ABSTRACT

Title of Document: MOLECULAR PHYLOGENY AND TAXONOMIC REVISION OF FUNGI IN THE GENUS *Thelonectria* AND RELATED SPECIES WITH *Cylindrocarpon*-LIKE ANAMORPHS.

Catalina Salgado-Salazar, Doctor of Philosophy, 2014.

Directed By: Associate Professor Dr. Priscila Chaverri, Department of Plant Science and Landscape Architecture.

The genus *Thelonectria* and related species with *Cylindrocarpon*-like anamorphs are a group of perithecial ascomycetes in the family Nectriaceae that occur as saprobes and in few cases as pathogens of hardwood trees, shrubs or other plant substrates. Despite of being a key component of forest ecosystems around the world, species relationships and distribution are largely unknown. The objectives of this study were to: 1) infer species level phylogenetic relationships of the genus *Thelonectria* and related species with *Cylindrocarpon*-like anamorphs with uncertain classification, testing monophyly of each one of the groups studied; 2) delimit taxa, establishing taxon circumscriptions and providing brief descriptions; 3) resolve nomenclatural issues by identifying redundantly used names and synonyms; 4) provide identification tools, specifically, diagnostic keys and molecular data that can be used further as

molecular barcodes; 4) provide distribution data and to take the first steps into the identification of speciation patterns observed in these fungi. To achieve these goals, herbarium materials, as well as freshly collected material obtained from the field or from fungal repositories were compared using phylogenetic analyses of multiple loci, morphology and geographic distribution. This research resulted in the narrower circumscription of the genus *Thelonectria*, not to contain one of the most common species in the group, *T. jungneri*. According to the results of the phylogenetic analyses it was found *T. jungneri* is a segregating clade that needs to be recognized as a different genus. For the genus *Thelonectria*, a total of 31 new species were described, and three new genera, closely related to *Thelonectria* were created to accommodate the diversity of other species with *Cylindrocarpon*-like anamorphs: *Cinnamonectria* gen nov. with *C. cinnamomea* as type taxon, *Macronectria* gen. nov. with *M. jungneri* as type taxon, and *Tumenectria* gen. nov. with *T. laetidisca* as type taxon. Species in this group of fungi present extensive morphological conservationism, representing a challenge for species identification without the use of molecular techniques, however offering a great opportunity to explore mechanisms of speciation and evolutionary diversification.

MOLECULAR PHYLOGENY AND TAXONOMIC REVISION OF FUNGI IN THE GENUS *Thelonectria* AND RELATED SPECIES WITH *Cylindrocarpon*-LIKE ANAMORPHS.

By

Catalina Salgado

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Advisory Committee:
Dr. Priscila Chaverri, Chair
Dr. Yilmaz Balci
Dr. Charles Mitter
Dr. Charles Delwiche
Dr. Amy Y. Rossman

Foreword

This thesis is based on the following articles, which are referred in the text as chapters.

1. **Salgado-Salazar C**, Rossman A, Samuels GJ, Capdet M, Chaverri P (2012) Multigene phylogenetic analyses of the Thelonectria coronata and T. veuillotiana species complexes. Mycologia 104:1325-1350.
2. **Salgado-Salazar C**, Rossman AY, Chaverri P (2013) Not as ubiquitous as we thought: taxonomic crypsis, hidden diversity and cryptic speciation in the cosmopolitan fungus Thelonectria discophora (Nectriaceae, Hypocreales, Ascomycota). PLoS ONE 8(10): e76737. doi:10.1371/journalpone.0076737.
3. **Salgado-Salazar C,** Rossman AY, Samuels GJ, Hirooka Y, Sanchez RM, Chaverri P (2013) Phylogeny and taxonomic revision of *Thelonectria discophora* (Ascomycota, Hypocreales, Nectriaceae) species complex.
4. **Salgado-Salazar C**, Rossman AY, Chaverri P (2013) Molecular phylogeny and taxonomic revision of the fungal genus *Thelonectria* (Nectriaceae, Hypocreales, Ascomycota) and related species with *Cylindrocarpon*-like anamorphs inferred from eight nuclear markers.

Dedication

For my beloved mother Maria I. Salazar, and my much loved husband Arthur V. Cresce.

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This dissertation is a milestone in my professional career. I have been very fortunate to have this opportunity to learn about fungi, my passion. I am grateful to a number of people who have guided and supported me throughout the research process and provided assistance for my venture.

I would first like to thank my advisor, Dr. Priscila Chaverri, who gave me this opportunity and guided me during my studies. I would not have been able to complete my research and achieve each one of my goals without her help. Her recommendations and advice have enabled me to assemble and finish the dissertation effectively.

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FIGURE 4.1. Majority rule Bayesian phylogram showing relationships among species in *Thelonectria* and related species with *Cylindrocarpon*-like anamorphs based on the concatenated analysis of eight loci. Number above branches indicate Bayesian posterior probabilities for analysis of data set after cleaning alignment of problematic regions with Gblocks setting 2. Number below branches indicate Bayesian posterior probabilities for analysis of original data set. Bionectriaceae was used as outgroup.

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FIGURE 4.12. *Cinnamonectria cinnamomea*. A. Perithecia (G.J.S. 86-300 = NY GJS4264). B. Perithecia (PC 1222 = BPI X). C-D. Longitudinal section of perithecia (G.J.S. 86-117 = NY GJS3619). E-G. Asci and ascospores in 3% KOH and cotton

blue (PC 1222 = BPI X). H. Macroconidia on SNA (CLLGUY12050 = CBS 133756). I. Chlamydospores on SNA (CLLGUY12050 = CBS 133756). J. Conidiophores on SNA (G.J.S. 86-300 = IMI 325253). K. Colony on PDA (PC 1222 = CBS 136783). L. Colony reverse on PDA (PC 1222 = CBS X). Bars: A-C = 500 μm; D = 100 μm; E-J = 50 μm.

FIGURE 4.13. *Macronectria jungneri* s. str. A. Perithecia (G.J.S. 10-148 = BPI X). B. Perithecia (PC 1219 = BPI X). C. Squash mount of perithecia with 3% KOH (G.J.S. 10-127 = BPI X). D. Squash mount of perithecia with after lactic acid (G.J.S. 10-127 = BPI X). E. Asci in 3% KOH (PC 1219 = BPI X). F-G. Asci and ascospores in 3% KOH and cotton blue (G.J.S. 10-148 = BPI X). H-I. Macroconidia on SNA (PC 1219 = CBS 136792). J. Colony on PDA (PC 1219 = CBS 136792). K. Colony reverse on PDA (PC 1219 = CBS 136792). L. Colony on PDA (G.J.S. 08-233 = CBS 124602). M. Colony reverse on PDA (G.J.S. 08-233 = CBS 124602). A-D = 500 μm; E-I = 50 μm.

FIGURE 4.14. *Macronectria asiatica*. A. Perithecia (94091407 = BPI X). B. Perithecia (94091601 = BPI X). C. Squash mount of perithecia with 3% KOH (94091601 = BPI X). D. Squash mount of perithecia after lactic acid (94091601 = BPI X). E-G. Asci and ascospore in 3% KOH and cotton blue (94091601 = BPI X). H. Conidiophores on SNA (MAFF 239833). I-J. Macroconidia and microconidia on SNA (MAFF 239833). K. Colony on PDA (MAFF 239833). L. Colony reverse on PDA (MAFF 239833 A-B = 500 μm; C-D = 100 μm; E-J = 50 μm.

FIGURE 4.15. *Macronectria magna*. A. Perithecia (CLLGUY12004 = BPI X). B. Squash mount of perithecia with 3% KOH (CLLGUY12004 = BPI X). C. Squash

mount of perithecia after lactic acid (CLLGUY12004 = BPI X). D-F. Asci and ascospores in 3% KOH and cotton blue (CLLGUY12004 = BPI X). G. Macroconidia on SNA (CBS 21359). H. Macroconidia on SNA (CLLGUY12004 = CBS 133489). I. Microconidia on SNA (CLLGUY12004 = CBS 133489). J. Colony on PDA (CBS 21359). K. Colony reverse on PDA (CBS 21359). L. Colony on PDA (CLLGUY12004 = CBS 133489). M. Colony reverse on PDA (CLLGUY12004 = CBS 133489). Bars: A = 500 μm; B-C = 100 μm; D-I = 50 μm.

FIGURE 4.16. *Macronectria neotropicalis*. A. Perithecia (PC 1213 = BPI X). B. Squash mount of perithecia with 3% KOH. Note the big and round perithecial apex (PC 1213 = BPI X). C. Squash mount of perithecia after lactic acid (PC 1213 = BPI X). D. Squash mount of perithecia with detail on the apex (PC 1213 = BPI X). E-J. Asci and ascospores with 3% KOH and cotton blue (PC 1213 = BPI X). H-I. Macroconidia on SNA (G.J.S. 10-125 = CBS 136790). J. Chlamydospores on SNA (G.J.S. 10-125 = CBS X). K. Colony on PDA (G.J.S. 10-125 = CBS 136790). L. Colony reverse on PDA (G.J.S. 10-125 = CBS 136790). Bars: A-C = 500 μm; D = 100 μm; E-J = 50 μm.

FIGURE 4.17. *Macronectria venezolana*. A-B. Perithecia (C.T.R. 71-244 = NY KPD-VE 1980). C. Squash mount of perithecia with detail on apex section in 3% KOH (C.T.R. 71-244 = NY KPD-VE 1980). D. Squash mount of perithecia with detail on apex section after lactic acid (C.T.R. 71-244 = NY KPD-VE 1980). E-G. Ascospores in 3% KOH and cotton blue (C.T.R. 71-244 = NY KPD-VE 1980). H-I. Macroconidia on SNA (G.J.S. 09-1343 = CBS X). J. Colony on PDA (C.T.R. 71-244 = CBS 122576). K. Colony reverse on PDA (C.T.R. 71-244 = CBS 122576). L.

Colony on PDA (G.J.S. 09-1343 = CBS X). M. Colony reverse on PDA (G.J.S. 09-1343 = CBS X). Bars: A-B = 500 μm; C=D = 100 μm; E-I = 50 μm.

FIGURE 4.18. *Tumenectria laetidisca.* A. *T. laetidisca* perithecia (C.T.R. 71-14 = CUP-MJ 770). B. Perithecia in CUP-MJ 768. C. Squash mount of perithecia with 3% KOH (CUP-MJ 768). D. Squash mount of perithecia after lactic acid (CUP-MJ 768). E-G. Ascospores in 3% KOH and stained with cotton blue (CUP-MJ 768). H-J. Conidiophores and macroconidia on SNA (CBS 100284). K. Colony on PDA (C.T.R. 71-14 = CBS 101909). L. Colony reverse on PDA (C.T.R. 71-14 = CBS 101909). Bars: A-B = 500 μm; C-D = 200 μm; E-J = 50 μm.

Introduction

Thelonectria P. Chaverri & C. Salgado (2011) in a recently created genus in the Nectriaceae family, designated to accommodate species in the previously described *Nectria mammoidea* and *N. veuillotiana* morphological groups (Brayford et al. 2004, Brayford and Samuels 1993, Chaverri et al. 2011). Before the onset of systematic studies using molecular DNA data, species in *Thelonectria* and related groups with *Neonectria/Cylindrocarpon*-like anamorphs were included in the genus *Neonectria* Wollen. 1917. Since the beginning of the taxonomic studies, when morphology was the foundation of systematics of fungi, morphological differences among the number of species in the group *Neonectria/Cylindrocarpon* became noticeable, leading to the recognition of several artificial groups, which in turn supported the hypothesis that the group could represent more than one genus (Brayford et al. 2004, Castlebury et al. 2006, Mantiri et al. 2001, Samuels and Brayford 1994). Even though differences existed, the hypothesis was only formally tested until 2011 (Chaverri et al. 2011), thanks to the accessibility molecular phylogenetics had gained in the previous years. Chaverri's et al. study (2011) revealed that indeed the previous classifications and species relationships in *Neonectria/Cylindrocarpon* were of polyphyletic nature. It was found that *Neonectria/Cylindrocarpon* species form five different lineages, today described as different genera. These genera cluster in two major groups, one containing the genera *Neonectria* (including the *'Nectria' coccinea/galligena* group) and *Ilyonectria* (formerly known as *'Nectria' radicicola* group), and the other containing the genera *Thelonectria*, *Rugonectria* (formerly known as the *'Nectria'*

rugulosa group), and a recently described genus, *Campylocarpon* (Chaverri et al. 2011, Halleen et al. 2004).

Higher taxonomic relationships of *Thelonectria* and related species with *Cylindrocarpon*-like anamorphs

The genus *Thelonectria* and related species with *Cylindrocarpon*-like anamorphs belong to the family Nectriaceae in the order Hypocreales. The Hypocreales Lindau 1897 (Kingdom Fungi, Phylum Ascomycota, Subphylum Pezizomycotina, Class Sordariomycetes, Subclass Hypocreomycetidae) is a vast order of fungi that includes seven families: *Bionectriaceae* Samuels & Rossman 1999, *Clavicipitaceae* (Lindau) Earle ex Rogerson 1971, *Cordycipitaceae* Kreisel 1969 ex G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, *Hypocreaceae* De Not. 1844, *Nectriaceae* Tul. & C. Tul. 1844, *Niessliaceae* Kirschst. 1939 and *Ophiocordycipitaceae* G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (Lumbsch and Huhndorf 2007, Rossman et al. 1999, Sung et al. 2007). Since the order was created, it has been the subject of many debate, reorganization and redefinitions, and has remained with an ever-growing number of reports of new species fitting these characteristics (Rogerson 1970, Rossman 1996, Rossman et al. 1999). Luttrell (1951) suggested that the Hypocreales should be restricted to fungi with unitunicate asci and a *Nectria*-type centrum. The *Nectria*-type centrum is characterized by the formation of downward growing sterile filaments known as 'apical paraphyses' and, in part, by the formation of basal hymenium of unitunicate asci. Traditionally, these fungi have light-to bright-colored perithecia and together with texture and type of development, have been commonly used to classify species in this order (Rogerson 1970, Rossman 1996). The ascal apex is simple or

contains a non-amyloid ring. Ascospores are usually colorless, less frequent yellowish-brown, often bicellular with a median septum although species with amerospores, phragmospores or dictyospores are also known (Samuels and Seifert 1987, Rossman et al. 1999).

Hypocrealean fungi are worldwide distributed although the greatest diversity of genera occurs in temperate and tropical regions. The Hypocreales are ubiquitous in nature and exhibit a wide diversity of teleomorph and anamorph morphology, ecology, and nutritional specialization (Rossman 1996, Rossman et al. 1999). The impacts hypocrealean fungi have in nature are diverse. They can be pathogens of important crops, for example species of *Calonectria/Cylindrocladium, Ilyonectria, Fusarium,* among others (Cabral et al. 2012, Hallen et al. 2006; Lombard et al. 2010, Rossman et al. 1999); pathogens of cultivated mushrooms, which include some *Trichoderma* species; sources of pharmaceutical compounds and mycotoxins e.g. *Acremonium, Cylindrocarpon, Fusarium,* and *Claviceps* species (Desjardins 2006, Rossman 1996, Yu et al. 2010). Some species of *Cylindrocarpon*, *Acremonium* and *Fusarium* can be pathogens of humans and animals (Summerbell and Schroers 2002). Hypocrealean fungi are also known as biological control agents and among the most intensively used species in diverse crop systems are *Beauveria* sp., *Metarrhizium* sp., *Paecilomyces* sp., *Trichoderma* sp. and *Clonostachys* sp. (Rossman 1996, Rossman et al. 1999). A high percentage of species in the Hypocreales include saprophytes and facultative parasites of plants, animals and other fungi (Rehner and Samuels 1995, Rossman 1996, Rossman et al. 1999, Rossman 2000).

The family *Nectriaceae* was described by Tulasne & C. Tulasne in 1865 (Tulasne and Tulasne 1865) with *Nectria cinnabarina* (Tode) Fr. 1849 as the type taxon. The *Nectriaceae* is a family of approximately 20 genera in which there is a close equivalency between sexual and asexual generic concepts (Rossman 2000). Some of the most well known genera are *Albonectria* (anamorph *Fusarium decemcellulare*), *Calonectria* (anamorph *Cylindrocladium*), *Cosmospora* (several anamorphs such as *Acremonium*, *Fusarium* and *Vollutella*), *Haematonectria* (anamorph *Fusarium solani*), *Giberella* (anamorph *Fusarium sambucinum*), *Nectria* (anamorph *Tubercularia*) and *Neonectria* (anamorph *Cylindrocarpon*) (Rossman et al. 1999, Rossman 2000). Members of the family *Nectriaceae* can be distinguished from the other families in the order Hypocreales based on their ascomata color and color reactions in KOH and lactic acid solution. Nectriaceous species have soft-textured, uniloculate perithecia, with light to bright color, usually red, orange-red or light brown, with color reaction when exposed to KOH (perithecial wall becomes purple) and subsequently to 100% lactic acid solution (perithecial wall becomes yellow) (Rossman et al. 1999). In the *Nectriaceae*, some exceptions to the color reaction occur, for example the genera *Albonectria,* which has white perithecial walls and *Gibberella,* which has bluish-purple perithecial walls, do not have the typical color reaction of the family *Nectriaceae* (Rossman et al. 1999, Samuels et al. 2009). *Nectriaceae* fungi have perithecial wall structure with characteristic particular to every genus. The asci are unitunicate with apical ring in those species that have ascospore sizes around 20 μm, species with elongate or larger ascospores generally lack an apical apparatus and ascospores are released when the asci deliquesce at

maturity (Rossman et al. 1999). There is variability in the shape of ascospores in the *Nectriaceae*, they can be ellipsoid, fusiform or allantoid; ascospore color is generally hyaline to golden yellow or golden brown. Ascospore surface can vary from smooth to verrucose, striate or spinulose, and is often characteristic of every genus (Rossman et al. 1999).

Ascomata in most members of the *Nectriaceae* develop solitary, or in clusters of few to numerous perithecia, and develop either without the presence of stroma or upon an inconspicuous or pseudoparenchymatous stroma. The pseudoparenchymatous stroma is often associated with the anamorph. Ascomata form in cracks of bark of woody tree species, in canker lesions or root tissues; other species develop superficial perithecia upon the stromata of other pyrenomycete fungi (for example some *Cosmospora* species). Species in this family are relatively common in tropical regions (Samuels et al. 2002) as well as in temperate regions (Rossman et al. 1999). In general, they can be weak to virulent plant and insect pathogens, parasites or saprophytes of other fungi, saprophytes in diverse kind of plant material or dung, and occasionally parasites of animals and humans (Rossman et al. 1999, Rossman 2000).

Overview of the genera in the Nectriaceae closely related to *Thelonectria* and other *Cylindrocarpon*-like anamorphs

One of the genera closely related to Thelonectria is Neonectria. Species of *Neonectria* s. str. were first known to belong the Booth's Coccinea group (Booth 1959), with *N. ramulariae* as the type taxon. The species were described as having ovate smooth-walled perithecia with erumpent stroma normally well developed; they can produce

clusters of few to up to 100 perithecia. The most well known species in this group are *N. coccinea*, *N. faginata*, *N. fuckeliana*, *N. punicea* and *N. ditissima*. Perithecial walls having two regions characterize species in *Neonectria s. str.* The outer region consists of globose cells with thick walls, which are a continuation of the stroma; very thin-walled and elongated cells form the inner layer. Ascospores can be smooth or finely ornamented, mostly two-celled (Booth 1959). The *Cylindrocarpon* anamorphs of *Neonectria* s. str. produce cylindrical macroconidia, typically 3–5-septate with rounded ends and microconidia and chlamydospores. Microconidia are formed in simple generally unbranched conidiophores and lack a prominent abscission scar (Booth 1966, Chaverri et al. 2011). *Neonectria* s. str. contains recognized plant pathogens of trees in Northern latitudes: *N. coccinea*, which causes cankers of *Fagus* species only in Europe, *N. faginata*, which causes cankers on North American *Fagus* species, *N. ditissima* causes serious canker diseases in apples and pears (Castlebury et al. 2006) and has a wide geographical range. These species are mostly found in temperate regions.

The genus *Rugonectria*, one two genera closest to *Thelonectria,* was described to contain species previously included in *'Nectria' rugulosa* group. *Rugonectria rugulosa* (Pat. & Gaill.) Chaverri, C. Salgado & Samuels (=*Neonectria rugulosa*) has been designated as the type taxon from the genus, based on material collected in Venezuela (Chaverri et al. 2011, Samuels and Brayford 1994). Species in this group can be found on bark of recently dead or killed trees or causing cankers and decay on several species of hardwood trees (Hirooka et al. 2005, Ko and Kunimoto 1991, Kobayashi et al. 2005) and are presumed to have tropical and subtropical distributions

(Chaverri et al. 2011, Samuels and Brayford 1994). *Rugonectria* species are characterized by having reddish to orange perithecia, globose to subglobose, not collapsing when dry, growing on a basal erumpent stroma. The outer region of the perithecial wall is formed by several layers of cells, including warts with small globose cells that are very thick-walled and merge with the surrounding stroma (Chaverri et al. 2011, Hirooka et al. 2005, Samuels and Brayford 1994). The anamorphic state produces Cylindrocarpon-like conidia that are somewhat different from those observed in *Neonectria* or *Thelonectria*. The most noticeable difference lies in the fusiform shape of macroconidia with tapering apical ends, similar to those produced by Fusarium species (Chaverri et al. 2011). Some representative species of this genus are *R. rugulosa*, *R. castaneicola, R. neobalansae* (Chaverri et al. 2011).

The second closest genus to Thelonectria is Campylocarpon. The type taxon of *Campylocarpon* is *C. fasciculare* Halleen, Schroers & Crous based on material collected in South Africa (Hallen et al. 2004). *Campylocarpon* species are only known for the anamorph, with no sexual state associated to date (Hallen et al. 2004). Conidia in *Campylocarpon* resemble morphologically species of *Thelonectria*, with septate curved macroconidia with round ends. Microconidia are not produced by species in the genus, however chlamydospores have been observed (Chaverri et al. 2011, Hallen et al. 2004). *Campylocarpon* species have been identified as one of the causal agents of black foot disease of grapevines, and even though it was first detected in South Africa (Hallen et al. 2004), however, it has also been reported in Spain (Alaniz et al. 2011) and Uruguay (Abreo et al. 2010) indicating it can be widely

distributed. Species in this group include *C. fasciculare* and *C. pseudofasciculare* (Chaverri et al. 2011, Hallen et al. 2004).

The genus *Ilyonectria*, together with *Neonectria*, constitute the more distantly genera related to *Thelonectria. Ilyonectria* was created to contain species previously known as part of the *'Nectria' radicicola* group. *Ilyonectria radicicola* (Gerlach and L. Nilsson) Chaverri & C. Salgado has been designated as the type taxon for the genus, based on material collected in Sweden (Chaverri et al. 2011, Samuels and Brayford 1990). Species in *Ilyonectria* are cosmopolitan and common soil borne-fungi, many times found associated with the roots or underground parts of a large number of woody and herbaceous hosts (Booth 1966, Samuels and Brayford 1990). The plant pathology literature contains a large number of reports of diseases caused by *Ilyonectria* species, which include seedling blights, basal rots and root roots (Nicot 1951, Samuels and Brayford 1990, Seifert et al. 2003). The species in this group have smooth to slightly warted, usually solitary perithecia with the outer region of the perithecial wall composed of large, thin-walled cells, and smooth ascospores (Chaverri et al. 2011, Samuels and Brayford 1990). The anamorph of *Ilyonectria* species produces cylindrical septate macroconidia with round ends, similar to those produced by species in the *Neonectria* sensu stricto group, and microconidia and chlamydospores. These species are mainly found in soil environments; consequently the production of chlamydospores can serve as overwintering structures associated with the survival of these species when conditions are not favorable (Chaverri et al. 2011). *Ilyonectria radicicola*, one of the most representative and well known species in this group is known better by the previously known the name of the anamorph,

Cylindrocarpon destructans. This species has been largely associated with root rots of wild and commercial plants, such as grapevines, ginseng, blackberry, among others (Cedeño et al. 2004, Petit and Gluber 2005, Samuels and Brayford 1990, Seifert et al. 2003,). *Ilyonectria radicicola* is a complex of species, probably with host and geographic structure; however, this hypothesis has not been tested in detail (Seifert et al. 2003). Other species included in this group are *I. coprosmae*, *I. liriodendri* and *I. macrodydima* (Chaverri et al. 2011).

Justification and impact

Systematics with its elementary particles, species, is the fundamental science of biodiversity (Systematics Agenda 2000, 1994a; 1994b). The efforts to estimate the diversity of life and the practical consequences of knowing our surrounding biodiversity are only possible when taxonomy, phylogenetic systematics, biogeography and evolutionary biology are integrated (Wiley and Mayden 2000). The first step to manage and use biological diversity lies on its discovery, description and posterior understanding, interpretation and communication, areas of the biological research traditionally covered by the discipline of taxonomy (Systematics Agenda 2000, 1994a; 1994b). The impacts that systematics research and its related disciplines, such as taxonomy and phylogenetics, are diverse. Fields such as conservation biology, public health and pest management are profoundly influenced by the systematics research and how accurate this work is done. There is worldwide concern at the loss of biodiversity and widespread popular support for efforts to conserve diversity before it disappears. However, efforts to prioritize individual species for conservation as well as areas rich in biodiversity are hampered by our lack

of knowledge of what species exist and where they are found (Systematics Agenda 2000, 1994a, 1994b, Ross et al. 2010, McLeod 2010, http://www.bionet-intl.org). Additionally, the accurate identification of causal agents of plant and animal diseases, disease vectors and alien or invasive species plays a significant role in the choice and/or improvement of control measures (Ebach et al. 2011, Rossman and Palm-Hernandez 2008, http://www.bionet-intl.org). Due to the complex connections among all forms of life on earth, the impacts of systematics research are larger and more important than can be circumscribed in a summarized way, however it is important to highlight that to be able to reach the benefits associated to the knowledge of biodiversity, the communication and accessibility to information generated by systematics studies is also crucial.

Fungi are not strangers to systematics research and the efforts to catalogue the diversity of life. In 1991, estimates about global number of fungal species were conservatively calculated to be close to 1.5 million (Hawksworth 1991). With the rapid development of new molecular biology techniques and phylogenetic methods, this number has risen to 5.1 million, with the possibility of being increased even more in the future (Blackwell 2011, O'Brien et al. 2005). The increased access to molecular characters (i.e. DNA data) resulting from more than two decades of research in the area (Taylor 2011), together with the consequent change from the traditional species delimitation criteria (morphological species criteria-MSC or biological species criteria-BSC) to species delimitation criteria that fit better the biological and evolutionary complexities of fungi (phylogenetic species criteria, Taylor et al. 2000, Taylor et al. 2006), have modernized the field, increasing the rate of discovery of

diversity seemingly endless. In the last few years, fungal systematics has reached a sense of organization many mycologists were looking for. Changes in the nomenclatural rules governing fungi by discarding the dual system of nomenclature, which used to be different for the sexual and asexual stages of one species, is only the beginning of a new era of enlightenment of fungal systematics (Hibbett and Taylor 2013).

Objectives

- Infer species level phylogenetic relationships of the genus *Thelonectria* and related species with *Cylindrocarpon*-like anamorphs with uncertain classification, testing monophyly of each one of the groups studied.
- Delimit taxa, establishing taxon circumscriptions and providing brief descriptions.
- Resolve nomenclatural issues by identifying redundantly used names and synonyms.
- Provide identification tools, specifically, diagnostic keys and molecular data that can be used further as molecular barcodes.
- Provide distribution data and to take the first steps into the identification of speciation patterns seen in these fungi.

Chapter 1: Multigene phylogenetic analyses of the *Thelonectria coronata* and *T. veuillotiana* species complexes.

Abstract

Thelonectria is a recently established genus of common and ubiquitous fungi on woody hosts, previously placed in the genus *Neonectria*. *Thelonectria coronata* and *T. veuillotiana* occur sympatrically in several geographical areas in tropical, subtropical, and temperate regions. Previous taxonomic studies including *T. coronata* and *T. veuillotiana* suggested these fungi could represent species complexes; however, the morphological features used to define species exhibited few differences useful for testing this hypothesis. In order to assess the status of *T. coronata* and *T. veuillotiana*, phylogenetic analyses of six genomic regions were combined with a morphological examination of specimens. A multigene phylogeny reconstructed using Maximum Parsimony, Maximum Likelihood, and Bayesian approaches identified five phylogenetic groups in *T. coronata* and six in *T. veuillotiana*. As is common for cryptic species, unequivocal diagnostic morphological characters could not be identified; however, average values of morphological traits correspond to the phylogenetic groups. An increased level of non-synonymous/synonymous substitutions in the β-tubulin gene and a decreased or absent production of conidia was detected within the *T. coronata* complex, possibly indicating the homothallic nature of these isolates. Here, *T. coronata* and *T. veuillotiana* and related species are described and illustrated; a dichotomous key to all species is provided.

Key words: Ascomycota, fungi, homothallic, Hypocreales, *Neonectria,* phylogenetic analyses, species concept, taxonomy.

Introduction

Thelonectria coronata (Penz. & Sacc.) P. Chaverri & C. Salgado (2011) and *T. veuillotiana* (Sacc. & Roum.) P. Chaverri & C. Salgado (2011) are sister species of nectriaceous fungi previously recognized in the genus *Neonectria* Wollenw. Recent studies showed that species of *Neonectria s. l.* constitute five lineages, which were recognized as distinct genera (Chaverri et al. 2011). One of those lineages is *Thelonectria* based on *T. discophora* (Mont.) P. Chaverri & C. Salgado. *Thelonectria* includes species previously recognized as the "*Nectria mammoidea"* and "*N. veuillotiana"* groups (Booth 1959, Brayford and Samuels 1993, Samuels and Brayford 1994), the latter group including *T. coronata* and *T. veuillotiana.*

Thelonectria coronata was first described by Penzig & Saccardo in 1897 from specimens collected in Indonesia (Penzig and Saccardo 1897) while *T. veuillotiana* was described by Saccardo & Roumeguère in 1880 from specimens collected in France (Debeaux et al. 1880). Even though *T. coronata* and *T. veuillotiana* have similar teleomorph and anamorph morphologies (Samuels et al. 1990, Brayford and Samuels 1993, Samuels and Brayford 1994), these species can be easily differentiated based on the morphology of the perithecial apex. In both species, the perithecial apex is composed of a palisade of hyphal elements arising from the inner region in the perithecial wall. The terminal cells of the hyphal elements at the exterior of the apical disc are conspicuously swollen and saccate, giving the apex a knobby appearance. However, in *T. coronata*, the perithecial apex is usually darkly pigmented and

swollen saccate cells form a distinctive fringe giving the apex a coronate aspect (Samuels et al. 1990, Brayford and Samuels 1993, Samuels and Brayford 1994).

Thelonectria coronata and *T. veuillotiana* are species of fungi abundant in temperate, tropical, and subtropical regions (Brayford and Samuels 1993, Guu et al. 2007, Zhuang et al. 2007) where they are found on bark of recently dead or dying trees, sometimes growing on canker lesions caused by other disease-causing organisms (Samuels et al. 1990, Brayford and Samuels 1993). Contrary to *T. veuillotiana*, which has been collected widely on bark of hardwood trees or shrubs, sometimes associated with pathogenic *Neonectria* species (Marra and Corwin 2009), *T. coronata* is inconspicuous, not often collected due to the small size and dark color of its perithecia (Brayford and Samuels 1993).

The most recent taxonomic treatments including these species suggest that *T. coronata* and *T. veuillotiana* may comprise species complexes (Samuels et al. 1990, Brayford and Samuels 1993, Samuels and Brayford 1994). These first taxonomic studies were based solely on morphological characters, thus this hypothesis could not be confidently tested because of the problems associated with the application of morphological approaches to species recognition in fungi (Taylor et al. 2000, 2006). In addition, the wide taxonomic range of hosts and distribution make efforts to separate taxa based on ecological preferences or geography futile. The development of species delimitation criteria suitable for fungi (Taylor et al. 2006) based on genetic characters whose rate of change is faster than that of morphological characters, have increased the confidence of species delimitation and diversity estimates. Such methods include those based on genealogical concordance criteria, i.e. monophyly

criteria for species delimitation (Dettman et al. 2003), genealogical sorting index (Cummings et al. 2008), clustering optimization methods (Stielow et al. 2011), genetic distances in monophyletic lineages (Del-Prado et al. 2010), and recombination methods (Carbone and Kohn 2004), among others. Phylogenetic species recognition based on genealogical concordance has been widely used to delimit species in fungi (Miller and Huhndorf 2004, Fournier et al. 2005, Pringle et al. 2005, Druzhinina et al. 2010, Taskin et al. 2010) and it has become a reliable method for assessing the diversity of those groups presumed to comprise species complexes. Recombination tests have been used for geographic differentiation and to identify putative biological species among different populations (Koufopanou et al. 1997, Fisher et al. 2002). In theory, if recombination occurs within strongly supported clades in gene genealogies but not between clades, then these clades define the boundaries of biological species, i.e. the populations are not interconnected by gene flow (Sites and Marshall 2003, Carbone and Kohn 2004). Several methods to detect recombination among populations using DNA sequence data are available (Cunningham 1997, Huson and Bryant 2006, Bruen et al. 2006, Martin et al. 2010). These techniques can be used to infer the occurrence of recombination either directly through sequence comparisons or indirectly through phylogenetic analysis, and can be used within closely or distantly related genotypes (Posada 2002, Bruen et al. 2006). In fungi, these methods have been mainly applied to detect cryptic recombination within species thought to be clonal in nature (Burt et al. 1996, Geiser et al. 1998, Pringle et al. 2005, Croll and Sanders 2009); however, its utility in the

recognition of limits between populations has been scarcely explored (Druzhinina et al. 2010).

In the present study, the existence of cryptic species in *T. coronata* and *T. veuillotiana* was investigated. For this purpose, cryptic species are identified using phylogenetic species recognition methods based on genealogical concordance between multiple phylogenies. Additional support for separation of taxa was provided by application of the genealogical sorting index method and recombination tests. Morphological studies of teleomorphs and anamorphs were combined with the molecular tools to examine the phylogenetic diversity of the species.

Materials and Methods

Fungal isolates

Nineteen isolates each of *T. coronata* and *T. veuillotiana* from diverse locations and substrates were studied. Data associated with these isolates, including GenBank accession numbers, are listed in TABLE 1.1. Additionally, two isolates of *Neonectria amamiensis* (Hirooka et al. 2006) and one isolate of *Nectria acrotyla* (Brayford and Samuels 1993) were included due to their relatedness with *T. veuillotiana*. Two isolates of *T. westlandica* were used as outgroup in the phylogenetic analyses. Specimens and cultures were obtained from U.S. National Fungus Collection (BPI), New York Botanical Garden (NY), CABI Bioscience (IMI), Centraalbureau voor Schimmelcultures (CBS), and Japanese Ministry of Agriculture, Fisheries and Food Collection (MAFF), as well as from freshly collected material.

DNA extraction, PCR, and DNA sequencing

Six nuclear loci were sequenced for this study: partial large nuclear ribosomal subunit (LSU, ca. 900 bp), complete internal transcribed spacers 1 and 2 (ITS, including 5.8S of the nuclear ribosomal DNA, ca. 600 bp), partial β-tubulin (*tub*, ca. 500 bp), α-actin (*act*, ca. 600 bp), RNA polymerase II subunit 1 (*rpb1*, ca. 700 bp), and translation elongation factor 1α (*tef1*, ca. 700 bp) (Chaverri et al. 2011). Genomic DNA was extracted using the PowerPlant™ DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, California, U.S.A.). Protocols for PCR were carried out as described by Chaverri et al. (2011). Clean PCR products were sequenced in both directions at the University of Maryland DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland, USA). Sequences were assembled and edited using Sequencher 4.8 (Gene Codes, Madison, Wisconsin, USA). Sequences were deposited in GenBank as listed in TABLE 1.1.

Phylogenetic analyses

DNA sequences corresponding to forty-three strains of *T. veuillotiana*, *T. coronata*, related species, and outgroups were analyzed using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI). All sequences were aligned using MAFFT version 6 (http://mafft.cbrc.jp/alignment/server/) using the E-INS-I strategy (Katoh and Toh 2008). In order to depict the phylogenetic relationships in *T. coronata* and *T. veuillotiana*, single gene genealogies were constructed to recognize phylogenetic species using the genealogical concordance criteria (Avise and Ball 1990, Dettman et al. 2003) using MP, ML, and BI approaches. As a final step and to observe the overall relationships among the different isolates, a concatenated dataset

containing all six loci was constructed and analyzed. Sequence alignment of concatenated dataset has been deposited at the DRYAD repository (http://datadryad.org) under study accession number doi:10.5061/dryad5d6p6.

In addition to the recognition of phylogenetic species by genealogical concordance, we applied the Genealogical Sorting Index (*gsi*) method (Cummings et al. 2008. For this, 100 trees produced from ML analyses and the last 100 trees produced from Bayesian analysis (50 trees per run) using the combined dataset were uploaded in a separate analysis into the online program available at www.genealogicalsortingindex.com. Each isolate was assigned to a cryptic clade according to the results obtained in the phylogenetic analysis. The *gsi* was calculated for all trees using 10,000 permutations (Cummings et al. 2008). MP analyses were carried out using PAUP* v. 4.0a114 (Swofford 2000); heuristic searches were conducted using 1000 random-addition-sequence replicates using tree bisection-reconnection (TBR) branch swapping, MULTrees option on, and gaps were treated as missing data. Non-parametric bootstrapping values (Felsenstein 1985) were generated as heuristic searches with 500 replicates, each with 10 random-addition replicates. The number of rearrangements was restricted to 10 million per replicate. When needed, trees were summarized in a strict consensus reconstruction. ML analyses were performed with the program RAxML v. 7.2.8 (Stamatakis 2006) using the GTRGAMMA model, which includes a parameter (τ) for rate heterogeneity among sites. Branch support was assessed with 1000 nonparametric bootstrapping replicates using the same model parameters settings. For MP and ML analyses, clades with bootstrap support $\geq$ 70% were considered well supported.

BI analyses were performed using MrBayes v. 3.1 (Huelsenbeck et al. 2001, Ronquist and Huelsenbeck 2003) and jModeltest v 0.1.1 (Posada 2008, Guindon and Gascuel 2003) was used to determine the best nucleotide substitution model (general time reversible —GTR— substitution model with gamma (G) and proportion of invariable site (I)). BI analyses were initiated from random starting trees, run for 10 million generations with four chains (Metropolis-coupled Markov Chain Monte Carlo; Ronquist and Huelsenbeck 2004) and sampled at intervals of 1000 generations. Two independent BI analyses were run. To evaluate stationarity and convergence between runs, log-likelihood scores were plotted using TRACER v. 1.5 (Rambaut and Drummond 2007). Trees generated prior to stationarity were discarded (Huelsenbeck et al. 2001). Trees were summarized in a majority-rule consensus tree from the remaining trees from the four independent runs. Bayesian posterior probabilities (PP) were assessed at all nodes, and clades with PP ≥ 0.95 were considered well supported (Huelsenbeck and Rannala 2004). MP, ML, and BI analyses were done for both single gene and combined datasets (MP and ML single gene trees not shown). Trees were visualized with FigTree v1.3.1 (Rambaut 2005).

Basic population statistics, such as nucleotide diversity (π) within and between populations, average number of nucleotide differences (k), nucleotide divergence (Dxy), and number of non-synonymous and synonymous substitutions per synonymous site (dN/dS) were calculated using DnaSP v5.10.01 (Rozas et al. 2003) and Mega5 (Tamura et al. 2011). For dN/dS analyses, only protein coding regions (exons) were used and each gene was analyzed individually.

Test for recombination

To test whether different sequence-based methods for detecting recombination can help resolve the limit of recombining populations in *T. coronata* and *T. veuillotiana*, several recombination tests were done using the concatenated sequences from six loci. These tests were run separately for *T. coronata* and *T. veuillotiana* isolates and did not include outgroup sequences. The partition homogeneity test (ILD/PHT) (Farris et al. 1994, 1995) implemented in PAUP* version 4.0a114 (Swofford 2000) was used to estimate the degree of incongruence among loci created by recombination. Because this test has a high rate of type I error, a conservative criterion for significance ($P < 0.001$) was used as a threshold to reject the null hypothesis of incongruence between loci (Cunningham 1997, Barker and Lutzoni 2002, Pringle et al. 2005). The Phi-test implemented in SplitsTree v4.11.3 (Huson and Bryant 2006), which uses the pairwise homoplasy index, PHI (= ϕ) statistic, was used to detect refined incompatibility indicating recombination. This test was run with default settings. The neighbour-net algorithm (Bryant and Moulton 2004) implemented by the same software, which is based on the split decomposition method (Bandelt and Dress 1992, Huson and Bryant 2006) and detects incompatible and ambiguous signals generated by recombination events, was used to construct a network based on the uncorrected p-distance using the concatenated dataset for all loci (Huson and Bryant 2006). This method gives graphical support when recombination is present among isolates in different populations. Robustness of the branching pattern was assessed with 1000 bootstrap replicates. Lastly, five miscellaneous tests to detect recombination (Geneconv, MaxChi, RDP, Bootscanning,

and Chimaera) implemented in the RDP3 software (Martin et al. 2010) were applied following settings implemented by Croll and Sanders (2009), in order to detect putative recombinant regions in the concatenated sequences. The significance threshold for all tests, except ILD/PHT, was set to $P < 0.05$.

Morphological studies

The morphology of *T. coronata*, *T. veuillotiana,* and related species, specimens and associated cultures was studied according to Chaverri et al. (2011). For the teleomorph, only the length and width of ascospores were recorded, as these are the most informative characters (Samuels et al. 1990, Brayford and Samuels 1993, Samuels and Brayford 1994). For the anamorph, if conidiation did not occur, isolates were grown on different media (potato dextrose agar, PDA; and SNA + 0.1% yeast extract) and/or incubated under a near UV light treatment for the required time to observe conidiation. Growth rates and colony characteristics were determined on plates containing 20 mL of PDA inoculated with 4 mm diam mycelium plugs. Five different temperatures were evaluated, 15, 20, 25, 30, and 35 C; cultures growing at 20 C were selected to describe colony characteristics and standard growth. Color of colonies was determined according to Rayner (1970).

Statistical analysis of morphological characters

Measurements of continuous characters for both anamorph and teleomorph structures (length, width) were made using Scion Image software v. 4.0.2 (Scion Corporation, Frederick, Maryland, U.S.A) and are based on 30 units per structure in each isolate. Descriptive statistics for the morphological characters, such as mean, minimum, and

maximum values, standard deviation and 95% confidence intervals were obtained using MYSTAT Software v. 12.02.00 (Systat Software Inc. Chicago, Illinois, U.S.A). Ranges are reported as the extremes (maximum and minimum) in brackets separated by the mean ± one standard deviation.

Results

Phylogenetic analyses

This study includes sequence data from 43 taxa in total, of which 239 sequences were newly generated. The combined data set with six genomic regions included 4123 characters of which 3471 were constant, 128 variable and parsimony-uninformative, and 524 variable and parsimony-informative. The single-gene genealogies showed no general pattern of conflict; despite clades with low bootstrap support, there were no supported conflicts, namely alternative topology with significant branch support compared with the tree for the combined dataset (FIG. 1.1). In general, individual gene genealogies obtained using MP, ML, and BI approaches showed significant support values yet some nodes are unresolved (FIG. S1.1). For ITS and LSU datasets most of the taxa revealed unique sequences that resulted in unresolved polytomies; however, protein-coding loci datasets (*act, tub, tef1, rpb1*) mostly produced well-supported nodes (FIG. S1.1). Clades in *T. coronata* and *T. veuillotiana* fit the criteria for phylogenetic species recognition by genealogical concordance (Avise and Ball 1990, Dettman et al. 2003), based on the results of the single gene genealogies (FIG. S1.1). Due to the general agreement and lack of conflict in the single gene genealogies, our interpretation of the phylogenetic structure in these species is based on the

phylogenies obtained from the analyses of the combined dataset. The multilocus phylogenetic relationships among genotypes in each species complex, *T. coronata* and *T. veuillotiana*, are shown in FIG. 1.1.

MP, ML, and BI analyses of the combined dataset produced similar trees with most nodes supported by high MP and ML bootstrap and Bayesian PP values; consequently, only a Bayesian majority rule-consensus tree is shown. In general, ML and BI analyses generated higher branch support compared to MP analyses (FIG. 1.1). Parsimony analysis of the combined dataset yielded one most parsimonious tree with length equal to 807 steps, CI= 0.768, RI= 0.943 and HI= 0.232. ML and BI analyses of the same combined dataset produced trees of log-likelihoods of -11327.84 and -12543.79, respectively. Four independent lineages within *T. coronata* and six within *T. veuillotiana* were supported by both bootstrap and Bayesian PP values greater than 90% and 0.95, respectively (FIG. 1.1). In addition, the *gsi* analyses, which provided a numerical value for the level of monophyly and statistical support (*P*) for separation of the labeled taxa, supported the independent lineages within *T. coronata* and *T. veuillotiana* as monophyletic groups, with gsi_T values > 0.9 (P <0.01). These support values are included in FIG. 1.1.

Neither of the lineages within the *T. coronata* or *T. veuillotiana* species complexes showed any association by host (FIG. 1.1, TABLE 1.1); however many hosts could not be identified unequivocally. None of the clades in the *T. coronata* complex showed strict association with geography. Isolates 93082102 and 94043006 (Clade TC5, FIG. 1.1) from Taiwan constitute the only clade with isolates from the same geographic region. *Thelonectria coronata* was originally described from a specimen

collected in Indonesia, and for that reason clade TC2 is designated here as corresponding to *T. coronata s. str*. and isolate G.J.S. 85-207 from Indonesia was chosen to epitypify this species (FIG. 1.1). Isolate C.T.R. 72-178 from Venezuela formed a single-isolate lineage closely related to *T. coronata s. str*. (FIG. 1.1).

In *T. veuillotiana* some isolates group according to the geographic origin of the strain; for example, Clades TV1 and TV4 are represented by isolates from the eastern USA and isolates in the Clade TV3 (*T. amamiensis)* were collected only in Japan (FIG. 1.1). Clades TV5 and TV6 comprise isolates collected in different and distant areas; the two isolates of Clade TV5 are from France and the Azores, and Clade TV6 consists of two isolates from the USA and Japan (FIG. 1.1). Three species of the *T. veuillotiana* complex were collected from the eastern USA, possibly suggesting that this area is an important center of diversification for these species. *Thelonectria veuillotiana* was originally described from France; consequently Clade TV5 is considered to be *T. veuillotiana* s. str. (FIG. 1.1, see also Taxonomy Section). Four isolates of *T. veuillotiana* formed single-isolate lineages dispersed throughout the tree. Two of these isolates from Japan fell within a larger clade consisting of Clades TV1, TV2 and TV3, and two are isolates from tropical regions; one strain from Costa Rica (G.J.S. 10-124) and one isolate from Venezuela (G.J.S. 90-171) (FIG. 1.1), which was previously described as *Nectria acrotyla* by Brayford and Samuels (1993). These authors stated that this species is "*Nectria veuillotianae* affinis" based on morphological characteristics of the teleomorphic and anamorphic states. *Nectria acrotyla* is here newly combined in the genus *Thelonectria*. The single isolate

lineages in *T. coronata* (C.T.R. 72-178) and *T. veuillotiana* (MAFF 241544, MAFF 241551, G.J.S. 10-124) are not described here as new species.

Among the genomic regions sequenced in this study, all except for ITS-LSU revealed the same cryptic lineages within the two species complexes. For *T. coronata* and *T. veuillotiana*, the nucleotide diversity (π) within each of the clades was low, ranging from 0 to 0.0155 in *T. coronata* (TABLE 1.2) and 0 to 0.0196 in *T. veuillotiana* (TABLE 1.3). In general, all loci included in this study showed low nucleotide diversity or low number of polymorphisms within and between clades, consequently the average numbers of nucleotide differences among the clades was also low. Pairwise nucleotide divergence between clades ranged from 0.12% to 4.62% in *T. coronata* and 0.097 % to 6.87% in *T. veuillotiana* and, on average, nucleotide divergence was higher among clades in *T. coronata* (TABLE 1.2) than among clades in *T. veuillotiana* (TABLE 1.3). As a whole, ITS and LSU regions exhibited low levels of interspecific nucleotide diversity, making the identification of cryptic lineages using these markers only possible when clades show a high genetic divergence (> 1%). In *T. coronata,* Clades TC1 and TC2 showed the smallest molecular divergence when compared with the more divergent Clades TC3 to TC5 (TABLE 1.2) and, similarly, in *T. veuillotiana*, clades TV1, TV2, and TV3 showed the smallest molecular divergence compared with clades TV4 to TV6 (TABLE 1.3). In *T. veuillotiana,* analyses of rates of sequence evolution in the protein coding regions showed a dN/dS ratio < 1. In *T. coronata* all protein coding regions showed a dN/dS < 1, except *tub*, which showed a dN/dS ratio > 1.

Tests for recombination detection

No significant evidence of recombination or incongruence within or among the different clades in *T. coronata* or *T. veuillotiana* was detected by any of the tests proposed. Results obtained with the split decomposition method to detect recombination (Bandelt and Dress 1992, Huson and Bryant 2006) are included in FIG. 2.1.

Morphological studies

Mean values of morphological characters observed among isolates of *T. coronata* and *T. veuillotiana* are included in TABLE 1.4. The mean values of characters such as size of ascospores, conidia, and phialides show considerable overlap among the different isolates within clades. Although the discreteness of the recognized clades is not evident at first glance, the different lineages have, on average, dissimilar values for the morphological characters (ascospore and conidial size) that are useful in their differentiation. For *T. coronata* isolates, only morphological characters of the teleomorph could be studied, as the isolates failed to produce the anamorph in culture even when grown on different media and under different light regimes. Interestingly, isolates in this group produced perithecial structures as early as one week in culture; all clades in *T. coronata* showed this characteristic. Ascospores from perithecia produced in culture germinated and formed new colonies however these also failed to produce the anamorph. Clades in this complex showed insignificant variation in the mean values of ascospore size; four clades have a mean ascospore length of 20 μm although varying in ascospore width, and one Clade (TC2) has an average ascospore length and width smaller than the other clades in the complex (TABLE 1.4). Growth

rates and culture characteristics showed insignificant differences among clades in *T. coronata*.

