosmospora khandalensis*	XXXXXXX	-	-	-
Diatrype stigma	Dst	UCDDCash 200	USA	*Dialonectria episphaeria*	DQ006945	-	DQ007003	-
Eutypa lata	Ela	CBS 289.87	France	*Pseudocosmospora eutypae*	DQ006928	-	DQ006973	-
Eutypella scoparia	Esc	DFMAL100	USA?	*Pseudocosmospora eutypellae*	GQ293962	-	GQ294029	-

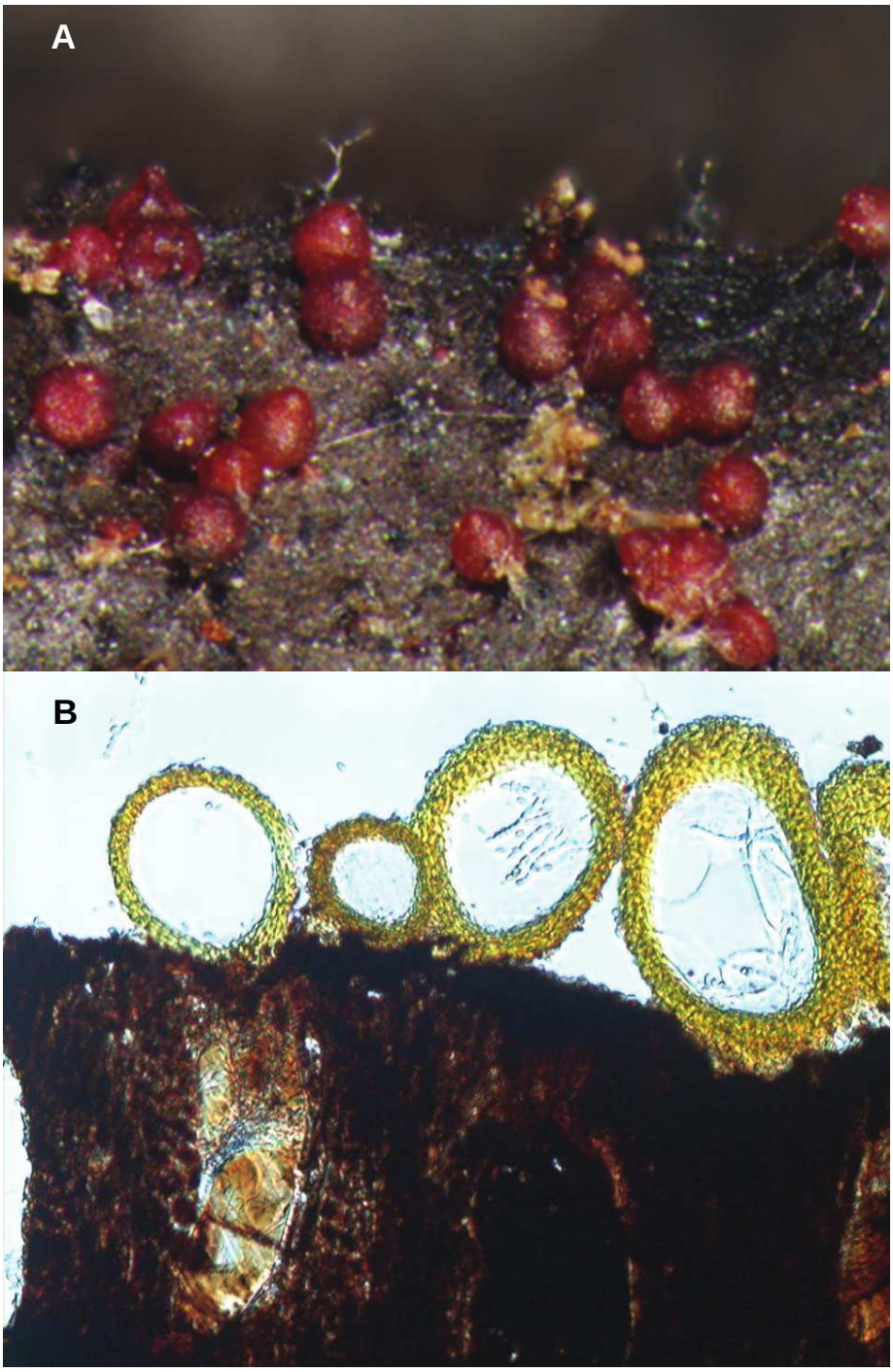
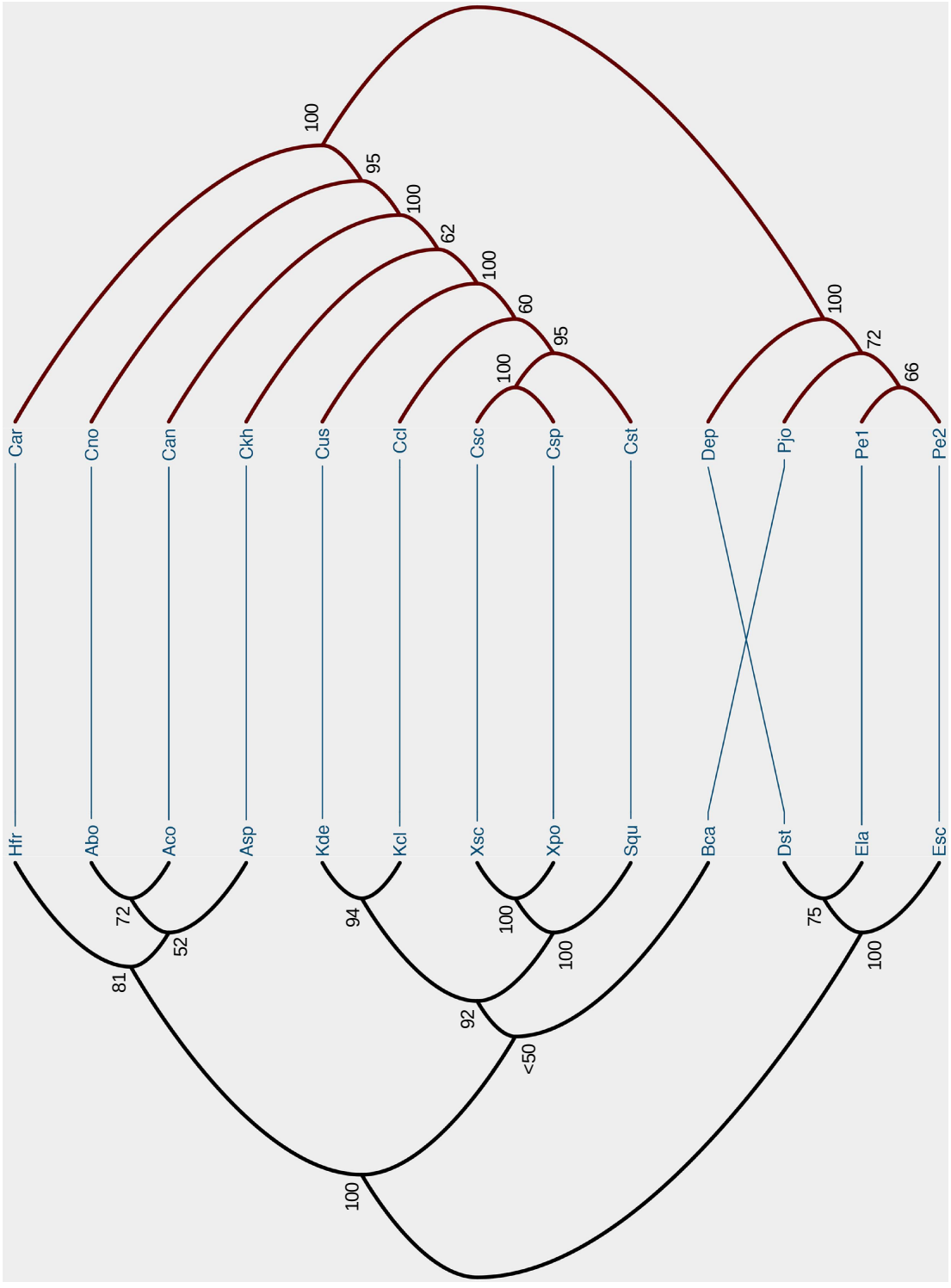