In the *T. veuillotiana* complex, data for ascospores, conidia, and phialide dimension were obtained; however, isolates collected in tropical regions (G.J.S. 10-124, G.J.S. 91-171) sporulated poorly in culture. Isolates in Clades TV1 and TV2 are characterized by having 3–5-septate conidia. Even though these two clades show overlapping average sizes of anamorphic characters, they can be differentiated based on ascospore size and geography; Clade TV1 consists of isolates collected in the United States and with ascospores having a mean length of 16 μm, while Clade TV2 consists of isolates from Argentina with a mean ascospore length of 14 μm (TABLE 1.4). Isolates in Clade TV3 (*T. amamiensis*) are characterized by having macroconidia with hooked, acute to rounded apical cells and rounded to truncate basal cells. This species is only known from Japan (Hirooka et al. 2006). Isolates in Clades TV4 and TV5 have on average smaller conidia and larger ascospores compared to the other clades; isolates in Clade TV4 are restricted to the southeastern U.S.A, i.e. North Carolina and Tennessee, while isolates in Clade TV5 are from the Azores and France. Isolates in Clade TV6 have on average smaller ascospores (14.4 × 6.1 μm) compared with the other clades in the group (TABLE 1.4). Isolates G.J.S. 10-124 and *Nectria acrotyla* (G.J.S. 90-171) have on average longer and wider ascospores and smaller conidia than the other clades in the *T. veuillotiana* complex. *Nectria acrotyla* G.J.S. 90-171 has longer and wider ascospores compared to G.J.S. 10-124 and produces microconidia and chlamydospores in culture. Isolates in Clades TV1, TV4, TV6, and *N. acrotyla* G.J.S. 90-171 produce perithecia in culture. Growth rates and colony

characteristics of the different clades in *T. veuillotiana* were not significantly different.

Taxonomy

The results obtained from the phylogenetic analyses, DNA sequence divergence tests and morphological observations indicate that *Thelonectria coronata* and *T. veuillotiana* are species complexes, and consequently the different lineages are here described as new species. For *T. coronata* and *T. veuillotiana* sensu stricto a full description is presented. However, for the newly described species only a brief description is provided, mainly including those characters that are different from the narrowly defined species of *T. coronata* and *T. veuillotiana*.

Thelonectria coronata (Penz. & Sacc) P. Chaverri & C. Salgado, Stud. Mycol. 68: 77. 2011.

FIG. 1.3, A–G.

MycoBank MB518568.

≡ *Nectria coronata* Penz. & Sacc., Malpighia 11: 510. 1897.

≡ *Neonectria coronata* (Penz. & Sacc.) Mantiri & Samuels, Can. J. Bot. 79: 339. 2001.

= *Cylindrocarpon coronatum* Brayford & Samuels, Sydowia 46: 91. 1993.

Holotype. INDONESIA. Java. Tjibodas, on dead rotten bark, associated with *Nectria ambigua* v. *pallenti*, [date not known], [?Penzig] 452 ex p.

Stromata inconspicuous. Perithecia globose to subglobose with smooth and shiny or slightly roughened surface, (210–)260–360(–500) μm high, (100–)240–300(–400) μm wide, gregarious in groups of 30 or less, or solitary, often crowded around small cankers in bark, superficial or with the base immersed in erumpent basal stroma, not collapsed when dry, rust to umber with an often darker ostiolar disk, little or no reaction to 3% KOH, yellow in lactic acid, ostiolar disk 125–200 μm wide, of thin-walled saccate cells forming a distinctive fringe around the perithecial apex giving the ostiolar ring a crown-like aspect. Cells at the surface of the perithecial wall lacking a definite outline or nearly circular; perithecial wall 15–30 μm wide, of a single cell layer, lumina 6–25 μm long, 2–4 μm wide, increasingly compacted toward the perithecial locule; perithecial apex formed by a palisade of hyphal elements continuous with the inner region of perithecial wall, tips of cells at the exterior of the apical disc swollen, saccate, at the interior increasingly narrower toward the ostiolar canal and merging with periphyses. Asci clavate to fusiform, (54–)60–90(–105) × (7–)11–15(–18) μm, 8-spored, lacking an apical ring. Ascospores ellipsoid to fusiform, (16.0–)17.5–20.3(–23.0) × (4.7–)6.0–8.5(–11.2) μm (mean 20.7 × 8.3 μm), two-celled, symmetrical or eccentric, sometimes with one side curved and one side flattened, not constricted at the septum, spinulose or striate, hyaline becoming yellowish. Colonies on PDA 22–24 mm diam after 7 d at 20 C, aerial mycelium floccose, ocher to buff, colony reverse also ocher to buff. Anamorph not observed in cultures. Perithecia produced after three weeks on SNA.

Habitat and distribution. On bark of shrubs and trees, sometimes associated with small cankers; known from Indonesia (type locality), Taiwan, and Costa Rica. Possibly pantropical.

Epitype designated here. INDONESIA. NORTH SULAWESI: Gn. Muajat, Danua Alia, 00°45'N, 124°25'E, elev. 1400 m, on decaying herbaceous stem, 26 Oct. 1985, G.J. Samuels (NY GJS 2400A, ex-epitype culture G.J.S. 85-207 = IMI325241).

Additional specimens examined. COSTA RICA. LIMON PROVINCE. Parque Nacional Braulio Carrillo, 10°02'N, 84°01'W, elev. 1562 m, on bark of decaying shrub, 13 Mar. 2010, C. Salgado A.Y. Rossman, G.J. Samuels, Y. Hirooka, C. Herrera, P. Chaverri P.C. 1006 (BPI 882597, culture G.J.S. 10-108 = CBS 132322). TAIWAN. TAIPEI COUNTY: Hsintien, Jhih-tan-shan, on bark, 9 Oct. 2003, J –R Guu 92100902 (culture J.-R. Guu 92100902 = CBS 132334).

Notes. This species corresponds to *T. coronata s. str*. A specimen with culture from Indonesia, the type locality, is designated as epitype. Cultures of this species produce few or no asexual reproductive structures, even on recently collected isolates. Possible reasons for this behavior are discussed above. Brayford and Samuels (1993) observed characteristics of the asexual reproductive structures for species other than *T. coronata s. str*.

Thelonectria diademata C. Salgado & M. Capdet, sp. nov.

FIG. 1.3, H–M.

MycoBank MB564258.

Similar to *Thelonectria coronata;* ascospores 19–22 × 6.8–9.8 μm, spinulose and/or striate; no conidia produced in culture.

Holotype. ARGENTINA. TUCUMAN PROVINCE: road to Catamarca, near Rio Cochuna, on decaying bark of a fallen tree, 18 Apr. 2011, C. Salgado, A.Y. Rossman (BPI 882585, ex-type culture A.R. 4765 = CBS 132331).

Etymology. Refers to the crown of saccate cells around the apex giving the perithecia a coronate aspect.

Stromata inconspicuous. Perithecia globose to subglobose with smooth and shiny or slightly roughened surface, gregarious in groups of 20 or less, or solitary, often crowded around small cankers or cracks in bark, superficial or with the base immersed in erumpent basal stroma, chestnut colored. Asci clavate to fusiform, (55–)59–90(–103) × (7–)11–14(–18) μm, 8-spored, lacking an apical ring. Ascospores ellipsoid to fusiform, (17.0–)19–22.2(–24.7) × (5.3–)6.8–9.8(–11.2) μm (mean 20.7 × 8.3 μm), two-celled, symmetrical or eccentric, sometimes with one side curved and one side flattened, not constricted at the septum, spinulose, sometimes spinulae arranged longitudinally giving ascospores a striate appearance, hyaline becoming yellowish. Colonies on PDA 21–24 mm diam after 7 days at 20 C, aerial mycelium floccose, pale luteous to buff, colony reverse buff. Anamorph not observed in culture. Chlamydospores not produced on SNA. Perithecia produced after three weeks on SNA.

Habitat and distribution. On bark of recently killed and decaying shrubs and trees, sometimes associated with small cankers and cracks on bark. Known from Argentina, Costa Rica, and Jamaica; possibly extending throughout Neotropical and subtropical regions in South America.

Additional specimens examined. ARGENTINA. TUCUMAN PROVINCE: road to San Javier, ca. 2 km, on decaying bark of fallen tree, 20 Apr. 2011, C. Salgado, A.Y. Rossman (BPI 882586, culture A.R. 4787 = CBS 132332). COSTA RICA. LIMON PROVINCE: Parque Nacional Braulio Carrillo, 10°3'N, 84°01W, elev. 1734, on decaying bark of decaying shrub, 14 Mar. 2010, C. Salgado, A.Y. Rossman, G.J. Samuels, Y. Hirooka, C. Herrera, P. Chaverri, P.C. 1080 (BPI 882584, culture G.J.S. 10-137 = CBS 132321). JAMAICA. St. Andrew Parish, Holywell Forestry Camp, on *Pinus patula*, 11 Jan. 1971, C.T. Rogerson (NY CUP-MJ 767, culture C.T.R. 71-52 = CBS 132333).

Notes. *Thelonectria diademata* is an inconspicuous species often collected by chance in association with other species in the genus *Thelonectria* such as *T. discophora* and *T. lucida*.

Thelonectria cidaria C. Salgado & P. Chaverri, sp. nov.

FIG. 1.4, A–F.

MycoBank MB564259.

Similar to *Thelonectria coronata;* ascospores 19–22 × 5.9–7.2 μm, spinulose; no conidia produced in culture.

Holotype. COSTA RICA. LIMON PROVINCE: Parque Nacional Braulio Carrillo, entrance to Quebrada González, 10°9'N, 83°56', elev. 697 m, on twigs of dead shrub, 15 Mar. 2010, C. Salgado, A.Y. Rossman, G.J. Samuels, Y. Hirooka, C. Herrera, P. Chaverri, P.C. 1110 (BPI 882589, ex-type culture G.J.S. 10-135 = CBS 132323).

Etymology. Refers to the crown of saccate cells around the apex giving the perithecia a coronate aspect.

Mycelium visible on some specimens, white. *Stromata* inconspicuous. Perithecia globose to subglobose with smooth and shiny or slightly roughened surface, gregarious in groups of 30 or less, or solitary, often crowded around small cankers or cracks in bark, superficial or with the base immersed in erumpent basal stroma, scarlet to rust. Asci clavate to fusiform, (57–)59–85(–101) × (7–)11–15(–18) μm, 8-spored, lacking apical ring. Ascospores ellipsoid to fusiform, (18.0–)19.4–22.6(–25.6) × (5.3–)5.9–7.2(–8.0) μm (mean 21 × 6.6 μm), two-celled, symmetrical or eccentric, sometimes with one side curved and one side flattened, not constricted at the septum, spinulose, sometimes spinulae arranged longitudinally giving ascospores a striate appearance, hyaline, becoming yellowish. Colonies on PDA 13–19 mm diam after 7 days at 20 C, aerial mycelium floccose to slimy and felty, white to buff, colony reverse buff with umber center. Anamorph not observed in culture. Chlamydospores not produced on SNA. Perithecia produced after three weeks on SNA.

Habitat and distribution. On bark of decaying shrubs and trees. Known from Costa Rica and Jamaica; possibly distributed throughout the Neotropical region.

Additional specimens examined. COSTA RICA. LIMON PROVINCE: Parque Nacional Braulio Carrillo, entrance to Quebrada González, 10°9'N, 83°56', elev. 697 m, on twigs of dead shrub, 15 Mar. 2010, C. Salgado A.Y. Rossman, G.J. Samuels, Y. Hirooka, C. Herrera, P. Chaverri, P.C. 1112 (BPI 882588, culture G.J.S. 10-136 = CBS 132324). JAMAICA. ST. THOMAS: between Wheelerfield and Johnson Mt., elev 150 m, on bark, C.T. Rogerson (NY CUP-MJ 874, culture C.T.R. 71-79 = IMI 325844).

Thelonectria stemmata C. Salgado & P. Chaverri, sp. nov.

FIG. 1.4, G–M.

MycoBank MB564260.

Similar to *Thelonectria coronata;* ascospores 18–23 × 7.2–9.2 μm, spinulose; no conidia produced in culture.

Holotype. JAMAICA. PORTLAND PARISH: along trail to Silver Hill Gap, near Woodcutter's Gap, vic. Newcastle, on wood, 9 Jan. 1971, G.J. Samuels, A.Y. Rossman (NY CUP-MJ 759, ex-type culture C.T.R. 71-19 = CBS 112468)

Etymology. Refers to the crown of saccate cells around the apex giving the perithecia a coronate aspect.

Stromata inconspicuous. Perithecia globose to subglobose with smooth and shiny or slightly roughened surface, gregarious in groups of 30 or less, or solitary, often crowded around small cankers or cracks in bark, superficial or with the base immersed in erumpent basal stroma, chestnut colored. Asci clavate to fusiform, (58–)60–87(–101) × (8–)11–15(–18) μm, 8-spored, lacking apical ring, partially to completely biseriate. Ascospores ellipsoid to fusiform, (16.0–)18.4–23.5(–29.0) × (5.6–)7.2–9.2(–11.0) μm (mean 20 × 8.2 μm), two-celled, symmetrical or eccentric, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, sometimes spinulae arranged longitudinally thus appearing striate, hyaline becoming yellowish. Colonies on PDA 19–22 mm diam after 7 d at 20 C, aerial mycelium floccose, white, colony reverse saffron to white. Anamorph not observed in culture. Chlamydospores not produced on SNA. Perithecia produced after three weeks on SNA.

Habitat and distribution. On bark of decaying shrubs and trees, sometimes associated with small cankers and cracks in bark. Known from Costa Rica, Jamaica, and Venezuela, possibly widespread in Neotropical regions.

Additional specimens examined. COSTA RICA. LIMON PROVINCE: Parque Nacional Braulio Carrillo, entrance from Calle Zurquí, 10°02'N, 84°01', elev. 1562, on bark of recently killed tree, 13 Mar. 2010, C. Salgado, A.Y. Rossman, G.J. Samuels, Y. Hirooka, C. Herrera, P. Chaverri, P.C. 997 (BPI 882587, culture G.J.S. 10-117 = CBS 132325); 10°03'N, 84°01', elev. 1734 m, on bark of decaying shrub, 14 Mar. 2010, C. Salgado, A.Y. Rossman, G.J. Samuels, Y. Hirooka, C. Herrera, P. Chaverri, P.C. 1058 (BPI 882596, culture G.J.S. 10-130 = CBS 132326). JAMAICA. PORTLAND PARISH: along trial to Silver Hill Gap, near Woodcutter's Gap vic. Newcastle, on wood, 9 Jan 1971, C.T. Rogerson (NY CUP-MJ 955, culture C.T.R. 71-27 = IMI 325840); A.Y. Rossman (NY CUP-MJ 751, culture C.T.R. 71-21 = CBS 132336). VENEZUELA. MERIDA PROVINCE: Parque Nacional Sierra Nevada, above Tabay, Qda. Coromoto, La Mucuy, 08°36'N, 71°02'W, elev. 2300 m, on bark, 9, 17 Nov. 1990, G.J. Samuels (BPI 1112753A, culture G.J.S. 90-141 CBS 125117).

Thelonectria coronalis C. Salgado & J.-R. Guu, sp. nov.

FIG. 1.5, A–G.

MycoBank MB564261.

Similar to *Thelonectria coronata*, ascospores 17–24 × 6–9 μm, striated, macroconidia scarcely produced 5–7 septate.

Holotype. TAIWAN. ILAN COUNTY: Fu-shan, Hapenshi, on bark, 21 Aug 2004, J. –R. Guu (BPI 884081, ex-type culture J.-R. Guu 93082102 = CBS 132337)

Etymology. Refers to the crown of saccate cells around the apex giving the perithecia a coronate aspect.

Mycelium visible, white. *Stromata* inconspicuous. Perithecia globose to subglobose with surface smooth and shiny or slightly roughened, gregarious in groups of 10 or less, or solitary, superficial or with the base immersed in erumpent basal stroma, bay to rust. Asci clavate to fusiform, (55–)60–76(–85) × (9–)11–15(–17) μm, 8-spored, lacking apical ring. Ascospores broadly ellipsoid, (17.4–)18.5–21.5(–24.0) × (6.2–)7.0–8.5(–9.2) μm (mean 20.0 × 7.7 μm), equally two-celled, not constricted at the septum, spinulose or striate, hyaline becoming yellowish. Colonies on PDA 35–40 mm diam after 7 d at 20 C, aerial mycelium floccose, white to buff with luteous drops of exudates towards center, colony reverse white to salmon. Cultures have little production of conidiophores and macroconidia, mostly 5–7 septate, microconidia not produced. Chlamydospores not produced on SNA. Perithecia produced after two or three weeks on SNA.

Habitat and distribution. On bark of decaying shrubs and trees. Known only from Taiwan.

Additional specimens examined. TAIWAN. TAIPEI COUNTY: Pingshi, Chou-tou-shan, on bark, 30 Apr 2005, J.–R. Guu (BPI 884082, culture J.–R Guu 94043006 = CBS 132338).

Notes. Although Guu et al. (2007) reported that one isolate of this species as *Neonectria coronata* (strain J.–R Guu 94043006) produced microconidia in culture, this was most likely due to a contaminant.

Thelonectria veuillotiana (Sacc. & Roum.) P. Chaverri & C. Salgado, Stud. Mycol. 68: 77. 2011.

FIG. 1.5, H–O.

MycoBank MB518574

≡ *Nectria veuillotiana* Sacc. & Roum. in Désmazières, Rev. Mycol. (Toulouse) 2: 189. 1880.

≡ *Dialonectria veuillotiana* (Sacc. & Roum.) Cooke, Grevillea 12: 110. 1884.

≡ *Cucurbitaria veuillotiana* (Sacc. & Roum.) Kuntze, Revis. Gen. Pl. (Leipzig) 3: 462. 1898.

≡ *Neonectria veuillotiana* (Sacc. & Roum.) Mantiri & Samuels, Can. J. Bot. 79: 339. 2001.

= *Sphaerostilbe sanguinea* Fuckel, Symb. Myc. App. 3: 22. 1877.

= *Cylindrocarpon candidulum* (Sacc.) Wollenw., Z. Parasitenk. 1: 160. 1928.

≡ *Atractium candiduli* Sacc., Syll. Fung. 2: 512.1883.

Isotype. FRANCE. "On the bark of dead *Gleditschia triacanthos*. J. Therry" *Roumeguere, Fungi Gallici exsiccati XI (1881): 1076* [BPI! (slide)!].

Perithecia solitary or gregarious in groups of many formed in cracks of bark, superficial or basally immersed in a inconspicuous yellow pseudoparenchymatous superficial stroma; perithecia red to scarlet, globose, 280–400 μm high, 250–360 μm wide, with a broad constricted "knobby" apex of same color as the perithecium, becoming dark red in 3% KOH, yellow in lactic acid, not collapsing when dry,

smooth to scaly. Cells at the surface of the perithecial wall circular to angular, 10–30 μm diam, walls 3 μm thick, perithecial wall 40–60 μm wide, comprising two or three regions, outer region when present 15–20 μm wide, composed by strongly pigmented thick walled globose cells, middle region 15–20 μm wide, comprising elongated to globose cells, inner region lining perithecial cavity, 10–15 μm wide, comprising layers of flattened cells, having thin, non pigmented walls. Perithecial apex formed of files of vertically elongated cells originating from the middle region of the perithecial wall and terminating in clavate cells 2–3 μm wide with pigmented walls. Asci cylindrical or clavate with conspicuous ring, (70–)76–97(–115) × 10–14(–16) μm, 8-spored. Ascospores ellipsoid to fusiform, (15.7–)18.0–21.0(–23.1) × (5.0–)5.6–6.8(–7.3) μm (mean 19.5 × 6.2 μm), hyaline, equally two-celled, sometimes with one side curved and one side flattened, slightly constricted at the septum, ascospore wall spinulose. Colonies on PDA 16.5–23.5 mm diam after 7 d at 20 C, aerial mycelium floccose, white to buff, colony reverse pale yellow to white with salmon center. Conidia on SNA forming after 3 d, hyaline, forming in slimy droplets in aerial mycelium or on agar surface, pionnotes sometimes formed close to filter paper. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen with periclinal thickening and collarette, (13.3–) 16.0–17.0(–21.8) × (2.5–)3.8 × 4.3(–5.3) μm. Macroconidia cylindrical to slightly fusiform, curved of rounded tips, 3–5(–6-septate): 3-septate (33.8–)40.3–53.4(–62.4) × (4.4–)5.1–6.3(–7.3) μm (mean 47.0 × 5.7 μm), 4-septate (24.0–)49.9–66.9(–79.0) × (4.6–)5.5–6.8(–7.8) μm (mean 58.4 × 6.2 μm), 5-septate (62.0–)63.2–69.8(–72.6) × (6.7–)7.0–7.6(–7.7) μm (mean 66.6 × 7.3 μm). Microconidia absent.

Chlamydospores produced on SNA. Perithecia produced after three weeks on SNA media.

Habitat and distribution. On bark of deciduous trees, *Eucalyptus* sp., *Fagus* sp., *Gleditschia triacanthos, Salix* sp.; Azores Islands, France, and Germany.

Additional type specimen examined. GERMANY. HATTENHEIM: on rooting trunk of *Salix*, *Fuckel, Fungi Rhenani 2655* (K!, syntype of *Atractium candidulum* Sacc., with *Sphaerostilbe sanguinea* Fuckel).

Additional specimens examined. AZORES. SÃO MIGUEL: 5.5 km south of Ribeira Grande toward Foga, on bark of *Eucalyptus* sp., 1 Apr 1978, A.Y. Rossman (CUP-MM 1706, culture A.R. 1751 = CBS 132341). FRANCE. OLORON-SAINTE-MARIE: Fôret de Bugangue, on bark of *Fagus* sp., G.J. Samuels (BPI 802643, culture G.J.S. 92-24 = CBS 125114); PYRENÉES ATLANTIQUES: vic. 64 Mauleon, Fôret de Cheraut, on bark of *Fagus* sp., 27 Oct 1998, G.J. Samuels (BPI 748323, culture G.J.S. 98-145 = CBS 123379).

Notes. *Thelonectria veuillotiana* is an inconspicuous species often collected in association with other species such as *T. discophora* and *T. lucida. Thelonectria veuillotiana* in the narrow sense seems to be distributed in the Old World, commonly found on species of *Fagus*.

Thelonectria nodosa C. Salgado & P. Chaverri, sp. nov.

FIG 1.6, A–H.

MycoBank MB564252.

Similar to *Thelonectria veuillotiana*. Species distributed in eastern regions of United States. Ascospores 15.7–16.2 × 6.2–6.3 μm. Chlamydospores present.

Holotype. UNITED STATES. TENNESSEE: Great Smoky Mountains National Park, vic. Cosby, Snake Den Rock Trail, 35°45'N, 83°13'W, elev. 940 m, on bark of *Thuja canadiensis*, 14 Jul 2004, G.J. Samuels (BPI 860484, ex-type culture G.J.S. 04-155 = CBS 132327).

Etymology. Refers to the flat, discoidal perithecial apex characteristic of this species.

Mycelium visible on specimens, white. Perithecia solitary or gregarious in groups of many, formed in cracks of bark, superficial or basally immersed in an inconspicuous yellow pseudoparenchymatous stroma; perithecia red to scarlet, globose with a broad constricted "knobby" apex of the same color as the rest of the perithecium, not collapsing when dry, smooth to scaly. Asci cylindrical or clavate with conspicuous ring, (68–)72–97(–115) × 10–14(–17) μm, 8-spored. Ascospores ellipsoid to fusiform, (13.0–)15.7–16.2(–20.2) × (4.8–)6.2–6.3(–7.7) μm (mean 16.0 × 6.3 μm), hyaline, equally two-celled, sometimes with one side curved and one side flattened, slightly constricted at the septum, ascospore wall spinulose. Colonies on PDA 17–21 mm diam after 7 d at 20 C, aerial mycelium floccose, apricot with white borders, colony reverse also apricot with white borders. Conidia on SNA media forming in hyaline, slimy droplets in aerial mycelium or on agar surface, pionnotes sometimes formed close to filter paper. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (10.7–)14.3–19.7(–27.0) × (2.8–)3.5 × 4.4(–5.3) μm, with periclinal thickening and collarette. Macroconidia cylindrical to slightly fusiform, curved with rounded tips, 3–5(–6)-septate: 3-septate (40.0–)49.8–63(–74.4) × (4.4–)5.2–6.3(–7.7) μm (mean 56.4 × 5.8 μm), 4-septate (46.2–)61.2–74.8(–85.4) × (4.6–)5.4–6.5(–7.3) μm (mean 68.0 × 6.0

μm), 5-septate (47.5–)67.0–81.6(–96.3) × (4.8–)5.6–6.7(–7.7) μm (mean 74.4 × 6.2 μm). Microconidia absent, chlamydospores produced on SNA by some isolates of this species. Fertile perithecia produced after three weeks on SNA.

Habitat and distribution. On bark of decaying shrubs and trees. Sometimes found growing on weak tissues surrounding canker lesions caused by pathogenic *Neonectria* species. Known from eastern regions of United States.

Additional specimens examined. UNITED STATES. CONNECTICUT: New Haven, West Rock Ridge State Park, on *Fagus grandifolia* bark, Oct 2007, R. Marra, A.Y. Rossman (BPI 880706, culture A.R. 4500 = CBS 124742); New Haven, West Rock Ridge SP, on bark of *Fagus grandifolia* standing tree, Oct 2007, R. Marra, A.Y. Rossman (BPI 878946, culture A.R. 4505 = CBS 125173). NEW YORK: Dutchess Co, East side of Pawling, Pauling Nature Reserve, Nature Conservancy, on bark of *Acer* sp., 6, 8 Oct. 1990, G.J. Samuels (BPI 1107130, culture G.J.S. 90-66 = CBS124352). VIRGINIA: Giles Co., Cascades Recreation Site, elev. 840 meters, 37°2'N, 80°35'W, 4 miles N. of Pembroke, Little Stony Creek, on bark of dead *Quercus* sp tree, 18 Sep 1991, G.J. Samuels (BPI 1112885, culture G.J.S. 91-116 = CBS 124740); Mt. Lake Biological Station, Alt 1170 meters, 37°22'N, 80°31W, Spruce Bog, on standing *Rhododendron* sp. base, 17 Sep 1991, G.J. Samuels (BPI 1112874, culture G.J.S. 91-105 = IMI 351445).

Notes. This species is often associated with pathogenic *Neonectria* species, such as *N. ditissima*. The core of the distribution of this species seems to be the eastern states of the USA, such as Connecticut, New York, Virginia and less frequently Tennessee.

Thelonectria torulosa C. Salgado & M. Capdet, sp. nov.

FIG. 1.6, I–P.

MycoBank MB564253.

Similar to *Thelonectria veuillotiana*. Known from subtropical areas in Argentina, possibly in other subtropical areas of South America.

Holotype. ARGENTINA. TUCUMAN PROVINCE: road to Catamarca, near Río Cochuna, on decaying bark of a fallen tree, 18 Apr. 2011, C. Salgado, A.Y. Rossman (BPI 882590, ex-type culture A.R. 4764 = CBS 132339).

Etymology. Refers to the flat, discoidal perithecial apex characteristic of this species.

Mycelium visible in some specimens, white. Perithecia solitary or gregarious in groups of many formed in cracks of bark, superficial or basally immersed in an erumpent inconspicuous yellow pseudoparenchymatous stroma, perithecia red to dark red, globose with a broad constricted "knobby" apex of same color as the rest of the perithecium, not collapsing when dry, smooth to scaly surface. Asci cylindrical or clavate, simple with conspicuous ring, (65–)72–94(–110) × 10–14(–16) μm, 8-spored. Ascospores ellipsoid to fusiform, (12.5–)13.6–16.2(–17.5) × (5.3–)5.7–6.8(–7.4) μm (mean 15.0 × 6.3 μm), hyaline, equally two-celled, sometimes with one side curved and one side flattened, slightly constricted at the septum, spinulose. Colonies on PDA 38–45 mm diam after 7 d at 20 C, aerial mycelium floccose, white with saffron center, colony reverse saffron with white borders. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface on slimy pionnotes sometimes formed close to filter paper. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen

(10.0–)15.3–19.7(–24.0) × (2.7–)3.5 × 4.4(–5.4) μm, with periclinal thickening and collarette. Macroconidia cylindrical to slightly fusiform, based truncate and tips slightly tapered, 3–5(–6)-septate, 3-septate (52.0–)51.7–62.7(–67.1) × (5.7–)5.8–6.8(–7.0) μm (mean 57.2 × 6.3 μm), 4-septate (54.5–)59.8–74.5(–84.4) × (5.3–)5.7–6.7(–7.3) μm (mean 67.2 × 6.2 μm), 5-septate (67.8–)73.4–86.3(–95.3) × (5.6–)6.3–7.2(–8.0) μm (mean 80.0 × 6.8 μm). Microconidia, chlamydospores, and perithecia not produced on SNA.

Host and distribution. On bark of recently dead shrubs and trees. Known from wet subtropical forest of Argentina, possibly occurring elsewhere in subtropical regions of South America.

Additional specimen examined. ARGENTINA. TUCUMAN PROVINCE: road to Catamarca, Las Lenguas trial, on decaying bark, 18 Apr 2011, C. Salgado A.Y. Rossman (BPI 882591, culture A.R. 4768A = CBS 132340).

Notes. This species is common in a wet subtropical forest in northern Argentina. It is often associated with other species such as *Thelonectria coronata*, *T. discophora,* and *T. lucida*.

Thelonectria amamiensis (Hirooka & Tak. Kobay.) C. Salgado & P. Chaverri, comb. nov.

Mycobank MB564254.

≡ *Neonectria amamiensis* Hirooka & Tak. Kobay., Mycoscience 47: 250. 2006.

= *Cylindrocarpon amamiense* Hirooka & Tak. Kobay., Mycoscience 47: 250. 2006.

Similar to *Thelonectria nodosa* and *T. torulosa*; ascospores 16.0–20.0 × 5.0–8.5 μm; chlamydospores absent; macroconidia with a strongly hooked and acute apical cell.

Habitat and distribution. On bark of recently dead trees in Japan.

Descriptions and illustrations. See Hirooka et al. (2006).

Thelonectria gongylodes C. Salgado & P. Chaverri, sp. nov.

FIG. 1.7, A–H.

MycoBank MB564255.

Similar to *Thelonectria nodosa* and *T. veuillotiana.* Ascospores 14.9–19.1 × 5.7–7.3 μm; chlamydospores absent; known from the eastern USA (North Carolina, Tennessee).

Holotype. UNITED STATES. TENNESSEE: Great Smoky Mts. National Park, vic. Crosby, Greenbriar Ranger Station, Ramsey Cascade Trail, elev. 940 m. 35°45'N 83°13'W, on decaying bark of *Acer* sp., 14 Jul 2004, G.J. Samuels (BPI 881093, ex-type culture G.J.S. 04-171 = CBS 124611).

Etymology. Refers to the flat, discoidal perithecial apex characteristic of the species

Perithecia solitary or gregarious in groups of many, in cracks of bark, superficial or basally immersed in an inconspicuous yellow pseudoparenchymatous stroma; perithecia orange to scarlet, globose with a broad constricted "knobby" apex of same color as perithecium, not collapsing when dry, smooth to scaly surface. Asci cylindrical or clavate, apex simple with conspicuous ring, (68–)72–97(–115) × 10–14(–17) μm, 8-spored. Ascospores ellipsoid to fusiform, (12.6–)15.0–19.0(–22.6) × (5.2–)5.7–7.3(–9.0) μm (mean 17.0 × 6.6 μm), hyaline, equally two-celled, sometimes with one side curved and one side flattened, slightly constricted at the septum, ascospore wall spinulose. Colonies on PDA 20–24 mm diam after 7 d at 20 C, aerial mycelium floccose, white with umber center, pigmented concentric rings

often observed, colony reverse saffron to white. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface on slimy pionnotes close to filter paper and forming concentric rings. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen, (12.0–)14.6–19.5(–26.0) × (2.5–)3.5–4.4(–5.0) μm, with periclinal thickening and collarette. Macroconidia cylindrical to slightly fusiform, curved with rounded tips, 3–5(–6)-septate: 3-septate (30.0–)38.5–53(–62.4) × (4.2–)5–6.2(–7.5) μm (mean 45.8 × 5.7 μm), 4-septate (34.0–)49.0–64.7(–70.3) × (4.0–)5.5–6.5(–7.3) μm (mean 57.0 × 6.0 μm), 5-septate (47.8–)56.7–69.8(–80.0) × (5.2–)5.8–6.8(–7.8) μm (mean 63.3 × 6.3 μm). Microconidia and chlamydospores not produced on SNA. In some isolates fertile perithecia are produced after three weeks on SNA.

Habitat and distribution. On decaying bark of *Acer* sp. and *Fagus* sp.; known from the United States (North Carolina, Tennessee).

Additional specimens examined. UNITED STATES. NORTH CAROLINA: Clay Co., Standing Indian Campground off Hwy. 64, on bark of dead *Fagus* sp. tree, 15 Oct 1990, G.J. Samuels (BPI 1107131, culture G.J.S. 90-50 = IMI 343571); Jackson Co., Nantahala National Forest, Bull Pen Road to Chattooga River, Ellicott Rock Trail from Fowler Creek, on bark of dead *Acer rubrum* tree, 28 Sep 1989, G.J. Samuels (BPI 1107265, culture G.J.S. 89-131 = IMI 336160); Macon Co., Ammons Branch Campground, off Bull Pen road, elev. 3000 ft. 35°1'N 83°8'W, on bark of dead *Quercus* sp. tree, 14 Oct 1990, G.J. Samuels (BPI 1107127, culture G.J.S. 90-48 = CBS 125118).

Notes. *Thelonectria gongylodes*, *T. nodosa,* and *T. torulosa* are the three species related to *T. veuillotiana* that ocurr in the United States. *Thelonectria nodosa* was found primarily in the northeastern USA (New York, Connecticut) and Appalachian areas in Virginia and Tennessee, while *T. gongylodes* was collected in the southeastern USA (North Carolina) and, although less frequently, in Appalachian areas of Tennessee.

Thelonectria truncata C. Salgado & P. Chaverri, sp. nov.

FIG. 1.7, I–Q.

MycoBank MB564256.

Similar to *Thelonectria gongylodes, T. nodosa*, and *T. veuillotiana*; ascospores 12–16 × 5–6.9 μm.

Holotype. UNITED STATES. TENNESSEE: Great Smoky Mountains National Park, vic. Crosby, Greenbriar Ranger Station, Ramsey Cascade Trail, elev. 480 m, 35°43'N 83°24'W, on bark of decaying tree, 13 Jul 2004, G.J. Samuels (BPI 881092, ex-type culture G.J.S. 04-357 = CBS 132329).

Etymology. Refers to the flat, discoidal perithecial apex characteristic of this species.

Perithecia solitary or gregarious in groups of many formed in cracks of bark, basally immersed in an inconspicuous yellow pseudoparenchymatous non erumpent stroma, perithecia orange to scarlet, globose with a broad constricted "knobby" apex of same color as the rest of perithecium, not collapsing when dry, smooth to scaly surface. Asci cylindrical or clavate, apex with conspicuous ring (70–)72–90(–110) × 10–14(–18) μm, 8-spored; ascospores completely to partially biseriate. Ascospores ellipsoid to fusiform, (11.3–)12.5–16(–18.2) × (4.6–)5.2–7(–7.6) μm (mean 14.4 × 6 μm),

hyaline, equally two-celled, sometimes with one side curved and one side flattened, slightly constricted at the septum, spinulose. Colonies on PDA 13–18 mm diam after 7 d at 20 C, aerial mycelium floccose, white to saffron, colony reverse saffron to white. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA agar. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.2–)16.0–20.6(–23.4) × (–2.5)3.5–4.4(–5.1) μm, with periclinal thickening and collarette. Macroconidia cylindrical to slightly fusiform, curved of rounded tips, 3–5(–6)- septate: 3-septate (40.5–)46.9–58.9(–71.4) × (4.4–)4.9–5.8(–7) μm (mean 53.3 × 5.4 μm), 4-septate (44.6–)55–67.5(–74.4) × (4.4–)5–6(–6.5) μm (mean 61.2 × 5.5 μm), 5-septate (60.0–)65.4–77(–82.5) × (4.4–)5.2–6.3(–6.6) μm (mean 71.2 × 5.8 μm). Microconidia and chlamydospores not produced on SNA. Fertile perithecia produced after three weeks on SNA.

Host and distribution. On bark of recently killed and decaying *Tsuga* sp. and other trees. Known from the United States and Japan.

Additional specimen studied. JAPAN. OKUTAMA-GUN: on twigs, 20 Nov 2003, Y. Hirooka H224 (BPI 882090, culture MAFF 241521).

Notes. This species has been collected from distant geographic locations suggesting that its distribution may be extensive throughout the Asian-American Pacific regions.

Thelonectria acrotyla (Brayford & Samuels) C. Salgado & P. Chaverri, comb. nov.

Mycobank MB564257.

≡ *Nectria acrotyla* Brayford & Samuels, Mycologia 85: 614. 1993.

= *Cylindrocarpon mirum* Brayford & Samuels, Mycologia 85: 614. 1993

Similar to *Thelonectria truncata* and *T. veuillotiana*; ascospores 23 × 9 μm; chlamydospores absent, microconidia present 6–9 × 3–5 μm.

Habitat and distribution. On bark of deciduous trees. Known from Brazil and Venezuela; possibly occurring throughout the Neotropics.

Descriptions and illustrations. See Brayford and Samuels (1993).

Discussion

Thelonectria coronata and *T. veuillotiana* are complexes of cryptic species as demonstrated by phylogenetic analyses of six nuclear loci and supported by population diversity measures. Dettman et al. (2003) proposed that cryptic species could be recognized if such groups meet either one of two criteria: a) genealogical concordance, or b) genealogical nondiscordance. For *T. coronata* and *T. veuillotiana* complexes, the cryptic lineages fit either the genealogical concordance or the genealogical non-discordance criteria. Cryptic species in *T. coronata* and *T. veuillotiana* complexes are characterized by genetically distinctive groups with little or no morphological differentiation indicating that most of the genetic variation among individuals is not yet expressed as variation in morphological and physiological traits (Rodriguez et al. 2005, Taylor et al. 2006, Bensch et al. 2010). With the development and advancement of methods of species recognition based on genetic isolation (phylogenetic species recognition, PSR), mycologists have an apparently easier and open way to recognize species. First individual organisms are sorted into species based on phylogenetic analyses and then variable phenotypic

characters that correlate with the cryptic species are sought (Taylor et al. 2006). In our study, the *T. coronata* and *T. veuillotiana* species complexes show significant overlap in morphological characters; however, careful observation of biometric data revealed differences in mean values that, together with the genetic divergence and other data such as geographic origin of the strains, help to differentiate clades. These differences are more evident in clades of the *T. veuillotiana* complex while the *T. coronata* complex represents a more difficult case. Despite the genetic divergence that exists among clades in this group, the morphological characters are mostly continuous making key construction and species diagnosis difficult. Regardless of this obstacle, the new lineages detected within these species complexes are here designated and described as new species. The taxonomic key provided for the *T. coronata* clades can help in the identification of the species based on the most informative morphological characters; however the individual species can only be unequivocally identified using molecular data. The ITS 1-2 and *rpb1* regions are useful for this purpose. Although single-isolate lineages are potential new species, they are not named here waiting more extensive taxon sampling.

Isolates in the *T. coronata* complex do not produce the anamorph in culture; instead, most of the isolates formed perithecia with ascospores as the primary reproductive propagule in culture. Even though these isolates were collected over decades, lack of conidial sporulation was not associated with the amount of time the strains had been maintained in culture; isolates obtained more recently, from 2010 and 2011, also exhibited this behavior. Specific nutritional requirements found in host plants but not in artificial media may be necessary to induce conidial sporulation in

these fungi. Interestingly, only teleomorphic state of species in the *T. coronata* complex are known from nature. No reports of a cylindrocarpon-like anamorph exist suggesting that asexual reproduction is not common or perhaps absent in these species. Based on the observation that fertile perithecia readily form in culture, it is probable that species in this group are homothallic. Several other fungi in the Sordariomycetes that do not produce conidia are known to be homothallic (Nauta and Hoekstra 1992, Davis 2000, Pöggeler et al. 2000, Whittle et al. 2011). One example is the model fungus *Neurospora*, in which homothallic species are recognized by the lack of conidia, a strategy that reduces or eliminates conidium-related mutations. Homothallic species of *Neurospora* also showed a significantly higher ratio of non-synonymous to synonymous substitution rates (dN/dS > 1) compared with heterothallic species (Nygren et al. 2011). In this study, we found that at least one of the protein-coding regions sequenced showed an elevated ratio of non-synonymous to synonymous substitution rate, suggesting a history of weakened purifying selection and genomic degeneration characteristic of some homothallic species of fungi (Nygren et al. 2011).

The cryptic lineages in the *T. veuillotiana* complex could not be resolved by using the ITS 1-2 region alone due to the low molecular and intraspecific nucleotide diversity exhibited by the isolates. On the other hand, cryptic lineages in the *T. coronata* complex could easily be differentiated using this genomic region as the isolates seem to be more genetically divergent compared to species in the *T. veuillotiana* complex (TABLES 1.2 and 1.3). Single gene phylogenetic analyses of the *tub, tef1*, and *rpb1* protein-coding regions distinguished all the lineages in both the *T.*

coronata and *T. veuillotiana* complexes. The intraspecific variation of these regions was always very low compared to the interspecific divergence (TABLES 1.2 and 1.3), and this clear distinction between intraspecific and interspecific nucleotide divergence (the barcode gap) makes them good potential candidates as secondary barcodes for diversity and phylogenetic studies of fungi in the family Nectriaceae (Seifert 2009, Jargeat et al. 2011, Schoch et al. 2012).

None of the tests to detect recombination resulted in assigning recombinant limits between the clades in *T. coronata* and *T. veuillotiana*. As observed in FIG. 1.2 (neighbour networks), a reticulate pattern suggesting recombination among genotypes in *T. coronata* and *T. veuillotiana* was not observed. In the *T. veuillotiana* network (FIG. 1.2B), the reticulate pattern observed between Clades TV1 to TV4 could be due to the lack of phylogenetic resolution, homoplasy, or incomplete lineage sorting (Croll and Sanders 2009). One possible reason why recombination was not detected may be related to the reproductive biology of these fungi. As mentioned above, isolates of *T. coronata* and *T. veuillotiana* produce perithecia in culture suggesting that these species may be homothallic. In homothallic species, sexual and asexual offspring have identical genotypes resulting in a genome-wide homozygosity (Bruggeman et al. 2003, Giraud et al. 2008). When a population is clonal, recombination tests give negative results and, if caution is not exercised, these negative results can be interpreted as indicative of reproductive isolation. Homothallism and clonality do not always define isolated populations, and homothallism does not indicate a complete lack of recombination since somatic recombination and parasexuality may occur (Ekins et al. 2006). Even though the

negative result from these tests may be attributable to the reproductive biology of these fungi, the power of the test statistics may also have been reduced due to the small sample size of taxa, and as a consequence, of molecular data (Wall 1999). Moreover, these tests depend on the amount of genetic variation within the dataset (Taylor et al. 1999); our dataset may not have had sufficient variation for recombination to be detected by the methods used here, which infer recombination based on permutation of sites and the number of phylogenetically informative sites (Posada et al. 2002, Posada 2002). Other studies have shown that recombination tests can be biased when isolates in the population sample are collected from widely separated geographic locations or over the course of many years (Douhan, 2007). However, this would not explain the failure to detect recombination among the isolates of the *T. coronata* and *T. veuillotiana* complexes. Even though recombination was not detected within clades formed by isolates from distant geographic locations, recombination among isolates from the same locality could not be detected as well, for example in Clades TC1 and TC4 in *T. coronata* and TV5 and TV1 and TV6 of *T. veuillotiana.* The usefulness of the particular recombination tests used in this study for geographic differentiation and to identify putative species among different populations of fungi may not be applicable in most cases.

The molecular phylogeny of the *T. coronata* and *T. veuillotiana* species complexes provided in this study contributes to increasing our understanding of the diversity of the genus *Thelonectria*. Single isolate lineages are not described here as new species. Ideally, new species should be described based on more than one specimen or culture (Seifert and Rossman 2011). Future work and more taxon

sampling are needed to find the necessary support to confidently assign these isolates to new taxa.

Key to species in the *T. coronata* and *T. veuillotiana* complexes

1a. Perithecia with a distinctive fringe of saccate cells around the perithecial apex giving the perithecium a coronate aspect……*T. coronata* complex 2

1b. Perithecia without a distinctive fringe of saccate cells around the perithecial apex, instead having a constricted, "knobby", or discoidal apex …*T. veuillotiana* complex 6

2a. Macroconidia mostly 5–7 septate. Ascospores striate, averaging 18.5–21.5 × 7.0–8.5 µm; Taiwan ……*T. coronalis*

2b. Macroconidia not produced ……3

3a. Ascospores averaging 18 × 7 µm, spinulose or striate; possibly pantropical (Indonesia, Taiwan and Costa Rica) ……...……..……...*T. coronata*

3b. Ascospores averaging more than 18 × 7 µm, spinulose or striate; known only from Costa Rica ……...……...……..4

4a. Ascospores averaging 20 × 8 µm, spinulose or striate; known from Argentina, Costa Rica, and Jamaica….……*T. diademata*

4b. Ascospores averaging more than 20 µm long, spinulose, not striate; known from Costa Rica, Jamaica, or Venezuela…….……...5

5a. Ascospores averaging 21 × 6 µm; known from Costa Rica, Jamaica……*T. cidaria*

5b. Ascospores averaging 21 × 8 µm; known from Costa Rica, Jamaica, and Venezuela ……*T. stemmata*

6a. Ascospores averaging 19 × 6 µm; chlamydospores and perithecia produced on SNA; known from the Azores, France, and elsewhere in Europe ……...*T. veuillotiana*

6b. Not known in Europe; ascospores averaging 15–23 × 6–9 µm …...……..7

7a. Macroconidia with hooked and acute to round apical cell and rounded to truncate basal cell; known only from Japan……...*T. amamiensis*

7b. Macroconidia without hooked apical cell; known outside of Europe and Japan…8

8a. Known in subtropical regions of Argentina…….*T. torulosa*

8b. Known from tropical regions of Brazil and Venezuela or temperate region of the eastern USA and Japan……...……...9

9a. Known from tropical regions of Brazil and Venezuela; microconidia produced on SNA, ascospores averaging 23 × 9 µm……..*T. acrotyla*

9b. Not from tropical regions, known from eastern USA and Japan ……10

10a. Chlamydospores produced on SNA; ascospores averaging 16 × 6 µm; known from the eastern USA, Connecticut, New York, Virginia, less frequently Tennessee…….*T. nodosa*

10b. Chlamydospores no produced on SNA; ascospores averaging longer or shorter than 16 µm; known from the eastern US and Japan……11

11a. Ascospores averaging 17 × 6 µm; known from the eastern USA, mostly North Carolina, less frequently Tennessee……..*T. gongylodes*

11b. Ascospores averaging 14 × 6 µm; known from northeastern Tennessee and Japan ……*T. truncata*

Acknowledgements

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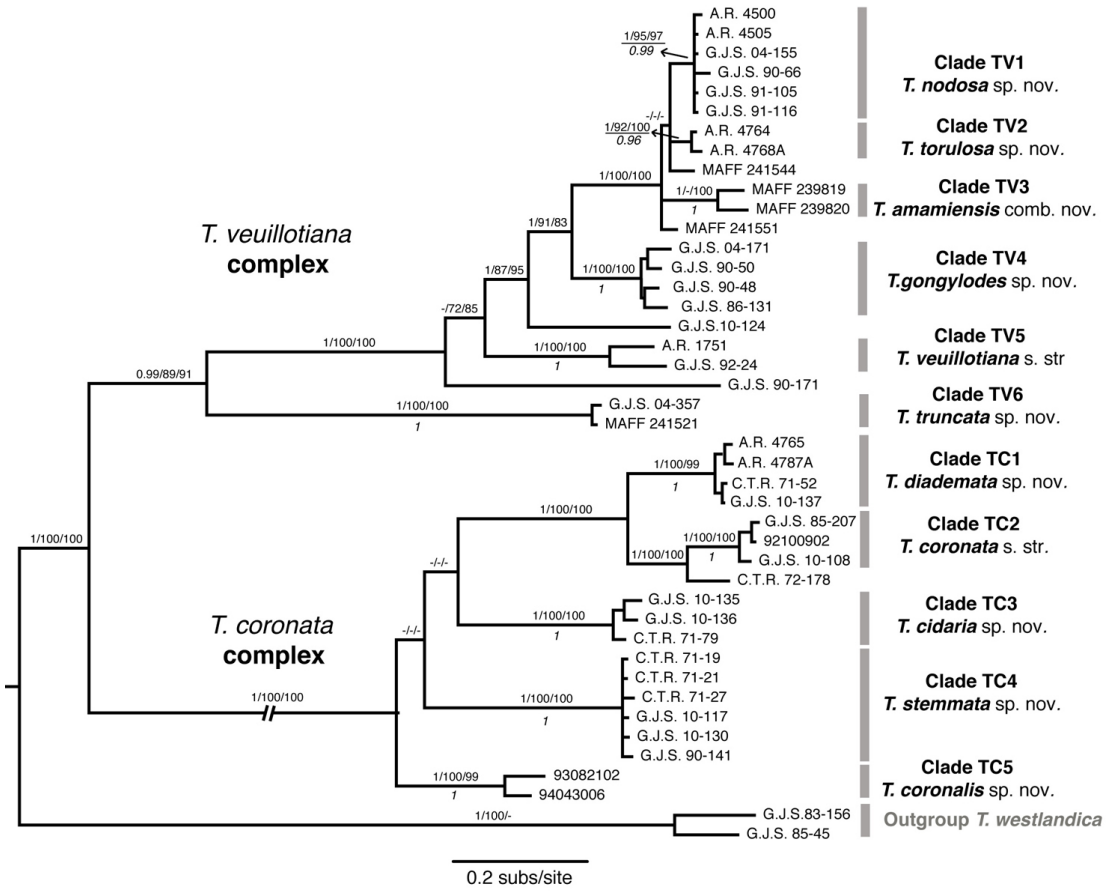

FIGURE 1.1. Bayesian majority rule-consensus tree of *Thelonectria coronata* and *T. veuillotiana* species complexes and outgroup taxa based on the concatenated data of six nuclear loci (*act*, LSU, ITS, *rpb1, tef1, tub*). Support values (posterior probability/ML bootstrap/MP bootstrap) are indicated above the branches, *gsi* vales are indicated below the branches. Only support values ≥ BS 70/PP 0.95 are indicated. Clade numbers plotted to the right of the tree indicate candidate species. *T. westlandica* was used as outgroup taxon.

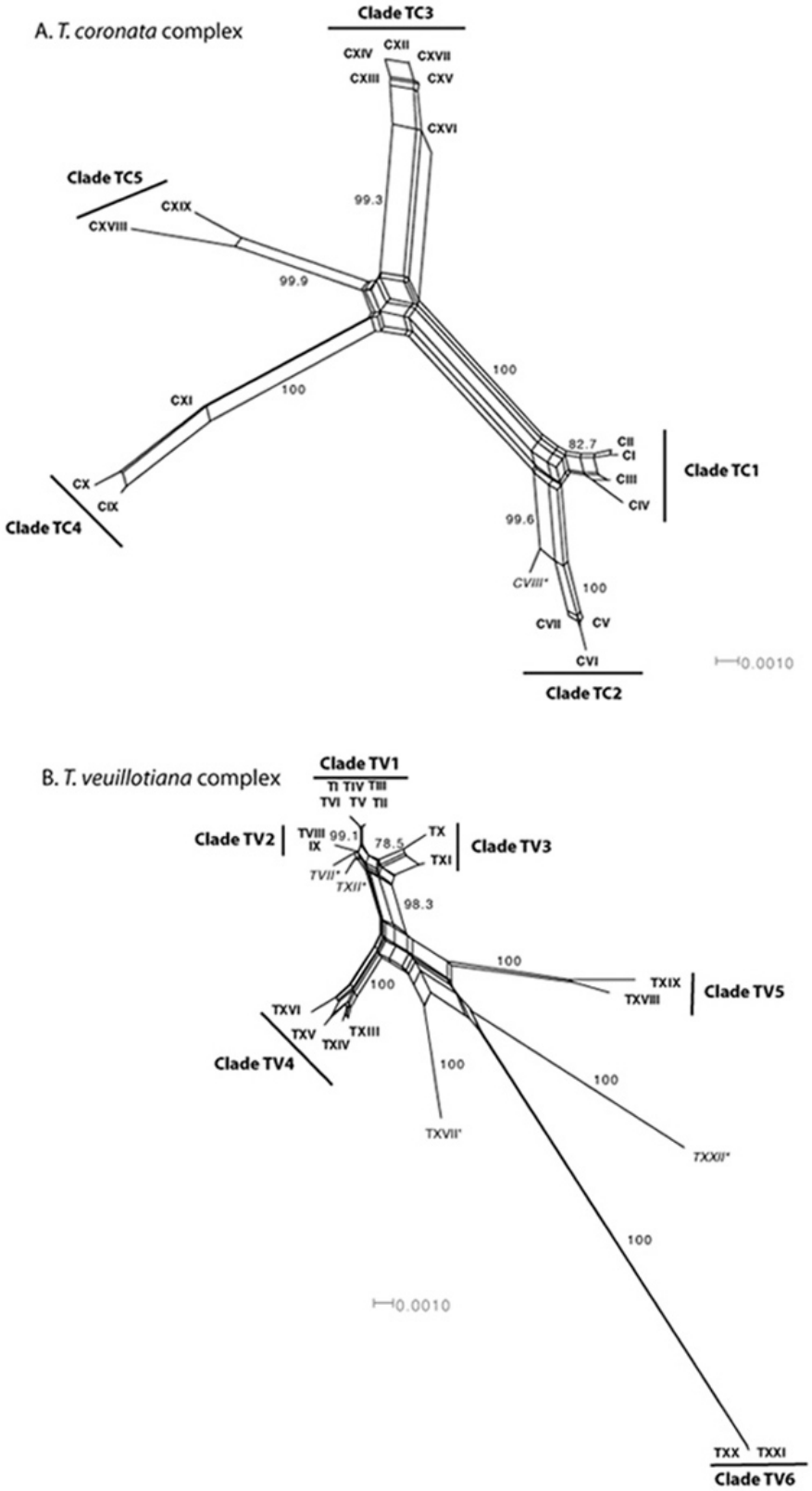
A. T. coronata complex
Clade TC3
CXII
CXIV
CXVII
CXIII
CXV
CXVI
Clade TC5
CXIX
CXVIII
99.3
99.9
100
CXI
100
82.7
CII
CI
CIII
CIV
Clade TC1
CX
CIX
Clade TC4
99.6
CVIII*
100
CVII
CV
CVI
Clade TC2
0.0010
B. T. veuillotiana complex
Clade TV1
TI TIV TIII
TVI TV TII
Clade TV2
TVIII
IX
99.1
78.5
TX
TXI
Clade TV3
TVII*
TXII*
98.3
100
TXIX
TXVIII
Clade TV5
100
TXVI
TXV
TXIV
TXIII
Clade TV4
100
100
TXVII*
TXXII*
100
TXX
TXXI
Clade TV6
0.0010

FIGURE 1.2. Neighbour-networks using uncorrected p-distance showing phylogenetic relationships among genotypes in (A) *Thelonectria coronata* and (B) *T. veuillotiana*. Bootstrap support for branches ≥ 90% is indicated. Roman numerals represent the different genotypes (for details see TABLE 1.1).

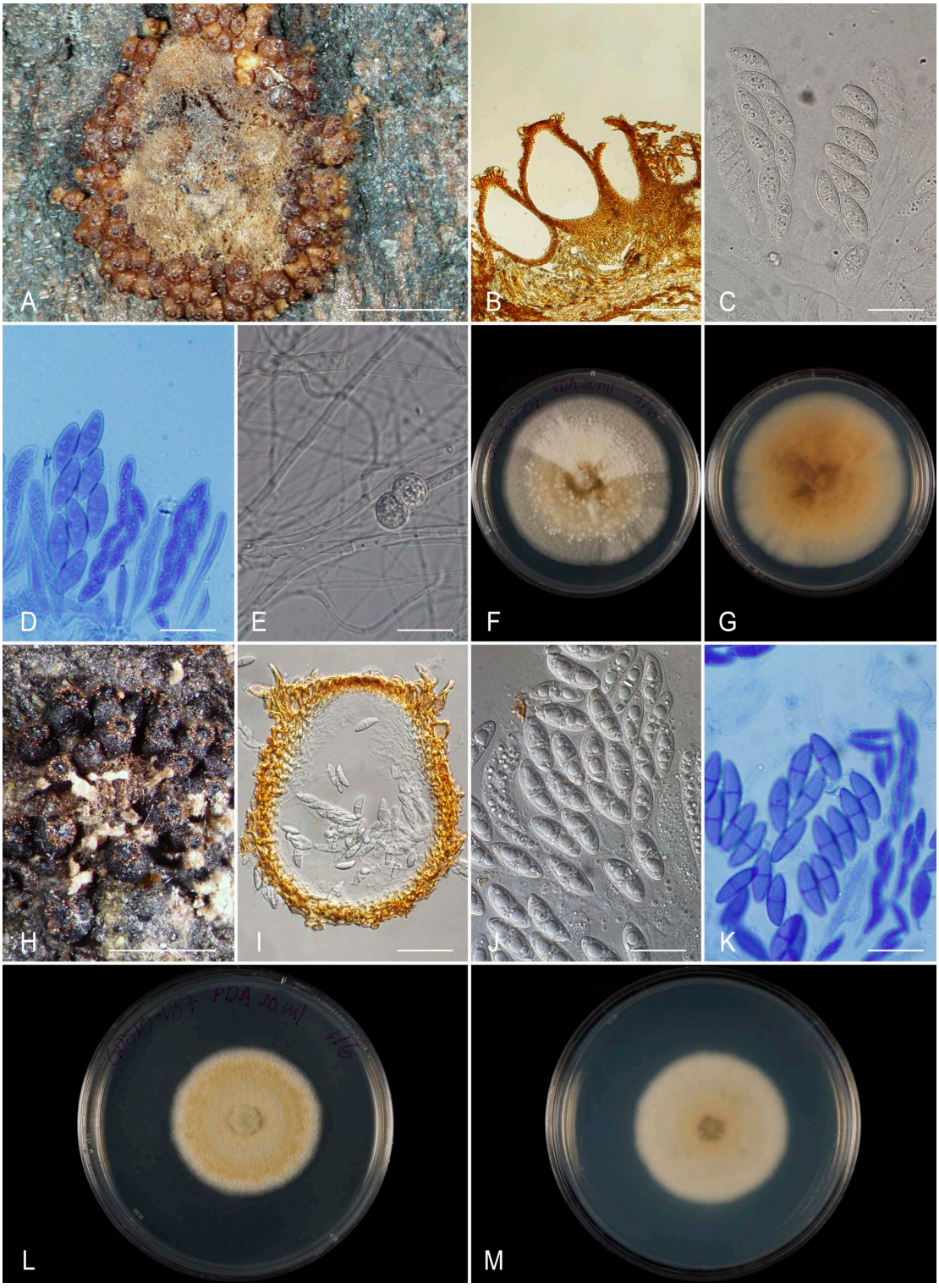

FIGURE 1.3. *Thelonectria coronata* s. str. A – C, *Thelonectria diademata* H – M. A. *T. coronata* s. str. perithecia (92100902). B. Longitudinal section of perithecia

(92100902). C – D. Asci and ascospores (G.J.S 85-207 = IMI 325241). E. Chlamydospores. F. Colony on PDA (G.J.S 85-207 = IMI 325241). G. Colony reverse on PDA (G.J.S 85-207 = IMI 325241). H. *T. diademata* perithecia (A.R. 4765 = BPI 882585). I. Longitudinal section of perithecia (A.R. 4765 = BPI 882585). J – K. Asci and ascospores (A.R. 4765 and C.T.R. 71-52, respectively). L. Colony on PDA (G.J.S. 10-137). M. Colony reverse on PDA (G.J.S. 10-137). Bars: A, H = 500 μm; B, I = 250 μm; C – E, J – K = 50 μm.

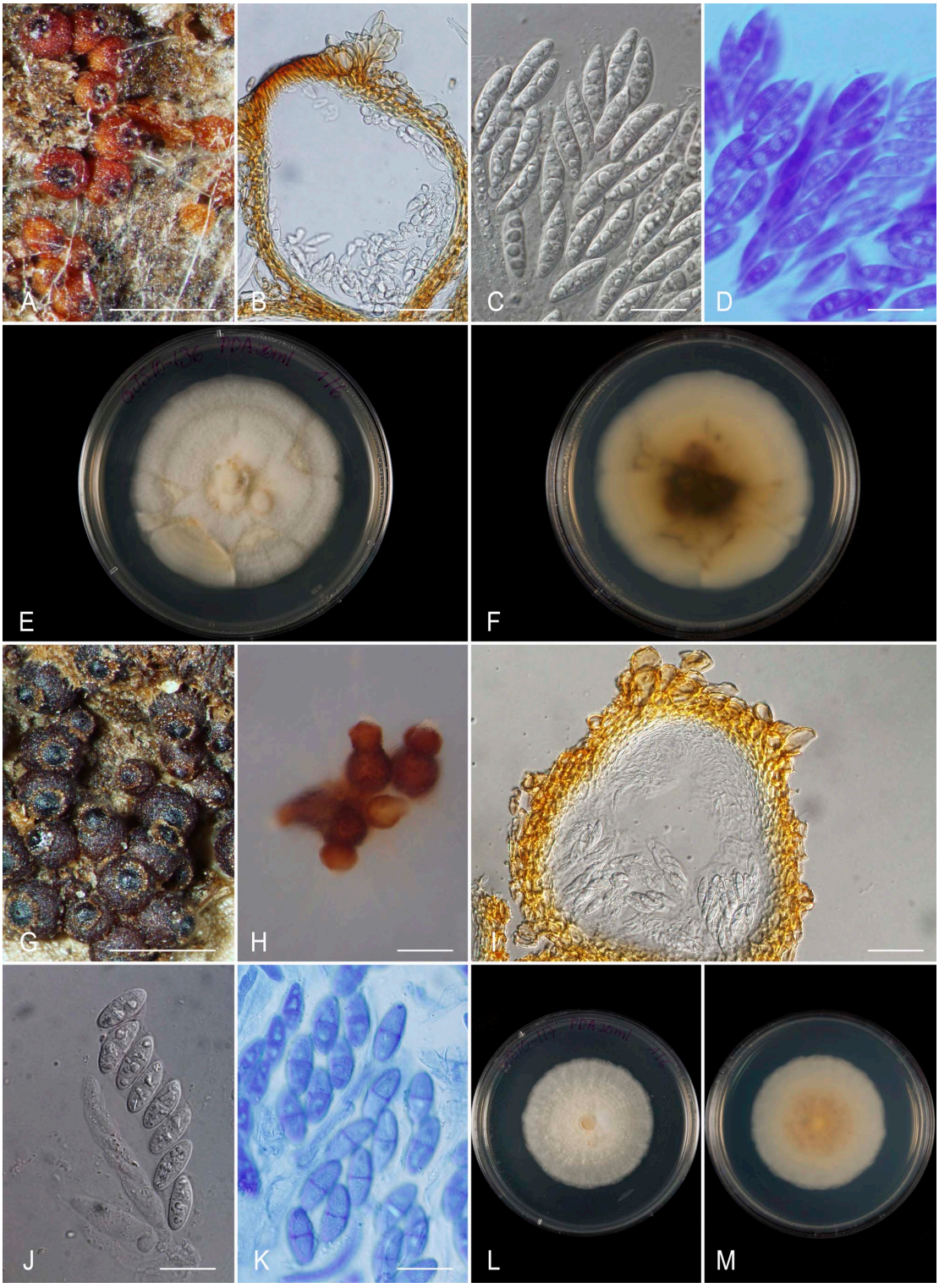

FIGURE 1.4. *Thelonectria cidaria* A – F, *Thelonectria stemmata* G – M. A. *T. cidaria* perithecia (G.J.S. 10-135 = BPI 882589)). B. Longitudinal section of perithecia

(G.J.S. 10-135 = BPI 882589). C – D. Asci and ascospores (G.J.S. 10-136 = BPI 882588). E. Colony on PDA (G.J.S. 10-136 = BPI 882588). F. Colony reverse on PDA (G.J.S. 10-136 = BPI 882588). G. *T. stemmata* perithecia (C.T.R. 71-19 = NYBG CUP-MJ 759). H. Perithecia produced on SNA (C.T.R. 71-19 = CBS 112468). I. Longitudinal section of perithecia (C.T.R. 71-27 = NYBG CUP-MJ 955). J – K. Asci and ascospores (C.T.R. 71-19 = CBS 112468). L. Colony on PDA (G.J.S 10-117). M. Colony reverse on PDA (G.J.S 10-117). Bars: A, G = 500 μm; B, I = 250 μm; C – D, J – K = 50 μm; H = 1 mm.

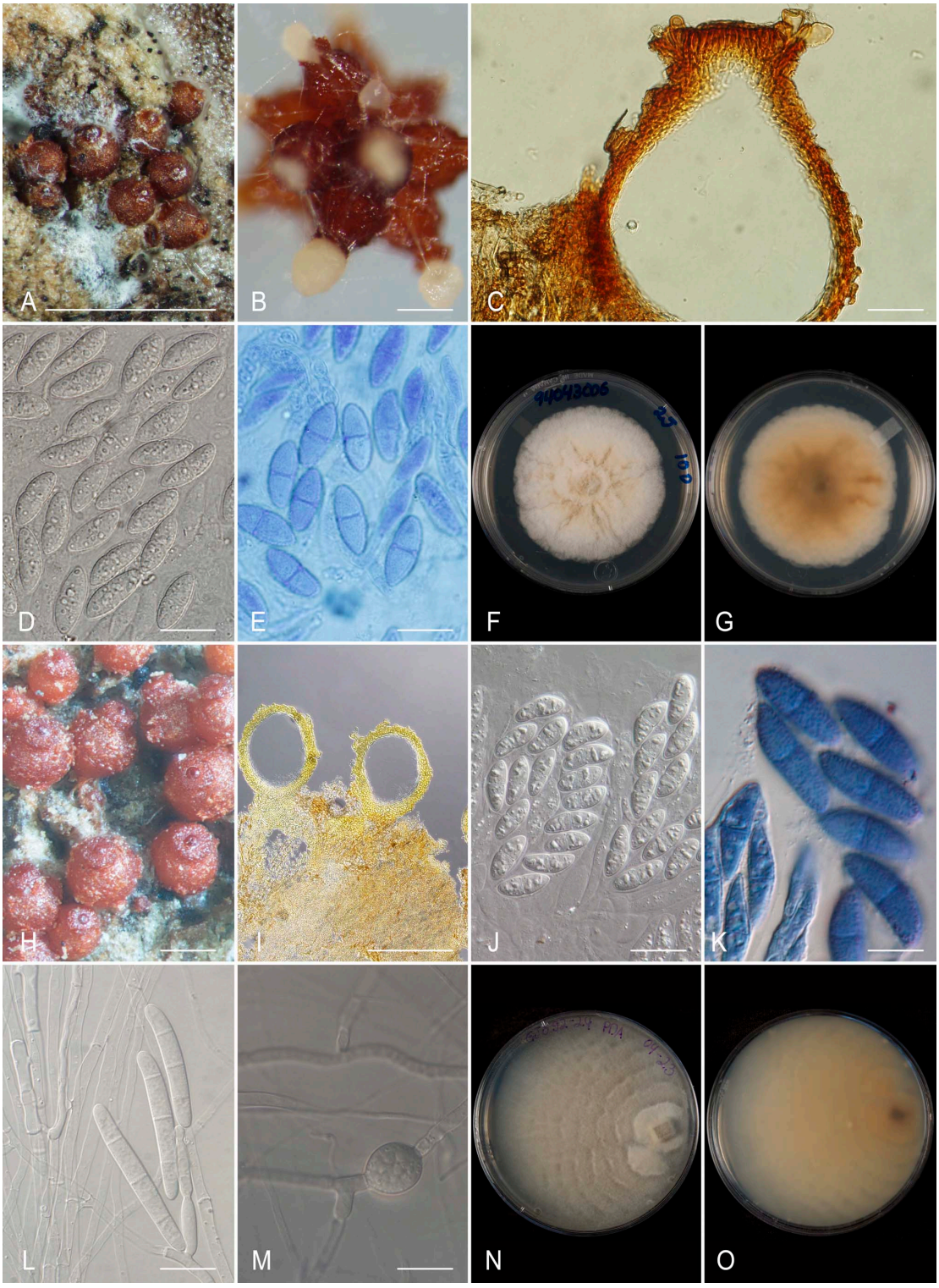

FIGURE 1.5. *Thelonectria coronalis* A – G, *Thelonectria veuillotiana* s. str. H – O. A. *T. coronalis* perithecia (94043006). B. Perithecia produced on SNA (94043006). C.

Longitudinal section of perithecia (93082102). D – E. Ascospores (93082102). F. Colony on PDA (94043006). G. Colony reverse on PDA (94043006). H. *T. veuillotiana* s. str. perithecia (A.R. 1751 = CUP-MM 1706). I. Longitudinal section of perithecia (A.R. 1751 = CUP-MM 1706). J – K. Asci and ascospores (A.R. 1751 = CUP-MM 1706). L. Conidiophores and macroconidia (G.J.S. 92-24 = CBS 124114). M. Chlamydospores (A.R. 1751). N. Colony on PDA (G.J.S. 92-24 = CBS 124114). O. Colony reverse on PDA (G.J.S. 92-24 = CBS 124114). Bars: A – B, H – I = 500 μm; C = 250 μm; D – E, J – M = 50 μm.

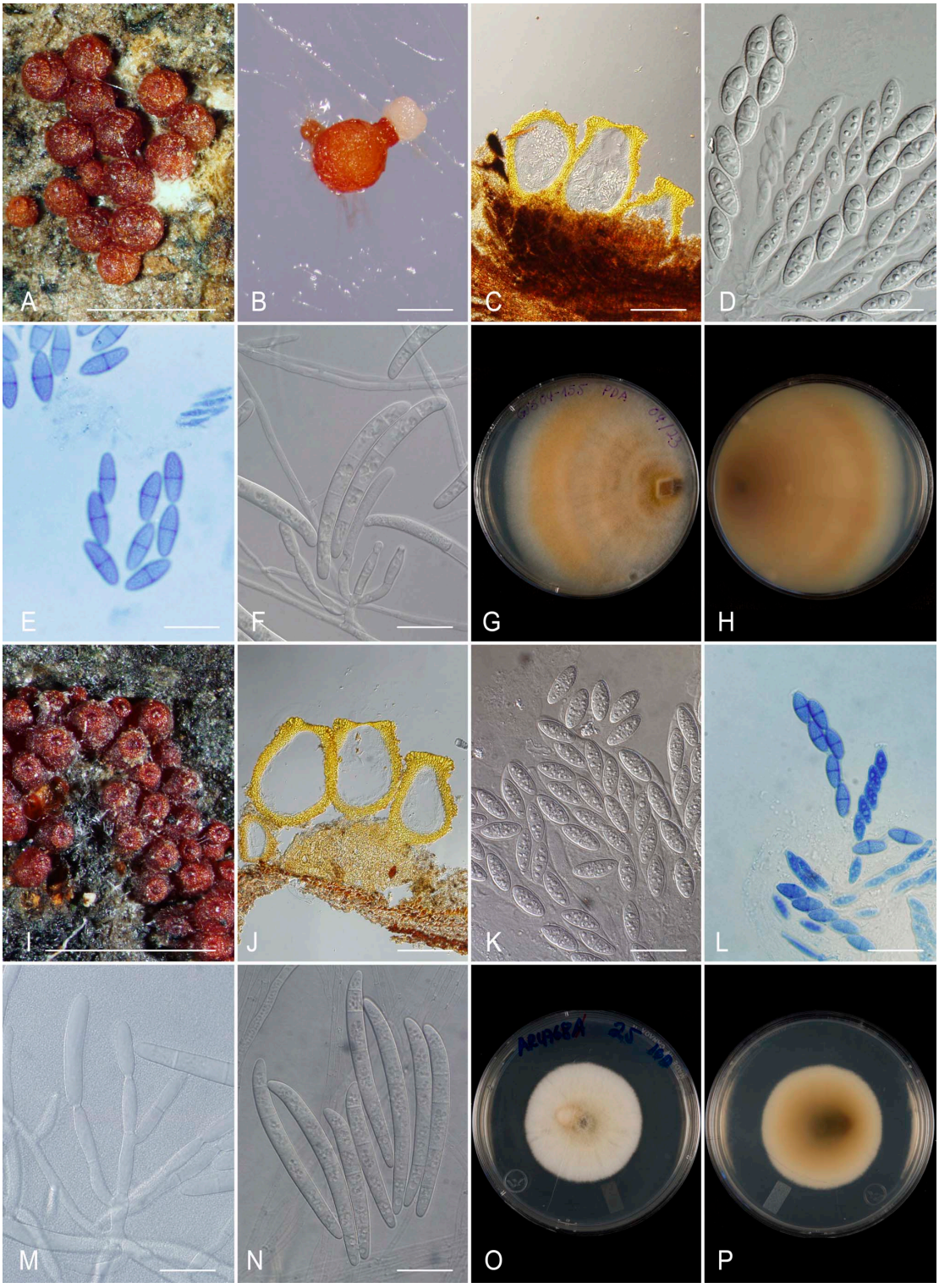

FIGURE 1.6. *Thelonectria nodosa* A – H, *Thelonectria torulosa* I – P. A. *T. nodosa* perithecia (G.J.S. 04-155 = BPI 860484). B. Perithecia produced on SNA (G.J.S. 90-

66 = CBS 124352). C. Longitudinal section of perithecia (G.J.S. 04-155 = BPI 860484). D – E. Asci and ascospores (G.J.S. 91-105 = IMI 351445). F. Conidiophores and macroconidia (A.R. 4500 = CBS 124742). G. Colony on PDA (G.J.S. 04-155). H. Colony reverse on PDA (G.J.S. 04-155). I. *T. torulosa* perithecia (A.R. 4764 = BPI882590). J. Longitudinal section of perithecia (A.R. 4764 = BPI882590). K – L. Asci and ascospores (A.R. 4764). M - N. Conidiophores and macroconidia (A.R.4768A = BPI 882591). O. Colony on PDA (A.R. 4768A). P. Colony reverse on PDA (A.R. 4768A). Bars: A, I = 500 μm; B – C, J = 250 μm; D – F, K – N = 50 μm.

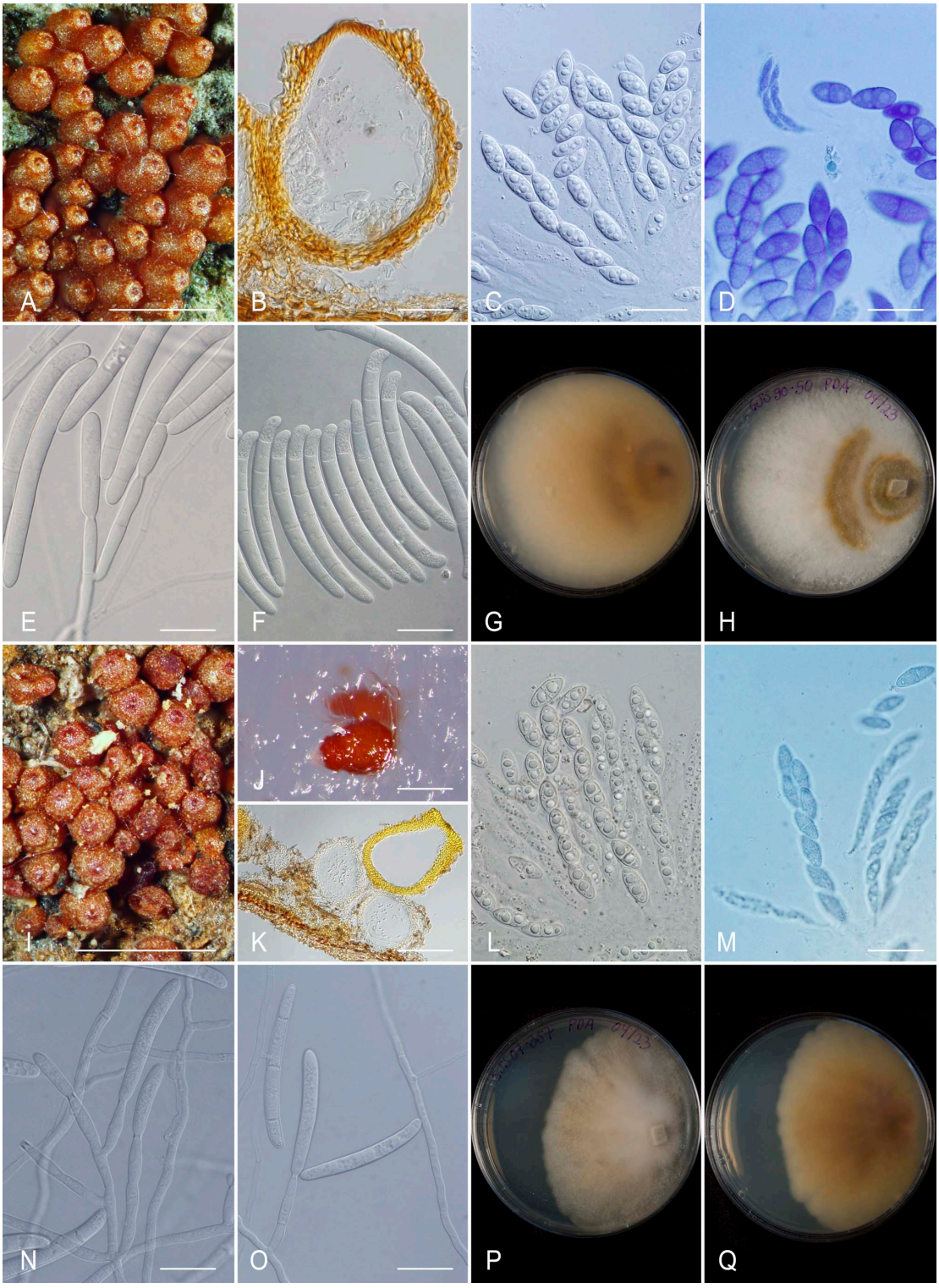

FIGURE 1.7. *Thelonectria gongylodes* A – H, *Thelonectria truncata* I – Q. A. *T. gongylodes* perithecia (G.J.S 04-171 = BPI 881093). B. Longitudinal section of

perithecia (G.J.S 04-171 = BPI 881093). C – D. Asci and ascospores (G.J.S. 04-171). E – F. Conidiophores and macroconidia (G.J.S. 04-171). G. Colony on PDA (G.J.S. 90-50 = IMI 343571). H. Colony reverse on PDA (G.J.S. 90-50 = IMI 343571). I. *T. truncata* perithecia (YH224 = BPI 882090). J. Perithecia produced on SNA (G.J.S 04-357). K. Longitudinal section of perithecia (G.J.S. 04-357 = BPI 881092). L – M. Asci and ascospores (MAFF241521). N – O. Conidiophores and macroconidia (G.J.S. 04-357). P. Colony on PDA (G.J.S. 04-357). Q. Colony reverse on PDA (G.J.S. 04-357). Bars: A, I – K = 500 μm; B = 250 μm; C – F, L – O = 50 μm.

TABLE 1.1. Taxa used in the phylogenetic analyses, including information about the origin of the fungal material, collection codes and GenBank accession numbers

Strain	Code	Code 2*	Host	Origin	GenBank accession numbers					
					act	**LSU**	**ITS**	**rpb1**	**tef1**	**tub**
T. acrotyla	G.J.S 90-171 (=CBS 123766)	TXXII	Unknown	Venezuela	JQ365047	JQ403368	JQ403329	JQ403407	JQ394751	JQ394720
T. amamiensis	MAFF239820	TX	*Pinus luchuensis*	Japan (Okinawa)	JQ365055	JQ403376	JQ403338	JQ403413		JQ394728
T. amamiensis	MAFF239819	TXI	*Pinus luchuensis*	Japan (Kagoshima)	JQ365054	JQ403375	JQ403337			JQ394727
T. diademata	A.R. 4765 (=CBS 132331)	CI	Unknown	Argentina (Tucuman)	JQ365029	JQ403348	JQ403308	JQ403384	JQ394736	JQ394700
T. diademata	A.R. 4787 (=CBS 132332)	CII	Unknown	Argentina (Tucuman)	JQ365032	JQ403351	JQ403311	JQ403387	JQ394738	JQ394703
T. diademata	C.T.R. 71-52 (CBS 132333)	CIII	*Pinus patula*	Jamaica		JQ403354	JQ403314	JQ403391	JQ394740	JQ394706
T. diademata	G.J.S. 10-137 (=CBS 132321)	CIV	Unknown	Costa Rica		JQ403364	JQ403325	JQ403403	JQ394748	JQ394716
T. coronata	G.J.S. 85-207 (=IMI325241)	CV	Unknown	Indonesia	JQ365044	JQ403365	JQ403326	JQ403404	JQ394749	JQ394717
T. coronata	92100902 (=CBS 132334)	CVI	Unknown	Taiwan (Taipei County)	JQ365059		JQ403342	JQ403417	JQ394760	JQ394731
T. coronata	G.J.S. 10-108 (=CBS 132322)	CVII	Unknown	Costa Rica	JQ365040	JQ403360	JQ403320	JQ403397	JQ394746	JQ394711
T. coronata	C.T.R. 72-178 (=CBS 132335)	CVIII	*Cecropia* sp.	Venezuela	JQ365036	JQ403356	JQ403316	JQ403393	JQ394742	JQ394708
T. cidaria	GJS10-135 (=CBS 132323)	CIX	Unknown	Costa Rica			JQ403324	JQ403401		JQ394714
T. cidaria	GJS10-136 (=CBS 132324)	CX	Unknown	Costa Rica	JQ365043			JQ403402		JQ394715
T. cidaria	CTR71-79 (=IMI 325844)	CXI	Unknown	Jamaica	JQ365035	JQ403355	JQ403315	JQ403392	JQ394741	JQ394707
T. stemmata	CTR71-19 (=CBS 112468)	CXII	Unknown	Jamaica	JQ365033	JQ403352	JQ403312	JQ403388	JQ394739	JQ394704

T. stemmata	CTR71-21 (=CBS 132336)	CXIII	*Cecropia* sp.	Jamaica	JQ365034	JQ403353	JQ403313	JQ403389		JQ394705
T. stemmata	CTR71-27 (=IMI325840)	CXIV	Unknown	Jamaica	JQ365052			JQ403390		
T. stemmata	GJS10-117 (=CBS 132325)	CXV	Unknown	Costa Rica		JQ403361	JQ403321	JQ403398		JQ394712
T. stemmata	GJS10-130 (=CBS 132326)	CXVI	Unknown	Costa Rica	JQ365042	JQ403363	JQ403323	JQ403400		
T. stemmata	GJS90-141 (=CBS 125117)	CXVII	Unknown	Venezuela	JQ365046	JQ403367	JQ403328	JQ403406		JQ394719
T. coronalis	93082102 (=CBS 132337)	CXVIII	Unknown	Taiwan (Taipei County)		JQ403380	JQ403343	JQ403418	JQ394761	JQ394732
T. coronalis	94043006 (=CBS 132338)	CXIX	Unknown	Taiwan (Taipei County)		JQ403381	JQ403344	JQ403419		JQ394733
T. nodosa	GJS90-66 (=CBS 124352)	TI	*Acer* sp.	USA, NY	JQ365049	JQ403371	JQ403332	JQ403409	JQ394753	JQ394722
T. nodosa	GJS04-155 (=CBS 132327)	TII	*Thuja canadiensis*	USA, TN	JQ365037	JQ403357	JQ403317	JQ403394	JQ394743	JQ394709
T. nodosa	GJS91-105 (=IMI 351445)	TIII	*Fagus grandifolia*	USA, VA	JQ365050	JQ403372	JQ403333	JQ403410	JQ394754	JQ394723
T. nodosa	AR4505 (=CBS 125173)	TIV	*Fagus grandifolia*	USA, CT	HM352862.1	JQ403347	JQ403307	HM364328.1	HM364348	HM352862
T. nodosa	AR4500 (=CBS 124742)	TV	*Fagus grandifolia*	USA, CT	JQ365028	JQ403346	JQ403306	JQ403383	JQ394735	JQ394699
T. nodosa	GJS91-116 (=CBS 124740)	TVI	*Quercus* sp.	USA, VA	JQ365051	JQ403373	JQ403334	JQ403411	HM054126.1	JQ394724
T. veuillotiana	MAFF241544	TVII	Unknown	Japan (Miyagi)	JQ365057	JQ403378	JQ403340	JQ403415	JQ394758	JQ394729
T. torulosa	AR4764 (=CBS 132339)	TVIII	Unknown	Argentina (Tucuman)	JQ365030	JQ403349	JQ403309	JQ403385		JQ394701
T. torulosa	AR4768A (=CBS 132340)	TIX	Unknown	Argentina (Tucuman)	JQ365031	JQ403350	JQ403310	JQ403386	JQ394737	JQ394702
T. veuillotiana	MAFF241551	TXII	Unknown	Japan (Yamanashi)	JQ365058	JQ403379	JQ403341	JQ403416	JQ394759	JQ394730
T. gongylodes	GJS89-131 (=IMI 336160)	TXIII	*Acer rubrum*	USA, NC	JQ365053	JQ403374	JQ403336	JQ403412	JQ394756	JQ394726
T. gongylodes	GJS90-48 (=CBS 125118)	TXIV	*Quercus* sp.	USA, NC	HM352888.1	JQ403369	JQ403330	HM364338.1	HM364357.1	HM352870.1

T. gongylodes	GJS90-50 (=IMI 343571)	TXV	*Fagus grandifolia*	USA, NC	JQ365048	JQ403370	JQ403331	JQ403408	JQ394752	JQ394721
T. gongylodes	GJS04-171 (=CBS 124611)	TXVI	*Acer* sp.	USA, TN	JQ365038	JQ403358	JQ403318	JQ403395	JQ394744	JQ394710
T. veuillotiana	GJS10-124 (=CBS 132328)	TXVII	Unknown	Costa Rica	JQ365041	JQ403362	JQ403322	JQ403399	JQ394747	JQ394713
T. veuillotiana	GJS92-24 (=CBS 125114)	TXVIII	*Fagus sylvatica*	France	GQ505980.1	GQ506005.1	JQ403335	GQ506034.1	JQ394755	JQ394725
T. veuillotiana	AR1751 (=CBS 132341)	TXIX	*Eucalyptus* sp.	Azores Island		JQ403345	JQ403305	JQ403382	JQ394734	JQ394698
T. veuillotiana	MAFF241521	TXX	Unknown	Japan	JQ365056	JQ403377	JQ403339	JQ403414	JQ394757	
T. truncata	GJS04-357 (=CBS 132329)	TXXI	Unknown	USA, TN	JQ365039	JQ403359	JQ403319	JQ403396	JQ394745	
T. westlandica	GJS83-156 (=CBS 112464)				HM352887.1	HM364321.1	HM484559.1	HM364337.1	HM364355.1	HM352868.1
T. westlandica	GJS85-45 (=CBS 132330)				JQ365045	JQ403366	JQ403327	JQ403405	JQ394750	JQ394718

*Codification used for Figures 2A and 2B

TABLE 1.2. Statistical data calculated at the intra- and intergroup levels for five clades in *T. coronata* (TC1-5 = *T. coronata* clades 1-5)

	Number of fixed differences						**π**						**k**						**Dxy (%)**					
	act	btub	tef	ITS	LSU	rpb1	act	btub	tef	ITS	LSU	rpb1	act	btub	tef	ITS	LSU	rpb1	act	btub	tef	ITS	LSU	rpb1
TC1							0	0.007	0.005	0.003	0	0.001	0	3.50	3.50	1.50	0	0.67	0	0.297	0.380	0.117	0	0.107
TC2							0	0.003	0	0	0	0.002	0	1.33	0	0	0	1.33	0	0.264	0	0	0	0.215
TC3							0	0.004	0	0	0	0.001	0	2.00	0	0	0	0.67	0	0.264	-	0	-	0.107
TC4							0	0	0	0	0	0.001	0	0	0	0	0	0.33	0	0	-	0	0	0.054
TC5							-	0.015	0	0.006	0.010	0.002	-	8.00	0	3.00	5.00	1.00	-	1.400	-	0.707	0.964	0.161
TC1-TC2	5	0	8	0	1	9	0.006	0.008	0.010	0.002	0.001	0.010	5.00	5.08	10.25	0.75	1.00	10.50	0.928	0.978	1.520	0.145	0.149	1.690
TC1-TC3	4	18	22	7	4	12	0.005	0.027	0.017	0.009	0.002	0.012	4.00	21.58	24.25	7.75	4.00	12.83	0.742	4.220	3.540	1.508	0.594	2.070
TC1-TC4	7	17	25	11	3	19	0.006	0.022	0.019	0.013	0.002	0.017	7.00	18.75	27.25	11.75	3.00	19.67	1.290	3.600	3.970	2.282	0.448	3.170
TC1-TC5	-	10	20	21	3	11	-	0.020	0.016	0.026	0.006	0.011	-	15.75	22.00	23.25	5.50	12.00	-	3.040	3.210	4.568	1.050	1.930
TC2-TC3	16	22	24	7	3	17	0.016	0.030	0.017	0.008	0.002	0.018	16.00	24.33	24.33	7.00	3.00	18.33	2.630	3.860	3.320	1.360	0.365	2.910
TC2-TC4	16	22	28	11	2	25	0.014	0.026	0.020	0.011	0.001	0.021	16.00	23.00	28.33	11.00	2.00	26.17	2.630	4.420	3.860	2.130	0.244	4.150
TC2-TC5	-	15	19	21	2	17	-	0.026	0.014	0.027	0.007	0.018	-	20.00	19.33	22.50	4.50	18.50	-	3.860	2.630	4.420	0.862	2.930
TC3-TC4	15	23	24	10	1	12	0.011	0.027	0.028	0.009	0	0.010	15.00	24.00	24.00	10.00	1.00	12.50	2.380	4.620	2.770	1.940	0.122	1.980
TC3-TC5	-	17	16	14	2	4	-	0.027	0.019	0.021	0.009	0.005	-	20.66	16.00	15.50	4.50	4.83	-	4.060	1.850	3.050	0.862	0.767
TC4-TC5	-	10	24	19	1	10	-	0.015	0.028	0.020	0.004	0.008	-	13.50	24.00	20.50	3.50	10.67	-	2.610	2.770	4.030	0.670	1.690

π, Nucleotide diversity; k, average number of nucleotide differences; Dxy (%), nucleotide divergence (average number of nucleotide substitutions per site between populations x 100); -, no data.

TABLE 1.3. Statistical data calculated at the intra- and intergroup levels for six clades in *T. veuillotiana* (TV1-6 = *T. veuillotiana* clades 1-6)

	Number of fixed differences						π						k						Dxy (%)					
	act	btub	tef	ITS	LSU	rpb1	act	btub	tef	ITS	LSU	rpb1	act	btub	tef	ITS	LSU	rpb1	act	btub	tef	ITS	LSU	rpb1
TV1							0	0	0.002	0	0	0	0	0	1.33	0	0	0	0	0	0.036	0	0	0
TV2							0.002	0	0	0	0	0	1.00	0	0	0	0	0	0.185	0	-	0	0	0
TV3							0.002	0	-	0.006	0.004	0	1.00	0	-	3.00	3.00	0	0.185	0	-	0.705	0.578	TD
TV4							0.001	0.001	0.007	0.002	0	0.007	0.67	0.67	5.33	1.00	0	4.50	0	0.132	0.509	0.234	0	0.650
TV5							0	0.013	0.011	0.002	0.001	0	0	7.00	9.00	1.00	1.00	0	-	1.196	0.876	0.234	0.192	0
TV6							0	-	0.001	0	0	0.020	0	-	1.00	0	0	24.00	0	-	0.108	0	0	0
TV1-TV2	1	2	6	0	1	0	0.001	0.002	0.003	0	0.001	0	1.50	2.00	6.67	0	1.00	0	0.241	0.377	0.787	0	0.126	0
TV1-TV3	3	6	-	0	1	2	0.002	0	-	0.001	0.001	0.001	3.50	6.00	-	1.50	2.50	2.00	0.563	1.130	-	0.296	0.304	0.317
TV1-TV4	9	11	9	0	1	2	0.008	0.011	0.011	0.001	0.001	0.007	9.50	11.50	13.17	0.50	1.00	6.75	1.530	2.110	1.800	0.010	0.122	1.070
TV1-TV5	3	27	15	3	3	8	0.001	0.024	0.011	0.003	0.002	0.005	3.00	30.00	20.17	3.50	3.50	8.00	0.482	5.540	2.380	0.684	0.426	1.270
TV1-TV6	19	-	36	35	14	5	0.013	-	0.020	0.030	0.007	0.013	19.00	-	37.17	35.00	14.00	17.00	3.060	-	4.380	6.900	1.700	2.690
TV2-TV3	4	6	-	0	0	2	0.006	0.008	-	0.003	0.002	0.002	5.00	6.00	-	1.50	1.50	2.00	0.797	1.130	-	0.341	0.189	0.317
TV2-TV4	10	11	8	0	2	2	0.010	0.012	0.011	0.002	0.001	0.009	11.00	11.50	12.00	0.50	2.00	6.75	1.770	2.160	1.650	0.114	0.252	1.070
TV2-TV5	4	26	13	3	2	8	0.005	0.039	0.017	0.006	0.003	0.008	4.50	29.00	17.50	3.50	3.00	8.00	0.718	5.480	2.060	0.795	0.379	1.270
TV2-TV6	21	-	34	28	14	5	0.023	-	0.028	0.043	0.012	0.024	21.50	-	34.50	28.00	14.00	17.00	3.430	-	4.070	6.400	1.760	2.690
TV3-TV4	12	12	-	0	2	4	0.012	0.013	-	0.003	0.003	0.010	13.00	12.50	-	2.00	3.50	8.50	2.090	2.360	-	0.394	0.427	1.340
TV3-TV5	3	27	-	1	2	8	0.007	0.040	-	0.005	0.005	0.008	6.50	30.00	-	3.00	4.50	8.00	1.030	5.690	-	0.592	0.547	1.270
TV3-TV6	23	-	-	33	12	6	0.025	-	-	0.047	0.012	0.032	23.50	-	-	34.50	13.50	18.00	3.740	-	-	6.870	1.630	2.850
TV4-TV5	8	22	13	3	4	5	0.006	0.026	0.019	0.005	0.003	0.011	8.50	25.50	20.75	4.00	5.00	9.25	1.370	4.710	2.850	0.780	0.611	1.460
TV4-TV6	14	-	27	34	16	2	0.013	-	0.026	0.038	0.010	0.021	14.50	-	31.50	35.00	16.00	18.25	2.340	-	4.320	6.900	1.950	2.890
TV5-TV6	20	-	29	34	15	0	0.021	-	0.028	0.046	0.013	0.019	20.00	-	34.00	34.50	16.00	12.00	3.170	-	3.950	6.800	1.940	1.900

π, Nucleotide diversity; k, average number of nucleotide differences; Dxy (%), nucleotide divergence (average number of nucleotide substitutions per site between populations x 100); -, no data.

TABLE 1.4. Mean values of morphological characters (in μm) of clades in the *T. coronata* and *T. veuillotiana* species complexes.

	Asc-L[1]	Asc-W[2]	Con3-L[3]	Con3W[4]	Con4-L[5]	Con4-W[6]	Con5-L[7]	Con5-W[8]
Clade TC1 *T. diademata*	20.7	8.3						
Clade TC2 *T. coronata*	18.9	7.2						
Clade TC3 *T. cidaria*	21	6.6						
Clade TC4 *T. stemmata*	21	8.2						
Clade TC5 *T. coronalis*	20	7.7						
Clade TV1 *T. nodosa*	16	6.3	56.4	5.8	68	6	74.4	6.2
Clade TV2 *T. torulosa*	15	6.3	57.2	6.3	67.2	6.2	80	6.8
Clade TV3 *T. amamiensis*	18	6.8	51.3	6.3	62.5	6.3	68.8	6.3
Clade TV4 *T. gongylodes*	17	6.6	45.8	5.7	57	6	63.3	6.3
Clade TV5 *T. veuillotiana*	19.5	6.2	47	5.7	58.4	6.2	66.6	7.3
Clade TV6 *T. truncata*	14.4	6	53.3	5.4	61.2	5.5	71.2	5.8
T. acrotyla	23	9.8	40.6	7.5	57	7.8	61.3	8

[1] ascospore length, [2] ascospore width, [3] conidia 3-septate length, [4] conidia 3-septate width, [5] conidia 4-septate length, [6] conidia 4-septate width, [7] conidia 5-septate length, [8] conidia 5-septate width

Chapter 2: Not as ubiquitous as we thought: taxonomic crypsis, hidden diversity and cryptic speciation in the cosmopolitan fungus *Thelonectria discophora* (Nectriaceae, Hypocreales, Ascomycota).

Abstract

The distribution of microbial species, including fungi, has long been considered cosmopolitan. Recently, this perception has been challenged by molecular studies in historical biogeography, phylogeny and population genetics. Here we explore this issue using the fungal morphological species *Thelonectria discophora,* one of the most common species of fungi in the family Nectriaceae, encountered in almost all geographic regions and considered as a cosmopolitan taxon. In order to determine if *T. discophora* is a single cosmopolitan species or an assemblage of sibling species, we conducted various phylogenetic analyses, including standard gene concatenation, Bayesian concordance methods, and coalescent-based species tree reconstruction on isolates collected from a wide geographic range. Results show that diversity among isolates referred as *T. discophora* is greatly underestimated and that it represents a species complex. Within this complex, sixteen distinct highly supported lineages were recovered, each of which has a restricted geographic distribution and ecology. The taxonomic status of isolates regarded as *T. discophora* is reconsidered, and the assumed cosmopolitan distribution of this species is rejected. We discuss how assumptions about geographically widespread species have implications regarding

their taxonomy, true diversity, biological diversity conservation, and ecological functions.

Introduction

The high plasticity of morphological characters in fungi led early taxonomists to group or "lump" similar-looking species into one individual species. Accordingly, as this species was found in a wide geographic range, it was then labeled as being cosmopolitan *i.e.,* having a worldwide distribution. This resulted in long-held assumptions about long-distance dispersal capabilities in fungi (Peay et al. 2010, Taylor et al. 2006), a trend that was also assumed for other microorganisms such as protozoa, protophytes, and other small organisms (*i.e.,* those less than 1 mm in length such as nematodes, rotifers and marine invertebrates) (Fenchel and Finlay 2004, Finlay et al. 1996, Finlay 2002, Staley and Gosink 1999, Tamames et al. 2010). Assumptions about long-distance dispersal, or the well-known Bass-Becking hypothesis (Bass-Becking 1934, Fenchel and Finlay 2004, Finlay 2002, Sato et al. 2012), implied that propagules could be easily carried by wind or water so they can be distributed everywhere. However these mechanisms of long-distance dispersal, as well as patterns of geographical distribution and factors driving species diversity, are less known for microorganisms when compared with those of macroorganisms, and have resulted in a drastic underestimation of the global species richness of microorganisms and fungi (Blackwell 2011, Leavitt et al. 2011). Cosmopolitanism also implies that the number of species has remained stable through time and is relatively small with virtually nonexistent extinction episodes (Fenchel and Finlay 2004, Finlay and Fenchel 2004). These are phenomena commonly seen at the family

and genus levels; however, at the species level, many cases were cosmopolitanism has been assumed have been disproven by molecular data (Tamames et al. 2010). While some empirical studies have supported the Bass-Becking hypothesis in some species of microorganisms (Fierer and Jackson 2006, Finlay 2002, Finlay and Fenchel 2004, Queloz et al. 2011, Rydholm et al. 2006), there are many examples, not only for the majority of fungi (Carriconde et al. 2008, Dettman et al. 2003, James et al. 1999, Pringle et al. 2005, Stielow et al. 2011, Taylor et al. 2006, Wedin et al. 2009), but also for other microbial eukaryotes, marine and fresh water invertebrates, and even for some protists (Bleidorn et al. 2006, Kalla et al. 2011, Klautau et al. 1999, Suatoni et al. 2006, Vanormelingen et al. 2007, Zufall et al. 2012), where this hypothesis has been disproven. Since the rise of molecular phylogenetics over two decades ago, the view that microorganisms have large-scale spatial distributional ranges has changed, mostly due to the use of molecular markers and phylogenetic analyses, which have increased the level of resolution in systematics studies (Taylor et al. 2006). Studies have found that there is a distinctive correlation between geographical distance and similarity in community composition (distance-decay relationship) observed in local to global-scale analyses (Sato et al. 2012).