Fig. 4.1. *Cosmospora* species. A. *Cosmospora* perithecia (reddish). **B.** Median section of *Cosmospora* perithecia (stained in Lactic Acid: yellow) and fruiting body of xylariaceous host (dark). *Cosmospora* perithecia growing directly above the host perithecia (empty spaces).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52
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92
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75
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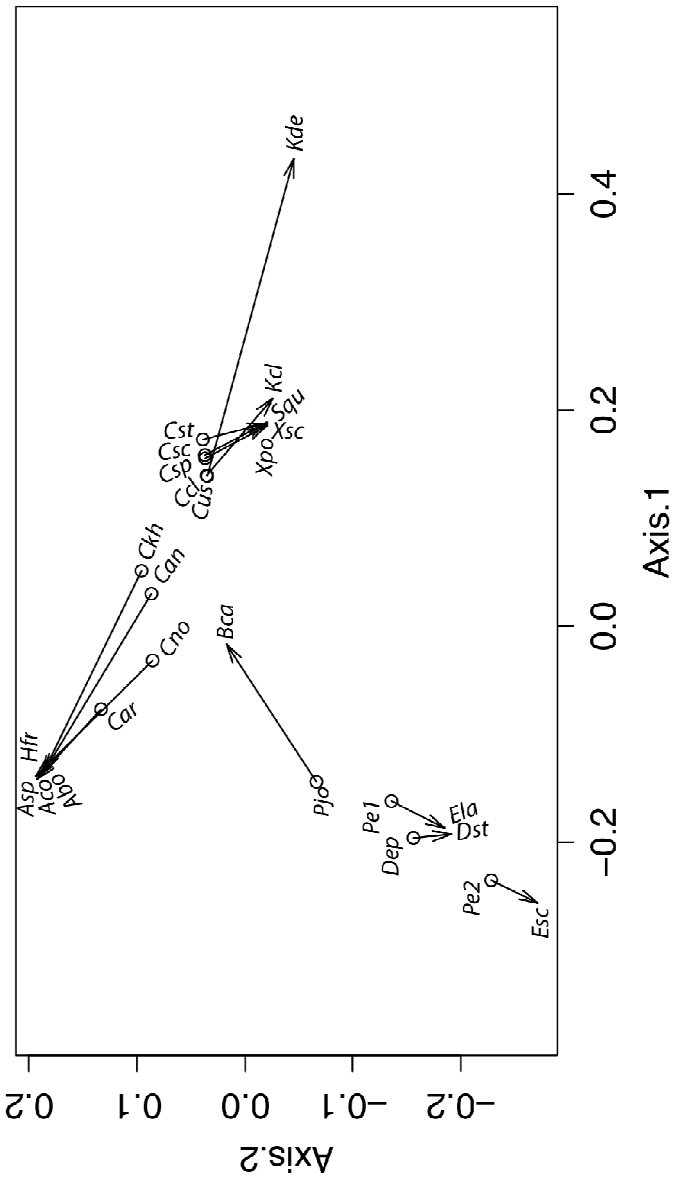
Fig. 4.2. Tanglegram between *Cosmospora* (red) and host (black) phylogenies. Solid lines between *Cosmospora* species and the associated host indicate host-parasite associations. ML bootstraps are provided for each node. See Tables 1 and 2 for abbreviations of taxa.