In the present study, we explore various issues concerning cosmopolitanism and conservative application of species names and the consequences these bring to taxonomy, true diversity estimates, biological species conservation efforts and ecological functions. We focused our study on *Thelonectria discophora* (Mont.) P. Chaverri & C. Salgado (2011), one of the most representative and common species of the fungal family Nectriaceae (Hypocreales, Ascomycota) and the type species of the

genus *Thelonectria*. *Thelonectria discophora* is a saprobe on newly dead, organic plant material where it is among the first colonizers (Brayford et al. 2004, Guu et al. 2007, Hirooka and Kobayashi 2007, Samuels et al. 1990). It is common in disturbed areas with recently fallen or cut plant material and is rarely found in old growth forests (Chaverri and Vilchez 2006), thus serving as an indicator of forest disturbance. This species has been reported to have a cosmopolitan distribution, being encountered on every continent, excluding Antarctica and the Arctic regions (Brayford et al. 2004, Guu et al. 2007, Hirooka and Kobayashi 2007, Samuels et al. 1990). It is found in a diverse set of habitats on plant substrata such as on the bark of twigs and branches or trunks of recently dead or dying trees, with little morphological variability (FIG. 2.1) (Booth 1966, Brayford et al. 2004, Chaverri et al. 2011, Guu et al. 2007, Samuels et al. 1990). This species has also been reported as the causal agent of a distinctive basal canker of cultivated *Rubus idaeus* and *R. fruticosus*. However, it has been regarded as a secondary or weak pathogen since disease outbreaks have been mostly correlated with stressed plants following wind damage or waterlogging (Brayford 1991, Brayford et al. 2004).

To determine if *T. discophora* is truly cosmopolitan or a complex of geographically and ecologically restricted species, we conducted a series of multi-locus phylogenetic and genetic divergence analyses. We studied representatives of *T. discophora*-like fungi, including taxa considered synonyms of *T. discophora,* collected from a wide geographic distribution. Data were subjected to Bayesian Inference and Maximum Likelihood analyses using 6-gene nuclear DNA sequence data sets. We compared the results of the phylogenetic analyses using standard gene

concatenation with those of Bayesian Concordance analyses (Ané et al. 2007) and Bayesian Inference of Species Trees (Heled and Drummond 2010). Combining these methods allows us to assess whether the genetic divergence among isolates in *T. discophora* is sufficient to consider them as separate units, and to obtain the concordance phylogeny and species tree that best describes the diversity of putative species in this complex.

Materials and Methods

Fungal isolates

A total of 66 isolates from different localities and hosts with morphology corresponding to *T. discophora*, including some with names that have been considered synonyms, were included in this study (TABLE 2.1). Five isolates representing *T. lucida* (Höhn.) P. Chaverri & Salgado (2011) were used as outgroup in the phylogenetic analyses. Specimens and cultures were obtained from CABI Bioscience (IMI); Centraalbureau voor Schimmelcultures (CBS); Japanese Ministry of Agriculture, Fisheries and Food Collection (MAFF); New York Botanical Garden (NY); and U.S. National Fungus Collection (G.J.S, A.R).

Polymerase chain reaction, sequencing, alignment and data compatibility

DNA extraction and PCR protocols were carried out as described by Chaverri et al. (2011). Six nuclear loci were sequenced for this study: partial large nuclear ribosomal subunit (LSU, *ca*. 900 bp), complete internal transcribed spacers 1 and 2 (ITS, including 5.8S of the nuclear ribosomal DNA, *ca*. 600 bp), partial β-tubulin (*tub*, *ca*. 500 bp), α-actin (*act*, *ca*. 600 bp), RNA polymerase II subunit 1 (*rpb1*, *ca*. 700 bp),

and translation elongation factor 1α (*tef1*, *ca*. 700 bp) (TABLE 2.2). These nuclear loci are commonly used for phylogenetic studies of fungi in the order Hypocreales proving useful for species level studies (Chaverri et al. 2011, Hirooka et al. 2012). Clean PCR products were sequenced in both directions at the University of Maryland DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland, U.S.A.). Sequences were assembled and edited using the program Sequencher 4.9 (Gene Codes, Madison, Wisconsin, U.S.A.). Alignments were performed using MAFFT version 6 (http://mafft.cbrc.jp/alignment/server/) using the E-INS-I strategy (Katoh and Toh 2008) or PRANK (Loytynoja and Goldman 2005) implemented by The GUIDANCE Server (http://guidance.tau.ac.il/index.html) (Penn et al. 2010), using default settings. The PRANK algorithm was used especially for multiple sequence alignments of nuclear loci with rapidly evolving regions and high incidence of insertions and deletions, such as *tef1*, *tub*, *rpb1* and ITS. This method is used to treat insertions correctly and avoid over-estimation of the number of deletion events in the alignments (Loytynoja and Goldman 2005). The program Concaterpillar v. 1.4 (Leigh et al. 2008), which performs hierarchical likelihood ratio tests for phylogenetic congruence, was used to test if the different loci used in this study support alternative topologies (gene-tree/species-tree conflicts; Leigh et al. 2008). For this, an alpha level of 0.01 and the WAG substitution model was used with a four-class discretized Γ model for rates across sites.

Gene tree reconstruction and concatenated phylogenetic analyses

Posterior distribution of gene trees was reconstructed from each of the individual nuclear data sets and from a data set of the combined nuclear genes using Bayesian Inference analysis (BI) in MrBayes v. 3.1 (Huelsenbeck et al. 2001, Ronquist and Huelsenbeck 2003). JModeltest v 0.1.1 (Posada 2008) was used to determine the best nucleotide substitution model using BIC criteria (TABLE 2.2). For the concatenated analyses, we used a partitioned approach with model parameters estimated previously. The analyses were initiated from random starting trees, run for 10 million generations with four chains with two independent repetitions (Metropolis-coupled Markov Chain Monte Carlo; Huelsenbeck and Rannala 2004) and sampled at intervals of 1000 generations. Default priors were used in all analyses. To evaluate stationarity and convergence between runs, log-likelihood scores were plotted using TRACER v. 1.5 (Rambaut and Drummond 2007). In addition, we examined the distribution of split frequencies using the online program AWTY (Nylander et al. 2008), in order to assess whether an MCMC analysis has run long enough such that tree topologies were sampled in proportion to their true posterior probability distribution, independent of the apparent stationarity detected in TRACER. Trees generated prior to stationarity were discarded and the rest of the trees were summarized in a majority-rule consensus tree from the four independent runs. For this, the estimated burn-in based on log likelihood plots was 1000 samples (100,000 generations) per chain leaving 9000 samples per chain (18,000 total) for inference. Bayesian posterior probabilities (PP) were assessed at all nodes, and clades with PP ≥ 0.95 were considered well supported (Huelsenbeck and Rannala 2004). Maximum

likelihood (ML) analyses were performed with the program RAxML v. 7.2.8 (Stamatakis 2006). Branch support was assessed with 1000 nonparametric bootstrapping replicates using the same model parameters settings as the BI analyses. Final trees were visualized with FigTree v1.3.1 (Rambaut 2005).

Bayesian concordance analysis of gene trees

Bayesian concordance analysis (BCA) (Ané et al. 2007) was used to provide an estimate of the level of concordance in reconstructed branches among the posterior distributions of gene trees generated for each nuclear gene. For these analyses, tree files from each single gene obtained in the BI analyses in MrBayes were summarized using the command mbsum included in the BUCKy program (Larget et al. 2010), with a burn-in of 1000 trees. The BCA was then performed in the program BUCKy v. 1.4.2, with four independent runs and four Markov chain Monte Carlo (MCMC) chains, each with 10 million generations with a burn-in period of 100,000. Five values of the alpha parameter (0.1, 0.5, 1, 5, 10) were tested, which correspond to the prior probability distribution for the number of distinct gene trees (Ané et al. 2007).

Since we observed in the single gene analyses that loci such as *act* and LSU have poor resolution at low taxonomic ranks, we also tested if their inclusion in the BCA affected the final outcome of the test. For this, two data sets were built: one containing all genes and another containing a subset of the more phylogenetically informative genes (*tub*, *tef1*, ITS, *rpb1*). Default settings were used for all other parameters. A primary concordance tree with clade concordance factors (CF) and their 95 % credibility intervals (sample and genome wide) were determined for each one of the two data sets.

Coalescent-based species tree analysis

We used the program Species Tree Ancestral Reconstruction / Bayesian Evolutionary Analysis by Sampling Trees (*BEAST v1.7.2) (Drummond et al. 2012) to estimate the species tree for the group. The species assignment for each one of the isolates was based on the groupings obtained in the concatenated phylogenetic analyses using BI and ML approaches. All six nuclear gene datasets were used and the nucleotide substitution model was the same used for BI and ML phylogenetic analyses. We repeated each analysis twice and MCMC analyses were run for a total of 30 million generations, sampled trees at intervals of 1000 generations, and a burn-in of 10 %. A Yule process was used for the species tree prior; the population size model was set to Piecewise linear and constant root. Default values were used for remaining priors. Convergence was assessed in TRACER v1.5, with the species tree reconstructed after a 25 % burn-in using Tree Annotator v1.7.2 (Drummond and Rambaut 2007).

Polymorphism and divergence

To better visualize differences within and between clades, we calculated basic nucleotide polymorphism statistics. The program DnaSP v.5 (Librado and Rozas 2009) was used to calculate nucleotide diversity (π, average number of nucleotide differences among sequences in a sample; Nei and Li 1979), number of haplotypes (H), and total number of polymorphic sites (N_{poly}) within clades, and nucleotide divergence (Dxy, pairwise average number of nucleotide substitutions per site between groups; Nei 1987) between clades. For these calculations, groups to be compared were defined based on clade assignment of each individual in the concatenated ML and Bayesian phylogeny. The randomization test to assess the

significance of Dxy values between groups of clades was calculated using 1000 permutations in DnaSP v.5 (Hudson et al. 1992, Librado and Rozas 2009). Singletons or orphan isolates were not included in these calculations since these methods compare clades of multiple isolates.

Results

For this study a total of 402 sequences were generated and are available in GenBank (TABLE S2.1). The number of variable sites (nucleotides) across the six loci ranged from 0.054 to 0.351. The *rpb1* region showed the highest sequence variability and number of parsimony informative sites, while the ribosomal genes (ITS, LSU) exhibited low levels of variation, approximately more than half that observed in *rpb1* (TABLE 2.2). The combined matrix consisted of 4136 aligned nucleotide positions of which 3361 were constant, 159 variable parsimony-uninformative, and 616 variable and parsimony-informative. Sequence alignments for each locus are deposited under doi:10.5061/dryad.q3s66 at the DRYAD data repository (http://datadryad.org/).

Bayesian and Maximum Likelihood analyses

By combining the evidence found in the concatenated phylogenetic analyses, a total of sixteen putative species were recovered, having significant ML bootstrap (>70%) and BI posterior probability (>0.95) support (FIG. 2.2). Bayesian PP values were usually higher than ML bootstrap support values. The analyses of the concatenated alignment converged quickly to a stationary distribution in the Bayesian analysis run in MrBayes. Effective sample sizes (ESS) of all parameters were at least 300, and most were greater than 1000. The MrBayes majority consensus tree topology shown

in FIG. 2 was the same as the best ML tree. Significant incongruence was found when the ITS region was included in the concatenated data set. Thus, based on the results obtained by Concaterpillar, ML and MrBayes analyses were also run using a concatenated data set in which ITS was excluded (FIG. S2.1). The branch support obtained when the ITS data set was excluded from the concatenated analysis is also included in FIG. 2.2.

The 16 putative species grouped into three major clusters: the first containing clades I to VI, the second containing clades VII to X, the third containing clades XIII to XV; clades XI and XII appear basal to clades I to X and are surrounded by singletons (single isolate lineages) (FIG. 2.2). Isolates in clade XVI were recovered as the most basal species, being distantly related to the rest of *T. discophora*-like putative species, and close to the outgroup species *T. lucida*. Since *T. discophora* was originally described from Chile, clade XIV constitutes the type locality clade and is thus recognized as true *T. discophora*. Eight isolates were found to form singletons. These singletons or orphan isolates either did not cluster with the closest related putative species having significant branch support, *i.e.,* the branch support decreases if they were included, or they were separated from them by a long branch (FIG. 2.2). The majority of the internal nodes in the phylogeny are resolved and well supported. When the ITS data set was not included in the concatenated analysis, the branch support for the majority of putative species remained significant in both ML and MrBayes analyses, with the exception of clade IV which lost branch support and was resolved as a polytomy (FIG. S2.1). Support for some internal nodes in the ML

analysis decreased due to the exclusion of ITS; yet, for the most part, support for internal nodes in MrBayes analyses remained the same (FIG. 2.2).

Single gene tree analyses

Single gene tree analyses recovered the 16 putative species as observed in the concatenated analyses. In the single gene phylogenies, MrBayes and ML analyses recovered the same clades; however, their positions and those of some singleton isolates differed from that seen in the combined analyses (*rpb1* genealogy shown; FIG. S2.2 and S2.3). Although some of the clades were not significantly supported, they were also not contradicting, consequently fitting the criteria for species delimitation using genealogical concordance (GCPSR) (Dettman et al. 2003).

According to the analyses carried out in Concaterpillar v.1.4 (Leigh et al. 2008), the inclusion of ITS sequences in the concatenated data set was rejected at $P < 0.01$, indicating its discordance with the rest of the data sets (*act,* LSU, *tef1*, *tub*, *rpb1*). This discordance was restricted to the position of putative species II and XVI (FIG. S2.4 and S2.5). In the ITS genealogy obtained from MrBayes and ML analyses, the putative species II clustered with the group of putative species IX and it was resolved as a polytomy, in contrast with the rest of the loci where clade II was always associated with the group of putative species I–V. On the other hand, in the MrBayes and ML ITS analyses, clade XVI appeared well supported and basal to clades I–XI. This is contrary to the other single gene topologies and concatenated analyses in which this clade was basal to all clades I–XV. Single gene trees obtained for evolutionarily conserved nuclear loci *act* and LSU showed that relationships among isolates were largely unresolved and resulted in large polytomies (Data not shown).

Bayesian concordance trees and coalescent-based species tree

Due to the increasing awareness that the sole use of concatenated sequence data for phylogenetic analyses can produce misleading results due to gene tree/species tree discordance (Weisrock et al. 2012), we compared the results of standard gene concatenation with those of Bayesian Concordance Analyses (BCA, Ané et al. 2007) and Bayesian Inference of Species Trees (Heled and Drummond 2010). The primary concordance tree obtained from the BCA was similar to the topology obtained in the phylogenetic analyses of the concatenated data set (FIG. 2.3A). However, concordance factor (CF) values for the putative species were much more conservative in this method than the posterior probabilities and bootstrap values obtained in the analyses of the concatenated alignment. CF values for thirteen out of the sixteen putative species were > 0.5; while for three clades the CF values were < 0.5 (FIG. 2.3A). There was agreement between the calculated sample-wide CF and the extrapolated genome-wide CF, although the genome-wide CF was slightly lower than the sample-wide CF (FIG. S2.6). The groups of putative species XIII-XVI obtained the highest CF values and 95% CI values (sample and genome wide) compared with the rest of clades.

The species tree topology estimated using the coalescent-based method (*BEAST) was somewhat different from that obtained in the concatenated and BCA analyses, even though the branch support for the putative species was significantly high. The main conflict between these two methods was observed in the relationships of putative species V and VI (FIG. 2.3B). The support for internal nodes or the "backbone" of the phylogeny from both BCA and *BEAST was particularly weak

(FIG. 2.3A-B). The use of different values for the alpha parameter (the *a priori* degree of incongruence) did not have any effect on the CF of each putative species in the primary concordance tree, given that the method is robust and with the exception of the ITS loci, there are no major conflicts in the data set.

The BCA and *BEAST analyses of all six genes and of the reduced data set excluding *act* and LSU resulted in differences in support values for the putative species. For BCA, concordance factors obtained from the analyses excluding *act* and LSU increased for 11 and decreased for 4 putative species (FIG. 2.3A). Despite the fact that branch support was lower for some putative species, this reduction was less than 20 %. On the other hand, the increase in branch support for 11 clades went up to 45.7 % (FIG. 2.3A). *BEAST analyses excluding *act* and LSU produced species trees with the same topology as the combined analyses and * BEAST runs with the complete data sets. However, the PP values were generally higher when *act* and LSU were excluded (FIG. 2.3A).

According to the software specifications, *BEAST and BUCKy are designed to identify the underlying species-level phylogeny while accounting for heterogeneity among gene genealogies due to incomplete lineage sorting (Ané et al. 2007, Edwards et al. 2007). The inclusion of a discordant locus data set such ITS would have little or no effect. However, *BEAST and BCA analyses without the ITS dataset resulted in a general decrease in branch support (FIG. 2.3A-B) and differences in tree topology (FIG. S2.7), possibly due to the exclusion of phylogenetically informative sites contained in the ITS region. The support for internal nodes was also low. This is a general trend observed even in the analyses with the complete data set, apparently

because the loci used in this study lack sufficient informative sites to resolve deep nodes.

Genetic divergence estimates

The greatest nucleotide diversity (π) within putative species in this complex was recovered from protein coding loci (including exon and intron regions) such as *tef1*, *tub* and *rpb1*, ranging from 0.0005 to 0.0189 (TABLE 2.3). In general, all nuclear loci used, either ribosomal or protein coding, showed a high number of unique haplotypes. Many of the putative species showed one single haplotype per nuclear locus. However, clades such as V, VII, VIII, IX, and XI show more than one unique haplotype for the majority of nuclear loci, which also coincides with their high nucleotide diversity. Additionally, genetic distance, as measured by Dxy values obtained between all pairs of putative species, ranged from 0.004 and 0.068 (TABLE 2.4). The highest average genetic distances were observed between clusters of putative species 1 (I–XII) and 2 (XIII–XV) with values ranging from 0.031 to 0.067. The putative species containing the pathogenic isolates (clade XVI) is the most distantly related to clusters 1 and 2, with genetic distances ranging from 0.045 to 0.051 (TABLE 2.4). The degree of genetic distance between pairs of clades was not related to geographic distance. For example, clades III and XIII contain isolates from New Zealand, which have no clear geographic but genetic boundaries.

Ecology and geographic distribution of species

Even though geographical segregation at various levels was observed, it is not a strong character for defining the putative species (TABLE 2.1, FIG. 2.2). From the

combined phylogenetic analyses we could observe putative species formed by isolates from the same geographic region (Clades III–V, IX, X); isolates from close-by regions (Clades I, VII, VIII, XII); and isolates coming from apparently distant regions (Clades II, VI, XI, XIII–XV, XVI) (TABLE 2.1, FIG. 2.2).

Interestingly, a correlation with ecology was observed for all putative species. Isolates assigned to the clades I–XI and XIII–XV were collected in their sexual state, *i.e.,* as fruiting bodies (perithecia) on decaying plant material. Isolates in clade XII were collected as saprobes on soil in the asexual state ('*Cylindrocarpon*'). Isolates in clade XVI are plant pathogens on several species of *Rubus*, also collected as the sexual state (TABLE 2.1; Brayford 1991). None of the rest of putative species has been found on *Rubus* species causing disease. Isolates in the outgroup and sister species *T. lucida* were collected as sexual fruiting bodies on decaying plant material. Based on our observations, no host specificity was shown by the putative species. However, the lack of information about the host on which some of the isolates were collected makes it almost impossible to reach a definite conclusion (TABLE 2.1).

Discussion

Species delimitation, diversity and taxonomy

Utilizing a combination of phylogenetic analyses, genetic distances and species tree inference, we have delineated within the morphospecies *T. discophora* at least 16 distinct putative species. The genetic distances between putative species mostly exceeded standard values of genetic distance (0.01–0.03) used to delimit operational taxonomic units (OTU), revealing them as independent entities. This indicates a > 16-fold increase in the species diversity in the genus *Thelonectria* and provides

additional evidence for the hyperdiversity of fungi. This high number of putative species is even more surprising considering the relatively few and scattered localities from where we obtained specimens, representing only a tiny portion of their potential geographic distribution. The conclusion that a species is not cosmopolitan has implications for its taxonomy and thus the true diversity of a group of organisms (Blackwell 2011, Hawksworth and Rossman, Taylor et al. 2006). Revealing the true distribution of a species also has implications in conservation programs, because some of the most important criteria used to assess the conservation status of a species are their geographic distribution, population size and information on how these features are changing over time (Dahlberg and Muller 2011).

Given the lack of diagnostic morphological characters than can be used for taxonomic circumscription within many fungal groups and especially species complexes, the inclusion of other kinds of data such as molecular characters is extremely helpful for establishing species limits (Roe et al. 2010, Stielow et al. 2011, Taylor et al. 2000). Our species delimitation criterion is the genealogical concordance phylogenetic species recognition (GCPSR) (Dettman et al. 2003), which is based on the convergence of evidence from phylogenetic analyses of unlinked loci, combined with species tree recovery and genetic divergence estimates. The approaches used in this study to reveal phylogenetic relationships among isolates of *T. discophora* uncovered the same relationships and confidently identified 16 previously undetected putative species. For Clades I–II and III–IV, the low values of genetic divergence and short branches may be indicative of their possible recent divergence. The rest of the

clades show divergence values higher than 0.01, demonstrating little genetic cohesiveness and independent evolutionary history.

Our phylogenetic analyses also detected eight single isolate lineages or singletons. In systematics, no consensus exists on how singleton lineages should be treated, even though they represent distinctive evolutionary units and make up a considerable portion of the global diversity of species in this group (Seifert and Rossman 2010). One possibility could be to include them as part of the closest clade; however, it has been seen that this causes a decrease in the putative species' support values. Further increase in taxon sampling would determine if they should be separate species and if should be named. Many of these singletons likely constitute rare taxa, which highlight even more the importance of preserving the habitat where they can be found (Dahlberg et al. 2011).

Species tree reconstruction

Bayesian concordance analyses (BCA) (Ané et al. 2007) and Bayesian inference of species trees (*BEAST) (Drummond et al. 2012, Heled and Drummond 2010) have been proven as useful complements to the phylogenetic analyses using standard concatenated data as they are able to account for gene tree/species tree discordance. Phylogenies with the concatenated data and BCA estimated the same species relationships, however, different tree topologies were obtained from the *BEAST analyses. The presence of other topologies in *BEAST may indicate that our data set do not contain enough information to accurately infer the species tree by this coalescent method or that different evolutionary histories can affect this method more than others (Cranston et al. 2009). Additional studies with larger taxon sampling and

data sets will allow further analysis of the results of *BEAST compared with BCA and concatenated analyses.

The concatenated phylogeny recovered much higher bootstrap and posterior support values compared to those obtained from the Bayesian Concordance tree estimation (CF values) and coalescent-based species tree strategy (PP values). This is a trend that has been observed in several similar studies (Leache and Rannala 2011, Lee et al. 2012). In the case of CF values in BCA, it is important to note that they are not equivalent to bootstrap percentage or Bayesian posterior probabilities, rather they are estimates of the proportion of sample genes for which a particular clade is true (Ané et al. 2007, Baum 2007). There is little agreement on what constitutes a significant CF value that would indicate a history of genetic isolation for a particular clade (Baum 2007). While we obtained clades with CF values higher than 0.5 (meaning, in an operational way, that the clade is present at least in 50 % of the gene genealogies; Baum 2007), clades with low CF values may indicate a difference in the stage of the speciation process (Baum 2007). Therefore, we have recovered the dominant phylogenetic history in spite of the low CF recovered for some putative species. The number of genes used in this study did not have a steady effect on CF values. The CF values might depend more on the biology of the organisms (*e.g.*, reticulation history), as this knowledge is a major determinant of the sampling effort for taxa and data needed to obtain clades with good CF estimates (Baum 2007, Lee et al. 2012). Even though the tree topologies estimated by *BEAST using the reduced data sets, *i.e.*, excluding *act* and LSU, and excluding ITS, differed from the concatenated and BCA, the putative species had, in general, high PP support (FIG.

2.3B and S2.6). This is particularly clear in the topology of the tree obtained with the four-locus data set excluding *act* and LSU, and it is likely to have been strongly influenced by the exclusion of these low-informative data sets. The different parameter models that are used for both reduced data sets can also explain the difference in tree topologies. It has been proven that this method is particularly sensitive to gene discordance and other coalescent processes such as ancestral population sizes (Heled and Drummond 2010).

In our study, we found that the ITS gene tree was discordant with the rest of the data sets. This incongruence was not restricted to weak or unresolved nodes as might be expected under a scenario of rapid diversification, evident in the group of putative species I–IV. Rather, this incongruence extends to conflicts involving strongly supported clades, in this case, the putative species XVI. The nodes supporting these clades created the conflict between the ITS locus and the rest of the nuclear data sets analyzed. Putative species XVI includes isolates of *T. discophora* known to be pathogenic to *Rubus* species. This divergence between saprobic and pathogenic species may be recent based on the incongruence found. Based on these results, we could conclude that this incongruence might be due to incomplete lineage sorting, admixture events, or both. Since these two events are often difficult to distinguish with traditional phylogenetic methods as they produce very similar genetic patterns (Qu et al. 2012), we do not rule out that either process or both are affecting the historical divergence of *T. discophora*-like species.

Geographic structure and cosmopolitanism

Cosmopolitan species are defined as those found all around the world or with a wide geographic distribution. To be truly cosmopolitan, a species needs to have at least the following characteristics: (1) it maintains its genetic cohesiveness mediated by gene flow throughout its distribution (Klautau et al. 1999), and consequently, (2) it possesses highly effective mechanisms for long distance dispersal (Taylor et al. 2006). In the case of microorganisms, it has been said that microbial taxa will not exhibit endemism because their enormous populations remove dispersal as an effective constraint on geographical range (Vanormelingen et al. 2007). The results from our research support previous studies with other microorganisms, including fungi, that there are very few truly cosmopolitan species (Carriconde et al. 2008, Pringle et al. 2005, Taylor et al. 2006). Because *T. discophora* is a species complex that includes species with restricted geography and ecology, we hypothesize that the limited effective dispersal mechanisms over long geographic distances is affecting their distribution.

The causes and effects of spore dispersal in the geographic distribution of fungi have been poorly studied (Bass-Becking 1934, Roper et al. 2010). Several factors such as spore size, shape, ejection force, and pigmentation can have a significant influence on cosmopolitanism, although fitting these criteria not always implies development of cosmopolitanism (Foissner 2006). Because of their microscopic size, the forcibly ejected sexual spores of ascomycetous fungi are quickly brought to rest by drag (the force exerted on a body moving in a fluid) (Roper et al. 2010). To avoid this, some fungi have evolved minute asexual spores (< 5 μm)

that are carried easily over long distances by wind currents (*e.g., Aspergillus* spp., *Fusarium oxysporum,* and *Penicillium* spp.) or have spores with drag-minimizing shapes (Roper et al. 2008). Other species such as *Ascobolus* spp. and *Sclerotinia sclerotiorum* have the ability to manipulate the local fluid environment surrounding their fruiting bodies to enhance spore dispersal (Roper et al. 2010). Finally, dark-pigmented spores protected with melanin against UV radiation and desiccation may survive long-distance dispersion (*e.g*., *Alternaria* spp. and *Sordaria* spp.).

Taking into account the dispersal capabilities and spore morphology in *Thelonectria*, it is unlikely that *T. discophora* has the ability to be a cosmopolitan species. This fungus has asexual spores (macroconidia) that are colorless and longer than 30 μm, sometimes reaching 100 μm; its sexual spores (ascospores) are also colorless or non-melanized (FIG. 2.1). More importantly, both sexual and asexual spores do not have shapes that could improve dispersal, possibly resting after traveling short distances. These characteristics together limit considerably the range of dispersal, and consequently populations experience independent evolutionary trajectories and, ultimately, species divergence. The marked genetic structure observed among the putative species in *T. discophora* likely reflects the interplay between their poor dispersal capabilities and the restrictions to gene flow, either imposed by geographical or reproductive barriers.

The *Thelonectria discophora* complex contains putative species that correlate to geographic origin, meaning one putative species can group isolates from the same or close-by regions. However, this complex also contains putative species that group isolates from apparently distant geographic locations. This phenomenon most

probably indicates that members of this species complex show a continuum from regional, indicated by the co-occurrence of putative species with possible niche overlap (Cothran et al. 2013), to large-scale distribution. According to our data, ten putative species were found only in temperate regions such as United States, Europe, Asia and New Zealand. Fewer putative species were found in tropical regions; however, this could be a result of the low taxon sampling that has been made in tropical regions. For example, collections from Venezuela represent at least four putative species. Thus, one may assume that *T. discophora*-like species can be found in tropical and temperate regions equally. Due to their small size and ecological preferences, these fungi have only been collected serendipitously translating into a limited taxon sampling. As it is the case with poorly studied organisms, increasing their collection can further support assumptions about the geographic range of the putative species or about their center of origin. The role of human mediated movement of species of *T. discophora* contributing to the actual geographic distribution of species, cannot be disregarded; however, because these species are not invasive or pathogens of commercial or forest plants, their presence can be overlooked and movement difficult to track.

Conclusions

The world's actual magnitude of fungal diversity remains largely under-documented. The continuing destruction and disturbance of natural ecosystems are increasing the chances of massive extinction of species, together with the knowledge about them (Blackwell 2011). Because of their small visible structures, fungi often remain undetected, increasing the potential loss of this group of highly diverse organisms.

Under these conditions, efforts to catalogue and explain fungal diversity need to be prioritized treating each diversity group accordingly (Bickford et al. 2007). A significant percentage of the under-documented diversity of fungi exists as cryptic complexes of species previously recognized as one single species with wide geographical distribution, which have been formally described as separate entities (Blackwell 2011, Qu et al. 2012). Based on the evidence presented in this study, the taxonomic status of isolates recognized as *T. discophora* should be reconsidered. This trend is not only found in *Thelonectria*, but also in other genera of the Hypocreales (Hirooka et al. 2012, Lombard et al. 2010), other ascomycetes (Leavitt et al. 2011, McDonald et al. 2012), and many other microorganisms. At the gross morphological level, *Thelonectria discophora*-like species might appear to be under morphological stasis or an example of many species showing a morphological convergence. However, as for other fungi in the genus *Thelonectria* (Salgado-Salazar et al. 2012) statistically significant morphological differences could exist. Analyses of populations from a wider geographical range are necessary to untangle the patterns of diversity within this curious species complex, and formal recognition of species in this complex has implications for the diversity of fungi in the family Nectriaceae. Future molecular phylogenetic investigations of geographically and ecologically widespread fungi will most likely uncover far greater levels of biodiversity than currently recognized, as cryptic speciation and regional endemism are revealed.

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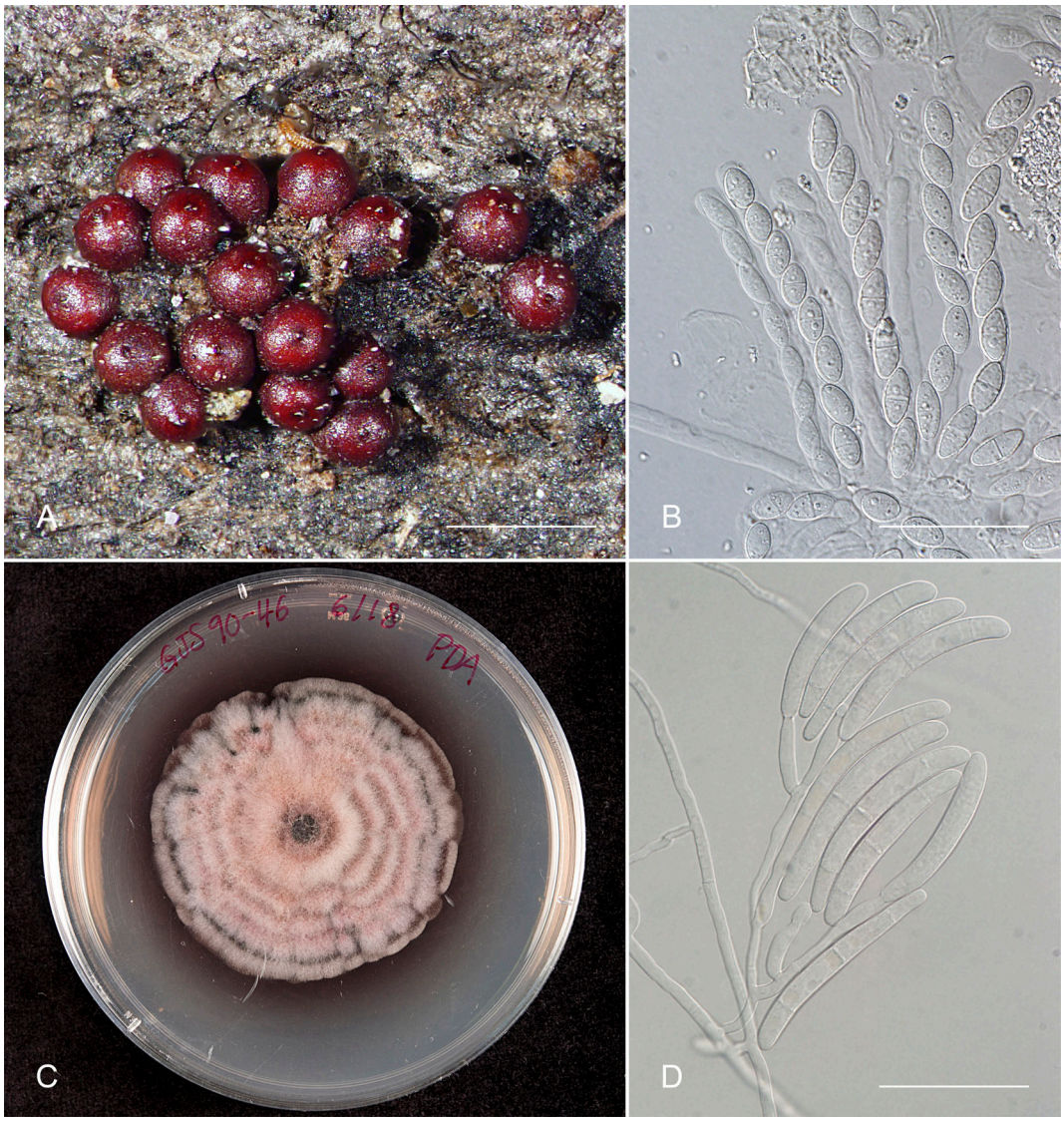

FIGURE 2.1. Typical morphology of *T. discophora*-like species. A. Perithecia (sexual fruiting bodies). B. Asci and ascospores (sac and sexual spores). C. Aspect of colony

on PDA (potato-dextrose agar). E. Conidiophores and conidia (asexual structures and spores). Bars A= 500 μm, B= 50 μm, D= 50 μm.

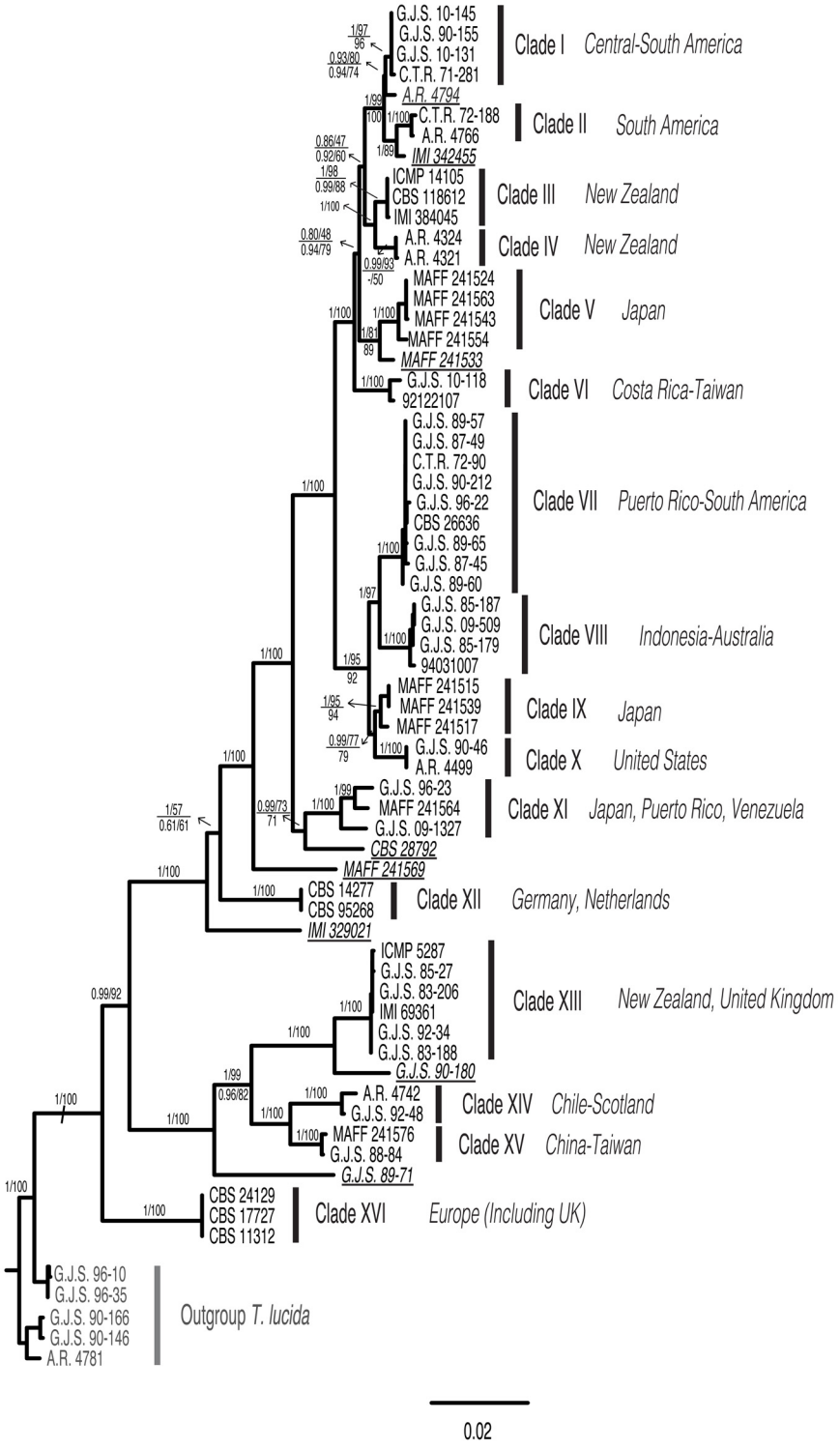

FIGURE 2.2. Bayesian phylogram showing relationships among isolates of *T. discophora*-like species based on the concatenated analysis of six loci. Bayesian posterior probabilities and ML bootstrap are indicated above each branch. Bayesian posterior probabilities and ML bootstrap from the concatenated analyses excluding ITS loci are indicated below each branch. No values below branches indicate equal support was found for the different analyses. "-" indicates branch was not recovered/supported.

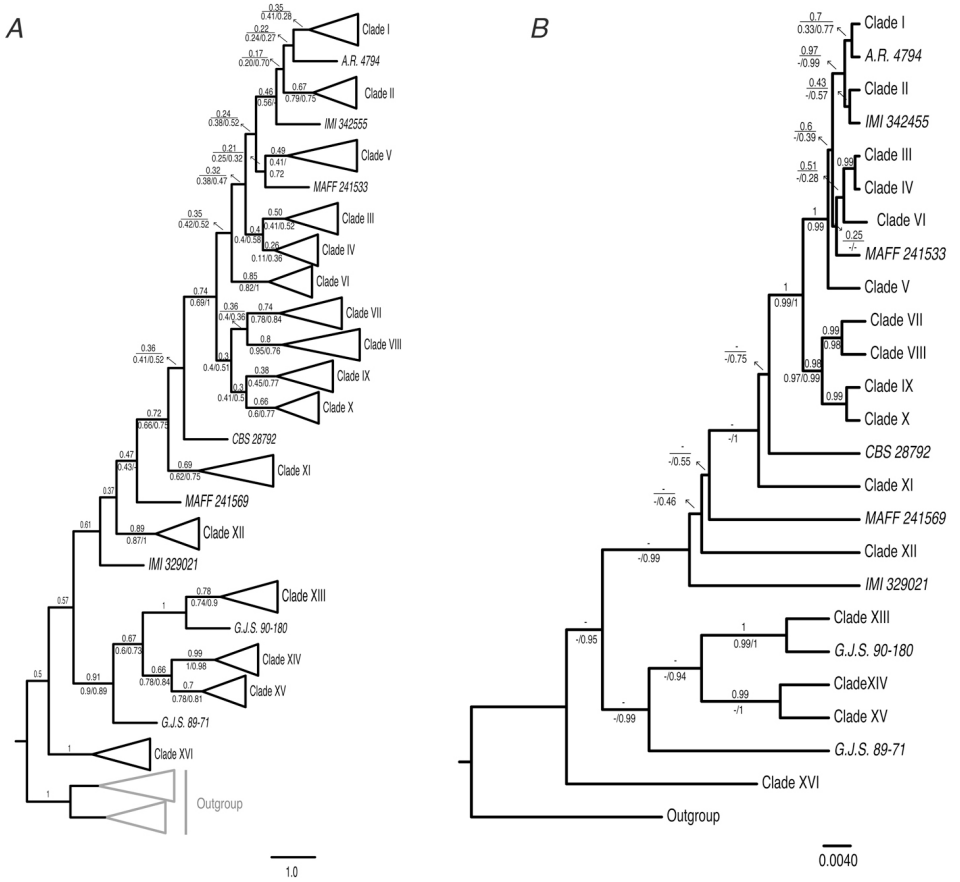

FIGURE 2.3. The Bayesian concordance analyses (BCA) tree and coalescent-based species trees. A. Primary concordance tree estimated by BCA; values above branches indicate sample-wide clade concordance factors (CF). Values below branches

correspond to sample-wide CF from the analyses of the data set excluding ITS and reduced data set (4-loci), respectively. The primary concordance tree with 95% credibility intervals is included in FIGURE S2.5. B. Maximum clade credibility tree from concatenated analyses in *BEAST. This tree represents the posterior sample with the maximum sum of clade posterior probabilities at the internal nodes. Branch lengths equal to expected substitutions per site. Posterior probabilities of each clade are shown above branches. Values below branches correspond to posterior probabilities obtained in the analyses of the data set excluding ITS and reduced data set (4-loci), respectively. "-" indicates clade not recovered/supported.

TABLE 2.1. Taxa used in this study, including information about the origin of the fungal material, collection codes and GenBank accession numbers

Strain	Code	Host	Origin	GenBank accession numbers					
				act	ITS	LSU	*rpb1*	*tef*	*tub*
Cyl. ianthothele	CBS 118612	*Quercus rubur*	New Zealand	KC121381	KC153719	KC121445	KC153912	KC153848	KC153784
Cyl. ianthothele var. majus	CBS 28792	On soil	Brazil	KC121386	KC153724	KC121450	KC153917	KC153853	KC153789
Cyl. ianthothele var. majus	CBS 95268	On soil	Germany	KC121387	KC153725	KC121451	KC153918	KC153854	KC153790
Neo. discophora var. rubi	CBS 11312	*Rubus idaeus*	Switzerland	KC121380	KC153718	KC121444	KC153911	KC153847	KC153783
Neo. discophora var. rubi	CBS 14277	On soil	Netherlands	KC121382	KC153720	KC121446	KC153913	KC153849	KC153785
Neo. discophora var. rubi	CBS 17727	*Rubus idaeus*	England	KC121383	KC153721	KC121447	KC153914	KC153850	KC153786
Neo. discophora var. rubi	CBS 24129	*Rubus idaeus*	Scotland	KC121384	KC153722	KC121448	KC153915	KC153851	KC153787
Neo. discophora var. rubi	ICMP 14105	Unknown	New Zealand	KC121420	KC153758	KC121484	KC153951	KC153887	KC153823
T. discophora	92122107 (=CBS 134038)	unknown	Taiwan	KC121373	KC153711	KC121437	KC153904	KC153840	KC153775
T. discophora	94031007 (=CBS 134039)	unknown	Taiwan	KC121374	KC153712	KC121438	KC153905	KC153841	KC153776
T. discophora	A.R. 4321 (=CBS 134033)	*Pinus radiata*	New Zealand	KC121375	KC153713	KC121439	KC153906	KC153842	KC153777
T. discophora	A.R. 4324 (=CBS 125153)	*Pinus radiata*	New Zealand	HM352875	HM364294	HM364307	HM364326	HM364345	HM352860
T. discophora	A.R. 4499 (=CBS 125172)	*Fagus grandifolia*	U.S	HM352877	HM364296	HM364309	HM364327	HM364347	HM364327
T. discophora	A.R 4742 (=CBS 134034)	*Tepualia stipularis*	Chile	KC121376	KC153714	KC121440	KC153907	KC153843	KC153779
T. discophora	A.R. 4766 (=CBS 134035)	Unknown	Argentina	KC121377	KC153715	KC121441	KC153908	KC153844	KC153780
T. discophora	A.R. 4794 (=CBS 134037)	Unknown	Argentina	KC121379	KC153717	KC121443	KC153910	KC153846	KC153782

T. discophora	C.T.R. 71-281 (=CBS 112458)	unknown	Venezuela	KC121388	KC153726	KC121452	KC153919	KC153855	KC153791
T. discophora	C.T.R. 72-188 (=CBS 134040)	Unknown	Venezuela	KC121389	KC153727	KC121453	KC153920	KC153856	KC153792
T. discophora	C.T.R. 72-90	Unknown palm	Venezuela	KC121390	KC153728	KC121454	KC153921	KC153857	KC153793
T. discophora	G.J.S. 09-1327 (=CBS 134022)	Unknown	Venezuela	KC121391	KC153729	KC121455	KC153922	KC153858	KC153794
T. discophora	G.J.S. 09-509	*Acacia celsa*	Australia	KC121392	KC153730	KC121456	KC153923	KC153859	KC153795
T. discophora	G.J.S. 10-118 (=CBS 134023)	Unknown	Costa Rica	KC121393	KC153731	KC121457	KC153924	KC153860	KC153796
T. discophora	G.J.S. 10-131 (=CBS 134024)	Unknown	Costa Rica	KC121394	KC153732	KC121458	KC153925	KC153861	KC153797
T. discophora	G.J.S. 10-145 (=CBS 134025)	Unknown	Costa Rica	KC121395	KC153733	KC121459	KC153926	KC153862	KC153798
T. discophora	G.J.S. 83-188 (=IMI 326256)	*Fuchsia exorticata*	New Zealand	KC121396	KC153734	KC121460	KC153927	KC153863	KC153799
T. discophora	G.J.S. 83-206 (=IMI 326258)	Unknown	New Zealand	KC121397	KC153735	KC121461	KC153928	KC153864	KC153800
T. discophora	G.J.S. 85-179 (=IMI 329113)	Unknown	Indonesia	KC121398	KC153736	KC121462	KC153929	KC153865	KC153801
T. discophora	G.J.S. 85-187 (=ATCC 76478)	Unknown	Indonesia	KC121399	KC153737	KC121463	KC153930	KC153866	KC153802
T. discophora	G.J.S. 85-27 (=CBS 112457)	unknown	New Zealand	KC121400	KC153738	KC121464	KC153931	KC153867	KC153803
T. discophora	G.J.S. 87-45 (=IMI 325855)	unknown	Guyana	KC121401	KC153739	KC121465	KC153932	KC153868	KC153804
T. discophora	G.J.S. 87-49 (=CBS 112461)	Unknown	Guyana	KC121402	KC153740	KC121466	KC153933	KC153869	KC153805
T. discophora	G.J.S. 88-84 (=IMI 348190)	Unknown	China	KC121403	KC153741	KC121467	KC153934	KC153870	KC153806
T. discophora	G.J.S. 89-57 (=CBS 112459)	Unknown	Guyana	KC121404	KC153742	KC121468	KC153935	KC153871	KC153807
T. discophora	G.J.S. 89-60	Unknown	Guyana	KC121405	KC153743	KC121469	KC153936	KC153872	KC153808
T. discophora	G.J.S. 89-65	Unknown	Guyana	KC121406	KC153744	KC121470	KC153937	KC153873	KC153809

	(=CBS 123970)								
T. discophora	G.J.S. 89-71 (= CBS 134026)	Unknown	Guyana	KC121407	KC153745	KC121471	KC153938	KC153874	KC153810
T. discophora	G.J.S. 90-155 (=CBS 123966)	Unknown palm	Venezuela	KC121409	KC153747	KC121473	KC153940	KC153876	KC153812
T. discophora	G.J.S. 90-180 (=CBS 134027)	Unknown	Venezuela	KC121411	KC153749	KC121475	KC153942	KC153878	KC153814
T. discophora	G.J.S. 90-212 (=CBS 134028)	Unknown	Venezuela	KC121412	KC153750	KC121476	KC153943	KC153879	KC153815
T. discophora	G.J.S. 90-46 (=CBS 134029)	*Quercus* sp.	U.S	KC121413	KC153751	KC121477	KC153944	KC153880	KC153816
T. discophora	G.J.S. 92-34 (=CBS 134030)	Unknown	Scotland	KC121414	KC153752	KC121478	KC153945	KC153881	KC153817
T. discophora	G.J.S. 92-48 (=CBS 134031)	*Aesculus* sp.	Scotland	KC121415	KC153753	KC121479	KC153946	KC153882	KC153818
T. discophora	G.J.S 96-22 (=IMI 370946)	*Ocoter* sp.	Puerto Rico	KC121417	KC153755	KC121481	KC153948	KC153884	KC153820
T. discophora	G.J.S. 96-23 (=IMI 370947)	Unknown	Puerto Rico	KC121418	KC153756	KC121482	KC153949	KC153885	KC153821
T. discophora	ICMP 5287	Unknown	New Zealand	KC121421	KC153759	KC121485	KC153952	KC153888	KC153824
T. discophora	IMI 329021	*Beilschmiedia tawa*	New Zealand	KC121422	KC153760	KC121486	KC153953	KC153889	KC153825
T. discophora	IMI 342455	Unknown	Kenya	KC121423	KC153761	KC121487	KC153954	KC153890	KC153826
T. discophora	IMI 384045	Unknown	New Zealand	KC121424	KC153762	KC121488	KC153955	KC153891	KC153827
T. discophora	IMI 69361	*Smyrnium olusatrum*	UK	KC121425	KC153763	KC121489	KC153956	KC153892	KC153828
T. discophora	MAFF 241515	Unknown	Japan	KC121426	KC153764	KC121490	KC153957	KC153893	KC153829
T. discophora	MAFF 241517	*Cryptomeria japonica*	Japan	KC121427	KC153765	KC121491	KC153958	KC153894	KC153830
T. discophora	MAFF 241524	Unknown	Japan	KC121428	KC153766	KC121492	KC153959	KC153895	KC153831
T. discophora	MAFF 241533	Unknown	Japan	KC121429	KC153767	KC121493	KC153960	KC153896	KC153832
T. discophora	MAFF 241539	Unknown	Japan	KC121430	KC153768	KC121494	KC153961	KC153897	KC153833

T. discophora	MAFF 241543	Unknown	Japan	KC121431	KC153769	KC121495	KC153962	KC153898	KC153834
T. discophora	MAFF 241554	Unknown	Japan	KC121432	KC153770	KC121496	KC153963	KC153899	KC153835
T. discophora	MAFF241563	*Fagus crenata*	Japan	KC121433	KC153771	KC121497	KC153964	KC153900	KC153836
T. discophora	MAFF 241564	Unknown	Japan	KC121434	KC153772	KC121498	KC153965	KC153901	KC153837
T. discophora	MAFF 241569	Unknown	Japan	KC121435	KC153773	KC121499	KC153966	KC153902	KC153838
T. discophora	MAFF 241576	Unknown	Japan	KC121436	KC153774	KC121500	KC153967	KC153903	KC153839
T. lucida	A.R 4781 (=CBS 134036)	Unknown	Argentina	KC121378	KC153716	KC121442	KC153909	KC153845	KC153781
T. lucida	G.J.S 90-146 (=CBS 134032)	Unknown	Venezuela	KC121408	KC153746	KC121472	KC153939	KC153875	KC153811
T. lucida	G.J.S 90-166 (=CBS 126099)	Unknown	Venezuela	KC121410	KC153748	KC121474	KC153941	KC153877	KC153813
T. lucida	G.J.S 96-10 (=IMI 370944)	Unknown	Puerto Rico	KC121416	KC153754	KC121480	KC153947	KC153883	KC153819
T. lucida	G.J.S 96-35 (=CBS 112456)	Unknown	Puerto Rico	KC121419	KC153757	KC121483	KC153950	KC153886	KC153822

TABLE 2.2. List of molecular markers and descriptive statistics for the six loci used in this study.