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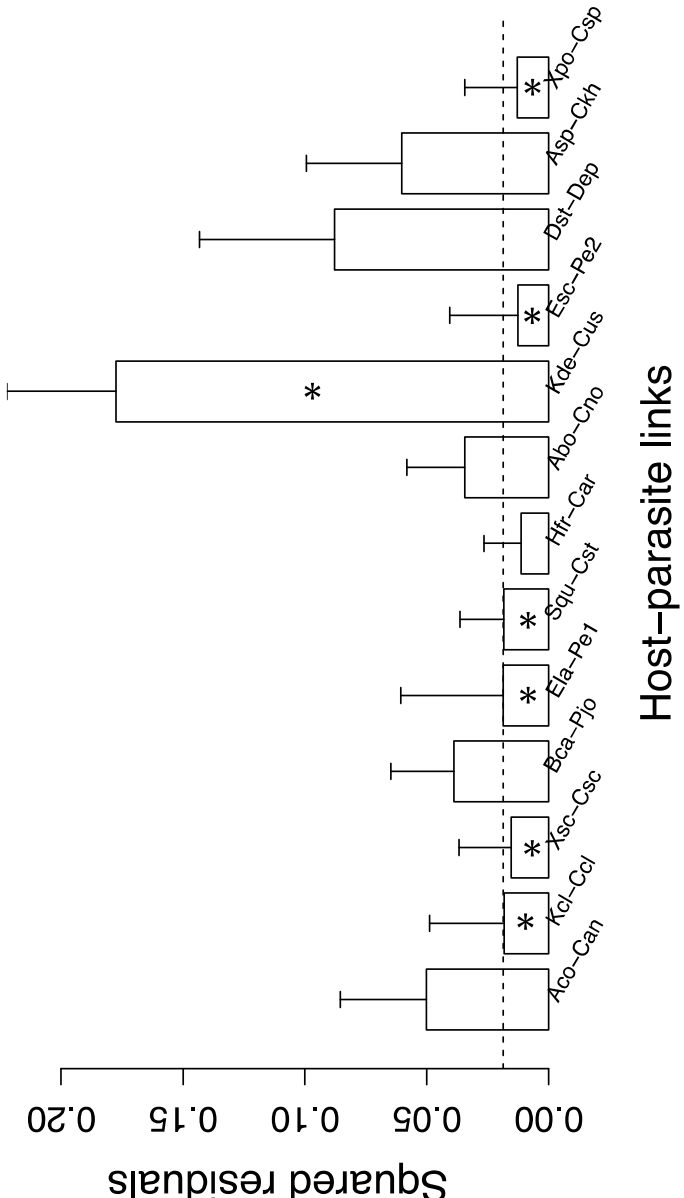