Locus	Substitution model	Aligned length	Variable sites (%)	Parsimony informative sites (%)	%GC	Primers	Reference
act	TrN + G	545	66 (12.11)	43 (7.88)	56.6	F 5'TGGCACCACACCTTCTACAATGA3' R 5'TCCTCCGCTTATTGATATGC3'	1
ITS	HKY + G	661	96 (14.52)	80 (12.10)	55.3	F 5'GGAAGTAAAAGTCGTAACAAGG3' R 5'TCCTCCGCTTATTGATATGC3'	2
LSU	TrNef + I	807	44 (5.45)	35 (4.33)	53.7	F 5'ACCCGCTGAACTTAAGC3' R 5'TCCTGAGGGAAACTTCG3'	Vilgalys n.d.
tef	HKY + I + G	921	183 (19.86)	135 (14.65)	55.8	F 5'CATCGAGAAGTTCGAGAAGG3' R 5'ACHGTRCCRATACCACCRAT3'	3
tub	TrN + G	556	159 (28.6)	126 (22.66)	56.6	F 5'AACATGCGTGAGATTGTAAGT3' R 5'TAGTGACCCTTGGCCCAGTTG3'	4
rpb1	TrNef + I	646	227 (35.13)	197 (30.5)	53.4	F 5'CAYCCWGGYTTYATCAAGAA3' R 5'CCNGCDATNTCRTTRTCCATRTA3'	5

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4. O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous. Mol Phylogenet Evol 7: 103-117.
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TABLE 2.3. Polymorphism statistic for putative species within the *T. discophora* species-complex.

	act		ITS		LSU		*rpb1*		*tef1*		*tub*	
	N/Npoly/h	π	N/Npoly/h	π	N/Npoly/h	π	N/Npoly/h	π	N/Npoly/h	π	N/Npoly/h	π
Clade I	4/0/1	0	4/0/1	0	4/0/1	0	4/0/1	0	4/4/3	0.00239	4/0/1	0
Clade II	2/0/1	0	2/0/1	0	2/0/1	0	2/0/1	0	2/4/2	0.00477	2/0/1	0
Clade III	3/0/1	0	3/0/1	0	3/0/1	0	3/1/2	0.00105	3/0/1	0	3/0/1	0
Clade IV	2/1/2	0.00186	2/0/2	0	2/0/2	0	2/0/1	0	2/0/1	0	2/0/1	0
Clade V	5/3/2	0.00233	5/5/2	0.00404	5/1/2	0.00050	5/12/3	0.00757	5/11/3	0.00645	5/13/3	0.01176
Clade VI	2/2/2	0.00371	2/0/1	0	2/0/1	0	2/3/2	0.00473	2/3/2	0.00359	2/2/2	0.00380
Clade VII	9/2/3	0.00082	9/0/1	0	9/0/1	0	9/0/1	0	9/3/4	0.00079	9/3/3	0.00158
Clade VIII	4/2/2	0.00186	4/0/1	0	4/0/1	0	4/0/1	0	4/2/2	0.00119	4/2/3	0.00222
Clade IX	3/2/2	0.00247	3/0/1	0	3/0/1	0	3/0/1	0	3/11/3	0.00876	3/1/2	0.00127
Clade X	2/0/1	0	2/0/1	0	2/0/1	0	2/0/1	0	2/0/1	0	2/0/1	0
Clade XI	3/4/2	0.00497	3/7/3	0.00970	3/4/3	0.00331	3/17/3	0.01893	3/19/3	0.01580	3/14/2	0.01895
Clade XII	2/0/1	0	2/0/1	0	2/0/1	0	2/0/1	0	2/0/1	0	2/0/1	0
Clade XIII	6/0/1	0	6/0/1	0	6/0/1	0	6/0/1	0	6/2/3	0.00105	6/1/2	0.00064
Clade XIV	2/0/1	0	2/0/1	0	2/0/1	0	2/1/2	0.00160	2/10/2	0.01215	2/0/1	0
Clade XV	2/2/1	0.00372	2/0/1	0	2/0/1	0	2/0/1	0	2/0/1	0	2/2/2	0.00391
Clade XVI	3/0/1	0	3/0/1	0	3/0/1	0	3/0/1	0	3/0/1	0	3/0/1	0
Total	54/43/25	0.01269	54/80/16	0.02597	54/35/13	0.00840	54/197/23	0.0731	54/135/32	0.0315	54/126/26	0.0478

N, number of sequences/individuals, Npoly, number of polymorphic sites, h, number of unique haplotypes, π, nucleotide diversity

TABLE 2.4. Nucleotide divergence (Dxy*) for all pairwise comparisons of putative species identified within *T. discophora* species-complex. All positions containing gaps were eliminated for a total of 3708 positions. Numbers across the top row correspond to putative species numbers in the first column.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
Clade I																
Clade II	0.004															
Clade III	0.006	0.008														
Clade IV	0.005	0.007	0.001													
Clade V	0.010	0.011	0.008	0.008												
Clade VI	0.013	0.014	0.010	0.009	0.014											
Clade VII	0.016	0.015	0.013	0.012	0.016	0.016										
Clade VIII	0.018	0.017	0.014	0.014	0.016	0.018	0.009									
Clade IX	0.014	0.013	0.011	0.011	0.014	0.015	0.010	0.010								
Clade X	0.014	0.014	0.013	0.012	0.015	0.016	0.010	0.011	0.006							
Clade XI	0.029	0.029	0.028	0.027	0.029	0.030	0.028	0.028	0.026	0.027						
Clade XII	0.040	0.040	0.038	0.037	0.038	0.040	0.038	0.039	0.037	0.038	0.034					
Clade XIII	0.068	0.069	0.066	0.065	0.066	0.068	0.068	0.067	0.065	0.066	0.068	0.061				
Clade XIV	0.065	0.065	0.063	0.062	0.063	0.065	0.065	0.064	0.063	0.063	0.064	0.058	0.034			
Clade XV	0.062	0.062	0.060	0.059	0.061	0.061	0.061	0.061	0.059	0.060	0.061	0.054	0.031	0.016		
Clade XVI	0.049	0.050	0.048	0.047	0.049	0.050	0.049	0.049	0.048	0.048	0.051	0.044	0.052	0.049	0.045	

* $p < 0.001$

Chapter 3: Phylogeny and taxonomic revision of *Thelonectria discophora* (Ascomycota, Hypocreales, Nectriaceae) species complex.

Abstract

Thelonectria discophora (*Thelonectria*, Nectriaceae, Hypocreales) is a conspicuous group of saprobic fungi on decaying plant material, characterized by red perithecia each with a broad mammiform (nipple-like) apex. The anamorphic state is characterized by a cylindrocarpon-like morphology, with 3–5 septate macroconidia, unicellular microconidia and chlamydospores are rarely produced in culture. In the past, *T. discophora* was regarded as one species with a wide geographic distribution. However, a recent study rejected the monophyly and cosmopolitan distribution of this species, and showed the existence of at least sixteen cryptic species based solely on molecular data. In the present paper, we revise the taxonomy of the *T. discophora* species complex by describing twelve new species and four new combinations based on historic names. Individual diagnostic morphological characters for each species could not be identified; however, discrete morphological traits corresponding to each of the three main groups of species were discovered. Lineages could be differentiated by average values of morphological traits as well as presence/absence of characteristic asexual propagules and colony growth at 30 C. Descriptions, illustrations and keys for identification are provided for the recognized species.

Key words: New species, Rubus canker, species concept, taxonomy.

Introduction

Thelonectria discophora (Mont.) P. Chaverri & C. Salgado 2011 is a complex of morphologically similar species in the Nectriaceae (Hypocreales, Ascomycota). First

described in 1835 from material collected in Chile, it is the type species of the genus *Thelonectria* P. Chaverri & C. Salgado. This species was previously considered to have a cosmopolitan distribution because it had been encountered on every continent, excluding Antarctica and the Arctic regions (Brayford et al. 2004), and has been found in fungal diversity surveys throughout the world (e.g., Brayford et al. 2004, Guu et al. 2007, Hirooka and Kobayashi 2007, Samuels et al. 1990). However, recent studies have discovered that even though truly cosmopolitan fungal species exist (Fierer and Jackson 2006, Finlay 2002, James et al. 1999, Pringle et al. 2005, Queloz et al. 2011, Rydholm et al. 2006), cosmopolitanism could not be found in *T. discophora* species complex, as it is an assemblage of morphologically similar, or even identical, but genetically divergent species (Salgado-Salazar et al. 2013).

Species in the *T. discophora* complex occur in a diverse set of habitats and plant substrates, such as bark of twigs, branches or trunks of recently dead or dying trees (Brayford et al. 2004, Guu et al. 2007, Samuels et al. 1990). These cryptic species show little intraspecific morphological variability. Perithecia occur singly or in groups and are smooth, shiny, red to dark-red colored, often with a broad mammiform (nipple-like) apex. The ascospores are bicellular and colorless or pale yellow with a spinulose surface. The anamorphic state produces long, curved, 3–5 septate macroconidia with round ends (Booth 1966, Brayford et al. 2004, Chaverri et al. 2011). Microconidia and chlamydospores are rarely reported. These morphological characters equally describe other species in *Thelonectria,* such as *T. lucida*, making the accurate identification of these species difficult when based solely on morphological characters (Brayford et al. 2004).

Thelonectria discophora is among the first colonizers on newly dead organic plant material (Brayford et al. 2004, Samuels et al. 1990). It is common in disturbed areas with recently fallen or cut plant material and is rarely found fruiting in old growth forests (Chaverri and Vílchez 2006). Rarely collected on living plant material, one variety of this species, "*Neonectria" discophora* var. *rubi* (Osterw.) Brayford & Samuels 2004, has been associated with a distinctive basal canker of cultivated *Rubus idaeus* and *R. fruticosus* (Brayford 1991, Cedeño et al. 2004). Even though this variety causes a disease, it has been regarded as a secondary or weak pathogen because the disease outbreaks have been mostly correlated with stressed plants following wind damage or waterlogging (Brayford 1991, Brayford et al. 2004). "*Neonectria" discophora* var. *rubi* belongs to *Thelonectria*; however, it has not been formally transferred to this genus and has not been included in molecular studies. Because *T. discophora* was assumed to be common and cosmopolitan, many names of morphologically similar species were considered taxonomic synonyms (see Brayford et al. 2004, Chaverri et al. 2011). Based on the modification of the code of nomenclature, the generic name "*Cylindrocarpon*", previously applied to the anamorphic name of *T. discophora*, should not be used. In spite of that, *C. ianthothele* and *C. ianthothele* var. *majus* have not been formally regarded as synonyms of *T. discophora.* The name *Cylindrocarpon* sensu stricto is regarded as the asexual state name for *Neonectria* sensu stricto (Chaverri et al. 2011, Rossman et al. 2013).

Because *Thelonectria discophora* was determined to be a species complex (Salgado-Salazar et al. 2013), the main goal of this paper is to provide a phylogenetic overview of the species in this complex. In addition, each species is defined using

molecular and informative morphological characters with descriptions, illustrations and a key to identify the species. Many recently collected and herbarium specimens with their anamorphic states were studied, and analyses were conducted using sequences from six nuclear loci. These analyses combined with morphological observations allowed us to assess the genetic and phenotypic diversity of the group and assign species limits.

Materials and methods

Fungal isolates

A total of 77 isolates from different localities and hosts were included in this study (TABLE 3.1). From those, 56 correspond to *Thelonectria* cf. *discophora*, five to "*Neonectria" discophora* var. *rubi*, two to "*Cylindrocarpon" ianthothele* var. *majus*, and one to *C. ianthothele. "Cylindrocarpon" ianthothele* var. *majus* and *C. ianthothele* are included as they belong to the *T. discophora* species complex (Salgado-Salazar et al. 2013). Eight isolates representing *T. lucida* (Höhn.) P. Chaverri & Salgado 2011, two representing *T. trachosa* (Samuels & Brayford) P. Chaverri, C. Salgado & Samuels 2011, and four representing *T. westlandica* (Dingley) P. Chaverri & C. Salgado 2011 were used as outgroups in the phylogenetic analyses. Specimens and cultures were obtained from CABI Bioscience (IMI); Centraalbureau voor Schimmelcultures (CBS); Japanese Ministry of Agriculture, Fisheries and Food Collection (MAFF); New York Botanical Garden (NY); and U.S. National Fungus Collection (BPI, G.J.S, A.R., now deposited in CBS).

DNA extraction, PCR, sequencing and alignments

Strains listed in TABLE 3.1 were grown in Petri dishes (6 cm diam.) containing Difco™ potato-dextrose broth. Plates were incubated at 25 ºC for *ca*. 1 wk. DNA was extracted from the mycelial mat harvested from the surface of the broth using the PowerPlant™ DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, California, USA). Six nuclear loci were sequenced for this study: partial large nuclear ribosomal subunit (LSU, *ca*. 900 bp), complete internal transcribed spacers 1 and 2 (ITS, including 5.8S of the nuclear ribosomal DNA, *ca*. 600 bp), partial β-tubulin (*tub*, *ca*. 500 bp), α-actin (*act*, *ca*. 600 bp), RNA polymerase II subunit 1 (*rpb1*, *ca*. 700 bp), and translation elongation factor 1α (*tef1*, *ca*. 700 bp) (TABLE 3.2). These nuclear loci are commonly used for phylogenetic studies of nectriaceous fungi proving useful for species level studies (Chaverri et al. 2011, Hirooka et al. 2012, Salgado-Salazar et al. 2012). Protocols for PCR were carried out as described by Chaverri et al. (2011). Clean PCR products were sequenced in both directions at the University of Maryland DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland, USA). Sequences were assembled and edited using the program Sequencher 4.9 (Gene Codes, Madison, Wisconsin, USA). Alignments were performed using PRANK (Loytynoja and Goldman 2005) implemented by The GUIDANCE Server (http://guidance.tau.ac.il/index.html, Penn *et al.* 2010) using default settings.

Concatenated phylogenetic analyses

Posterior distributions of trees were reconstructed from the combined nuclear genes using Bayesian Inference analysis (BI) in MrBayes v. 3.1 (Huelsenbeck et al. 2001

Ronquist and Huelsenbeck 2003). Previous studies by Salgado-Salazar et al. (in press) using the program CONCATERPILLAR v1.4 (Leigh et al. 2008) detected phylogenetic incongruence between the ITS loci and the rest of the data sets (*act,* LSU, *tef1*, *tub*, *rpb1*) (See FIG. S2.4 and S2.5). However it was detected that this incongruence did not affect the recovery of the true species relationships, as it has no effect in the species tree reconstruction using different approaches (Salgado-Salazar et al. 2013). Consequently, a combined data set was directly created to obtain the total evidence phylogeny from which to infer the relationship between cryptic species. JModeltest v 0.1.1 (Posada 2008) was used to determine the best nucleotide substitution model using AIC criteria (TABLE 3.2). For the concatenated analyses, we used a partitioned approach with model parameters estimated previously. The analyses were initiated from random starting trees, run for 15 million generations with four chains (Metropolis-coupled Markov Chain Monte Carlo, Huelsenbeck and Rannala 2004) and sampled at intervals of 1000 generations. Default priors were used in all analyses. Two independent BI analyses were run. To evaluate stationarity and convergence between runs, log-likelihood scores were plotted using TRACER v. 1.5 (Rambaut and Drummond 2007). In addition, we examined the distribution of split frequencies using the online program AWTY (Are We There Yet, Nylander et al. 2008) in order to assess whether an MCMC analysis has run long enough such that tree topologies were sampled in proportion to their true posterior probability distribution, independent of the apparent stationarity detected in TRACER. Trees generated prior to stationarity were discarded and the rest of the trees were summarized in a majority-rule consensus tree from the four independent runs

(Huelsenbeck et al. 2001). Bayesian posterior probabilities (PP) were assessed at all nodes, and clades with PP ≥ 0.95 were considered well supported (Huelsenbeck and Rannala 2004). Maximum likelihood (ML) analyses were performed in RAxML (Stamatakis 2006), using the RAxML GUI vs. 1.1.1 (Silvestro and Michalak 2011). Branch support was assessed with 1000 nonparametric bootstrapping replicates using the same model parameters settings than BI analyses. Final trees were visualized with FigTree v1.3.1 (Rambaut 2005).

In order to better visualize differences among clades, we calculated nucleotide divergence (Dxy, pairwise average number of nucleotide substitutions per site between groups; Nei 1987) using the program DnaSP v.5 (Librado and Rozas 2009). For these calculations, groups to be compared were defined based on clade assignment of each individual in the concatenated ML and Bayesian phylogeny. The randomization test to assess the significance of Dxy values between groups of clades was calculated using 1000 permutations in DnaSP v.5 (Hudson et al. 1992, Librado and Rozas 2009). Singletons or orphan isolates were not included in these calculations since these methods compare clades of multiple isolates.

Morphological studies and statistical analyses of morphological characters

The morphology of species in the *T. discophora* complex, including specimens and associated cultures, measurements of continuous characters for both anamorph and teleomorph structures (length, width) were done as described in materials and methods section of Chapter 1 (Pag. 10).

Results

Phylogenetic analyses

The nucleotide sequences generated in this study were deposited in GenBank (TABLE 3.1). The concatenated data set including the six loci contained 4306 characters of which 3317 were constant, 173 parsimony-uninformative, and 816 parsimony informative (TABLE 3.2). The data from the concatenated data set have been deposited under doi:10.5061/dryad.q3s66 at the DRYAD data repository (http://datadryad.org/).

Based on the concatenated phylogenetic analyses, a total of sixteen cryptic species in the *Thelonectria discophora* species complex were recovered, having significant ML bootstrap (>70%) and BI posterior probability (>0.95) support (FIG. 3.1). The ML best tree topology shown in FIGURE 1 was the same as the majority rule consensus tree from MrBayes analysis; consequently only ML best tree topology is shown in FIG. 3.1. The majority of the internal nodes in the phylogeny are resolved and well supported, and the topology of the tree obtained using the combined data set corresponds to the species tree inferred in previous studies (Salgado-Salazar et al. 2013). The 16 cryptic species group into three large clusters: the first containing clades I to VI, the second containing clades VII to X, the third containing the clades XIII to XV. Clades XI and XII appear basal to clades I to X and are surrounded by singletons (single-isolate lineages) (FIG. 3.1). Isolates of "*Cylindrocarpon" ianthothele* var. *ianthothele (=Neonectria discophora* var. *rubi)*, known to be pathogenic to *Rubus* spp. (clade XVI), were recovered as the most basal clade of the group being distantly related to the rest of *T. discophora*-like species and even to the outgroup species *T. lucida*. Since the type specimen of *T. discophora* was originally

described from Chile, clade XIV (FIG. 3.1) is here recognized as the type clade thus as true *T. discophora*. Three other cryptic species and three singletons are closely related to the type clade (XV), which include isolates from China and Japan (clade XVI), and New Zealand, Scotland and Switzerland (clade XIII).

In total, nine isolates were found to be singletons. These singletons or orphan isolates either do not cluster with the closest related putative species having significant branch support, *i.e.,* the branch support decreases if they are included, or they are separated from them by a long branch (FIG. 3.1). Single gene tree analyses recovered the 16 putative species and singletons as observed in the concatenated analyses (see Salgado-Salazar et al. 2013). In the single gene phylogenies, the Bayesian and ML analyses recovered the same clades. However, the species positions in the trees and those of some singleton isolates differ from the positions seen in the combined analyses (data not shown). Although some of the clades were not significantly supported, they were also not contradicting the general clustering, consequently fitting the criteria for species delimitation using genealogical concordance (Dettman et al. 2003).

Zeng and Zhuang (2013) reported two new species with affinities to the *T. discophora* species complex, *T. beijingensis* Z.Q. Zeng, J. Luo & W.Y. Zhuang 2013 and *T. yunnanica* Z.Q. Zeng & W.Y. Zhuang 2013*,* based on a phylogenetic analysis of ITS and partial b-tubulin regions. To investigate the relationship of these species to *T. discophora* species complex, we constructed a two-loci (ITS+*tub*) dataset of the isolates used in this study and those by Zeng and Zhuang (2013) and analyzed it using ML analysis following the settings included in Materials and Methods. As depicted

by FIG. S3.1, *T. beijingensis* cluster with isolate MAFF241569, here identified as a singleton lineage, and *T. yunnanica* cluster with isolates in the *T. purpurescens* species.

Genetic distances, as measured by Dxy values obtained between all pairs of species, ranged from 0.002 to 0.064 (TABLE 3.3). The highest average genetic distances were observed between clusters 1 (I–XII) and 2 (XIII–XV) with values ranging from 0.031 to 0.064. The species containing the pathogenic isolates (clade XVI) is the most distantly related to clusters 1 and 2, with genetic distances ranging from 0.039 to 0.048 (TABLE 3.3). The species *T. brayfordii* and *T. pinea* showed the lowest genetic divergence in the group of species (0.002). The degree of genetic distance between pairs of species was not related to geographic distance. For example, *T. brayfordii* and *T. mammoidea* contain isolates from New Zealand, with no geographic restrictions however genetically divergent (TABLE 3.3).

Ecology and geographic distribution of species

Even though geographical segregation at various levels was observed, it is not a strong character for defining species in this complex (TABLE 3.1, FIG. 3.1). From the combined phylogenetic analyses we could observe species formed by isolates from the same geographic region (*T. brayfordii, T. japonica, T. pinea, T. porphyria, T. tyrus*); isolates from close-by regions (*T. blattea, T. conchyliata, T. phoenicea, T. purpurea*); and isolates from distant regions (*T. ianthina, T. mammoidea, T. purpurescens, T. violaria,* among others) (TABLE 3.1, FIG. 3.1). Interestingly, a correlation with ecology was observed for all species. With the exception of *T. blattea*, the isolates of most species were collected in their sexual state, *i.e.,* as

fruiting bodies (perithecia) on decaying plant material. Isolates in *T. blattea* were collected as saprobes in soil in their asexual state. One isolate of *T. mammoidea* (CBS 32881) was also collected in its asexual state (TABLE 3.1; Brayford 1991). *Thelonectria rubi* a plant pathogen on several species of *Rubus*, was also collected in its sexual state. None of the remaining species has been found on *Rubus* and causing disease. Isolates in the outgroup and sister species *T. lucida, T. trachosa* and *T. westlandica* were collected as sexual fruiting bodies on decaying plant material. Based on our observations, no host specificity was shown by the putative species, except for *T. pinea*, which here is redefined to include *T. discophora*-like isolates collected on *Pinus radiata* in New Zealand. Since one more isolate belonging to *T. mammoidea* was collected on *Pinus radiata* (ICMP 5287), their segregation can be based on growth rate at 25 C, morphology and genetic divergence estimates. The lack of information about the host on which some of the species were collected makes it almost impossible to reach a definite conclusion about host specificity or preference (TABLE 3.1).

Morphological analyses: Teleomorph

We could not detect significant differences in the morphology of the teleomorph. The variation in perithecial morphology, such as size, formation of flattened ostiolar disk or appearance of the external layer of cells in the wall of perithecia, and the size of asci and ascospores do not present discontinuities. A high intraspecific variation of these characters could be seen in each one of the species, including those by Zeng and Zhuang (2013). Ascospore size ranges from 10–15 × 4–6 μm in all species. *Thelonectria phoenicea* has on average the smallest ascospores (10.6 × 4.7 μm), and

T. mammoidea and *T. pinea* have on average the largest ascospores (16.5 × 7.4 μm and 18 × 7.5 μm, respectively).

Morphological analyses: Anamorph

As the published descriptions of *T. discophora* have stated (Booth 1966, Brayford et al. 2004, Chaverri et al. 2011, Guu et al. 2007, Samuels et al. 1990), the anamorph produces characteristic purple to cinnamon-colored colonies and pigments in the media, with the intensity of the color varying from species to species and dependent on growth temperature (See Taxonomy section). Higher quantities of pigments are produced when colonies are grown at low temperatures (15 C) and none of the species, except *T. tyrus*, produced pigment at high temperatures such as 30 C. Zeng and Zhaung (2013) did not report pigment diffusing in media by *T. beijingensis* and *T. yunnanica*. In all species in the *T. discophora* complex optimum temperatures for colony growth range between 20 and 25 C; however, reduced colony growth can be observed at 15 C. Only six species (*T. conchyliata, T. ianthina, T. phoenicea, T. porphyria, T. purpurescens, T. tyrus*) showed the ability to grown at 30 C (Online Resource 3). There are no significant differences among the species growing at 15 C, 20 C and 30 C; however, significant differences at 25 C among *T. asiatica, T. discophora, T. mammoidea* and *T. rubi* and the rest of the species could be observed (FIG. S3.2). These species mentioned above have low growth rates at 25 C when compared with the rest of the species in the complex.

The morphological characters of the anamorph are more informative than those of the teleomorph. As opposed to the previously published description of members in the genus *Thelonectria* (Booth 1966, Brayford et al. 2004, Chaverri et al.

2011, Samuels et al. 1990), we observed that, although rare, some species produce microconidia and chlamydospores in culture. Two species (*T. asiatica* and *T. rubi*) in the *T. discophora* complex produce microconidia and *T. blattea* produces chlamydospores in culture. None of the remaining species in the complex produce microconidia or chlamydospores in culture.

Macroconidia in the *T. discophora* complex present the typical 'cylindrocarpon' shape, with long conidia that can be cylindrical and straight or curved with round ends. The septation pattern, which varies from 1–6-septate, is a characteristic of groups of species. Thus, macroconidia of *Thelonectria asiatica* and *T. violaria* are 1–3 septate; *T. pinea* 1–4; *T. tyrus* 2–5 septate; *T. brayfordii*, *T. mammoidea, T. phoenicea* and *T. purpurea* 1–5 septate;, *T. conchyliata*, *T. discophora, T. ianthina* and *T. porphyria* 3–5 septate; and *T. blattea*, *T. japonica* and *T. purpurescens* 3–6 septate. The differences in septation among species are not correlated to biogeography or genetic divergence. Among these species, the ability to produce a certain number of septa has been gained and lost multiple times over the course of their evolutionary history (FIG. 3.1). *T. asiatica* and *T. violaria* are the only species to produce 1–3 septate macroconidia; however, *T. asiatica* produces microconidia in culture. Among the species that produce 1-septate macroconidia, *T. phoenicea* and *T. rubi* showed length sizes significantly smaller than the other species with this character. *Thelonectria rubi* has the smallest 1-septate macroconidia, significantly different from *T. phoenicea*. Among the species that produce them, *T. japonica* and *T. purpurescens* have significantly longer macroconidia 6-septate compared to *T. asiatica* and *T. blattea*. In species having 2–5 septate macroconidia

the size is overlapping with values that are not significantly different, even though average values are slightly different. There are no differences in width of micro- and macroconidia whose values range from 4–7 μm, as well as in length and width of phialides.

Thelonectria beijingensis and *T. yunnanica* have clear genetic affinities with species in the *T. discophora* complex; however, discrepancies were observed in the morphology presented by Zeng and Zhuang (2013) and the one provided here. In the phylogenetic analyses, *T. beijingensis* clusters with *T. purpurescens*; however, the description of the morphological characters of *T. beijingensis* does not agree with that of *T. purpurescens. Thelonectria beijingensis* produces microconidia and macroconidia 1–3 septate in culture. On the other hand, *T. purpurescens* described here does not produce microconidia and macroconidia are 3–6 septate. *Thelonectria yunnanica* is also reported to produce microconidia in culture; however, the isolate MAFF241569 from Japan does not produce them in culture.

Taxonomy

We use a combination of phylogenetic analyses, DNA sequence divergence tests and morphological observations to define species in the *Thelonectria discophora* complex. Sixteen lineages that correspond to species are described here as new. For the newly described species only a brief description is provided, mainly including those characters that are different or diagnostic from the narrowly defined species of *T. discophora*. Based on the modification of the code of nomenclature (Hawksworth 2011), the generic name "*Cylindrocarpon"*, which was previously applied to the anamorph of *T. discophora*, should not be used (Rossman et al. 2013).

Cylindrocarpon names related to *T. discophora* are here regarded as synonyms of the *T. discophora* species described below. For a detailed description of the perithecial anatomy of *T. discophora* see Chaverri et al. (2011) and Samuels et al. (1990).

Thelonectria discophora (Mont.) P. Chaverri & C. Salgado, Stud. Mycol. 68: 77. 2011.

FIG. 3.2, A–H.

Mycobank MB518569.

Basionym: *Sphaeria discophora* Mont., Ann. Sci. Nat. Bot. II 3: 353. 1835.

≡ *Neonectria discophora* (Mont.) Mantiri & Samuels var. *discophora*, Canad. J. Bot. 79: 339. 2001.

= *Nectria tasmanica* Berk. in Hooker, Flora Tasmaniae 2: 279. 1860.

= *Nectria umbilicata* Henn., Hedwigia 41: 3. 1902.

= *Creonectria discostiolata* Chardón, Bol. Soc. Venez. Ci. Nat. 5: 341. 1939.

Holotype of *Sphaeria discophora:* CHILE. Juan Fernandez, sur cortice arborum, date unknown, Bertero 1700 (PC!; ISOTYPE, PC!).

Mycelium not visible on host. Perithecia globose to subglobose, (250–)300–550(–650) μm high, (100–)240–300(–400) μm wide, surface smooth and shiny or slightly roughened, solitary or gregarious in groups of 20 or less, superficial or with the base immersed in substratum on a minute stroma, not collapsed when dry, red to rust with the ostiolar area often darker (chestnut), red to rose in 3% KOH, yellow in lactic acid, nonpapillate or with a broad mammiform apex 150–200 μm wide. Cells at surface of perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina irregular in shape 2–2.5 μm, 2–4 μm thick. Perithecial wall 30–50 μm wide of

two intergrading regions: outer region 20–30 μm wide, continuous over perithecium to form a uniform palisade of hyphal cells perpendicular to surface of perithecium, lumina <1 μm wide and tips rounded; inner region of perithecial wall 10–20 μm wide, cells lacking a definite outline but with long axes parallel to surface of perithecial wall, cells increasingly more compacted, thin-walled towards perithecial locule; perithecial apex of vertically elongated cells, continuous with lateral perithecial wall forming a disk around perithecial opening. Asci cylindrical to clavate, (66–)72–95(–119) × 7–10(–15) μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (10.4–)11.5–15.6(–16.7) × (4.5–)4.9–6.3(–7.0) μm (mean 13.6 × 5.6 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline becoming yellowish. Colonies on PDA 17–28 mm diam (mean 23 mm) after 12 d at 20 C, aerial mycelium floccose, mauve to pale vinaceous, producing purple pigment into media at 15–20 C, no pigment produced at > 20 C, colony reverse mauve to dark vinaceous. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.2–)16.0–20.6(–23.4) × (–2.5)3.5–4.4(–5.1) μm (mean 18 × 4), with periclinal thickening and collarette. Macroconidia slightly fusiform, curved with round ends, 3–5(–6)-septate: 3-septate (40.5–)46.9–58.9(–71.4) × (4.4–)4.9–5.8(–7) μm (mean 53.3 × 5.4 μm), 4-septate (44.6–)55–67.5(–74.4) × (4.4–)5–6(–6.5) μm (mean 61.2 × 5.5 μm), 5-septate (60–)65.4–77(–82.5) × (4.4–)5.2–6.3(–6.6) μm (mean 71.2 × 5.8 μm). Microconidia and chlamydospores not produced on SNA.

Habitat and distribution. Saprobic on decaying bark of shrubs and trees. Known from Chile (type locality) and Scotland; possibly distributed in temperate regions of Europe and America.

Additional specimens examined: CHILE. LLANQUIHUE PROVINCE: Los Lagos Region, Vicente Perez Rosales National Park, on wood of a recently killed *Tepualia stipularis* tree, April 2011, Andrés de Errasti (BPI 892687, culture A.R. 4742 = CBS 134034). SCOTLAND. COWAL PENINSULA: Argyll Forest Park, ca. 10 km north of Dunoon, Yourger Botanic Garden 50–100 m, on *Aesculus* sp. dead branchlets, 11-13 April 1992, G.J. Samuels, D. Brayford (BPI 802901, culture G.J.S. 92-48 = CBS 134031).

Notes. As a result of the phylogenetic analysis, the following names are no longer considered as synonyms of *T. discophora* because of their distinctive genetic divergence: *Nectria mammoidea* (≡ *Creonectria mammoidea*), *Nectria nelumbicola*, *Nectria mammoidea* var. *rugulosa*, *Nectria mammoidea* var. *minor*, and *Nectria pinea*.

Thelonectria asiatica C. Salgado & Hirooka, sp. nov.

FIG. 3.2, G–M.

Mycobank TBD.

Similar to *T. discophora*, microconidia present, macroconidia 1–3 septate.

Holotype. JAPAN. NAGANO PREFECTURE: Sugadaira, Ueda City, on twigs, 02 Sept 2006, Y. Hirooka (TPP-h548, BPI 881963, ex-type culture MAFF 241576).

Etymology. Refers to the geographic range where this species is found.

Mycelium not visible on host. Perithecia globose to subglobose, 300–600 μm high, 200–300 μm wide, surface smooth and shiny or slightly roughened, solitary or

gregarious in groups of 20 or less, superficial or with the base immersed in substratum on a minute stroma, not collapsed when dry, peach to orange with ostiolar area often darker (rust), red to rose in 3% KOH, yellow in lactic acid, nonpapillate or with a small mammiform apex 50–100 μm wide. Cells at surface of perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina, irregular in shape 2–2.5 μm, 2–4 μm thick. Perithecial wall 30–50 μm wide. Asci cylindrical, (68–)75–98(–119) × 7–10 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (13–)14–15.4(–15.8) × (4.6–)5.2–5.9(–6.2) μm (mean 14.7 × 5.5 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline. Colonies on PDA 16–17 mm diam after 12 d at 20 C, aerial mycelium floccose, pale vinaceous to lilac, producing purple to cinnamon pigment into media at temperatures ≤ 25 C, no pigment produced at > 25C, colony reverse sienna to livid purple. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.3–)18.3–26.4(–30.7) × (–2.6)3.1–4(–4.5) μm (mean 22.3 × 3.5), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 1–3- septate: 1-septate (20.1–)28.5–40.3(–49.8) × (3.7–)4.2–5.4(–6.6) μm (mean 34.4 × 4.8 μm), 2-septate (32.1–)37.5–48.3(–53.9) × (3.6–)4.4–5.5(–6.1) μm (mean 42.7 × 5 μm), 3-septate (34.1–)42.7–51.8(–57.4) × (3.9–)4.6–5.9(–6.8) μm (mean 47.2 × 5.2 μm). Microconidia produced in culture, cylindrical with round ends, (6.4–)7.2–8.8(–9.6) μm (mean 8 × 4.1 μm). No chlamydospores formed in culture.

Habitat and distribution. Saprobic on decaying bark of shrubs and trees. Known from Japan (type locality) and China. Possibly distributed throughout Asia.

Additional specimens examined. CHINA. YUNNAN PROVINCE: Lijiang region, on bark submerged in stream, 4 Nov 1988, R.P. Korf (only culture examined G.J.S. 88-84 = IMI 348190).

Notes. This species is sister to the type clade. It produces microconidia in culture and 1–3-septate macroconidia, both of which are lacking in true *T. discophora*. Two additional newly described species from Taiwan, *T. beijingensis* and *T. yunnanica,* are reported to be related to *T. discophora* and produce microconidia in culture (Zeng and Zhuang 2013). As explained in the results and discussion section, there is disagreement about the species reported in this study and those by Zeng and Zhuang (2013).

Thelonectria blattea C. Salgado & P. Chaverri, sp. nov.

FIG. 3.3, A–F.

Mycobank TBD

Only known from the asexual state. Anamorph similar to *T. discophora*. Occurs in soil.

Holotype. GERMANY. KIEL-KITZEBERG: in wheat field soil, Dec 1968, W. Gams (ex-type culture CBS 95268).

Etymology. Refers to the purple color the colony that this fungus produces.

Colonies on PDA 32–35 mm diam (mean 33 mm) after 12 d at 20 C, aerial mycelium floccose, white to livid vinaceous, no pigment produced into media, colony reverse also white to livid vinaceous. Conidia on SNA forming in hyaline, slimy droplets in

aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.2–)16–20.6(–23.4) × (–2.5)3.5–4.4(–5.1) μm (mean 18 × 4 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 3–5(–6)-septate: 3-septate (40.5–)46.9–58.9(–71.4) × (5.1–)6.2–7(–7.9) μm (mean 54.4 × 6.4 μm), 4-septate (42–)55–67.5(–74.2) × (6.2–)6.4–6.8(–7) μm (mean 60.3 × 6.6 μm), 5-septate (45.3–)60–70(–84) × (6–)6.4–6.8(–7) μm (mean 63 × 6.6 μm), 6-septate (55–)58.5–63.5(–76) × (3.5–)53.8–4.2(–5) μm (mean 60 × 6.4 μm). Microconidia not produced in culture. Chlamydospores formed in culture (mean 8.7 × 7.4 μm),

Habitat and distribution. Isolated from soil in agricultural settings. Known from Germany and The Netherlands; possibly distributed across Europe.

Additional culture/specimens examined. THE NETHERLANDS. WAGENINGEN: on roots in clay soil, Jan 1977, J.W. Veenbaas-Rijks (culture CBS 14277).

Thelonectria brayfordii C. Salgado & Samuels, sp. nov.

FIG. 3.3, G–L.

Mycobank TBD

Similar to *T. discophora*. Macroconidia 1–5-septate, found only in New Zealand on hosts other than *Pinus radiata*.

Holotype. NEW ZEALAND. AUCKLAND: on *Quercus robur*, March 2005, C.F. Hill (ex-type culture CBS 118612).

Etymology. In honor of David Brayford, a British mycologist who contributed greatly to the taxonomy of hypocrealean fungi.

Colonies on PDA 31–34 mm diam (mean 32 mm) after 12 d at 20 C, aerial mycelium floccose, white to mauve, purple to cinnamon pigment diffusing into media at temperatures ≤ 25 C, no pigment produced at > 25 C, colony reverse rosy vinaceous to rust. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.6–)14.5–22.7(–29.5) × (–3.5)3.7–4.6(–4.9) μm (mean 18.5 × 4.2 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 1–5-septate: 1-septate (22.8–)27.9–38.4(–44.1) × (3–)3.3–4.4(–4.9) μm (mean 33.1 × 3.8 μm), 2-septate (30.2–)36.7–56.6(–58.2) × (4–)4.6–6.1(–6.3) μm (mean 51.5 × 5.3 μm), 3-septate (65.7–)50.5–59.4(–65.7) × (4.7–)5.2–6.1(–6.8) μm (mean 54.9 × 5.6 μm), 4-septate (49.3–)56.3–65.8(–72.5) × (4.7–)5.3–6.3(–6.8) μm (mean 61 × 5.8 μm), 5-septate (55.3–)63.3–71.5(–77.7) × (5.1–)5.7–6.7(–7.5) μm (mean 67.4 × 6.2 μm). Chlamydospores and microconidia not produced in culture.

Habitat and distribution. On decaying bark of *Quercus robur* and roots of various plants. Only known from New Zealand.

Additional specimens examined. NEW ZEALAND. BAY OF PLENTY: Tauranga locality, on rotting root of unknown proteaceus plant, April 1 2000, C.F. Hill (culture only ICMP 14105). Ibid. on root of unknown dead plant, Apr 2000, H.M. Dance, LYN10 (culture only IMI 384045).

Notes. The description of this species is based only on characters obtained from the anamorph. Two species in the *T. discophora* complex occur in New Zealand, *T.*

brayfordii and *T. pinea; T. pinea* occurs only on *P. radiata* and has macroconidia 1–4-septate.

Thelonectria conchyliata C. Salgado & P. Chaverri, sp. nov.

FIG. 3.4, A–G.

Mycobank TBD.

Similar to *T. discophora*. Macroconidia 3–5 septate, average colony diameter on SNA at 30 C > 13 mm after 12 days.

Holotype. GUYANA. CUYUNI-MARAZUNI: Mazaruni Subregion, VII-2, along Koatse river, ca. 2 km east of Pong River, ca. hr walk west of Chinoweing, 05°28'N 60°04'W, 600-650 m, Feb-March 1987, on wood, G.J. Samuels, J. Pipoly, G. Gharbarran, J. Chin, R. Edwards (BPI 747133, ex-type culture G.J.S. 87-45 = IMI325855).

Etymology. Refers to the purple color of the colony and pigment that this species produces in culture.

Mycelium not visible on host of some specimens. Perithecia globose to subglobose, 400–500 μm high, 340–400 μm wide, surface smooth, shiny or slightly roughened, solitary or gregarious in groups of 20 or less, superficial or with base immersed in substratum on a minute stroma, not collapsed when dry, peach to bay with ostiolar area often darker (rust), red to rose in 3% KOH, yellow in lactic acid, nonpapillate or with a broad mammiform apex 150–200 μm wide. Perithecial wall 30–50 μm wide of two intergrading regions: outer region 20–30 μm wide. Asci cylindrical, (75–)85–100(–120) × 8–10 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (8.4–)9.4–15.7(–20) × (3.1–)4.3–6.5(–8.2) μm (mean 12.5 × 5.4 μm),

symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline becoming yellowish in mature ascospores. Colonies on PDA 27–44 mm diam (mean 33 mm) after 12 d at 20 C, aerial mycelium floccose, white to lilac, producing purple to cinnamon pigment to media in some isolates at temperatures < 30 C, colony reverse mauve to bay. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (9.5–)12.5–17.6(–23.2) × (–2.6)3.6–4.7(–5.8) μm (mean 15 × 4.2 μm), with periclinal thickening and collarette. Macroconidia slightly fusiform, curved with round ends, 3–5-septate: 3-septate (39.1–)45.4–55.3(–63.8) × (4.5–)5.8–6.9(–7.8) μm (mean 50.3 × 6.4 μm), 4-septate (47.7–)51.7–60.5(–66.2) × (5.4–)5.9–7(–8.1) μm (mean 56.1 × 6.5 μm), 5-septate (52.3–)57.2–66.4(–74.1) × (5.2–)6.1–7.2(–8.7) μm (mean 61.8 × 6.7 μm). Microconidia and chlamydospores not produced on SNA.

Habitat and distribution. Saprobic on decaying bark of *Ocotea* sp., palms and possibly other hardwood species. Distributed in tropical South America and Central America.

Additional specimens examined. GUYANA. CUYUNI-MARAZUNI: Mazaruni Subregion, VII-2, along Koatse river, ca. 2 km east of Pong River, ca hr walk west of Chinoweing, 05°28'N 60°04'W, 600-650 m, 28 Feb 1987, on branchlets of recently dead tree, G.J. Samuels, J. Pipoly, G. Gharbarran, J. Chin, R. Edwards (BPI 744725, culture G.J.S. 87-49 = CBS 112461); POTARO-SIPARUNI REGION: base of Mt. Wokomung, ca 5.5 hr walk NE of Kopinang Village in legume-dominated forest,

05°05’N 59°49’W, 27 Jun 1989, on bark of recently fallen tree, G.J. Samuels 6269A, B.M. Boom, G. Bacchus (NY, culture G.J.S. 89-57 = CBS 112459); 720 m, G.J. Samuels 6281, B.M. Boom, G. Bacchus (NY, culture G.J.S. 89-60); Mt. Wokomung, Wokomung Base Camp, ca. 8 hr walk NE of Kopinang Village in wet forest dominated by Euphorbiaceae, 05°05’N 59°50’W, 1070 m, Jun-Jul 1989, G.J. Samuels 6318, B.M. Boom, G. Bacchus (NY, culture G.J.S. 89-65 = CBS 123970). PUERTO RICO. 350-400 m, on *Ocotea* sp. twigs, 20 Feb 1996, G.J. Samuels, H.J. Schroers, D.J. Lodge (BPI 744683, culture G.J.S. 96-22 = IMI 370946). VENEZUELA. SUCRE STATE: NW of Irapa, trail between Los Pocitos and peak of Cerro Humo, on stem of unidentified palm, 12 Jul 1972, K.P. Dumont VE 4769, R.F. Cain, G.J. Samuels, G. Morillo, J. Farian (NY, culture C.T.R. 72-90); ARAGUA STATE: Henri Pittier National Park, Rancho Grande Biological Station, trail to Guacamayo, 1250-1400m, 10°21’N, 67°41’W, on bark of unidentified tree, 04 Dec 1990, G.J. Samuels, B. Hein, S.M. Huhndorf (BPI 842123, culture G.J.S. 90-212 = CBS 134028).

Notes. This species is similar to *T. ianthina*, which also produces 3–5 septate macroconidia. On average, *T. conchyliata* has a faster growth rate on PDA at 30 C than *T. ianthina*.

Thelonectria ianthina C. Salgado & J.-R Guu, sp. nov.

FIG. 3.4, H–N.

Mycobank TBD

Similar to *T. discophora*. Macroconidia 3–5-septate, average colony growth on SNA at 30 C < 13 mm after 12 days.

Holotype. COSTA RICA. HEREDIA PROVINCE: Braulio Carrillo National Park, Zurquí

Street entrance, 10°02'N 84°01'W, 1562 m, on bark, 13 March 2010, C. Salgado, C. Herrera, Y. Hirooka, A. Rossman, G.J. Samuels, P. Chaverri PC1001 (BPI 892691, ex-type culture G.J.S. 10–118 = CBS 134023).

Etymology. Refers to the purple color of the colony and pigment produced by this species.

Mycelium not visible on host. Perithecia globose to subglobose, 300–600 μm high, 200–350 μm wide, surface smooth and shiny or slightly roughened, solitary or gregarious in groups of 15 or less, superficial or with base immersed in substratum on a minute stroma, not collapsed when dry, red to bay with ostiolar area often darker (bay to umber), red to rose in 3% KOH, yellow in lactic acid, papillate or with a small mammiform apex 50–100 μm wide. Cells at surface of perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina irregular in shape 2–2.5 μm, 2–4 μm thick. Perithecial wall 35–50 μm wide. Asci cylindrical or slightly clavate, (60–)70–90(–111) × 7–10 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (11.7–)12.2–13.99(–15) × (5.3–)5.6–6.1(–6.4) μm (mean 13.1 × 5.8 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline. Colonies on PDA 31–35 mm diam (mean 33 mm) after 12 d at 20 C, aerial mycelium floccose, white to purple, producing purple pigment in media at temperatures ≤ 25 C, colony reverse white to bay. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA agar. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (12.2–)13.7–20.4(–25.9) × (–2.7)3.2–4.2(–4.9)

μm (mean 17 × 3.7 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 3–5-septate: 3-septate (37.9–)45.9–57.1(–64.7) × (4.7–)5.4–7(–8.7) μm (mean 51.5 × 6.2 μm), 4-septate (49.6–)55.1–63(–68.2) × (5.8–)6.2–7.5(–8.3) μm (mean 59.1 × 6.8 μm), 5-septate (52–)59.4–62.3(–69.7) × (3.5–)6.2–8(–8.7) μm (mean 60.9 × 7.1 μm). No microconidia or chlamydospores produced in culture.

Habitat and distribution. Saprobic on decaying bark of trees and shrubs. Known from Costa Rica (type locality) and Taiwan.

Additional specimens examined. TAIWAN. TAIPEI COUNTY: Jingtung, Jungtung historical trail, on bark, 21 Dec 2003, J.-R. Guu 92122107 (BPI 892688, culture Guu 92122107 = CBS 134038).

Notes. This species is similar to *T. conchyliata*, which also produces macroconidia 3–5-septate. They can be distinguished by the slower average growth rate on PDA at 30 C of *T. ianthina*.

Thelonectria japonica C. Salgado & Hirooka, sp. nov.

FIG. 3.5, A–F.

Mycobank TBD.

Similar to *T. discophora*. Macroconidia 3–6-septate. Only known from Japan.

Holotype. JAPAN. OKUTAMA-GUN: on twigs of undetermined plant, 20 Nov 2003, Y. Hirooka TPP-h-229-2 (BPI 882092, ex-type culture MAFF 241524).

Etymology. Refers to the geographic location where this species was found.

Mycelium not visible on host. Perithecia globose to subglobose, 300–610 μm high, 200–350 μm wide, surface smooth and shiny or slightly roughened, solitary or

gregarious in groups of 15 or less, superficial or with base immersed in substratum on a minute stroma, not collapsed when dry, peach to sienna with ostiolar area often darker (rust), red to rose in 3% KOH, yellow in lactic acid, papillated. Cells at surface of perithecial wall lacking a definite outline, appearing to be intertwined hyphae with lumina irregular in shape, 2–2.5 μm, 2–4 μm thick. Perithecial wall 25–40 μm wide. Asci cylindrical, (50–)68–90(–100) × 7–11 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (10.6–)11.6–13.7(–15.4) × (4.7–)5.2–6.1(–6.7) μm (mean 12.7 × 5.6 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline. Colonies on PDA 33–44 mm diam (mean 38 mm) after 12 d at 20 C, aerial mycelium floccose, white to purple, no pigment produced in media, colony reverse also white to purple. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (9.3–)11.5–18.2(–22.5) × (–3)3.5–4.8(–5.2) μm (mean 14.8 × 4.1 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 3–6-septate: 3-septate (45.2–)54–67.3(–79.8) × (4.6–)5.3–6.3(–7.2) μm (mean 60.6 × 5.8 μm), 4-septate (57–)64.3–75.6(–88.8) × (4.2–)5.4–6.7(–7.5) μm (mean 69.9 × 6.1 μm), 5-septate (64.3–)72.2–82.5(–90.7) × (5.2–)5.6–6.6(–7.4) μm (mean 77.3 × 6.1 μm), 6-septate (86.9–)86.5–90.2(–90.4) × (5.7–)5.8–6.7(–6.7) μm (mean 88.3 × 6.2 μm). No microconida or chlamydospores formed in culture.

Habitat and distribution. Saprobic on decaying bark of *Fagus crenata,* and possibly

on bark of other shrubs and trees. Known only from Japan.

Additional specimens examined. JAPAN. MIYAGI PREFECTURE: Kenminnomori, Rifu-cho, Miyagi-gun, on twigs of unknown plant, 5 Aug 2004, Y. Hirooka TPP-305-2 (BPI 882109, culture MAFF 241543). KANAGAWA PREFECTURE: Yamakitagawayose, Ashigarakami-gun, on bark of undetermined dead tree, 30 Oct 2004, Y. Hirooka TPP-h374-2 (BPI 881926, culture MAFF 241554); on bark of dead *Fagus crenata*, 17 Apr 2005, Y. Hirooka TPP-h-433-2 (BPI 881944, culture MAFF 241563).

Notes. There is one other species that can also be found in Japan, *T. porphyria*. However *T. japonica* produces macroconidia 3-6-septate, while *T. porphyria* produces macroconidia 3-5-septate.

Thelonectria mammoidea (W. Phillips & Plowr.) C. Salgado & R.M. Sanchez, comb. nov.

FIG. 3.5, G–L.

Mycobank TBD.

Basionym: *Nectria mammoidea* W. Phillips & Plowr., Grevillea 3: 126. 1875

≡ *Creonectria mammoidea* (W. Phillips & Plowr.) Seaver. Mycologia 1: 188. 1909.

≡ *Cucurbitaria mammoidea* (W. Plowr. & Plowr.) Kuntze, [as '*mammodea*'] Revis. gen. pl. (Leipzig) 3: 461. 1898.

= *Nectria mammoidea* var. *rugulosa* Weese, Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 125(7 & 8): 552. 1916.

= *Nectria nelumbicola* Henn., Verh. Bot. Vereins. Prov. Brandenburg 40: 151. 1898.

= *Cylindrocarpon ianthothele* var. *majus* Wollenw., Z. Parasitenk. (Berlin) 1: 161. 1928.

= *Cylindrocarpon ianthothele* var. *rugulosum* C. Booth, Mycol. Pap. 104: 25. 1966. *Type specimen/culture.* ENGLAND. NORFOLK COUNTY: North Wootton, on bark of unknown plant, Jan 1897, C.B. Plowright (Holotype E 00456070); Surlingham City, on *Smyrnium olusatrum* seeds, 1957, E.A. Ellis (ex-epitype culture IMI 69361). *Mycelium* sometimes visible on host. Stroma arising from cortex of host, cells angular to circular in outline, continuous with cells of outer region of perithecial wall. Perithecia globose to subglobose, 400–700 μm high, 240–340 μm wide, surface smooth and shiny or slightly roughened, solitary or gregarious in groups of 20 or less, superficial or with base immersed in substratum on a minute stroma, not collapsed when dry, peach to sienna with the ostiolar area often darker (rust), red to rose in 3% KOH, yellow in lactic acid, nonpapillate or with a broad mammiform apex 150–190 μm wide. Perithecial wall 30–50 μm wide of two intergrading regions; outer region 20–30 μm wide, continuous over perithecium to form a uniform palisade of hyphal cells perpendicular to surface of perithecium, lumina <1 μm wide and tips rounded; inner region of perithecial wall 10–25 μm wide, cells lacking a definite outline but with long axis parallel to surface of perithecial wall, cells increasingly more compacted, thin- walled towards perithecial locule; perithecial apex of vertically elongated cells, continuous with lateral perithecial wall, forming disk around perithecial opening. Asci cylindrical, (70–)80–100(–130) × 8–10 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (12.6–)15.3–17.7(–18.8) × (6–)6.7–8.0(–9.7) μm (mean 16.5 × 7.4 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline becoming yellowish. Colonies on PDA 25–27 mm diam (mean 26 mm) after

12 d at 20 C, aerial mycelium floccose, white to lilac or rosy buff, with cinnamon pigment produced in media, colony reverse white to mauve or cinnamon. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen, (13–)16.5–20.9(–23.7) × (–2.9)3.5–4.4(–5.1) μm (mean 17.5 × 4 μm), with periclinal thickening and collarette. Macroconidia slightly fusiform, curved with round ends, 1–5-septate: 1-septate (28.7–)29.5–36.9(–39.3) × (5.5–)5.6–6.2(–6.3) μm (mean 33.2 × 5.9 μm), 2-septate (35.9–)39.7–48.1(–50.5) × (5.8–)6–7(–7.5) μm (mean 43.9 × 6.5 μm), 3-septate (41.1–)45.8–57.9(–64) × (5–)5.7–7.0(–7.9) μm (mean 51.9 × 6.4 μm), 4-septate (52–)57–67.2(–73.7) × (5–)5.9–6.9(–7.6) μm (mean 62.1 × 6.4 μm), 5-septate (70.3–)69.6–74.2(–75) × (6.3–)6.3–6.4(–6.5) μm (mean 71.9 × 6.4 μm). No microconidia or chlamydospores produced on SNA.

Habitat and distribution. Saprobic on *Fuchsia excorticata*, *Pinus radiata* and possibly on diverse hardwood trees. Also on herbaceous plants (e.g., *Smyrnium olusatrum*) and decaying plant organic matter. Known from Europe and New Zealand, probably distributed throughout temperate regions.

Additional specimens examined. NEW ZEALAND. SOUTHLAND: Catlin's State Forest Park, Lake Wilkie, on bark of unidentified tree, 18 Apr 1985, G.J. Samuels, P.K. Buchanan, L.M. Kohn (PDD 50050, BPI 802469, culture G.J.S. 85-27 = CBS 112457); SOUTH ISLAND: Westland, Franz Joseph, track to Lake Wombat, on bark of *Fuchsia excorticata*, 10 Apr 1983, G.J. Samuels, R.H. Petersen (PDD 46365, BPI 1109329, culture G.J.S. 83-188 = IMI 326256); Waitomo, on bark of indetermined

tree, 26 Apr 1983, G.J. Samuels, P.R. Johnston, R.H. Petersen (PDD 46410, culture G.J.S. 83-206 = IMI 326258); on *Pinus radiata*, 01 Nov 1965, J. M. Dingley (culture ICMP 5287). SCOTLAND. COWAL PENINSULA: Argyll Forest Park, ca 5 km south of Strachur along river Cur, vic Glenbranter Village, Lauder Broadleaf Walk ca 50 m, on bark of unidentified dead hardwood tree, 12 Apr 1992, G.J. Samuels, D. Brayford (BPI 802649, culture G.J.S. 92-34 = CBS 134030). SWITZERLAND. May 1981, O. Petrini (culture CBS 32881).

Notes. The isolate ICMP 5287 was collected in New Zealand on *Pinus radiata*; however, it is genetically divergent from isolates in *T. pinea* (5.9 % Dxy) and produces macroconidia 1–5-septate. *Thelonectria pinea* produces macroconidia 1–4-septate.

Thelonectria phoenicea C. Salgado & P. Chaverri, sp. nov.

FIG. 3.6, A–F.

Mycobank TBD.

Similar to *T. discophora*. Macroconidia 1–5-septate. Found in Australia, Indonesia and Taiwan.

Holotype. INDONESIA. NORTH SULAWESI: Eastern Dumoga-Bone National Park, at confluence of Toraut and Tumpa Rivers, Project Wallace Base Camp, 0°34'N 123°57'E, 211 m, on twig of unidentified tree, Sep-Nov 1985, G.J. Samuels (NY 2222A, ex-type culture G.J.S. 85-179 = IMI 329113).

Etymology. Refers to the purple coloration of the anamorph and pigment produced in culture.

Mycelium not visible on host. Perithecia globose to subglobose, 300–600 μm high,

200–350 μm wide, surface smooth and shiny or slightly roughened, solitary or gregarious in groups of 15 or less, superficial or with base immersed in substratum on a minute stroma, not collapsed when dry, peach to sienna with ostiolar area often darker (bay), red to rose in 3% KOH, yellow in lactic acid, papillated. Cells at surface of perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina irregular in shape 2–2.5 μm, 2–4 μm thick. Perithecial wall 25–40 μm wide. Asci cylindrical, (50–)66–89(–105) × 7–11 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (9.2–)9.7–11.6(–13.2) × (3.9–)4.3–5(–5.7) μm (mean 10.6 × 4.7 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline. Colonies on PDA 29–30 mm diam after 12 d at 20 C, aerial mycelium floccose, white to purple, producing purple pigment at ≤ 25 C, colony reverse white to bay. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (10.7–)11–21.8(–59.2) × (–2.8)3.6–4.7(–6.4) μm (mean 16.4 × 4.1 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved of round ends, 1–5-septate: 1-septate (17.8–)22.6–30.6(–31.6) × (3.8–)4.2–5(–5.4) μm (mean 26.6 × 4.6 μm), 2-septate (28.6–)28.3–37.1(–37.3) × (5–)5.1–5.5(–5.5) μm (mean 32.7 × 5.3 μm), 3-septate (31.5–)44.9–58.5(–64.8) × (4.2–)5.4–6.5(–7.2) μm (mean 51.7 × 5.9 μm), 4-septate (49.8–)59.1–67.4(–72.7) × (4.9–)5.9–6.9(–7.6) μm (mean 63.3 × 6.4 μm), 5-septate (58.1–)62.7–70.4(–76.6) × (5–)6.1–7.2(–7.9) μm (mean 66.5 × 6.6 μm). No microconidia or chlamydospores formed in

culture.

Habitat and distribution. Saprobic on decaying *Acacia celsa* and other plants. Distributed in the type locality (Indonesia), Australia and Taiwan.

Additional specimens examined. AUSTRALIA. QUEENSLAND: Atherton City, Davis Creek, on *Acacia celsa*, 2 Feb 2009, A.Y. Rossman, P. Chaverri PC 883 (BPI 879019, culture G.J.S. 09-509). INDONESIA. SULAWESI: Demoga-Bone National Park, 0o28'N, 1230N, 47'E, ca. 810m, 18 Oct. 1985, G.J. Samuels GJS 2278 (NY GJS 2278, culture G.J.S. 85-187 = ATCC 76478). TAIWAN. KAOHSIUNG COUNTY: Liou-guei, Shan-ping, on bark of unidentified tree, 10 Mar 2005, J. –R. Guu (BPI 892688, culture Guu 94031007 = CBS 134039).

Notes. Species other than *T. phoenicea* produce 1–5-septate macroconidia that can be distinguished based on their geographic locations: *T. phoenicea* in Australia, Indonesia and Taiwan; *T. purpurea* in Central and northern South America.

Thelonectria pinea (Dingley) C. Salgado & P. Chaverri, comb. nov.

FIG. 3.6, G–L.

Mycobank TBD.

≡ *Nectria pinea* Dingley, Trans. Roy. Soc. New Zealand 79: 198. 1951.

= *Cylindrocarpon pineum* C. Booth, Mycol. Pap. 104: 26. 1966.

Type specimens. NEW ZEALAND. BAY OF PLENTY: Rotorua, Whakarewarewa, on bark of *Pinus radiata*, Sept 1949, G.B. Rawlings (Holotype PDD 7510); Bay of Plenty, Rotorura, on *Pinus radiata*, coll. Margaret Dick; Epitype designated here BPI XXX = NZFS 1793, ex epitype culture A.R. 4324 = CBS 125153).

Mycelium visible on host. Perithecia globose to subglobose, 400–800 μm high, 200–

350 μm wide, surface smooth and shiny or slightly roughened, solitary or gregarious in groups of 3–25, superficial or with base immersed in substratum on a minute stroma, not collapsed when dry, orange to sienna with ostiolar area often darker (bay) mainly in young perithecia, red to rose in 3% KOH, yellow in lactic acid, papillated. Cells at surface of perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina irregular in shape 2–2.5 μm, 2–4 μm thick. Perithecial wall 40–50 μm wide. Asci cylindrical, 100–130 × 8–9 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, 17–19 × 7–8 μm (mean 18 × 7.5 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline. Colonies on PDA 31 mm diam after 12 d at 20 C, aerial mycelium floccose, white to lilac, producing purple pigment in media at temperatures ≤ 20 C, colony reverse also white to lilac. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.3–)15.1–22.3(–27.5) × (–3.1)3.4–4.3(–4.8) μm (mean 18.7 × 3.8 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 1–4-septate: 1-septate (22.8–)27.9–38.4(–44.1) × (3–)3.3–4.4(–4.9) μm (mean 33.1 × 3.8 μm), 2-septate (30.2–)36.7–45.4(–56.4) × (3.8–)4–5.2(–5.8) μm (mean 41 × 4.7 μm), 3-septate (34.6–)41.4–48.2(–60.6) × (3.5–)4.3–5.4(–6.2) μm (mean 46.3 × 4.9 μm), 4-septate (44–)47.9–61.7(–71.2) × (4.8–)5.2–6.4(–6.9) μm (mean 54.8 × 5.8 μm). No microconidia or chlamydospores formed in culture.

Habitat and distribution. Saprobic on decaying bark of *Pinus radiata*. This species has only been reported from New Zealand.

Additional specimens examined. NEW ZEALAND. NORTHLAND: on *Pinus radiata*, 10 Sep 2003, Margaret Dick NZFS 1069 (culture only, A.R. 4321 = CBS 134033)

Notes. This species is only known from New Zealand, however, the original author, Dingley (1951) described this species as occurring in New Zealand as well as Europe and North America. Here the name *T. pinea* is circumscribed to include species only found on *Pinus radiata* in New Zealand. Isolates of *Nectria*-like fungi on *Pinus* in other parts of the world probably constitute different species.

Thelonectria porphyria C. Salgado & Hirooka, sp. nov.

FIG. 3.7, A–F.

Mycobank TBD.

Similar to *T. discophora*. Macroconidia 3–5-septate, known only from Japan.

Holotype. JAPAN. KOCHI PREFECTURE: Tosa-cho, on bark of dead tree, 04 Aug 2004, Y. Hirooka TPP-h171-1 (BPI 882162, ex-type culture MAFF 241515).

Etymology. Refers to the purple coloration of the anamorph and pigment produced in culture conditions.

Mycelium not visible on host. Perithecia globose to subglobose, 300–600 μm high, 200–350 μm wide, surface smooth and shiny or slightly roughened, solitary or gregarious in groups of 20 or less, superficial or with base immersed in substratum on a minute stroma, not collapsed when dry, orange to sienna with ostiolar area of same color than the rest of the perithecium, red to rose in 3% KOH, yellow in lactic acid, papillated. Cells at surface of perithecial wall lacking a definite outline appearing to

be intertwined hyphae with lumina irregular in shape 2–2.5 μm, 2–4 μm thick. Perithecial wall 25–40 μm wide. Asci cylindrical, (55–)69–89(–100) × 7–11 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (12–)12.8–14.7(–16.1) × (5–)5.7–6.9(–7.9) μm (mean 13.7 × 6.3 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline. Colonies on PDA 30–35 mm diam (mean 33 mm) after 12 d at 20 C, aerial mycelium floccose, white to purple, producing purple to cinnamon pigment diffusing into media at temperatures ≤ 25 C, colony reverse also white to bay. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (11.4–)15.1–20.4(–25.1) × (–3.1)3.7–4.6(–5.2) μm (mean 17.7 × 4.1 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 3–5-septate: 3-septate (42.7–)47.9–59.2(–68.8) × (4.5–)5.4–6.6(–7.5) μm (mean 53.6 × 6 μm), 4-septate (44.3–)51.2–66.3(–79.3) × (4.5–)5.5–6.7(–7.5) μm (mean 58.7 × 6.1 μm), 5-septate (50.9–)61.8–77.6(–88.4) × (5–)5.7–6.9(–7.4) μm (mean 69.7 × 6.3 μm). No microconidia or chlamydospores formed in culture.

Habitat and distribution. Saprobic on decaying bark of *Cryptomeria japonica* and other woody substrates. Found only in Japan.

Additional specimens examined. JAPAN. KOCHI PREFECTURE: Tosakitakaido, Tosa-cho, on twigs of *Cryptomeria japonica*, 04 Aug 2003, Y. Hirooka TPP-h178-2 (BPI 882164, culture MAFF 241517); MIYAGI PREFECTURE: Akiuootaki, Aki-cho,

Taihaku-ku, on twigs of undetermined dead tree, 04 Aug 2004, Y. Hirooka TPP-h292-2 (BPI 882106, culture MAFF 241539).

Notes. See notes for *T. japonica*.

Thelonectria purpurea C. Salgado & P. Chaverri, sp. nov.

FIG. 3.7, G–L.

Mycobank TBD.

Similar to *T. discophora*. Macroconidia 1–5-septate, known only from Central America (Costa Rica) and northern South America (Venezuela).

Holotype. COSTA RICA. HEREDIA PROVINCE: Braulio Carrillo National Park, Zurquí Street entrance, 10°03'N 84°01'W, 1734 m, on bark of undetermined twigs, 14 March 2010, C. Salgado, C. Herrera, Y. Hirooka, A. Rossman, G.J. Samuels, P. Chaverri PC1060 (BPI 892689, ex-type culture G.J.S. 10-131 = CBS 134024).

Etymology. Refers to the purple coloration of the colony of the anamorph and pigment produced under culture conditions.

Mycelium visible on host. Perithecia globose to subglobose, 350–740 μm high, 210–350 μm wide, surface smooth and shiny or slightly roughened, solitary or gregarious in groups of up to 20, superficial or base immersed in substratum on a minute stroma, not collapsed when dry, sienna with ostiolar area often darker (umber) mainly in mature perithecia, red to rose in 3% KOH, yellow in lactic acid, papillate. Cells at surface of perithecial wall lacking a definite outline, appearing to be intertwined hyphae with lumina irregular in shape, 2–2.5 μm, 2–4 μm thick. Perithecial wall 43–52 μm wide. Asci cylindrical, (55–)68–82(–98) × 7–10 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (10.8–)12–14.1(–15.4) × (3.8–)4.8–

6.1(–6.6) μm (mean 13.0 × 5.5 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline. Colonies on PDA 33–38 mm diam (mean 35 mm) after 12 d at 20 C, aerial mycelium floccose, white to purple or saffron, producing purple pigment in media at temperatures ≤ 25 C, colony reverse white to purple or saffron. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (11.4–)15.9–22.8(–30.6) × (–3.8)3.8–4.7(–5.4) μm (mean 19.4 × 4.3 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 1–5-septate: 1-septate (21.1–)22–44.6(–67) × (4.3–)4.6–6.2(–7.1) μm (mean 32.6 × 5.4 μm), 2-septate (31.9–)32.8–46.2(–47.9) × (5.2–)5.3–5.7(–5.8) μm (mean 39.5 × 5.5 μm), 3-septate (4.2–)47.3–60.7(–69) × (4.7–)5.4–6.4(–7.3) μm (mean 54.5 × 5.9 μm), 4-septate (49.9–)57.6–67.4(–76.4) × (4.5–)5.4–6.7(–7.6) μm (mean 62.5. × 6.1 μm), 5-septate (57.7–)62.1–71.9(–81.2) × (5–)5.7–7(–7.7) μm (mean 67 × 6.4 μm). No microconidia or chlamydospores formed in culture.

Habitat and distribution. Saprobic on decaying bark of woody substrates. Known from Venezuela and Costa Rica, possibly widely distributed in the Neotropics.

Additional specimens examined. COSTA RICA. HEREDIA PROVINCE: Braulio Carrillo National Park, Zurquí Street entrance, 10°03'N 84°01'W, 1734 m, on bark of undetermined dead tree, 14 March 2010, C. Salgado, C. Herrera, Y. Hirooka, A. Rossman, G.J. Samuels, P. Chaverri PC1081 (BPI 882335, culture G.J.S. 10-145 =

CBS 134025). VENEZUELA. ARAGUA STATE: Henry Pittier National Park, ca. 20 km above Maracay, on Maracay-Choroni road, on wood of unidentified dead tree, 13 Jul 1971, K.P. Dumont, J.H. Haines, G.J. Samuels (NY Dumont-VE 2173, culture C.T.R. 71-281 = CBS 112458). MERIDA STATE: Sierra Nevada National Park, above Tabay, Qda. Coromoto, La Mucuy, 08°36'N 71°02'W, ca. 2000 m, on palm fruit, G.J. Samuels et al. GJS 7244A (BPI 1109900, culture G.J.S. 90-155 = CBS 123966)

Notes. See notes for *T. phoenicea*.

Thelonectria purpurescens C. Salgado & P. Chaverri, sp. nov.

FIG. 3.8, A–F.

Mycobank TBD.

Similar to *T. discophora*. Macroconidia 3–6-septate, collected in the sexual state, saprobic, from Puerto Rico, Venezuela and Japan.

Holotype. PUERTO RICO. Caribbean National Forest, Luquillo Mountains, Rio Grande, trail to El Toro from rt 186, 650–750 m, on bark of unidentified recently dead tree, 24 Feb 1996, G.J. Samuels, H.-J Schroers, D.J. Lodge (BPI 745542, culture G.J.S. 96-23 = IMI 370947).

Etymology. Refers to the purple coloration of the colony of the anamorph in culture conditions.

Mycelium not visible on host. Perithecia globose to subglobose, 300–600 μm high, 200–350 μm wide, surface smooth and shiny or slightly roughened, solitary or gregarious in groups of 20 or less, superficial or with base immersed in substratum on a minute stroma, not collapsed when dry, peach to sienna with ostiolar area darker (bay), red to rose in 3% KOH, yellow in lactic acid, papillate. Cells at surface of

perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina irregular in shape 2–2.5 μm, 2–4 μm thick. Perithecial wall 25–40 μm wide. Asci cylindrical, (56–)67–86(–98) × 7–12 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (10.3–)11.1–12.4(–13.6) × (4–)4.7–5.6(–13.6) μm (mean 11.8 × 5.2 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline. Colonies on PDA 31–34 mm diam (mean 32 mm) after 12 d at 20 C, aerial mycelium floccose, white to purple, not producing pigment in media, colony reverse white to purple. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA agar. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (11.8–)14.9–19.5(–22.8) × (–3.4)4–4.9(–5.8) μm (mean 17.2 × 4.4 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 3–5-septate: 3-septate (40–)45.5–66.4(–69.1) × (4.8–)5.5–7.6(–9) μm (mean 55.9 × 6.5 μm), 4-septate (53.7–)62.6–76(–82.2) × (5.2–)5.9–7.8(–9.2) μm (mean 69.3 × 6.8 μm), 5-septate (66.2–)68.1–88.4(–107.8) × (5.2–)6.2–7.9(–9.2) μm (mean 78.2 × 7 μm). No microconidia or chlamydospores formed in culture.

Habitat and distribution. Saprobic on woody substrates. Found at the type locality in Puerto Rico, Venezuela and Japan; possibly widely distributed in the tropics and subtropics around the world.

Additional specimens examined. JAPAN. TOKYO: Sakaigatake, Hahajima, Ogasawara-mura, on bark of unidentified dead tree, 22 Jun 2005, Y. Hirooka TPP-

h488-2 (BPI 881951, culture MAFF 241564). VENEZUELA. BOLIVAR STATE: The Gran Sabana National Park, 1139 m, on wood of unidentified dead tree, 29 Jun 2009, C. Salgado, Y. Hirooka YH09-124 (BPI 882679, culture G.J.S. 09-1327 = CBS 134022).

Notes. This species is similar to *T. blattea*. *Thelonectria blattea* has been collected only in the anamorphic state on soil while *T. purpurescens* has been collected in the teleomorphic state as a sabrobe of decaying plant material.

Thelonectria rubi (Osterw.) C. Salgado & P. Chaverri, stat. nov. et comb. nov. FIG. 3.8, G–L.

Mycobank TBD.

≡ *Nectria rubi* Osterw., Ber. Deutsch. Bot. Ges. 29: 620. 1911.

≡ *Hypomyces rubi* (Ostew.) Wollenw., Phytopathology 3: 224. 1913.

= *Neonectria discophora* var. *rubi* (Osterw.) Brayford & Samuels, Mycologia 96: 572. 2004.

= *Cylindrocarpon ianthothele* var. *ianthothele* Wollenw., Ann. Mycol. 15: 56. 1917.

= *Cylindrocarpon ianthothele* Wollenw., Ann Mycol. 15: 56. 1917.

Holotype. SWITZERLAND. HORGEN DISTRICT: Wadenswill Locality, on *Rubus idaeus* roots, 1911, A. Osterwalder (only ex-type culture CBS 113.12 = IMI 113918).

Mycelium not visible on host. Perithecia globose to subglobose, 300–500 μm diam, smooth, solitary or gregarious in groups up to 5 growing on poorly developed erumpent stroma, not collapsed when dry, bright red to umber with ostiolar area the same color than the rest of the perithecium, red to rose in 3% KOH, yellow in lactic acid, papillate. Perithecial wall 80–100 μm. Asci cylindrical to clavate 80–120 x 5–6

μm, 8-spored, ascospores ellipsoid, 1-septate (11.5–)12–16(–23) × (4.5–)5.5–6.5(–8) μm (mean 14 × 6 μm), not constricted at septum, becoming pale yellow when mature, spinulose. Colonies on PDA 23–29 mm diam (mean 24 mm) after 12 d at 20 C, aerial mycelium floccose, white to purple, no pigment produced in media, colony reverse white to purple. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (15.1–)16.8–23.3(–28.8) × (–3.2)3.8–4.8(–5.5) μm (mean 20 × 4.3 μm), with periclinal thickening and collarette. Macroconidia cylindrical or curved with round ends, with one end thicker than the other, 1–6-septate (except 2-septate): 1-septate (10–)10.1–12.9(–14.2) × (4.2–)4.4–5.6(–5.7) μm (mean 11.5 × 5 μm), 3-septate (37.9–)47.1–61.8(–68.6) × (5.1–)5.8–(–7) μm (mean 54.4 × 6.4 μm), 4-septate (42–)56.5–67.2(–74.2) × (5.5–)6–7.2(–7.7) μm (mean 63 × 6.6 μm), 5-septate (45.3–)56.2–69.7(–84) × (5.1–)6–7.2(–7.7) μm (mean 60.3 × 6.3 μm), 6-septate (45.9–)46–77.3(–79.1) × (4.9–)5.8–6.9(–8) μm (mean 60.3 × 6.3 μm). Microconidia produced in culture, cylindrical with round ends, (7.3–)7.9–10(–11.5) × (4.2–)4.6–5.5(–6.3) μm (mean 9 × 5 μm). Chlamydospores formed in culture, 4 × 5 μm.

Habitat and distribution. On roots of diseased *Rubus* species. Found in the type locality (Wadenswill, Switerland); also Europe and one report from Venezuela (Cedeño et al. 2004).

Additional cultures examined. SCOTLAND. Locality unknown, 1929, H.M. Wollenweber (CBS 241.29 = IMI 113919). UNITED KINGDOM. ENGLAND: location

unknown, R.M. Nattrass (culture CBS 177.27 = IMI 113917).

Notes. Brayford et al. (2004) observed that perithecia of *T. rubi* were morphologically and anatomically indistinguishable from those of *T. discophora*. However, data suggest that true *T. rubi* is only found in association with roots and crowns of *Rubus* species. Although the type locality is Wadenswill, Switzerland, it is possible to find this species wherever *Rubus* species are found.

Thelonectria tyrus C. Salgado & P. Chaverri, sp. nov.

FIG. 3.9, A–F.

Mycobank TBD

Similar to *T. discophora*. Macroconidia 3–5-septate, only found in the eastern United States.

Holotype. UNITED STATES. NORTH CAROLINA: Macon County, Ellicott Rock Trail, off of Bull Pen Road, 35°02'N 83°08'W, 915 m, on bark of living *Quercus* sp., G.J. Samuels, A.Y. Rossman, Y. Doi (BPI 1107126, ex-type culture G.J.S. 90-46 = CBS 134029).

Etymology. Refers to the purple coloration of the culture of the anamorph and pigment diffusing into the media in culture conditions.

Mycelium not visible on host. Perithecia globose to subglobose, 300–600 μm high, 200–400 μm wide, surface smooth and shiny or slightly roughened, solitary or gregarious in groups of 20 or less, superficial or with base immersed in substratum with no visible stroma, not collapsed when dry, peach to sienna with ostiolar area same color as the rest of the perithecium, red to rose in 3% KOH, yellow in lactic acid, papillate. Cells at surface of perithecial wall lacking a definite outline, appearing

to be intertwined hyphae with lumina irregular in shape 2–2.5 μm, 2–4 μm thick. Perithecial wall 35–42 μm wide. Asci cylindrical, (57–)60–81(–100) × 7–12 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (12–)13–14(–15) × (5.5–)6–6.5(–7) μm (mean 13.8 × 6.2 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline. Colonies on PDA 21–27 mm diam (mean 24 mm) after 12 d at 20 C, aerial mycelium floccose, white to purple, producing purple pigment in media at temperatures ≤ 30 C, colony reverse white to bay. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.3–)15.8–21.2(–24.2) × (–3.2)3.6–4.5(–4.9) μm (mean 18.5 × 4 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 2–5-septate: 2-septate (35–)34.7–36.4(–36.7) × (5.6–)5.6–5.8(–5.9) μm (mean 35.7 × 5.7 μm), 3-septate (41.9–)47.6–56.2(–60.9) × (4.9–)5.6–6.5(–7) μm (mean 51.9 × 6.1 μm), 4-septate (50.9–)53.9–60.7(–65) × (5.6–)6.1–6.9(–7.1) μm (mean 57.3 × 6.5 μm), 5-septate (57–)59.8–65.5(–67.4) × (6.5–)6.6–7.5(–8) μm (mean 62.7 × 7.1 μm). No microconidia or chlamydospores formed in culture.

Habitat and distribution. Saprobic on decaying bark of woody substrates including *Quercus* sp. and *Fagus grandifolia*. Found in Connecticut and North Carolina.

Additional specimens examined. UNITED STATES. CONNECTICUT: New Haven, West Rock Ridge State Park, on bark of dead *Fagus grandifolia*, Oct 2007, R. Marra (BPI 878945, culture A.R. 4499 = CBS 125172).

Notes. This species is similar to *T. conchyliata, T. ianthina* and *T. porphyria* in that they also produce 3–5-septate macroconidia. However, only *T. tyrus* is found in the eastern United States.

Thelonectria violaria C. Salgado & R.M. Sanchez, sp. nov.

FIG. 3.9, G–L.

Mycobank TBD

Similar to *T. discophora*. Macroconidia 1–3 septate only, no microconidia produced in culture.

Holotype. ARGENTINA. TUCUMAN PROVINCE: road to Catamarca, Camino Las Lenguas, near to Rio Cochuna, 400 m, on bark of a rotting fallen tree, C. Salgado, A.Y. Rossman, A. Romero (BPI 892690, ex-type culture A.R. 4766 = CBS 134035).

Etymology. Refers to the purple coloration of the colony of the anamorph under culture conditions.

Mycelium not visible on host. Perithecia globose to subglobose, 300–600 μm high, 200–350 μm wide, surface smooth and shiny or slightly roughened, solitary or gregarious in groups of 20 or less, superficial or with base immersed in substratum with no visible stroma, not collapsed when dry, sienna to chestnut with the ostiolar area darker (blood color), red to rose in 3% KOH, yellow in lactic acid, papillate. Cells at surface of perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina irregular in shape 2–2.5 μm, 2–4 μm thick. Perithecial wall 35–45 μm wide. Asci cylindrical, (57–)68–86(–98) × 7–11 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (9.9–)10.5–12.8(–14) × (4.1–)4.6–6(–7) μm (mean 11.7 × 5.3 μm), symmetrically two-celled,

sometimes with one side curved and one side flattened, not constricted at septum, spinulose, hyaline. Colonies on PDA 30–35 mm diam (mean 33 mm) after 12 d at 20 C, aerial mycelium floccose, white to mauve, producing purple to cinnamon pigment in media at temperatures < 20 C colony, reverse white to bay. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.2–)14.3–20.1(–24.5) × (3.2–)3.8–4.8(–5.3) μm (mean 17.2 × 4.3 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 1–3-septate: 1-septate (31.4–)38.9–47.7(–49) × (3.4–)4.4–5.6(–6) μm (mean 43.3 × 5 μm), 2-septate (48.1–)48–54.3(–56.9) × (5–)5.1–5.5(–5.7) μm (mean 51.1 × 5.3 μm), 3-septate (36.7–)47.6–57.8(–68.4) × (4.6–)4.9–5.9(–7) μm (mean 52.7 × 5.4 μm). No microconidia or chlamydospores formed in culture.

Habitat and distribution. Saprobic on decaying bark woody substrates. Found in the type locality (Tucuman, Argentina) and Venezuela; possibly distributed widely in tropical and subtropical regions of South America.

Additional specimens examined. VENEZUELA. 13 km NE of Colonia Tovar on road between Colonia Tovar and El Tigre, Dto. Fed., on bark of unidentified tree, 19 Jul 1972, K.P Dumont, R.F. Cain, G.J. Samuels, B. Manara (NY Dumont-VE 6503, culture C.T.R. 72-188 = CBS 134040).

Notes. This species is similar to *T. asiatica* and *T. rubi* in that 1–3-septate macroconidia are produced in culture. However, *T. violaria* can be distinguished, as it

does not produce microconidia, and macroconidia with septations >4 are not observed.

Possible additional species

Thelonectria beijingensis Z.Q. Zeng, J. Luo & W.Y. Zhuang

Mycobank MB564936

See Zeng and Zhuang (2013) for description and illustrations.

Thelonectria yunnanica Z.Q. Zeng & W.Y. Zhuang

Mycobank MB564937.

See Zeng and Zhuang (2013) for description and illustrations.

Discussion

Historically, specimens with similar teleomorphic and anamorphic morphology to that of the type species were labeled *Thelonectria discophora* sensu lato. Because collections having this morphology have been found in many regions of the world, *T. discophora* was assumed to have a cosmopolitan distribution. Our phylogenetic and morphological analyses of a large number of specimens and cultures of *T. discophora* revealed at least sixteen previously unrecognized cryptic species. Using the genealogical concordance phylogenetic species concept principles (Dettman et al. 2003), we have formally described those sixteen cryptic species as separate and independent entities. The genetic distances between putative species mostly exceeded standard values of genetic distance (0.01–0.03) used to delimit operational taxonomic units (OTU), revealing them as independent entities (Salgado-Salazar et al. 2013). This indicates a > 16-fold increase in the species diversity in the genus *Thelonectria,*

a genus recently established for nectriaceous fungi that typically have a broad mammiform (nipple-like) perithecial apex.

Our phylogenetic analyses also detected nine single isolate lineages or singletons. These lineages, together with those found by Zeng and Zhuang (2013), *T. beijingensis* and *T. yunnanica* (FIG. S3.1), constitute additional distinctive evolutionary entities that make up a considerable portion of the diversity of species in this complex. In systematics, no consensus exists on how singleton lineages should be treated (Seifert and Rossman 2011). In phylogenetic analyses, singleton lineages are located in branches with unknown support (i.e., bootstrap, posterior probability), as a branch should have at least two tips to obtain statistic support. Even though *T. beijingensis* and *T. yunnanica* were found to have affinities to the species here described, their lack of agreement in the morphological characters of the anamorphic states prevents us from reaching a definite conclusion. Further increase in taxon and molecular data sampling would determine if the singleton lineages here described and those by Zeng and Zhuang (2013) represent different species and if they should be formally described. In spite of this, many of these singletons likely constitute rare taxa, which highlight even more the importance of preserving the habitat where they can be found (Dahlberg and Mueller 2011).

Species in the *T. discophora* complex appear not only to be consistent with allopatric but also sympatric speciation (Giraud et al. 2008). The *T. discophora* complex contains species that correlate with geographic origin, that is, the putative species group isolates from the same or close-by regions. However, this complex also includes species with isolates from distant geographic locations. Since no definite

explanation can be given for this phenomenon, it probably means that members of these species have not been sampled thoroughly and by adding more samples, a better geographic structure might be observed. According to our data, ten species were found only in temperate regions such as the United States, Europe, Asia and New Zealand. Fewer species were found in tropical regions. However, this could be a result of the lower taxon sampling as for example, collections from Venezuela represent at least four species. Thus, one may assume that *T. discophora*-like species can be found in tropical and temperate regions equally. Due to their small size and ecological preferences, these fungi have only been collected serendipitously translating into limited taxon sampling. As is the case with poorly studied organisms, increasing their collection can further support assumptions about the geographic range of the putative species or about their center of origin. The role of human-mediated movement of species of *T. discophora*, contributing to the actual geographic distribution of species, cannot be discarded. However, because these species do not include invasive or pathogens of commercial or forest plants, their presence can be overlooked and movement difficult to track.

The results from our research support previous studies with other microorganisms, including fungi, suggesting that there are very few truly cosmopolitan species (Carriconde et al. 2008, Pringle et al. 2005, Salgado-Salazar et al. 2013, Taylor et al. 2006). Because *T. discophora* is a species complex that includes various species with limited geographic distribution and ecology, we hypothesize that the lack of mechanisms for long-distance spore dispersal could be affecting their distribution. Only two species in this group have been found to

produce microconidia, which could be easily carried by wind currents. However, they are also found to be geographically restricted. The majority of *T. discophora* species have asexual spores (macroconidia) that are colorless and longer than 30 μm, sometimes reaching 100 μm; their sexual spores (ascospores) are also colorless or non-melanized. More importantly, both sexual and asexual spores do not have shapes that could improve dispersal, possibly landing after traveling short distances (Roper et al. 2008, Roper et al. 2010). These characteristics together may limit considerably the range of dispersal of these fungi, and consequently populations undergo independent evolutionary trajectories and ultimately, species divergence. The marked genetic structure observed among the species in *T. discophora* likely reflects the interplay between their poor dispersal capabilities and the restrictions to gene flow, either imposed by geographical or reproductive barriers.

With the exception of T. *rubi*, all the species in the *T. discophora* complex are saprobic on decaying plant material or soil. Species occurring in unconnected, but similar habitats and under similar selection pressures often display strikingly comparable morphology, behavior and life history and, without a phylogeny, it is often difficult to separate whether similar traits are a result of *in situ* diversification or independent colonization. Based on the results obtained in this study we determined that shared morphological and ecological characters of these species represent a case of convergence, and are the result of their similar habitat and selection pressures. As other species in the genus *Thelonectria*, such as *T. coronata*, *T. jungneri*, *T. lucida* and *T. veuillotiana*, can also be found in the same habitat as members of the *T. discophora* species group, it is possible that the ability of these species to produce

extracellular secondary metabolites, such as the characteristic purple pigment, could represent an adaptive advantage for the species (Yu and Keller 2005). Studies on the nature of pigments produced by these species are lacking, and further investigations may help elucidate their role in adaptation.

Since many historical species shared morphological characters with *T. discophora*, several monographic accounts regarded these species as synonyms. With this study, we revised the taxonomic synonyms and updated the current status of the names, using them when appropriate. For example, *Nectria mammoidea*, *N. pinea* and *Neonectria discophora* var. *rubi*, were redefined and epytypified. Anamorphic names in the case of *Thelonectria* are no longer used, consequently the names *Cylindrocarpon ianthothele* and *C. ianthothele* var. *ianthothele* are synonyms of *Thelonectria rubi*, *C. ianthothele* var. *majus* is synonym of *T. mammoidea*, and *C. pineum* is synonym of *T. pinea*. According to several taxonomic revisions (Booth 1966, Brayford et al. 2004, Chaverri et al. 2011, Samuels et al. 1990) no *Cylindrocarpon* name was ever correctly assigned to *T. discophora*, as all *Cylindrocarpon ianthothele* names were based on the conidial state of *T. mammoidea* (=*Nectria mammoidea*). One isolate of *Nectria tasmanica* was checked during this study (ICMP 5290). However, it was found to produce an anamorph with morphology similar to *Fusarium* and consequently it needs further revision to determine if it is related to the *T. discophora* species complex.

In conclusion, we demonstrate that a combination of both morphological and phylogenetic analyses is effective for the clarification of taxonomic status of species, especially those that have been difficult to resolve using morphological characters

alone. The unlinked nuclear markers and phylogenetic analyses enabled us to resolve the species level relationships in this group, despite the presence of recently diverged clades. Our results confirm high lineage diversity in this group of fungi, and highlight the importance of a comprehensive delimitation of species within highly diverse groups to better understand the factors that drive the diversification of biota, and for correctly identifying targets for conservation.

Key to the species in the *Thelonectria discophora* complex

1. Micro- and macroconidia produced on SNA…….2

1. Only macroconidia produced on SNA……...3

2. Found causing a distinctive basal canker in *Rubus* sp. in Europe, including Switzerland (type locality and United Kingdom…..…… *T. rubi*

2. Not found causing canker in *Rubus* sp., saprobe distributed in China and Japan (type locality), macroconidia 1–3-septate…….. *T. asiatica*

3. Macroconidia 1–3-septate, found in Argentina (type locality) and Venezuela ……*T. violaria*

3. Macroconidia 1–4- or 1–5- septate, known from temperate and tropical regions…4

4. Macroconidia 1–4- septate, known from New Zealand (type locality), saprobe, restricted to plant material of *Pinus radiata*……..……. *T. pinea*

4. Macroconidia 1–5-septate, from New Zealand, not on *P. radiata*……5

5. Macroconidia 1–5-septate, from New Zealand (type locality) on decaying plant material different than *P. radiata*, including *Quercus* sp……*T. brayfordii*

5. Macroconidia 1–5-septate, known from New Zealand and elsewhere……..6

6. Microconidia 1–5-septate, from New Zealand, United Kingdom (type locality) and

Switzerland…….. *T. mammoidea*

6. Macroconidia 1–5-septate, from Central and South America and Australasia……..7

7. Macroconidia 1–5-septate, from Central America (Costa Rica, type locality) and northern South America (Venezuela) only……...*T. purpurea*

7. Macroconidia 1–5-septate, not from Central and South America…….8

8. Macroconidia 1–5-septate, from Australia, Indonesia and Taiwan……*T. phoenicea*

8. Macroconidia 3–6-septate, widely distributed……...9

9. Macroconidia 3–6-septate, known from Japan (type locality)……..*T. japonica*

9. Macroconidia 3–6-septate, found other than Japan…….10

10. Macroconidia 3–6-septate, collected as the asexual state in soil in Germany (type locality) and The Netherlands……...*T. blattea*

10. Macroconidia 3–6-septate, collected as the sexual state on bark of decaying plant material……11

11. Macroconidia 3–6-septate, collected as the sexual state, known from Japan, Puerto Rico (type locality) and Venezuela……*T. purpurescens*

11. Macroconidia 3–5 septate, from temperate regions ……..12

12. Macroconidia 3–5-septate, from Chile (type locality) and Scotland...*T. discophora*

12. Macroconidia 3–5 septate, not from Chile nor Scotland……...13

13. Macroconidia 3–5 septate, only known from Japan (type locality)…*T. porphyria*

13. Macroconidia 3–5 septate, known from regions other than Japan…….14

14. Macroconidia 3–5-septate, known from eastern United States…….*T. tyrus*

14. Macroconidia 3–5-septate, known from other than eastern United States……15

15. Macroconidia 3–5-septate, average colony growth on PDA at 30 C > 13 mm after

12 days…… *T. conchyliata*

15. Macroconidia 3–5-septate, average colony growth on PDA at 30C < 13 mm after 12 days ……*T. ianthina*

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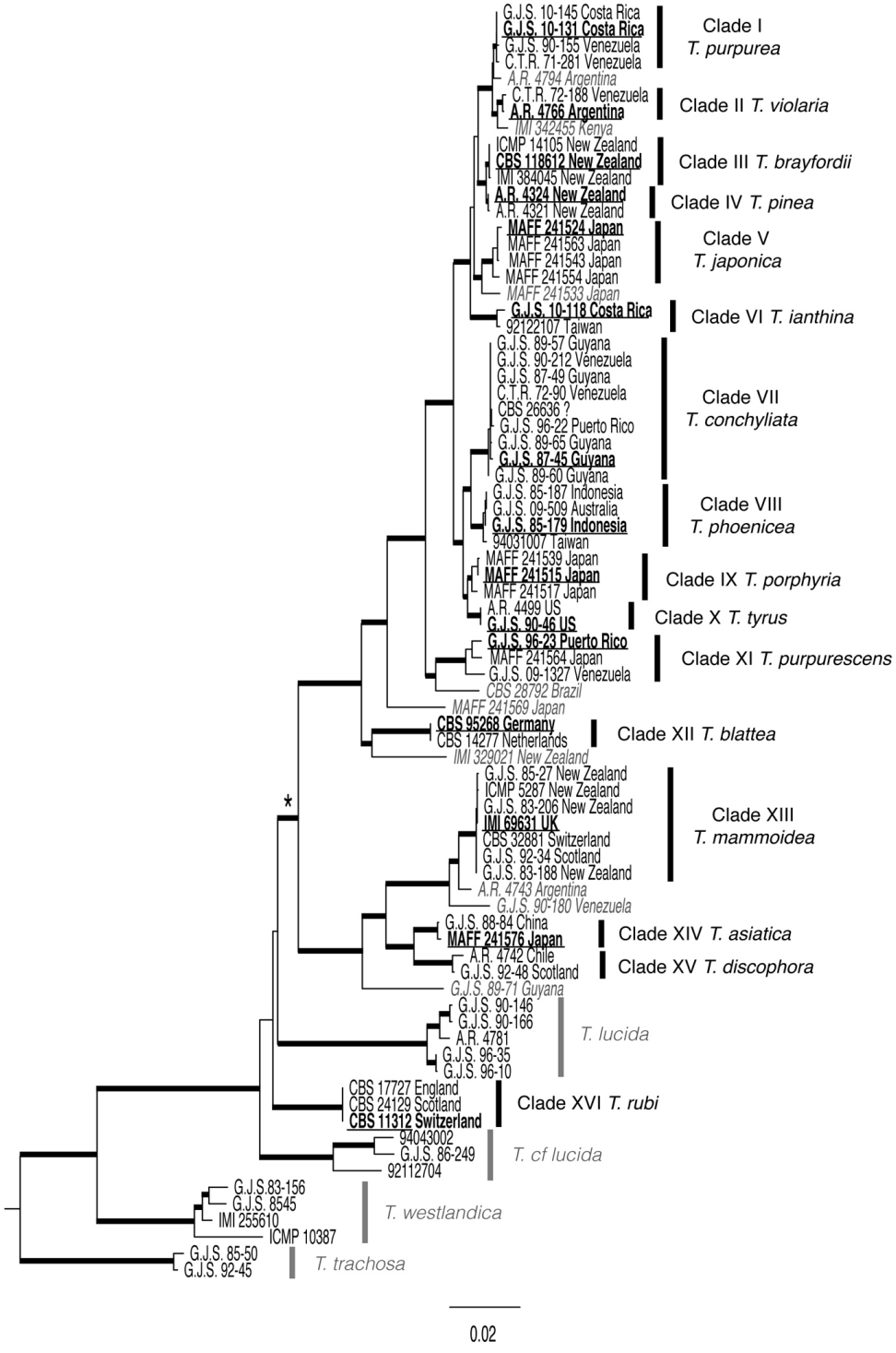
G.J.S. 10-145 Costa Rica
G.J.S. 10-131 Costa Rica
G.J.S. 90-155 Venezuela
C.T.R. 71-281 Venezuela
A.R. 4794 Argentina
Clade I
T. purpurea
C.T.R. 72-188 Venezuela
A.R. 4766 Argentina
IMI 342455 Kenya
Clade II T. violaria
ICMP 14105 New Zealand
CBS 118612 New Zealand
IMI 384045 New Zealand
Clade III T. brayfordii
A.R. 4324 New Zealand
A.R. 4321 New Zealand
Clade IV T. pinea
MAFF 241524 Japan
MAFF 241563 Japan
MAFF 241543 Japan
MAFF 241554 Japan
MAFF 241533 Japan
Clade V
T. japonica
G.J.S. 10-118 Costa Rica
92122107 Taiwan
Clade VI T. ianthina
G.J.S. 89-57 Guyana
G.J.S. 90-212 Venezuela
G.J.S. 87-49 Guyana
C.T.R. 72-90 Venezuela
CBS 26636 ?
G.J.S. 96-22 Puerto Rico
G.J.S. 89-65 Guyana
G.J.S. 87-45 Guyana
G.J.S. 89-60 Guyana
Clade VII
T. conchyliata
G.J.S. 85-187 Indonesia
G.J.S. 09-509 Australia
G.J.S. 85-179 Indonesia
94031007 Taiwan
Clade VIII
T. phoenicea
MAFF 241539 Japan
MAFF 241515 Japan
MAFF 241517 Japan
Clade IX T. porphyria
A.R. 4499 US
G.J.S. 90-46 US
Clade X T. tyrus
G.J.S. 96-23 Puerto Rico
MAFF 241564 Japan
G.J.S. 09-1327 Venezuela
Clade XI T. purpurescens
CBS 28792 Brazil
MAFF 241569 Japan
CBS 95268 Germany
CBS 14277 Netherlands
Clade XII T. blattea
IMI 329021 New Zealand
*
G.J.S. 85-27 New Zealand
ICMP 5287 New Zealand
G.J.S. 83-206 New Zealand
IMI 69631 UK
CBS 32881 Switzerland
G.J.S. 92-34 Scotland
G.J.S. 83-188 New Zealand
Clade XIII
T. mammoidea
A.R. 4743 Argentina
G.J.S. 90-180 Venezuela
G.J.S. 88-84 China
MAFF 241576 Japan
Clade XIV T. asiatica
A.R. 4742 Chile
G.J.S. 92-48 Scotland
Clade XV T. discophora
G.J.S. 89-71 Guyana
G.J.S. 90-146
G.J.S. 90-166
A.R. 4781
G.J.S. 96-35
G.J.S. 96-10
T. lucida
CBS 17727 England
CBS 24129 Scotland
CBS 11312 Switzerland
Clade XVI T. rubi
94043002
G.J.S. 86-249
92112704
T. cf lucida
G.J.S.83-156
G.J.S. 8545
IMI 255610
ICMP 10387
T. westlandica
G.J.S. 85-50
G.J.S. 92-45
T. trachosa
0.02

FIGURE 3.1. Majority rule Bayesian phylogram showing relationships among isolates of *Thelonectria discophora*-like species based on the concatenated analysis of six loci. Thick branches indicate Bayesian posterior probabilities >0.95 and ML bootstrap >70%. No thick branches indicate branch was not recovered/supported. "*" indicates where the *T. discophora* species complex starts. Underlined isolates indicate type specimen. *Thelonectria lucida*, *T. trachosa* and *T. westlandica* were used as outgroups.