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value is shown as a dashed line. See Tables 1 and 2 for abbreviations of taxa.

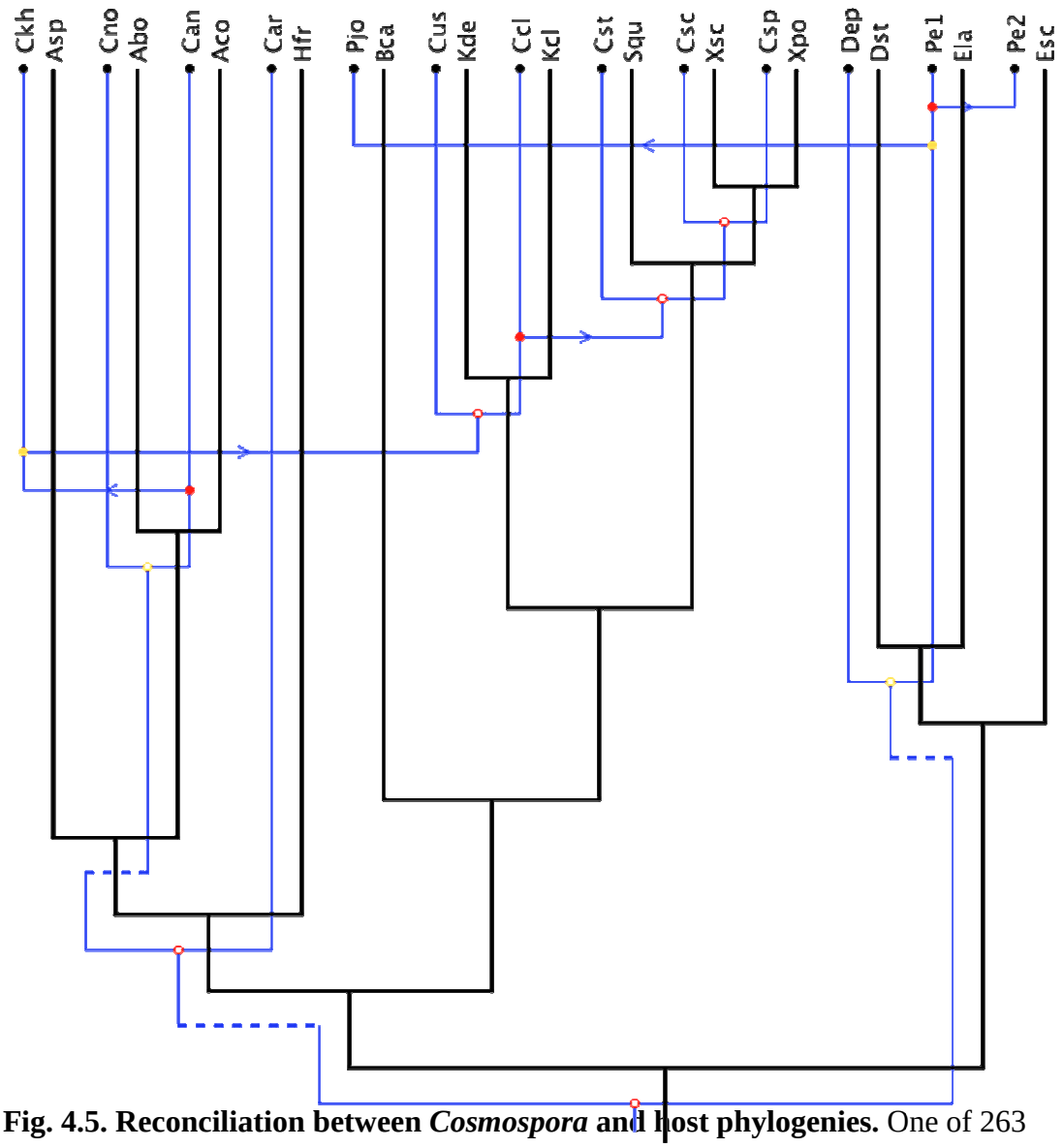

Fig. 4.5. Reconciliation between *Cosmospora* and host phylogenies. One of 263 isomorphic solutions with seven cospeciations, five duplications & host-switches, and three losses (total cost = 13). The reconciliation of *Cosmospora* and host trees was generated with Jane v.4. Blue and black lines represent *Cosmospora* and their fungal hosts, respectively. Empty circles represent cospeciations; Arrows represent host-switches; dash lines represent sorting events. See Tables 1 and 2 for abbreviations of taxa.