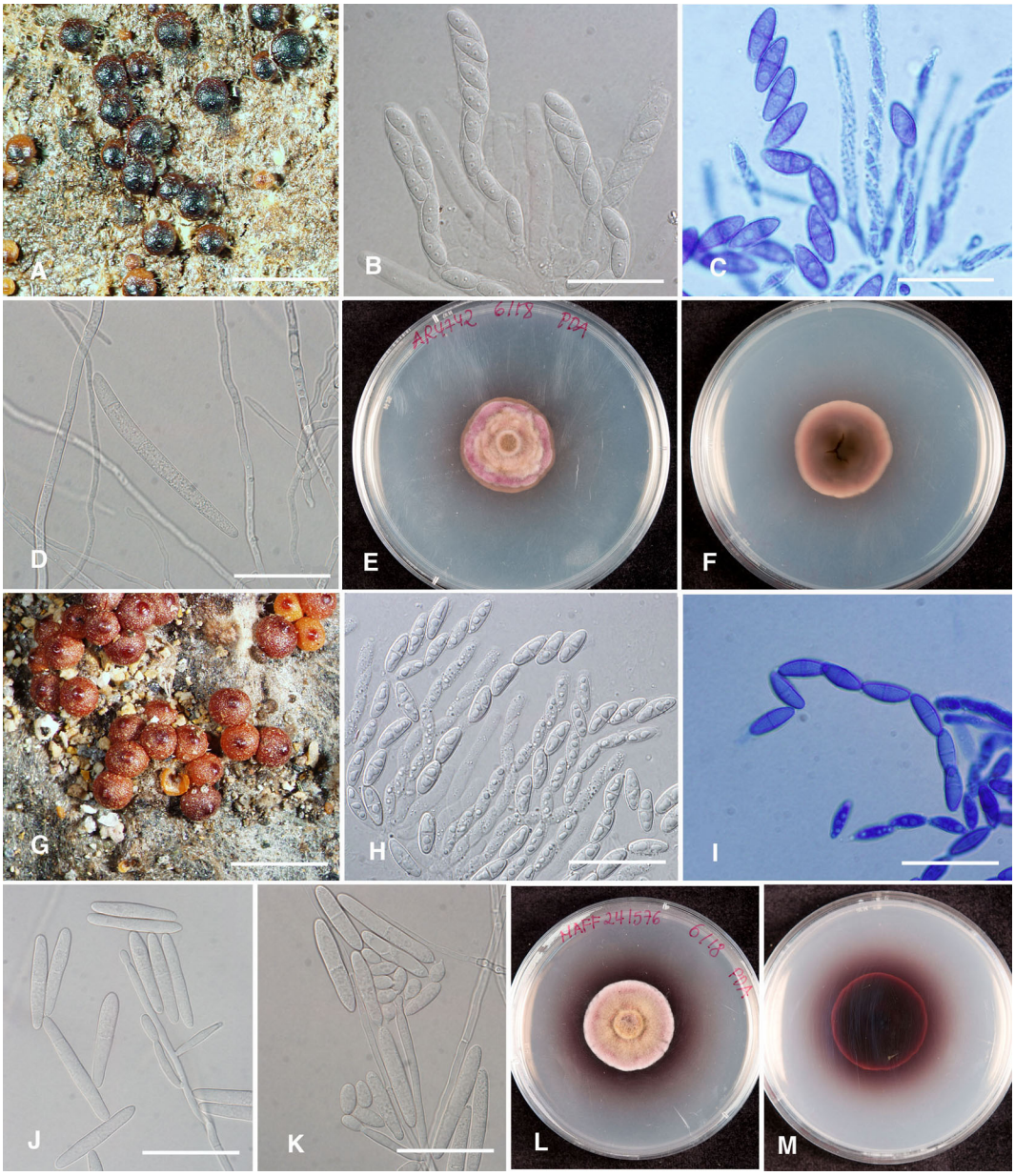

FIGURE 3.2. A–F. *Thelonectria discophora* s. str. G–M. *Thelonectria asiatica*. A. T. discophora s. str. perithecia (A.R. 4742 = BPI 892687). B, C. Asci and ascospores in KOH and cotton blue (G.J.S. 92-48 = BPI 802901). D. Macroconidia on SNA (G.J.S. 92-48 = CBS 134031). E. Colony on PDA (A.R. 4742 = CBS 134034). F. Colony reverse on PDA (A.R. 4742 = CBS 134034). G. *T. asiatica* perithecia (MAFF 241576

= BPI 881963). H, I. Asci and ascospores in KOH and cotton blue (MAFF 241576 = BPI 881963). J, K. Conidiophores, macroconidia and microconidia on SNA (G.J.S. 88-84 = IMI 348190). L. Colony on PDA (MAFF 241576). M. Colony reverse on PDA (MAFF241576). Bars: A, G = 500 μm; B–D, H–K = 50 μm.

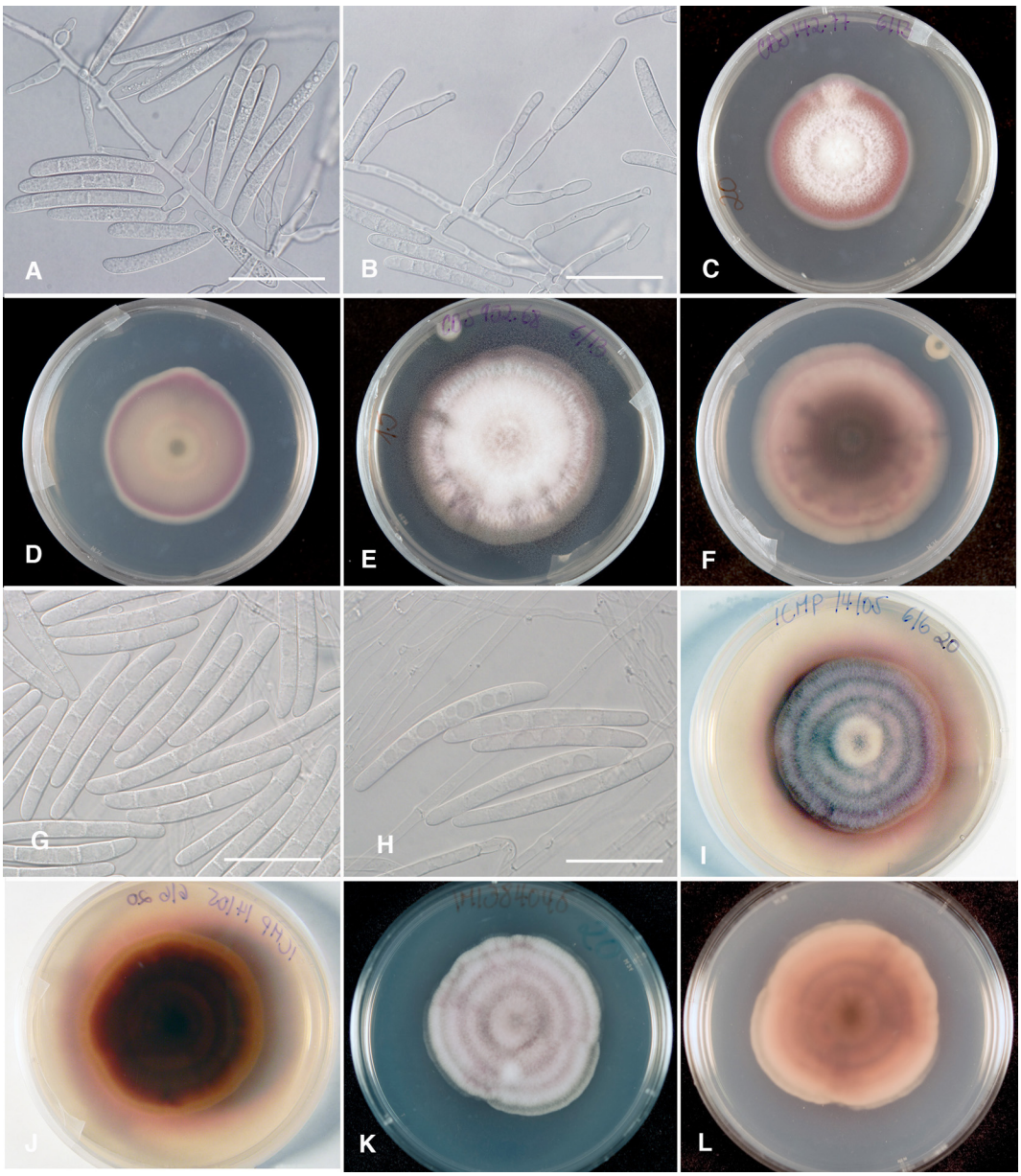

FIGURE 3.3. A–F. *Thelonectria blattea*. G–L. *Thelonectria brayfordii*. A–B. *T. blattea* conidiophores and macroconidia on SNA (CBS 14277). C. Colony on PDA (CBS

14277). D. Colony reverse on PDA (CBS 14277). E. Colony on PDA (CBS 95268). F. Colony reverse on PDA (CBS 95268). G–H. *T. brayfordii* macroconidia on SNA (ICMP 14105). I. Colony on PDA (ICMP 14015). J. Colony reverse on PDA (ICMP 14105). K. Colony on PDA (IMI 384045). L. Colony reverse on PDA (IMI 384045). Bars: A–B, G–H = 50 µm.

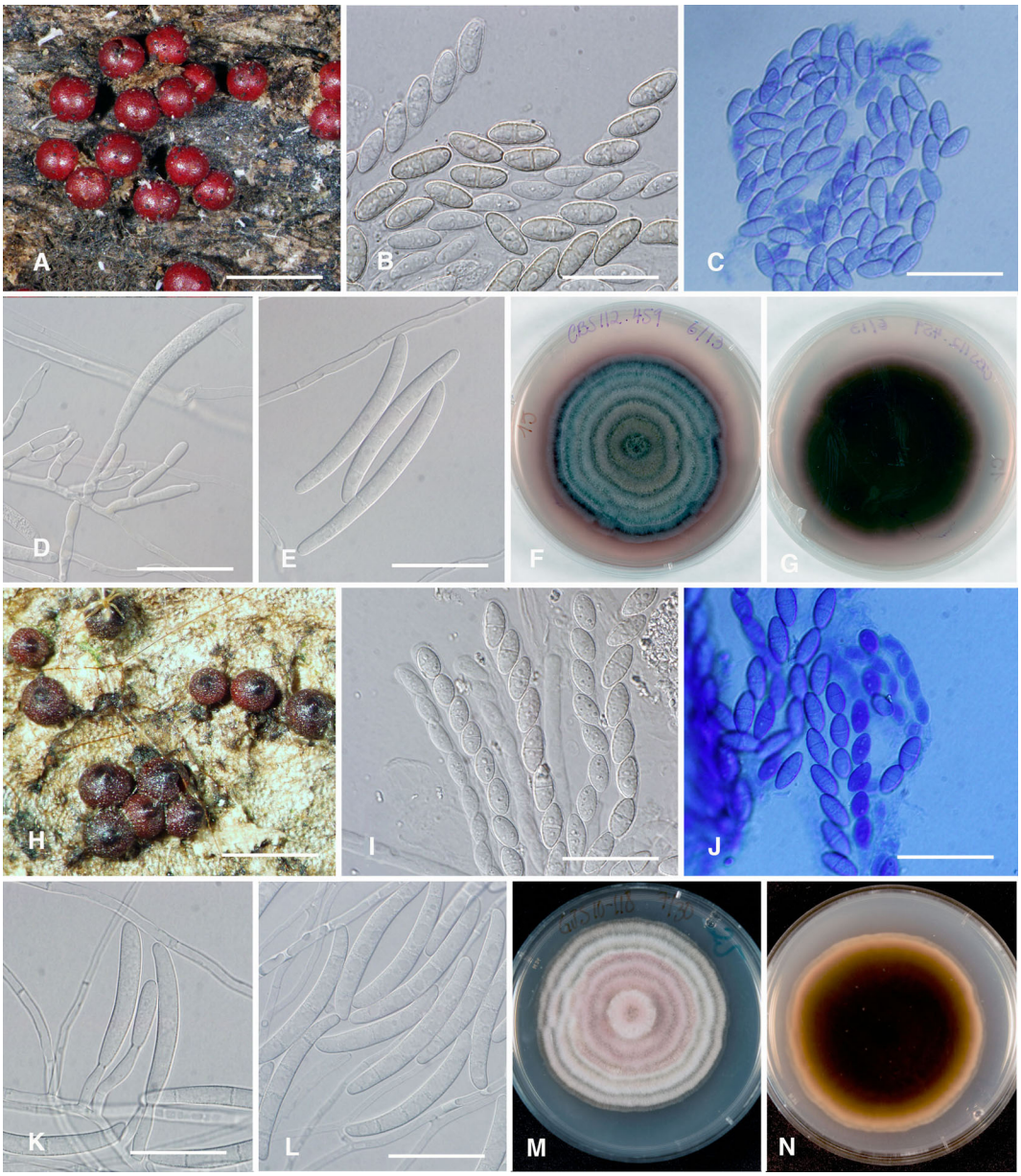

FIGURE 3.4. A–G. *Thelonectria conchyliata*. H–N. *Thelonectria ianthina*. A. *T. conchyliata* perithecia (G.J.S. 89-57 = NY Samuels 6269A). B, C. Asci and ascospores in KOH and cotton blue (G.J.S. 87-49 = BPI 744725). D–E. Conidiophores and macroconidia on SNA (G.J.S. 89-57 = CBS 112459). F. Colony on PDA (G.J.S. 87-49 = CBS 112461). G. Colony reverse on PDA (G.J.S. 87-49 = CBS 112461). H. *T. ianthina* perithecia (G.J.S. 10-118 = BPI 892691). I–J. Asci and ascospores in KOH and cotton blue (Guu 92122107 = BPI 892688). K–L. Conidiophores and macroconidia on SNA (G.J.S. 10-118 = CBS 134023). M. Colony on PDA (G.J.S. 10-118 = CBS 134023). N. Colony reverse on PDA (G.J.S. 10-118 = CBS 134023). Bars: A, H = 500 μm; B–E, I–L = 50 μm.

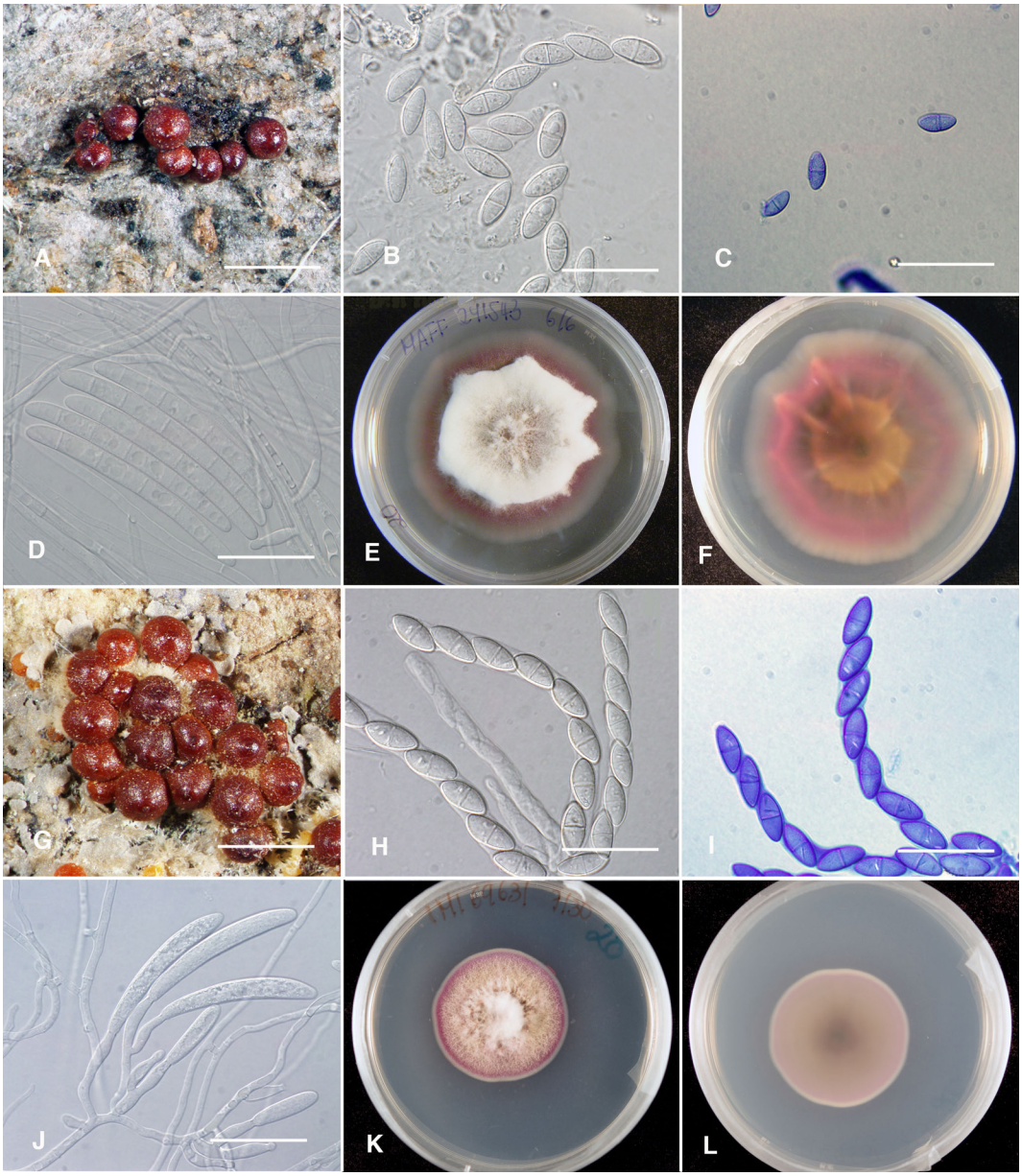

FIGURE 3.5. A–F. *Thelonectria japonica*. G–L. *Thelonectria mammoidea*. A. *T. japonica* perithecia (MAFF 241524 = BPI 882092). B, C. Asci and ascospores in KOH and cotton blue (MAFF 241524 = BPI 882092). D. Conidiophores and macroconidia on SNA (MAFF 241554). E. Colony on PDA (MAFF 241543). F. Colony reverse on PDA (MAFF 241543). G. *T. mammoidea* perithecia (G.J.S. 83-206 = PDD 46410). H, I. Asci and ascospores in KOH and cotton blue (G.J.S. 83-206 =

PDD 46410). J. Conidiophores and macroconidia on SNA (G.J.S. 86-206 = IMI 326258). L. Colony on PDA (IMI 69361). M. Colony reverse on PDA (IMI 69361). Bars: A, G = 500 µm; B–D, H–J = 50 µm.

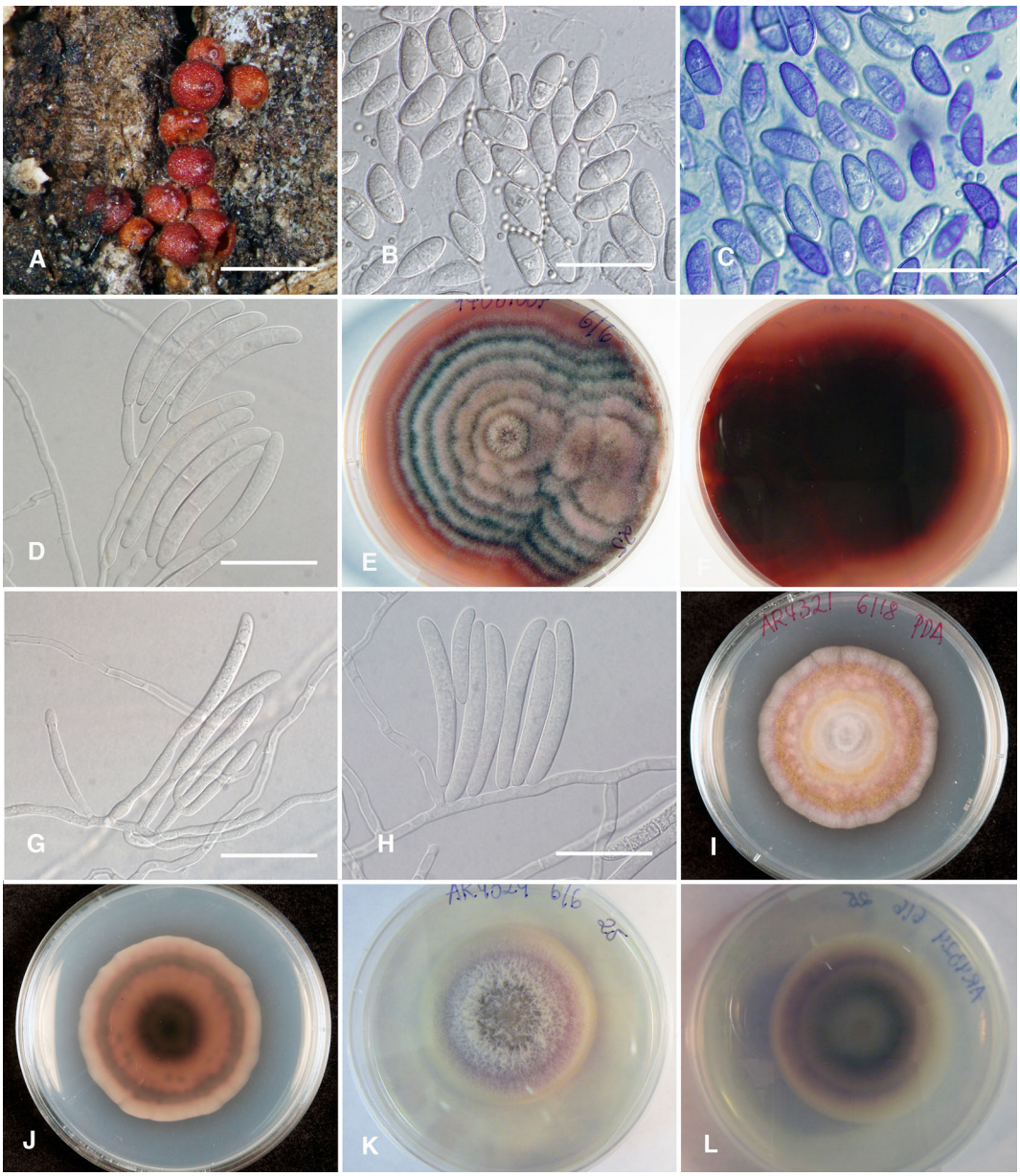

FIGURE 3.6. A–F. *Thelonectria phoenicea*. G–M. *Thelonectria pinea*. A. *T. phoenicea* perithecia (Guu 94031007 = BPI 892685). B, C. Asci and ascospores in KOH and cotton blue (Guu 94031007 = BPI 892685). D. Conidiophores and macroconidia on

SNA (Guu 94031007 = CBS 134039). E. Colony on PDA (Guu 94031007 = CBS 134039). F. Colony reverse on PDA (Guu 94031007 = CBS 134039). G–H. *T. pinea* conidiophores and macroconidia perithecia (A.R. 4321 = CBS 134033). I. Colony on PDA (A.R. 4321 = CBS 134033). J. Colony reverse on PDA (A.R. 4321 = CBS 134033). K. Colony on PDA (A.R. 4324 = CBS 125153). L. Colony on PDA (A.R. 4324 = CBS 125153). Bars: A = 500 μm; B–D, G–H = 50 μm.

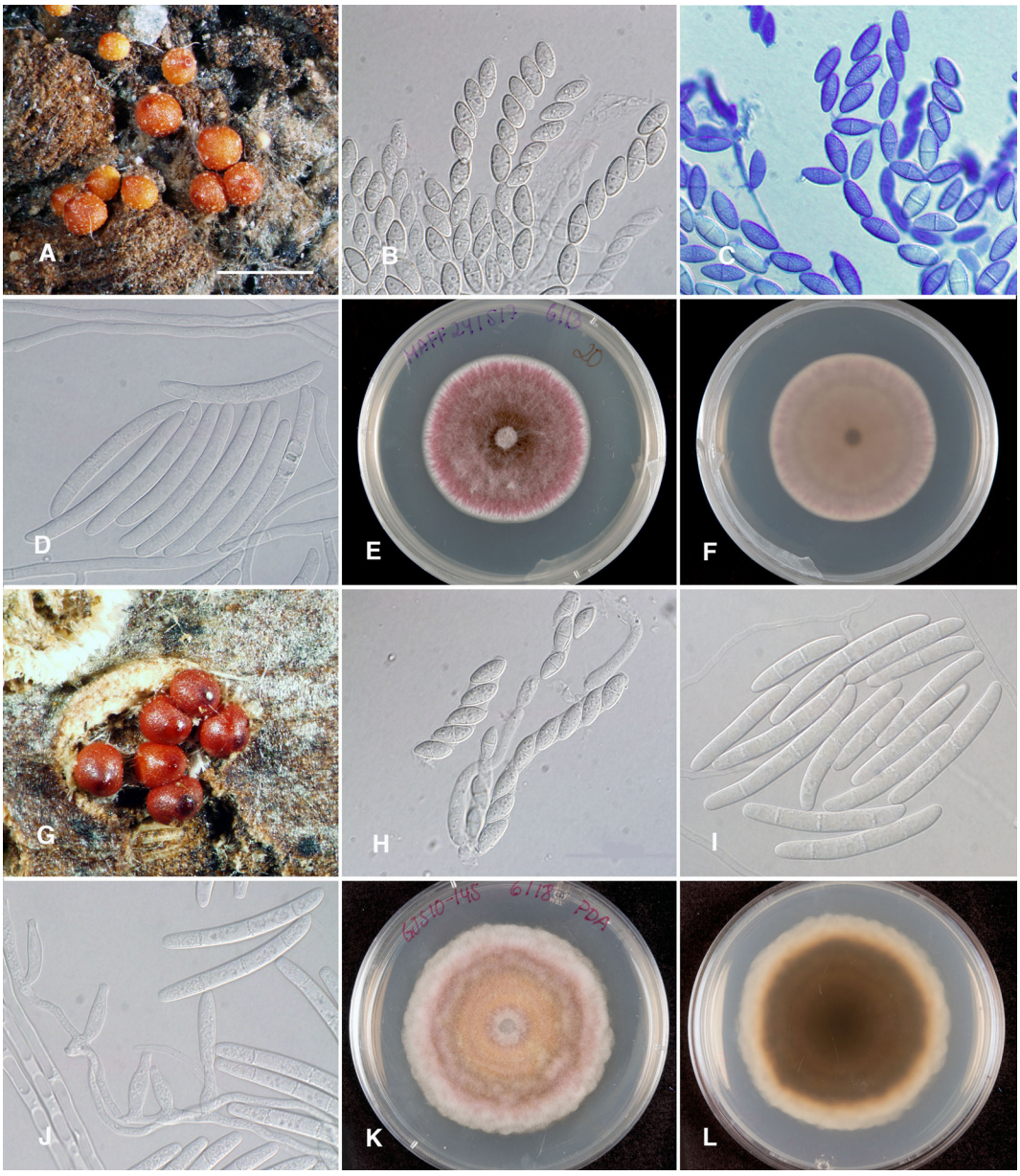

FIGURE 3.7. A–F. *Thelonectria porphyria*. G–L. *Thelonectria purpurea*. A. *T. porphyria* perithecia (MAFF 241515 = BPI 882162). B, C. Asci and ascospores in KOH and cotton blue (MAFF 241515 = BPI 882162). D. Macroconidia on SNA (MAFF 241539). E. Colony on PDA (MAFF 241517). F. Colony reverse on PDA (MAFF 241517). G. *T. purpurea* perithecia (G.J.S. 10-131 = BPI 892689). H. Asci and ascospores in KOH (G.J.S. 10-131 = BPI 892689). I, J. Conidiophores and macroconidia on SNA (G.J.S. 90-155 = CBS 123966). K. Colony on PDA (G.J.S. 10-145 = CBS 134025). M. Colony reverse on PDA (G.J.S. 10-145 = CBS 134025). Bars: A, G = 500 μm; B–D, H–J = 50 μm.

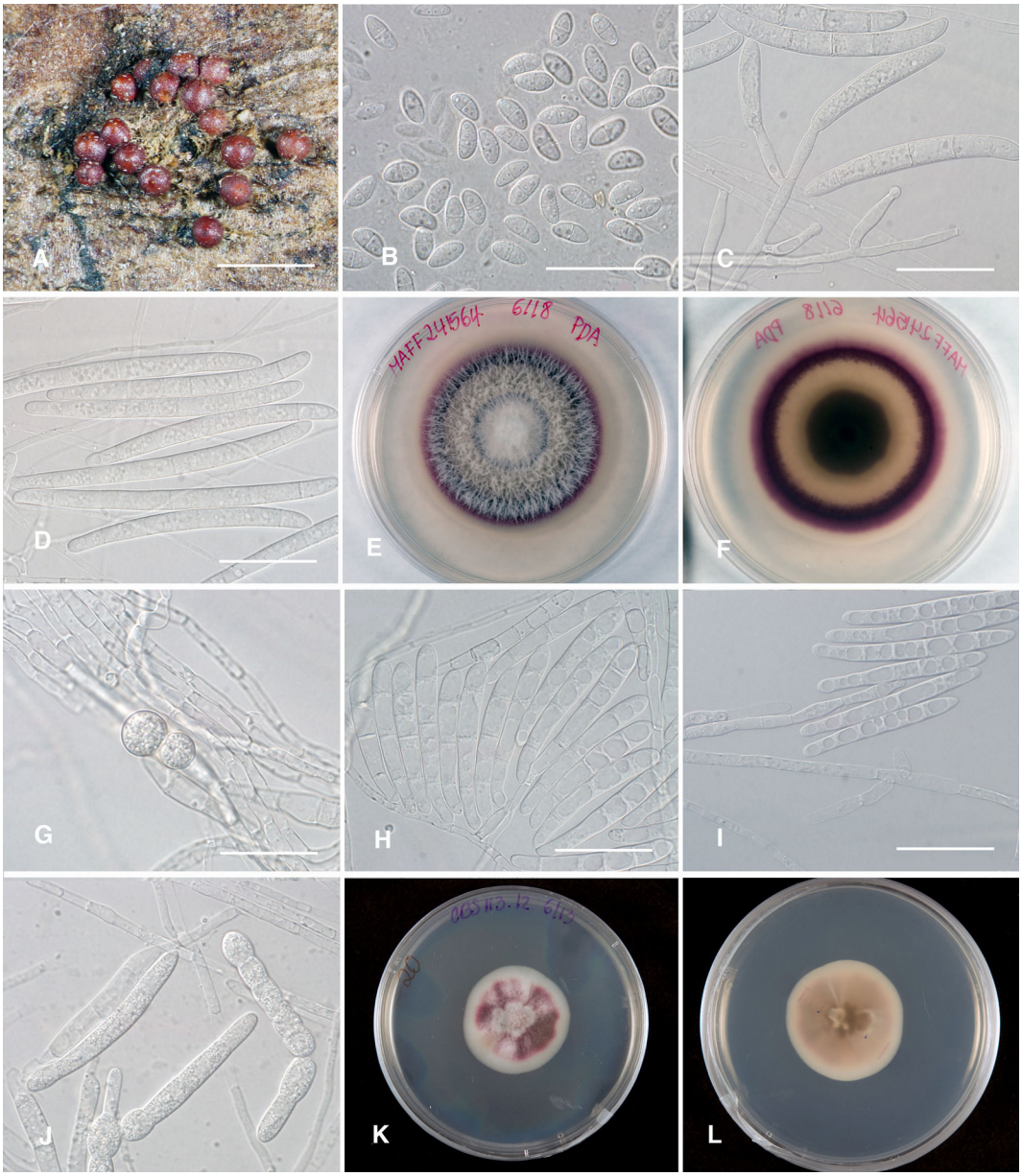

FIGURE 3.8. A–F. *Thelonectria purpurescens*. G–L. *Thelonectria rubi*. A. *T. purpurescens* perithecia (G.J.S. 96-23 = BPI 745542). B. Ascospores in KOH (G.J.S. 96-23 = BPI 745542). C–D. Conidiophores and macroconidia on SNA (MAFF 241564). E. Colony on PDA (MAFF 241564). F. Colony reverse on PDA (MAFF 241564). G. *T. rubi* chlamydospores on SNA (CBS 11312). H–I. Conidiophores and macroconidia on SNA (CBS 11312). J. Chlamydospores forming on macroconidia on

SNA (CBS 17727). K. Colony on PDA (CBS 11312). M. Colony reverse on PDA (CBS 11312). Bars: A = 500 µm; B–D, G–J = 50 µm.

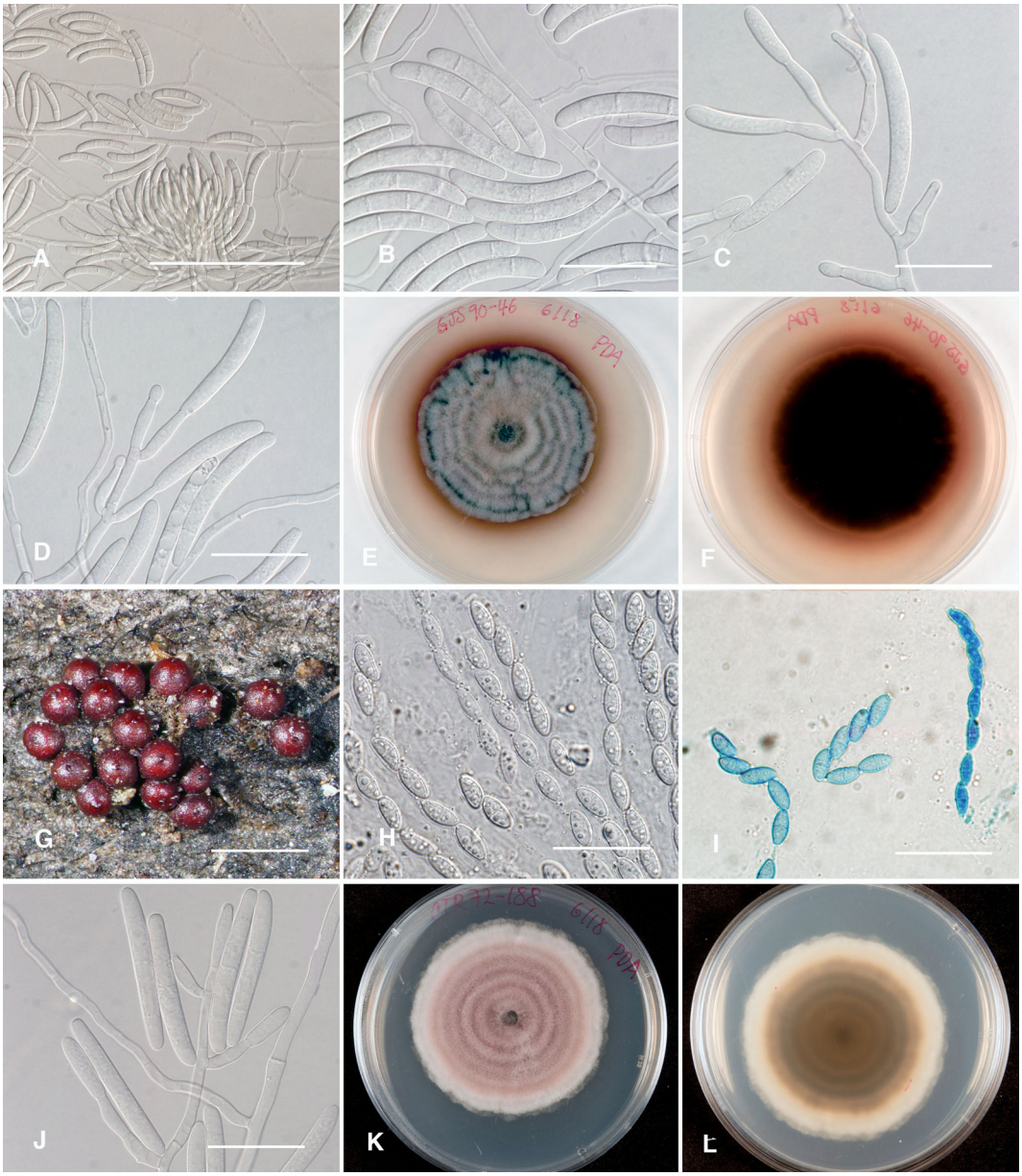

FIGURE 3.9. A–F. *Thelonectria tyrus*. G–L. *Thelonectria violaria*. A. *T. tyrus* pionnotes on SNA (G.J.S. 90-46 = CBS 134029). B–D. Conidiophores and macroconidia (G.J.S. 90-46 = CBS 134029). E. Colony on PDA (G.J.S. 90-46 = CBS 134029). F. Colony reverse on PDA (G.J.S. 90-46 = CBS 134029). G. *T. violaria*

perithecia (A.R. 4766 = BPI 892690). H–I. Asci and ascospores in KOH (A.R. 4766 = BPI 892690). J. Conidiophores and macroconidia on SNA (A.R. 4766 = CBS 134035). K. Colony on PDA (C.T.R. 72-188 = CBS 134040). M. Colony reverse on PDA (C.T.R. 72-188 = CBS 134040). Bars: A = 250 μm; B–D, H–J = 50 μm; G = 500 μm.

TABLE 3.1. Taxa used in this study, including information about the origin of the fungal material, collection codes and GenBank accession numbers.

Strain	Code	Host	Origin	GenBank accession numbers					
				act	ITS	LSU	*rpb1*	*tef1*	*tub*
Cyl. ianthothele var. *ianthothele*	CBS 11312	*Rubus idaeus*	Switzerland	KC121380	KC153718	KC121444	KC153911	KC153847	KC153783
Cyl. ianthothele	CBS 118612	*Quercus robur*	New Zealand	KC121381	KC153719	KC121445	KC153912	KC153848	KC153784
Cyl. ianthothele var. *ianthothele*	CBS 14277	On soil	Netherlands	KC121382	KC153720	KC121446	KC153913	KC153849	KC153785
Cyl. ianthothele var. *ianthothele*	CBS 17727	*Rubus idaeus*	England	KC121383	KC153721	KC121447	KC153914	KC153850	KC153786
Cyl. ianthothele var. *ianthothele*	CBS 24129	*Rubus idaeus*	Scotland	KC121384	KC153722	KC121448	KC153915	KC153851	KC153787
Cyl. ianthothele var. *minus*	CBS 26636	unknown	Germany	KC121385	KC153723	KC121449	KC153916	KC153852	KC153788
Cyl. ianthothele var. *majus*	CBS 28792	On soil	Brazil	KC121386	KC153724	KC121450	KC153917	KC153853	KC153789
Cyl. ianthothele var. *majus*	CBS 32881	Unknown	Switzerland	KF569826	KF569836	KF569845	KF569873	KF569854	KF569863
Cyl. ianthothele var. *majus*	CBS 95268	On soil	Germany	KC121387	KC153725	KC121451	KC153918	KC153854	KC153790
Neonectria mammoidea	C.T.R. 72-188 (= CBS 134040)	Unknown	Venezuela	KC121389	KC153727	KC121453	KC153920	KC153856	KC153792
Neonectria mammoidea	IMI 69361	*Smyrnium olusatrum*	UK	KC121425	KC153763	KC121489	KC153956	KC153892	KC153828
Nectria rubi	ICMP 14105	Unknown	New Zealand	KC121420	KC153758	KC121484	KC153951	KC153887	KC153823
Nectria pinea	ICMP 5287	Unknown	New Zealand	KC121421	KC153759	KC121485	KC153952	KC153888	KC153824
T. discophora	92122107 (=CBS 134038)	unknown	Taiwan	KC121373	KC153711	KC121437	KC153904	KC153840	KC153775
T. discophora	94031007 (=CBS 134039)	unknown	Taiwan	KC121374	KC153712	KC121438	KC153905	KC153841	KC153776
T. discophora	A.R. 4321 (=CBS 134033)	*Pinus radiata*	New Zealand	KC121375	KC153713	KC121439	KC153906	KC153842	KC153777

	(=CBS 125153)								
T. discophora	A.R. 4499 (=CBS 125172)	*Fagus grandifolia*	U.S	HM352877	HM364296	HM364309	HM364327	HM364347	HM364327
T. discophora	A.R 4742 (=CBS 134034)	*Tepualia stipularis*	Chile	KC121376	KC153714	KC121440	KC153907	KC153843	KC153779
T. discophora	A.R 4743 (CBS=XXX)	*Laureliopsis philipiana*	Argentina	KF569827	KF569837	KF569846	KF569874	KF569855	KF569864
T. discophora	A.R. 4766 (=CBS 134035)	Unknown	Argentina	KC121377	KC153715	KC121441	KC153908	KC153844	KC153780
T. discophora	A.R. 4794 (=CBS 134037)	Unknown	Argentina	KC121379	KC153717	KC121443	KC153910	KC153846	KC153782
T. discophora	C.T.R. 71-281 (=CBS 112458)	Unknown	Venezuela	KC121388	KC153726	KC121452	KC153919	KC153855	KC153791
T. discophora	C.T.R. 72-90	Unknown palm	Venezuela	KC121390	KC153728	KC121454	KC153921	KC153857	KC153793
T. discophora	G.J.S. 09-1327 (=CBS 134022)	Unknown	Venezuela	KC121391	KC153729	KC121455	KC153922	KC153858	KC153794
T. discophora	G.J.S. 09-509	*Acacia celsa*	Australia	KC121392	KC153730	KC121456	KC153923	KC153859	KC153795
T. discophora	G.J.S. 10-118 (=CBS 134023)	Unknown	Costa Rica	KC121393	KC153731	KC121457	KC153924	KC153860	KC153796
T. discophora	G.J.S. 10-131 (=CBS 134024)	Unknown	Costa Rica	KC121394	KC153732	KC121458	KC153925	KC153861	KC153797
T. discophora	G.J.S. 10-145 (=CBS 134025)	Unknown	Costa Rica	KC121395	KC153733	KC121459	KC153926	KC153862	KC153798
T. discophora	G.J.S. 83-188 (=IMI 326256)	*Fuchsia exorticata*	New Zealand	KC121396	KC153734	KC121460	KC153927	KC153863	KC153799
T. discophora	G.J.S. 83-206 (=IMI 326258)	Unknown	New Zealand	KC121397	KC153735	KC121461	KC153928	KC153864	KC153800
T. discophora	G.J.S. 85-179 (=IMI 329113)	Unknown	Indonesia	KC121398	KC153736	KC121462	KC153929	KC153865	KC153801
T. discophora	G.J.S. 85-187 (=ATCC 76478)	Unknown	Indonesia	KC121399	KC153737	KC121463	KC153930	KC153866	KC153802
T. discophora	G.J.S. 85-27 (=CBS 112457)	Unknown	New Zealand	KC121400	KC153738	KC121464	KC153931	KC153867	KC153803
T. discophora	G.J.S. 87-45 (=IMI 325855)	Unknown	Guyana	KC121401	KC153739	KC121465	KC153932	KC153868	KC153804

T. discophora	G.J.S. 87-49 (=CBS 112461)	Unknown	Guyana	KC121402	KC153740	KC121466	KC153933	KC153869	KC153805
T. discophora	G.J.S. 88-84 (=IMI 348190)	Unknown	China	KC121403	KC153741	KC121467	KC153934	KC153870	KC153806
T. discophora	G.J.S. 89-57 (=CBS 112459)	Unknown	Guyana	KC121404	KC153742	KC121468	KC153935	KC153871	KC153807
T. discophora	G.J.S. 89-60	Unknown	Guyana	KC121405	KC153743	KC121469	KC153936	KC153872	KC153808
T. discophora	G.J.S. 89-65 (=CBS 123970)	Unknown	Guyana	KC121406	KC153744	KC121470	KC153937	KC153873	KC153809
T. discophora	G.J.S. 89-71 (= CBS 134026)	Unknown	Guyana	KC121407	KC153745	KC121471	KC153938	KC153874	KC153810
T. discophora	G.J.S. 90-155 (=CBS 123966)	Unknown palm	Venezuela	KC121409	KC153747	KC121473	KC153940	KC153876	KC153812
T. discophora	G.J.S. 90-180 (=CBS 134027)	Unknown	Venezuela	KC121411	KC153749	KC121475	KC153942	KC153878	KC153814
T. discophora	G.J.S. 90-212 (=CBS 134028)	Unknown	Venezuela	KC121412	KC153750	KC121476	KC153943	KC153879	KC153815
T. discophora	G.J.S. 90-46 (=CBS 134029)	*Quercus* sp.	U.S	KC121413	KC153751	KC121477	KC153944	KC153880	KC153816
T. discophora	G.J.S. 92-34 (=CBS 134030)	Unknown	Scotland	KC121414	KC153752	KC121478	KC153945	KC153881	KC153817
T. discophora	G.J.S. 92-48 (=CBS 134031)	*Aesculus* sp.	Scotland	KC121415	KC153753	KC121479	KC153946	KC153882	KC153818
T. discophora	G.J.S 96-22 (=IMI 370946)	*Ocotea* sp.	Puerto Rico	KC121417	KC153755	KC121481	KC153948	KC153884	KC153820
T. discophora	G.J.S. 96-23 (=IMI 370947)	Unknown	Puerto Rico	KC121418	KC153756	KC121482	KC153949	KC153885	KC153821
T. discophora	IMI 329021	*Beilschmiedia tawa*	New Zealand	KC121422	KC153760	KC121486	KC153953	KC153889	KC153825
T. discophora	IMI 342455	Unknown	Kenya	KC121423	KC153761	KC121487	KC153954	KC153890	KC153826
T. discophora	IMI 384045	Unknown	New Zealand	KC121424	KC153762	KC121488	KC153955	KC153891	KC153827
T. discophora	MAFF 241515	Unknown	Japan	KC121426	KC153764	KC121490	KC153957	KC153893	KC153829
T. discophora	MAFF 241517	*Cryptomeria japonica*	Japan	KC121427	KC153765	KC121491	KC153958	KC153894	KC153830

T. discophora	MAFF 241524	Unknown	Japan	KC121428	KC153766	KC121492	KC153959	KC153895	KC153831
T. discophora	MAFF 241533	Unknown	Japan	KC121429	KC153767	KC121493	KC153960	KC153896	KC153832
T. discophora	MAFF 241539	Unknown	Japan	KC121430	KC153768	KC121494	KC153961	KC153897	KC153833
T. discophora	MAFF 241543	Unknown	Japan	KC121431	KC153769	KC121495	KC153962	KC153898	KC153834
T. discophora	MAFF 241554	Unknown	Japan	KC121432	KC153770	KC121496	KC153963	KC153899	KC153835
T. discophora	MAFF241563	*Fagus crenata*	Japan	KC121433	KC153771	KC121497	KC153964	KC153900	KC153836
T. discophora	MAFF 241564	Unknown	Japan	KC121434	KC153772	KC121498	KC153965	KC153901	KC153837
T. discophora	MAFF 241569	Unknown	Japan	KC121435	KC153773	KC121499	KC153966	KC153902	KC153838
T. discophora	MAFF 241576	Unknown	Japan	KC121436	KC153774	KC121500	KC153967	KC153903	KC153839
T. lucida	92112704 (=CBSXXX)	Unknown	Taiwan	KF569828	KF569838	KF569847	KF569875	KF569856	KF569865
T. lucida	94043002 (=CBSXXX)	Unknown	Taiwan	KF569829	KF569839	KF569848	KF569876	KF569857	KF569866
T. lucida	A.R 4781 (=CBS 134036)	Unknown	Argentina	KC121378	KC153716	KC121442	KC153909	KC153845	KC153781
T. lucida	G.J.S 86-249 (=IMI 325261)	*Philodendron* sp.	French Guiana	KF569830	KF569840	KF569849	KF569877	KF569858	KF569867
T. lucida	G.J.S 90-146 (=CBS 134032)	Unknown	Venezuela	KC121408	KC153746	KC121472	KC153939	KC153875	KC153811
T. lucida	G.J.S 90-166 (=CBS 126099)	Unknown	Venezuela	KC121410	KC153748	KC121474	KC153941	KC153877	KC153813
T. lucida	G.J.S 96-10 (=IMI 370944)	Unknown	Puerto Rico	KC121416	KC153754	KC121480	KC153947	KC153883	KC153819
T. lucida	G.J.S 96-35 (=CBS 112456)	Unknown	Puerto Rico	KC121419	KC153757	KC121483	KC153950	KC153886	KC153822
T. trachosa	G.J.S 85-50 (=CBS 119608)	Unknown	New Zealand	KF569831	KF569841	KF569850	KF569878	KF569859	KF569868
T. trachosa	G.J.S 92-45 (=CBS112467)	Unknown	Scotland	KF569832	KF529842	KF569851	KF569879	KF569860	KF569869

T. westlandica	G.J.S 83-156 (=CBS 112464)	*Dacrydium cupressinum*	New Zealand	KF569835	JQ403305	JQ403345	JQ403382	JQ394734	JQ394698
T. westlandica	G.J.S 85-45 (=CBS 132330)	Unknown	New Zealand	JQ365056	JQ403339	JQ403377	JQ403414	JQ394757	KF569872
T. westlandica	IMI 255610	*Metrosideros* sp.	New Zealand	KF569833	KF569843	KF569852	KF569880	KF569861	KF569870
T. westlandica	ICMP 10387	*Rosa* sp.	New Zealand	KF569834	KF569844	KF569853	KF569881	KF569862	KF569871

TABLE 3.2. List of molecular markers and descriptive statistics for the six loci used in this study.