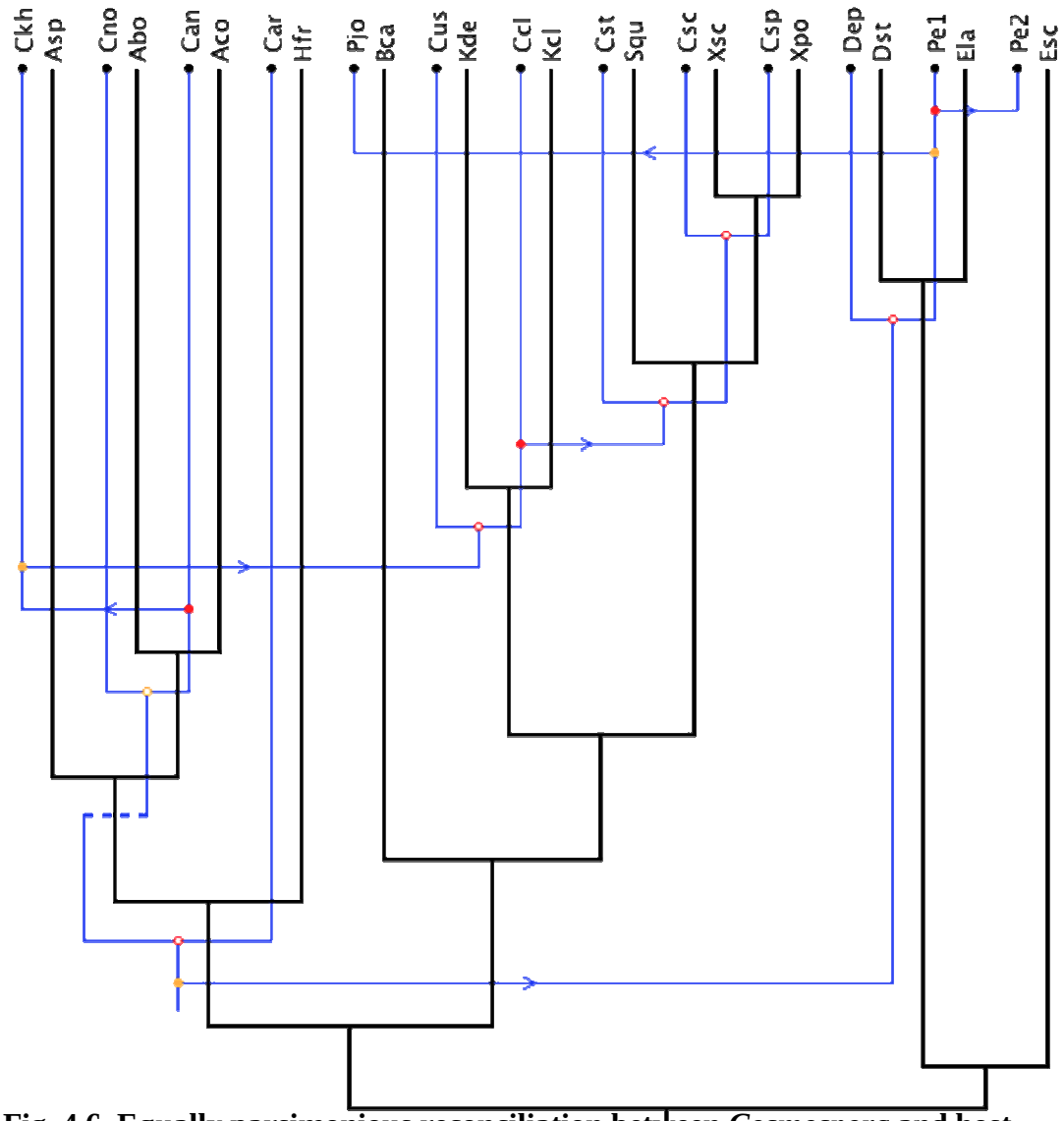

Fig. 4.6. Equally parsimonious reconciliation between *Cosmospora* and host phylogenies. One of 68 isomorphic solutions with six cospeciations, six duplications & host-switches, and one loss (total cost = 13). The reconciliation of *Cosmospora* and host trees was generated with Jane v.4. Blue and black lines represent *Cosmospora* and their fungal hosts, respectively. Empty circles represent cospeciations; Arrows represent host-switches; dash lines represent sorting events. See Tables 1 and 2 for abbreviations of taxa.

Conclusions

This dissertation represents one of the most comprehensive systematic works performed on the genus *Cosmospora* (in the broad sense) so far. It included more taxa than any other previous study. Many of which have been recognized as new species. Additionally, a few names (e.g. *Cosmospora vilior*) were stabilized with the selection of an epitype, which enabled determining correct classification of the fungus based on phylogeny. However, a high proportion of singleton lineages were also observed in the phylogenies. Singleton lineages do not conform to the species recognition concept (i.e., Genealogical Concordance Phylogenetic Species Recognition, GCPSR) used in this dissertation. By definition, a singleton lineage cannot be called a clade; a clade is composed by two or more specimens/isolates (reviewed in Vinuesa 2010). It was decided to recognize selected singleton lineages as species if the lineages were morphologically and/or ecologically distinct that sets them apart from other species (reviewed in Lim et al. 2012). The host of *Cosmospora* species, in particular, has been recognized in this dissertation to be a very informative character to diagnose *Cosmospora* species. Because *Cosmospora* species appear to have a high fidelity to their associated hosts, the recognized singleton species are likely to represent species. By recognizing singleton species, it was thought that it would make it easier to recognize the species again in nature, and fresh samples can be used to expand the morphological ranges of the species.

Two new genera, nine new combinations, and eleven new species were described in the taxonomic work included in this dissertation. Although progress has been made in increasing fungal diversity knowledge, diversity of cosmospora-like fungi is likely to be much higher that our current knowledge. The unrecognized singleton lineages, the many herbarium specimens, and fresh collections with new hosts are evidence that more work is needed. Unfortunately, extracting DNA from the fleshy insides of fruiting bodies stored in herbaria has been unsuccessful, and their phylogenetic position could not be determined. Either, the DNA of herbarium specimens has been degraded, or a better DNA extraction method needs to be found for this material. Additionally, DNA should be extracted from the fungal host for future collections of cosmospora-like fungi. It would provide a more accurate identity of the fungal hosts, and a better dataset for coevolutionary studies between *Cosmospora* species and their associated fungal hosts.

Chapter 4 represents one of the first fungal studies studying evolutionary relationships between a mycoparasite and the associated fungal host (e.g., Currie et al. 2003; Millanes et al. 2014). The results suggested that host switch events were common early in the evolution of *Cosmospora* species, while cospeciation events more prevalent late in the evolution of *Cosmospora* species. This phylogenetic pattern is consistent with pseudocospeciation reported in other systems (e.g., Hafner and Nadler 1988; Charleston and Robertson 2002; Sorenson et al. 2004; Huyse and volckaert 2005). Given that cosmospora-like fungi represent the only group of fungi in the family Nectriaceae that have fungicolous, insecticolous, and lichenicolous life-

style habits, future work will probably focus on evolution of life of lifestyle habits at the molecular level.

The data generated in this dissertation can be use to identify *Cosmospora* species. The dichotomous keys inside the dissertation are useful in the identification of *Cosmospora* species based on based on morphological and host characters. DNA sequences were deposited to GenBank, and unknown samples can be blasted against the deposited sequences. Sequence alignments were also deposited to a public online database, TreeBASE. Additionally, isolates were deposited to The Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, for long-term storage. CBS is a public fungal repository of strain cultures, and the strain cultures are available to all scientists for a relatively small fee that is attributed to the costs of handling and maintenance of cultures. New specimen collections were deposited to the U.S. National Fungus Collections (BPI), USDA-ARS, Beltsville, Maryland, and the specimens can be borrowed from BPI herbarium for study.

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