Locus	Substitution model	Aligned length	Variable sites (%)	Parsimony informative sites (%)	%GC	Primers	Reference
act	GTR + I + G	550	20 (3.6)	60 (10.9)	56.6	F 5'TGGCACCACACCTTCTACAATGA3' R 5'TCCTCCGCTTATTGATATGC3'	Samuels *et al*. 2006
ITS	TPM2uf + I + G	723	24 (3.3)	107 (14.8)	55.4	F 5'GGAAGTAAAAGTCGTAACAAGG3' R 5'TCCTCCGCTTATTGATATGC3'	White *et al*. 1990
LSU	TrN + I + G	808	7 (0.8)	53 (6.5)	53.7	F 5'ACCCGCTGAACTTAAGC3' R 5'TCCTGAGGGAAACTTCG3'	Vilgalys n.d.
tef	TPM3uf + I + G	955	49 (5.1)	194 (20.3)	55.7	F 5'CATCGAGAAGTTCGAGAAGG3' R 5'ACHGTRCCRATACCACCRAT3'	Carbone & Kohn, 1999
tub	TrN + G	588	44 (7.4)	172 (29.2)	56.5	F 5'AACATGCGTGAGATTGTAAGT3' R 5'TAGTGACCCTTGGCCCAGTTG3'	O'Donnell & Cigelnik, 1997
rpb1	TrN + I + G	642	29 (4.5)	230 (35.8)	53.3	F 5'CAYCCWGGYTTYATCAAGAA3' R 5'CCNGCDATNTCRTTRTCCATRTA3'	Castlebury *et al*. 2004

TABLE 3.3. Nucleotide divergence (Dxy*) for all pairwise comparisons of putative species identified within *T. discophora* species-complex. All positions containing gaps were eliminated for a total of 3559 positions. Numbers across the top row correspond to putative species numbers in the first column.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1. *T. purpurea*																
2. *T. violaria*	0.004															
3. *T. brayfordii*	0.006	0.008														
4. *T. pinea*	0.006	0.008	0.002													
5. *T. japonica*	0.008	0.010	0.007	0.007												
6. *T. ianthina*	0.013	0.015	0.009	0.009	0.013											
7. *T. conchyliata*	0.014	0.013	0.012	0.012	0.013	0.015										
8. *T. phoenicea*	0.016	0.015	0.014	0.013	0.013	0.017	0.008									
9. *T. porphyria*	0.012	0.012	0.011	0.010	0.011	0.014	0.009	0.008								
10. *T. tyrius*	0.013	0.012	0.012	0.011	0.012	0.016	0.009	0.009	0.005							
11. *T. purpurescens*	0.026	0.026	0.025	0.025	0.026	0.028	0.023	0.024	0.022	0.023						
12. *T. blattea*	0.035	0.036	0.034	0.033	0.033	0.037	0.034	0.034	0.031	0.033	0.034					
13. *T. mammoidea*	0.061	0.062	0.060	0.059	0.059	0.063	0.061	0.060	0.058	0.058	0.064	0.057				
14. *T. discophora*	0.057	0.058	0.056	0.055	0.055	0.058	0.057	0.055	0.054	0.056	0.059	0.052	0.031			
15. *T. asiatica*	0.054	0.055	0.053	0.052	0.052	0.054	0.053	0.052	0.051	0.052	0.056	0.048	0.028	0.015		
16. *T. rubi*	0.044	0.045	0.043	0.042	0.044	0.046	0.043	0.044	0.042	0.043	0.048	0.039	0.046	0.044	0.040	

* $p < 0.001$

Chapter 4: Molecular phylogeny and taxonomic revision of the fungal genus *Thelonectria* (Nectriaceae, Hypocreales, Ascomycota) and related species with *Cylindrocarpon*-like anamorphs inferred from eight nuclear markers.

Abstract

Species in the genus *Thelonectria* and related clades with cylindrocarpon-like anamorphs are among the most commonly collected members of the family Nectriaceae in the order Hypocreales. These species are characterized by having red globose to pyriform perithecia with a prominent, areolate and darkened papilla, and by producing cylindrocarpon-like morphologies with macroconidia 3–7-septate. They can be found in tropical, subtropical and temperate regions where they are among the first colonizers of decaying and dead plant matter. Even though efforts are being made to discover and describe microfungi, the diversity of these species and their relationships is still poor known. In this study we present a phylogenetic analysis of the genus *Thelonectria* and related species with cylindrocarpon-like anamorphs or with uncertain taxonomic classification. We sequenced eight partial nuclear loci (*act*, ITS, LSU, *tef*, *tub*, *rpb1*, *rpb2*, SSU) for a total of 5707 bp from 204 taxa, including outgroups and analyzed them using Maximum Likelihood and Bayesian Inference approaches. We determined that the genus *Thelonectria* is paraphyletic as it is presently known. One of its most common species, *T. jungneri*, as well as *T. viridispora* do not belong to *Thelonectria*. Based on these results, we transferred *T.*

jungneri to a new genus and address the possible phylogenetic position of *T. viridispora*. *Thelonectria* is primarily composed of complexes of morphologically similar species, for example *T. coronata, T. discophora, T. lucida* and *T. veuillotiana*. Only one species complex, *T. lucida*, has not been included in previous studies and it is here described, together with three additional taxonomic novelties for the genus. Additionally, two new genera are created to accommodate *Nectria laetidisca* and *Neonectria cinnamommea*, two groups of species closely related to *Thelonectria*, which also produce cylindrocarpon-like anamorphs. This study helps to clarify species relationships, and the systematic and taxonomic implications stemming from morphologically similar species are discussed.

Key words: Biodiversity, cryptic species, genera, Nectriaceae, molecular systematics, taxonomy.

Introduction

Thelonectria P. Chaverri & C. Salgado (2011) is a genus in the Nectriaceae described to accommodate species in the morphological groups *Nectria mammoidea* and *N. veuillotiana* (Brayford et al. 2004, Brayford and Samuels 1993, Chaverri et al. 2011). *Thelonectria discophora* (Mont.) P. Chaverri & C. Salgado (2011) was designated as the type species of the genus, based on material collected in Chile. Traditionally, this genus groups species of fungi having a sexual state producing bright red, uniloculate perithecia with a prominent, sometimes darkened papilla, unitunicate asci and one-septate ascospores (Chaverri et al. 2011, Salgado-Salazar et al. 2012). The prominent, sometimes darkened papilla is the character from which the name *Thelonectria* is derived. The cylindrocarpon-like asexual states of species in this genus were once

segregated by Booth (1966) in the morphological groups 2 and 4, whose main characteristics were the absence of microconidia and chlamydospores (Booth 1966, Brayford et al. 2004). Even though recent molecular phylogenetic studies (Chaverri et al 2011, Salgado-Salazar et al. 2012, Salgado-Salazar et al. 2013) supported previous morphological classification of species based on the sexual state morphology, they rejected the classification based on the asexual state, as more variability in morphology is evident in these species than previously reported. At present the morphology of *Cylindrocarpon* sensu stricto is restricted to species of *Neonectria*, thus names of *Cylindrocarpon* previously applied in a broad sense, for example, those related to species in the genus *Thelonectria*, need to be redefined (Rossman et al. 2013). Since the description of *Thelonectria* (Chaverri et al. 2011), 22 species have been described to date (http://www.indexfungorum.com), with most of the species known previously belong to a cryptic group of morphologically similar but distinct species, for example *T. coronata* and *T. veuillotiana* complexes (Salgado-Salazar et al. 2012).

Thelonectria is a cosmopolitan genus, with species abundant in temperate, subtropical and tropical regions, where they can be found as saprobes on bark of recently dead or dying trees, roots and soil (Chaverri et al. 2011, Salgado-Salazar et al. 2012, 2013, Guu et al. 2007). Species of *Thelonectria* are frequently found in areas and seasons of high humidity and warm temperatures, mostly in disturbed forests with decaying plant material (Chaverri and Vilchez 2006). So far, only one species, *T. rubi*, as been reported as pathogen of *Rubus* species as it causes a distinctive root and crown canker (Brayford 1990, Cedeño et al. 2004, Salgado-Salazar et al. 2013). Even

though isolates of *T. olida* can be found on rotting roots of various plants (Booth 1966, Chaverri et al. 2011), there is no indication that the species is involved in any pathogenesis. The majority of species in *Thelonectria* have been collected in the sexual state, i.e. perithecial fructifications not associated with plant disease symptoms. Species with cylindrocarpon-like asexual states isolated from plants and soil and thought to be the causal agent of several plant diseases belong in the related genus *Ilyonectria*, for example, *I. liriodendra* and *I. radicicola* (Cabral et al. 2012). The cylindrocarpon-like asexual states of *Thelonectria*, such as *T. blattea* (Salgado-Salazar et al. *submitted*) and '*Cylindrocarpon*' *theobromicola* (Booth 1966) are not associated with any plant disease.

After examining closely the species of *Thelonectria*, no cosmopolitanism has been found, and to date, several of species previously labeled as cosmopolitan (*T. coronata*, *T. discophora* and *T. veuillotiana*) have been determined to be complexes of genetically divergent but morphologically similar species (Salgado-Salazar et al. 2012, 2013). As a result, these three species have been redefined and a total of four, six, and fifteen segregate species with morphology similar to the type species have been described, respectively. The geographic distribution of species in these groups varies from local to regional or continental (Salgado-Salazar et al. 2012, 2013). Interestingly, in the studies of species complexes it was determined that, although rare, some monophyletic species include isolates from distant geographic locations (See Salgado-Salazar et al. 2012; 2013). Even though species known from close geographic regions, such as China, Japan or Taiwan, are easily considered to be

capable of limited dispersal, the dispersal mechanism for species known from distant geographic locations is obscure.

In the last decade with the aid of molecular phylogenetic techniques, studies by Chaverri et al. (2011) and Salgado-Salazar et al (2012, 2013) have begun to clarify species in the genus *Thelonectria* including those with cylindrocarpon-like morphology. In the above-mentioned studies, and in the majority of cases in fungi, a phylogenetic species concept derived from the general lineage concept (GLC) (de Queiroz, 2007) is applied, as it can define evolutionarily independent lineages in taxonomic groups with little morphological variation (Linde et al. 2013). Following the overview study by Chaverri et al. (2011), species having a neonectria/cylindrocarpon-like morphology that were not included in the first accounts needed further circumscription at both the generic and species level. In this section, we present a comprehensive phylogeny of *Thelonectria* and other closely related species with *cylindrocarpon*-like anamorphs of uncertain taxonomic classification. Based on the analyses of eight molecular loci, we tested the monophyly of newly defined genera and species, and examined species relationships comparing genetic diversity and morphology within these groups of fungi. Based on these results, we describe here three novel genera and eight previously undescribed species, emphasizing the diversity of mostly cryptic species in this group and its importance in understanding patterns of diversity within fungi in the Nectriaceae, Hypocreales.

Materials and Methods

Fungal isolates

Specimens and cultures were obtained from CABI Bioscience (IMI), Centraalbureau voor Schimmelcultures (CBS), International Collection of Microorganisms from Plants (ICMP), New Zealand Fungal and Plant Disease Collection (PDD), Japanese Ministry of Agriculture, Fisheries and Food Collection (MAFF), New York Botanical Garden (NY), and U.S. National Fungus Collection (BPI, G.J.S, A.R., now deposited in CBS). A total of 204 isolates were used in this study, including recognized species in the genus *Thelonectria*, and also other species with cylindrocarpon-like anamorphs and with uncertain classification (TABLE 4.1). In order to accurately assess the relationships among members of this group, sequences from species in the genera *Campylocarpon*, *Ilyonectria*, *Neonectria*, *Rugonectria*, among others were obtained from publicly available databases. Representative members of the sister family Bionectriaceae, specifically *Nectriopsis exigua*, *Hydropisphaerea fungicola*, *Selinia pulchra* and *Verrucostoma freycinetiae*, were used as outgroup in the phylogenetic analyses.

DNA extraction, PCR, loci and DNA sequencing

Eight sequence loci were amplified and sequenced for *Thelonectria* species: partial large nuclear ribosomal subunit (LSU, *ca*. 900 bp), complete internal transcribed spacers 1 and 2 (ITS, including 5.8S of the nuclear ribosomal DNA, *ca*. 600 bp), partial β-tubulin (*tub*, *ca*. 500 bp), α-actin (*act*, *ca*. 600 bp), RNA polymerase II subunit 1 (*rpb1*, *ca*. 700 bp), RNA polymerase II subunit 2 (*rpb2*, *ca*. 600 bp),

translation elongation factor 1α (*tef1*, *ca*. 700 bp), and small nuclear ribosomal subunit (SSU, *ca.* 600 bp) (TABLE 4.2). Protocols for PCR for *act*, ITS, LSU, *rpb1*, *tef* and *tub* were carried out as described by Chaverri et al. (2011), for *rpb2* as described by Liu et al. (1999) and for SSU as described by Gargas and Taylor (1992). Clean PCR products for *act*, ITS, LSU, *rpb1*, *tef* and *tub* were sequenced in both directions at the University of Maryland DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland, USA). PCR products for *rpb2* and SSU were sequenced with the BigDye Terminator 3.1 cycle sequencing kit (Applied Biosystems, Foster City, California) on an Applied Biosystems 3130xl Genetic Analyzer at the Systematic Mycology and Microbiology Laboratory in Beltsville, Maryland, USA. Sequences were assembled and edited using the program Sequencher 4.9 (Gene Codes, Madison, Wisconsin, USA).

Sequence alignment

Sequences with different rates of evolution represent a challenge during alignment and phylogeny reconstruction. Even though the dataset contains representatives of just one taxonomic family (Nectriaceae), highly divergent sequences could be found, especially for the non-coding regions of the ITS, *tef*, *tub* and *rpb1*. To account for this, we used SATé-II (Liu et al. 2012) to reconstruct alignments, as it is a highly efficient approach to produce accurate alignments. For each locus, we used MAFFT as aligner, MUSCLE as merger and FASTTREE as tree estimator, GTR + G20 as models, with an extra RAxML search. The rest of SATé settings were left to default. We used the program Gblocks v.0.91 (Talavera and Castresana 2007) in order to examine whether phylogenetic reconstruction improves after problematic regions of

the alignment (very divergent sequence regions with many gaps and/or differences in length) have been deleted. For this, each locus alignment obtained using SATe-II was profiled using two different parameters in Gblocks, one with the default parameters (stringent, Gblocks-1) and the other with relaxed selection (less stringent, Gblocks-2), following Talavera and Castresana (2007). Parameters and resulting characteristics of alignments are found in TABLE 4.3. The resulting alignments after problematic regions have been deleted were concatenated and the phylogenetic analyses results were compared with those obtained when the alignments have not been cleared of problematic regions.

Phylogenetic reconstruction

JModeltest v 0.1.1 (Posada 2008) was used to determine the best nucleotide substitution model using AIC criteria (TABLE 4.2). For the concatenated analyses, we used a partitioned approach with model parameters estimated previously. Total evidence phylogenetic trees from the original and two different Gblocks alignments were estimated by Maximum Likelihood and Bayesian approaches. Bayesian phylogenetic trees were obtained using MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003). The analyses were initiated from random starting trees, run for 10 million generations with four chains (Metropolis-coupled Markov Chain Monte Carlo, Huelsenbeck and Rannala 2004) and sampled at intervals of 1000 generations. Default priors were used in all analyses. Two independent BI analyses were run. To evaluate stationarity and convergence between runs, log-likelihood scores were plotted using TRACER v. 1.5 (Rambaut and Drummond 2007). Maximum likelihood (ML) analyses were performed in RAxML (Stamatakis 2006), using the RAxML GUI

vs. 1.1.1 (Silvestro and Michalak 2011). Branch support was assessed with 1000 nonparametric bootstrapping replicates using the same model parameters settings than BI analyses. Final trees were visualized with FigTree v1.3.1 (Rambaut 2005).

Morphological studies and statistical analyses of morphological characters

For the morphology of species in the *Thelonectria* and related groups, measurements of continuous characters of both sexual and asexual structures (length, width) were taken as described in materials and methods section of Chapter 1 (Pag. 10).

Results

Sequence data

The Bayesian and Maximum Likelihood model of sequence evolution is displayed in TABLE 4.2. The concatenated dataset was 5707 bp and all novel sequences were submitted to Genbank (TABLE 4.1). Characteristics of the alignments for each of the eight loci before and after cleaning with Gblocks are presented in TABLE 4.3. When the alignments were cleaned of problematic regions using the stringent setting in Gblocks (Gblocks-1, TABLE 4.3), a reduction of the length of the alignment from 17 to 82% was generally observed. This is particularly evident for loci recognized to have gaps and uncertain positions (ITS, *rpb1*, *tef*, *tub*), due to the presence of highly divergent sequences. Alignments cleaned of problematic regions using the less stringent setting in Gblocks (Gblocks-2, TABLE 4.3) also resulted in the reduction of the total length of the alignment, however to lower extent, from 11 to 30%.

Phylogenetic reconstruction

The quality of a sequence alignment has a large impact on the final phylogenetic tree such that the inferred phylogeny may be more dependent on the methods of alignment than on the methods of phylogenetic reconstruction (Wu et al. 2012). In this section we observed that the phylogenetic reconstruction of relationships obtained using the original concatenated alignment of loci i.e. before cleaning and that obtained after cleaning of problematic regions using Gblocks-2 settings produced the same tree topology and species relationships (FIG. 4.1, S4.1). However, when the alignments were cleaned using Gblocks-1 settings, the tree topology, species relationships and resolution for some terminal groups differed from those of the original alignment and Gblocks-2, using both Bayesian Inference and Maximum Likelihood approaches (FIG. S4.1). In general, stringent parameters (Gblocks-1) used to remove uncertain positions produced a decrease in the number of phylogenetically informative sites across the alignment (TABLE 4.3). Interestingly, even though relationships among species were different using of Gblocks-1 alignments compared to those obtained with the original alignment, the resulting clades had significant bootstrap support (FIG. S4.1). Three loci (LSU, *rpb2*, SSU) had no alteration in the number of parsimony informative sites after cleaning of alignments using Gblocks-2 settings, and *act*, *tef* and *tub* increased from 2 to 10 % the number of informative sites after G-blocks-2 cleaning (TABLE 4.3). Although some internal branches showed a decrease in support when comparing results of the analyses of the original alignment before cleaning and after cleaning with Gblocks-2 settings, support for the terminal (species) nodes remained significant. Branch support for species relationships determined using

Bayesian Inference and Maximum Likelihood were different, with bigger values observed for the Bayesian Inference (Maximum Likelihood tree not shown).

Monophyly of the genus Thelonectria *and relationships among species with* Cylindrocarpon*-like anamorphs*

The genus *Thelonectria* as it was defined by Chaverri et al (2011) could not be recovered as a monophyletic group because two species, *T. jungneri* and *T. viridispora,* were separated from the type and other species (FIG. 4.1). The remaining species of *Thelonectria* form a monophyletic group, in which two main subgroups are evident. One subgroup consists of species in the *T. discophora* complex, *T. lucida* complex and *T. trachosa* while the other subgroup includes species in the *T. coronata* and *T. veuillotiana* complexes and *T. westlandica*. All clades within the *Thelonectria* ingroup have significant bootstrap (≥ 90%) and posterior probability (≥0.95) support (FIG. 4.1). Additionally, three previously recognized species in *Cylindrocarpon* and *Nectria* (*C. arcuatum*, *C. theobromicola* and *Nectria platycephala*) were included in *Thelonectria* and should be recognized in this genus. One species, *T. rubi*, previously described as part of the *T. discophora* complex (Salgado-Salazar et al. *submitted*), was here determined to be more closely related to species in the *T. lucida* complex, distant from species in *T. discophora* complex. Even though *T. jungneri* shows the typical *Thelonectria* morphology in its sexual and asexual states, such as the mammiform round perithecial apex, it does not appear to have been derived from the same common ancestor as other species in *Thelonectria* (FIG. 4.1). In this study, we determined that the *T. jungneri* complex is genetically divergent from *Thelonectria.* In addition, the related genera *Campylocarpon, Rugonectria*, and one new genus for

isolates of *Nectria laetidisca* (Rossman 1983, FIG. 4.1) appear basal to *Thelonectria* and separate them from the distinct *T. jungneri* species complex. As determined for other species in *Thelonectria* such as *T. coronata*, *T. discophora* and *T. veuillotiana* (Salgado-Salazar et al. 2012, 2013), *T. jungneri* and *T. lucida* form a complex of species, with few morphological characters useful for species delimitation. *Thelonectria viridispora* (≡*Neonectria viridispora*), a species known only from the type collection (Ecuador), falls completely outside of *Thelonectria* sensu lato. Previous studies by Chaverri et al (2011) suggested that this species was related to *Thelonectria* based on the morphology of the cylindrocarpon-like macroconidia and lacking microconidia (Brayford et al. 2004). Our phylogenetic analyses determined that *T. viridispora* may be more closely related to *Fusarium* (FIG. 4.1). Among the clades separating *T. jungneri* from *Thelonectria* sensu stricto, a strongly supported monophyletic group was formed by isolates of *Nectria laetidisca* (Rossman 1983). Species in this group produce cylindrocarpon-like morphologies as the asexual state, in a similar way that in *T. jungneri*, perithecia of the sexual state have a mammiform perithecial apex (FIG. 4.18). Lastly, *Neonectria cinnamomea* also appears basal to *Thelonectria* and other species having cylindrocarpon-like anamorphs. Its branch support and position in the tree suggests it constitutes a distinct genus (FIG. 4.1). *Viridispora alata* appears as a sister genus to *N. cinnamomea*, however, *V. alata* is characterized by having *Penicilifer* anamorphs (Rossman et al. 1999). Only a few were detected in *Thelonectria* and related genera in addition to those undescribed singletons in Salgado-Salazar et al. (2012, 2013). The majority of singletons fell in

the *T. jungneri* group and two singletons closely related to *T. trachosa* and *T. westlandica* (FIG. 4.1).

Comparative morphology

In this study, we refer to the morphology of the asexual state as cylindrocarpon-like, because the species included here display morphologies similar to species in the genus *Neonectria* sensu stricto that have true *Cylindrocarpon* asexual states. The asexual spores of species in *Thelonectria* and related groups are produced on cylindrical conidiogenous cells (phialides) that can be solitary and apical, or arranged in groups on branching conidiophores arising from vegetative hyphae. The morphology of conidiogenous cells is conserved across species having a cylindrocarpon-like morphology as well as in those having true *Cylindrocarpon* morphologies. Septate macroconidia and microconidia are produced by these species, however, microconidia are produced in culture by only a few species. Macroconidia can be cylindrical or slightly curved with round apical and basal cells, and contrary to microconidia, they do not show a basal abscission scar. Macroconidial septation varies among species serving as a useful morphological character for defining species within a single group. For example, *Neonectria cinnamomea, T. rubrococca* and *T. theobromicola* produce macroconidia that are exclusively 3-septate (FIG. 4.8, 4.9, 4.18), while isolates in *T. jungneri* produce 5-8-septate macroconidia, and *T. lucida* isolates produce 3-7-septate macroconidia. Although hypothetically these species have the potential to produce any macroconidial septation, the evolutionary pressures that favor one septation pattern over another are not known. Macroconidial septations have been lost and gained several times over time. Colony appearance and coloration

patterns vary from group to group, however, they are not exclusive to one group or another. For example, species in the *T. discophora* complex generally exhibit a purple coloration of the colony, however, some isolates can display colors like white-green to white-brown, which are also typical of species in the *T. coronata* and *T. veuillotiana* groups. Species in the *T. jungneri* group have colonies that range from white to yellow and green, and species in *Neonectria cinnamomea* produce a colony whose color ranges from white to cinnamon. Asexual states of all species present some degree of pigmentation and exudates when grown in culture that usually correspond to the dominant color of the colony. In this way, species in *T. discophora* complex produce purple pigments, species in *T. coronata* and *T. veuillotiana* produce green-brown pigments, species in *N. cinnamomea* produce cinnamon pigments.

Some differences in the anatomy of the perithecial cell wall can be observed among *Thelonectria* and related species. A perithecial wall consisting of an outer region with a palisade structure formed by short hyphae perpendicular to the locule is typical of species in the *T. discophora* and *T. lucida* complexes. In some species such as *N. cinnamomea*, *T. trachosa* and *T. westlandica*, a superficial layer of large, angular cells sometimes resembling warts occurs near the perithecial apex obscuring the palisade-like layer. In the case of *N. cinnamomea*, these cells are loosely connected and can be easily detached (FIG. 4.12). The lack of this layer of angular cells gives the perithecia a smooth and shiny appearance, that otherwise looks roughened and matte. Species in the *T. coronata* and *T. jungneri* complexes have a perithecial wall lacking any apparent cellular structure and formed by intertwined hyphae having a seemingly random arrangement. Most of the species in *Thelonectria*,

except *T. trachosa* and *T. westlandica* have a swollen perithecial apex of varying size, a characteristic reflected in the generic name. Species in the *T. coronata* and *T. veuillotiana* complexes are very distinctive due to this character. In the *T. veuillotiana* complex, the apex is flat, knobby and constricted at the base, most of the times reaching 3/4 of the perithecial diameter. In the *T. coronata* complex, a layer of saccate cells form a distinctive fringe around the perithecial apex giving it a coronate aspect (Salgado-Salazar et al. 2012). *Thelonectria jungneri* and "*Nectria" laetidisca* also display this feature. In the *T. jungneri* complex species have a darker color at the apex that the rest of the perithecia, and in *N. laetidisca* the perithecia become rounded and more constricted at the base (FIG. 4.13–4.17). After the observation that species of the *T. jungneri* complex and *N. laetidisca* did not belong in *Thelonectria* sensu stricto, it became evident that a knobby, nipple-like perithecial apex is not a synapomorphic character exclusive of *Thelonectria*, as it was present in the ancestor of all these species, and has been lost at least three times in the course of species diversification. With the exception of *N. laetidisca*, which has 3-septate ascospores, all species of *Thelonectria* and related genera have 1-septate ascospores. Ascospore sizes show differences that correlate with groups of species. The smallest ascospores are found in *T. coronata* and *T. veuillotiana*, followed by species in the *T. discophora* complex. The largest ascospores occur in *N. cinnamomea*, *T. jungneri* and *T. lucida* species complexes. With the exception of *N. cinnamomea*, ascospores are hyaline, often displaying a yellowish coloration as ascospores grow old, and have a surface covered by spinules, sometimes closely packed and arranged linearly, giving the appearance of a striated surface. '*Neonectria' cinnamomea* produces ascospores with

a conspicuous yellow-brown sheath, appearing wrinkled and giving the ascospores a rugose appearance (FIG. 4.12).

Geographic distribution, ecology and host specificity

Species of *Thelonectria* and related groups are distributed over a wide range of geographical locations, including temperate, subtropical and tropical regions (TABLE 4.1). The regions where these species have been found are characterized by being climatically and geomorphologically stable, and have been hypothesized to more likely accumulate cryptic diversity than more spatio-environmentally dynamic regions (Singhal and Moritz 2013). According to the geographic distribution of individuals within species, three different categories are recognized. The first one includes species with restricted geographic distribution. In *Thelonectria* only a few species, such as *T. amamiense*, *T. acrotyla*, *T. brayfordii*, *T. japonica*, *T. pinea*, *T. trachosa*, *T. tyrus*, and *T. westlandica* have a restricted geography, encountered in only one or several regions in one country or a restricted location (FIG. 4.1, TABLE 4.1). The second category includes species whose range expands to two or several adjacent regions or countries. In this category, species such as *T. conchyliata* in the *T. discophora* complex, and species in *T. veuillotiana*, *T. jungneri* complexes and '*Neonectria' cinnamomea* complex were found in several adjacent countries or regions, e.g. *N. cinnamomea* was collected in Guyana, French Guiana, and northern Brazil. The third category includes species with isolates from different and distant geographic locations. In this category falls, for example, *T. discophora*, *T. ianthina*, *T. mammoidea*, and *T. purpurescens*. Possible explanations for this type of distribution are highlighted in the discussion.

With the exception of *T. rubi*, which can cause root canker on *Rubus* species, all species studied have been found as saprobes on fresh organic plant material or soil. Even though species such as *T. olida*, which are known from rotting tissues of *Asparagus* sp., *Solanum tuberosum* and others, and '*Cylindrocarpon' theobromicola*, which has been associated with *Theobroma cacao* (Booth 1966), there is no evidence that they are pathogenic on the associated plants. As for most species in *Thelonectria* and related groups, these fungi colonize senescent tissues or plants affected previously by pathogens, possible becoming secondary pathogens, or just simply taking advantage of weakened tissues. Due to the lack of detailed information about host associations it is difficult to test any hypotheses of host specificity. Only three species out of this large group, "*Nectria" laetidisca*, *T. pinea* and *T. rubi,* show host specificity being encountered on bamboo (subfamily Bambusoideae), *Pinus radiata* and raspberry (*Rubus* sp.), respectively. Other species in *Thelonectria* and related taxa along with many species in the family Nectriaceae have the potential to grow on any organic plant material if environmental conditions are favorable.

Taxonomy

In this section, we describe formally three new genera, nine new species, including four in the *T. lucida* complex and one in the *T. discophora* complex, and make three new combinations based on the new generic circumscriptions. In addition, species with no change in taxonomy are described and illustrated. Singleton species i.e. single isolate lineages are not described unless the isolate is the type isolate of a previously named species, for example, *Cylindrocarpon arcuatum* (FIG. 4.1), for which the taxonomic classification is updated. Species in the *T. coronata* and *T. veuillotiana*

complexes are described in Chapter 1, and species in the *T. discophora* complex are described in Chapter 3.

***Thelonectria discophora* species complex**

Thelonectria mamma C. Salgado & P. Chaverri, sp. nov.

FIG. 4.2

Mycobank TBD.

Similar to *T. discophora*, microconidia present, macroconidia 1–6 septate.

Holotype. TAIWAN. TAIPEI COUNTY: Chou-tou-shan, on unidentified bark, 30 Apr 2005, Guu, J.-R (BPI XX, ex-type culture 994043002 = CBS 136787).

Etymology. Refers to the papillated aspect of perithecial apex.

Mycelium not visible on host. Perithecia globose to subglobose, 300–650 μm high, 200–300 μm wide, surface smooth, shiny or slightly roughened, solitary or gregarious in groups up to five, superficial or with the base immersed in substratum on a minute stroma, not collapsed when dry, luteous to sienna with ostiolar area often darker (bay) than rest of perithecium, red in 3% KOH, yellow in lactic acid, nonpapillate or with a small mammiform apex 80–150 μm wide. Cells at surface of perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina, irregular in shape 2–2.5 μm, 2–4 μm thick. Perithecial wall 30–60 μm wide. Asci cylindrical, (78–)80–98(–115) × 7–10 μm, 8-spored, apex simple. Ascospores ellipsoid, (10.5–)11.6–14.1(–15.1) × (4.6–)5.1–6.1(–6.5) μm (mean 12.8 × 5.6 μm), symmetrically two-celled, not constricted at septum, spinulose, hyaline, turning luteous when old.

Colonies on PDA 35–37 mm diam after 15 d at 25 C, aerial mycelium floccose, white

to saffron, colony reverse white to luteous, no pigment released into media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (14.9–)15.3–19.2(–20.5) × (–3.8)4–5.2(–5.8) μm (mean 17.3 × 4.6 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 1–6- septate: 1-septate (13.5–)15.3–28(–33.6) × (5.4–)5.5–6.9(–7.7) μm (mean 21.6 × 6.2 μm), 2-septate (41.4–)41.9–48.8(–50.8) × (6.2–)6.4–7.1(–7.2) μm (mean 45.1 × 6.8 μm), 3-septate (39.1–)47.3–58.1(–60.8) × (5.–9)6.4–7.9(–8.5) μm (mean 52.7 × 7.1 μm), 4-septate (50.5–)59.6–68.4(–71.3) × (5.2–)6.6–8.1(–8.5) μm (mean 64.2 × 7.4 μm), 5-septate (60.1–)66.4–76.6(–82.7) × (6.9–)7.4–8.4(–8.8) μm (mean 71.5 × 7.9 μm), 6-septate (74–)74.9–79.9(–80.1) × (7.7–)7.8–8.5(–8.6) μm (mean 77.4 × 8.2 μm). Microconidia produced in culture, cylindrical with round ends, (5.9–)6.2–9.5(–12.6) μm (mean 7.8 × 5.9 μm). No chlamydospores observed.

Habitat and distribution. Saprobic on decaying bark of shrubs and trees. Known from Taiwan (type locality) and French Guiana. Possibly widely distributed in tropical and subtropical regions.

Additional specimens examined. TAIWAN. TAIPEI COUNTY: Jingtung historical trail, on bark submerged in stream, 27 Nov 2003, Guu J.-R (BPI XX, culture 92112704). FRENCH GUIANA. Orstom Research Area “Ecerex”, km 16 on road between Sinnamary and St. Elie, on decaying stem of *Philodendron* sp., 1 March 1986, G.J. Samuels (NY G.J.S. 3987, culture G.J.S. 86-249 = IMI 325261)

Notes. Isolates of *T. mamma* were first identified as *T. lucida*, however, in this study we determined that they are more closely related to species in the *T. discophora* complex. This species does not show the typical purple colony of the typical of the *T. discophora* complex, and produces microconidia and 1-6-septate macroconidia. Macroconidia in this septation range are not produced by any of the species in the *T. discophora* complex.

***Thelonectria lucida* species complex**

A total of three new species are described in this complex. Previous descriptions of *T. lucida* s.str. based on morphology found it produces ascospores whose size ranges from 10–14 × 5–6 μm, and macroconidia 3-5 septate (Booth 1966, Brayford et al. 2004). Since no extype culture is available nor does our data set contains an isolate from the type locality (Java, Indonesia), we designated here a *T. lucida* s. str. that resembles more closely the morphology of the type specimen.

Thelonectria lucida (Hohn.) P. Chaverri & C. Salgado, Stud. Mycol. 68: 77. 2011.

FIG. 4.3.

Mycobank MB518571.

Basionym: Nectria lucida Hohn., Akad. Wiss. Wien. math. Naturw. Kl., Abt. 1, 118: 289. 1090

≡ *Neonectria lucida* (Hohn.) Samuels & Brayford, Mycologia 96: 590. 2004.

= *Cylindrocarpon lucidum* Booth, Mycol. Pap. 104: 21. 1966.

Holotype: INDONESIA. Java. Tjibodas, Tjiburrum, an labenden Zw., 1907–1098, Hohnel (FH-Hohnel 2899!).

Mycelium not visible on host. Perithecia globose, 400 – 500 μm diam, solitary or gregarious in groups of 5 or more, papillate, apex slightly flattened and mammiform, not collapsing when dry, orange to red, with ostiolar area darker bay to blood, dark red in 3% KOH, yellow in lactic acid, wall smooth and shiny, superficial or partially immersed in substratum. Cells at surface of perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina, irregular in shape 1.5–2.5 μm, 2–5 μm thick. Perithecial wall 30–55 μm wide. Asci cylindrical to clavate, (65–)75–95(–119) × 7–10.5 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (10.2–)11.2–12.9(–14) × (4–)4.6–5.8(–6.7) μm (mean 12.1 × 5.2 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, slightly constricted at septum, spinulose, hyaline. Colonies on PDA 45–49 mm diam after 15 d at 25 C, aerial mycelium floccose, pale white to cinnamon, colony reverse white to fawn, not producing pigment into media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (14.3–)17.3–24.4(–28.7) × (–2.6)3.2–4(–4.6) μm (mean 21.5 × 3.5), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 3–6- septate: 3-septate (48–)51.3–64.4(–67.1) × (6–)6.2–6.8(–7) μm (mean 57.9 × 6.5 μm), 4-septate (57.9–)63–72.7(–77.3) × (6–)6.2–6.9(–7.2) μm (mean 67.9 × 6.6 μm), 5-septate (63.3–)69.4–79.2(–83.5) × (6–)6.3–7.2(–7.5) μm (mean 74.3 × 6.7 μm), 6-

septate (82.9–)83.3–98.1(–98.2) × (8–)8.1–8.5(–8.7) μm (mean 90.7 × 8.3 μm). Microconidia and chlamydospores not produced in culture.

Habitat and distribution. Saprobe on decaying bark of shrubs and trees. Known from Java (type locality), Cameroon, and Costa Rica. Possibly widely distributed in tropical regions.

Additional specimens examined. CAMEROON. SOUTH WEST REGION: Ndian Division, Mundemba Subdivision, Korup National Park, trail between Mana bridge and Chimpanzee Camp, alt. 166 m. 5°4'0"N 8°51'0"E, 11 Dec 2008, G.J. Samuels GJS 9613A (BPI 882041, culture G.J.S. 08-232). COSTA RICA. HEREDIA PROVINCE: Braulio Carrillo National Park, Zurquí Street entrance, 10°03'N 84°01'W, 1734 m, on bark of undetermined dead tree, 14 March 2010, C. Salgado, C. Herrera, Y. Hirooka, A. Rossman, G.J. Samuels, P. Chaverri (BPI X, culture G.J.S. 10-146 = CBS 136788).

Notes. Isolates of *T. lucida* s. str. have the smallest ascospores in the species complex, ranging from 10–14 × 4–6 μm. Other species in the group have ascospores that range from 17–24 × 8–10 μm. Differences in presence and size of macroconidia were also observed as true *T. lucida* has 3-6-septate macroconidia on average of smaller size than the other species in the complex.

Thelonectria elata C. Salgado & P. Chaverri, sp. nov.

FIG. 4.4.

Mycobank TBD.

Similar to *T. lucida*, macroconidia 4–7 septate, ascospores, ascospores 20.8 × 9.4 μm.

Holotype. VENEZUELA. LARA STATE: along road, 12-17 km. S. & E. of Sanare,

Parque Nacional Yacambu, on bark of unidentified twig, K.P. Dumont, J.H. Haines, J. Leal, G.J. Samuels (NY KPD-VE 1702, ex-type culture C.T.R. 71-241 = CBS 112454).

Etymology. Refers to the papillated-humpy aspect of perithecial apex.

Mycelium visible on host as hyphal hairs arising from the surface near perithecia. Perithecia globose, 400 – 520 μm diam, mostly solitary or in groups up to three, papillate, apex slightly flattened and mammiform, not collapsing when dry, peach to sienna, with ostiolar area darker bay to blood, dark red in 3% KOH, yellow in lactic acid, wall smooth and shiny, superficial or partially immersed in substratum. Cells at surface of perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina, irregular in shape 1.5–2.5 μm, 2–5 μm thick. Perithecial wall 30–56 μm wide. Asci cylindrical to clavate, (68–)73–91(–115) × 7–11 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (17.4–)19.2–22.4(–24.6) × (7.5–)8.4–10.5(–11.2) μm (mean 20.8 × 9.4 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, slightly constricted at septum, spinulose, hyaline. Colonies on PDA 38–40 mm diam after 15 d at 25 C, aerial mycelium floccose, white to fawn, colony reverse white to peach, not producing pigment into media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (15.5–)16–19.9(–21.4) × (–4.5)4.8–5.6(–5.9) μm (mean 18 × 5.2), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 4–7- septate: 4-septate

(81.8–)81.9–88.2(–88.3) × (8.4–)8.6–10.2(–10.3) μm (mean 85 × 9.4 μm), 5-septate (76.1–)85.6–99.2(–101.7) × (8.6–)9.1–10.2(–11) μm (mean 92.4 × 9.7 μm), 6-septate (83.4–)90.9–102.3(–108.7) × (8.6–)9.2–10.4(–10.9) μm (mean 96.6 × 9.8 μm), 7-septate (93.2–)97.4–109.4(–111.5) × (9.4–)9.8–10.9(–11.3) μm (mean 103.4 × 10.4 μm). Microconidia and chlamydospores not produced in culture.

Habitat and distribution. Saprobe on bark of decaying trees. Known from the type locality (Venezuela) and Costa Rica.

Additional specimens examined. COSTA RICA. LIMON PROVINCE: Parque Nacional Braulio Carrillo, entrance from Calle Zurquí, 10°02'N, 84°01', elev. 1562, on bark of recently killed tree, 13 Mar. 2010, C. Salgado, A.Y. Rossman, G.J. Samuels, Y. Hirooka, C. Herrera, P. Chaverri (BPI X, culture G.J.S. 10-122 = CBS 136784).

Notes. Macroconidia 3-septate were not observed in culture for this species as compare to *T. lucida* and *T. papilla*. *Thelonectria elata* only produces 4–7 septate macroconidia, and it is different from *T. papilla* as it does not produce microconidia and 1-7-septate macroconidia.

Thelonectria gibba C. Salgado & P. Chaverri, sp. nov.

FIG. 4.5.

Mycobank TBD.

Similar to *T. lucida*, macroconidia 5–7 septate, ascospores 22 × 10.4 μm, only known from Puerto Rico.

Holotype. PUERTO RICO. Caribbean National Forest, Luquillo Mountains, El Verde Research Area, 19 Feb 1996, on decaying bark of *Casearia arborea,* G. J. Samuels,

H. J. Schroers (BPI 744634, ex-type culture G.J.S. 96-10 = IMI 370944/CBS 112469).

Etymology. Refers to the papillated-humpy aspect of perithecial apex.

Mycelium visible on host as hyphal hairs arising from the surface near perithecia. Perithecia globose, 410 – 520 μm diam, mostly solitary or in groups up to three, papillate, apex slightly flattened and mammiform, not collapsing when dry, peach to sienna, with ostiolar area darker umber to bay, dark red in 3% KOH, yellow in lactic acid, wall smooth and shiny, superficial or partially immersed in substratum. Cells at surface of perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina, irregular in shape 1.5–2.5 μm, 2–5 μm thick. Perithecial wall 30–55 μm wide. Asci cylindrical to clavate, (68–)73–90(–114) × 7–10 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (18.4–)20.2–24.4(–25.8) × (8.5–)9.4–11(–11.6) μm (mean 22 × 10.4 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, slightly constricted at septum, spinulose, hyaline. Colonies on PDA 31–44 mm diam after 15 d at 25 C, aerial mycelium floccose, white to cinnamon, colony reverse white to rosy buff, some cinnamon to honey pigment produced into media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.6–)12.2–14.9(–15.2) × (–4.2)4.2–5.2(–5.5) μm (mean 13 × 4.7), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 5–7- septate: 5-septate (66.4–)71.7–82.7(–89.4) × (8.6–)9–10.2(–10.8) μm (mean 77.2 ×

9.6 μm), 6-septate (71.5–)77.7–84.6(–86.7) × (8.5–)9.1–9.8(–10.3) μm (mean 81.2 × 9.5 μm), 7-septate (79.2–)81.5–88(–96.9) × (8.5–)9.3–10.5(–11.1) μm (mean 103.4 × 10.4 μm). Microconidia and chlamydospores not produced in culture.

Habitat and distribution. On decaying bark of *Casearia arborea* and *Cecropia* sp. To date only known from type locality, Puerto Rico.

Additional specimens examined. PUERTO RICO. Caribbean National Forest, Luquillo Mountains, trail to El Toro from route 186, on bark of recently dead Cecropia sp. 24 Feb 1996, G.J. Samuels (8098), H.J. Schroers, D.J. Lodge (BPI 745543, culture G.J.S. 96-35 = CBS 112456).

Notes. In this species, only 5-7-septate macroconidia were observed. This together with the restricted geographic origin (Puerto Rico) makes this species different from the other species in the complex.

Thelonectria papillata C. Salgado & A. Romero, sp. nov.

FIG. 4.6.

Mycobank TBD.

Similar to *T. lucida*, microconidia present, macroconidia 1–7 septate, ascospores 19.3 × 8.3 μm.

Holotype. VENEZUELA. MERIDA STATE: Parque Nacional Sierra Nevada, above Tabay, Coromoto Creek, La Mucuy, 1 hr steady walk toward Coromoto Lake, 2700-3000 m, 8 8°36'N 71°1'W, on bark of undetermined tree, 10-12 Nov 1990, Samuels, G. J.; Hein, B.; Huhndorf, S. M. et al. (6839) (BPI 842126, ex-type culture G.J.S. 90-146 = CBS 134032).

Etymology. Refers to the papillated-humpy aspect of perithecial apex.

Mycelium visible on host as hyphal hairs arising from the surface near perithecia. Perithecia globose, 420 – 520 μm diam, mostly solitary or in groups up to three, papillate, apex slightly flattened and mammiform, not collapsing when dry, peach to bay, with ostiolar area darker orange to red, dark red in 3% KOH, yellow in lactic acid, wall smooth and shiny, superficial or partially immersed in substratum. Cells at surface of perithecial wall lacking a definite outline appearing to be intertwined hyphae with lumina, irregular in shape 1.5–2.5 μm, 2–5 μm thick. Perithecial wall 35–55 μm wide. Asci cylindrical to clavate, (78–)80–90(–100) × 7–12 μm, 8-spored, apex with a refractive ring. Ascospores ellipsoid to fusiform, (16.7–)17.9–20.7(–23.2) × (6.5–)7.3–9.3(–10) μm (mean 19.3 × 8.3 μm), symmetrically two-celled, sometimes with one side curved and one side flattened, slightly constricted at septum, spinulose, hyaline. Colonies on PDA 25–31 mm diam after 15 d at 25 C, aerial mycelium floccose, white to ochreous, colony reverse white to saffron, some pale luteous pigment produced into media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.1–)15–20.7(–22.5) × (–3.8)4.2–5.3(–5.8) μm (mean 17.8 × 4.7), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved with round ends, 1–7- septate: 1-septate (20.1–)28.5–40.3(–49.8) × (3.7–)4.2–5.4(–6.6) μm (mean 34.4 × 4.8 μm), 2-septate (32.1–)37.5–48.3(–53.9) × (3.6–)4.4–5.5(–6.1) μm (mean 42.7 × 5 μm), 3-septate (34.1–)42.7–51.8(–57.4) × (3.9–)4.6–5.9(–6.8) μm (mean 47.2 × 5.2 μm), 4-septate (72.6–)73.1–82(–83.1) × (8.5–)8.5–9.4(–9.6) μm (mean 77.5 × 9 μm), 5-septate

(68.9–)76.3–91.4(–96.2) × (7.6–)8.1–9.6(–10.4) μm (mean 83.8 × 8.9 μm), 6-septate (80–)87.4–104.2(–109.6) × (8.2–)8.8–10(–10.3) μm (mean 95.8 × 9.4 μm), 7-septate (92.1–)95.9–106.8(–109.2) × (7.9–)8.8–10.7(–11.1) μm (mean 101.3 × 9.8 μm). Microconidia produced in culture (6.4–)7.2–8.8(–9.6) μm (mean 8 × 4.1 μm). Chlamydospores mean 10 × 11.5 μm.

Habitat and distribution. Saprobe on decaying bark of trees. Known from the type locality (Venezuela, Parque Nacional Sierra Nevada) and Argentina.

Additional specimens examined. ARGENTINA. TUCUMAN PROVINCE: road to Catamarca, Camino Las Lenguas, near to Rio Cochuna, 400 m, on bark of a rotting fallen tree, C. Salgado, A.Y. Rossman, A. Romero (BPI X, culture A.R. 4781 = CBS 134036). VENEZUELA. MERIDA STATE: Parque Nacional Sierra Nevada, above Tabay, Coromoto Creek, 2300 m, 8°36'N 71°2'W, on bark of undetermined tree, 9-17 Nov 1990, Samuels, G. J.; Hein, B.; Huhndorf, S. M. et al. (6763) (BPI 842125, culture G.J.S. 90-166 = CBS 126099).

Notes. *T. papillata* is the only species producing microconidia and macroconidia 1-7 septate in the group.

Other species in the genus *Thelonectria* P. Chaverri & C. Salgado, 2011.

Thelonectria olida (Wollenw.) P. Chaverri & C. Salgado, Stud. Mycol. 68: 77. 2011.

Mycobank MB518572

Basionym: *Ramularia olida* Wollenw., Phytopathology 3:223. 1913.

≡ *Cylindrocarpon olidum var. olidum* (Wollenw) Wollenw., Fus. Autogr. Del., 1:471.

1916.

= *Cylindrocarpon curvatum* Hochapfel in Wollenw., Z. Parasitenk. 3:495. 1931.

Holotype. GERMANY. BERLIN: on rotting rhizome of *Asparagus officinalis*, W. Gerlach 8720 (ex-type culture CBS 215.67).

Descriptions and illustrations. See Booth (1966) and Brayford (1987).

Host and distribution. Saprobe on asparagus and other vegetables. Known from Germany, Ghana, probably widely distributed.

Notes. This species has only been collected in the asexual state. In our study we found it is phylogenetically related to species in the *T. veuillotiana* complex. Besides the production of chlamydospores, natural for a species found in soil and associated with plants, species in *T. veuillotiana* complex and *T. olida* share many morphological similarities that make difficult their identification without the use of molecular data.

Thelonectria platycephala (Brayford & Samuels) C. Salgado & P. Chaverri, comb. nov.

FIG. 4.7.

Mycobank TBD

Basionym: *Nectria platycephala* Brayford & Samuels, Mycologia, 85: 625. 1993.

= *Cylindrocarpon permirum* Brayford & Samuels, Mycologia, 85: 625. 1993.

Holotype. JAMAICA. ST. ANDREW PARISH: vicinity of Dick's Pond, west of Hardwar Gap, near Holywell Recreation Area and Wag Water River, 850–1000 m, on bark, 11 Jan 1971, R.P. Korf et al. (NY CUP-MJ 790, ex-type culture C.T.R. 71-25 = IMI 329100).

Mycelium not visible on host. Perithecia solitary or gregarious in groups formed in

cracks of bark, basally immersed in an inconspicuous yellow pseudoparenchymatous non erumpent stroma, perithecia orange to scarlet, globose with a broad constricted "knobby" apex of same color as the rest of perithecium, not collapsing when dry, smooth to scaly surface. Asci cylindrical or clavate, apex with conspicuous ring (68–)72–90(–100) × 10–14(–18) μm, 8-spored. Ascospores ellipsoid, (9.6–)10.3–12(–13.8) × (3.9–)4.3–5.3(–6.2) μm (mean 11.2 × 4.8 μm), hyaline, equally two-celled, sometimes with one side curved and one side flattened, slightly constricted at the septum, spinulose. Colonies on PDA 51 mm diam after 15 d at 25 C, aerial mycelium floccose, salmon to umber, colony reverse salmon to luteous. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper on SNA agar. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.2–)16.0–20.6(–23.4) × (–2.5)3.5–4.4(–5.1) μm, with periclinal thickening and collarette. Macroconidia fusiform with rounded ends, 3–5(–6)- septate: 3-septate (40.5–)46.9–58.9(–71.4) × (4.4–)4.9–5.8(–7) μm (mean 53.3 × 5.4 μm), 4-septate (44.6–)55–67.5(–74.4) × (4.4–)5–6(–6.5) μm (mean 61.2 × 5.5 μm), 5-septate (60.0–)65.4–77(–82.5) × (4.4–)5.2–6.3(–6.6) μm (mean 71.2 × 5.8 μm). Microconidia and chlamydospores not produced on SNA.

Habitat and distribution. Saprobe on woody substrates, known only from the type locality (Jamaica).

Notes. The original description of this species suggests that is related to the *T. veuillotiana* species complex based on the morphology of the perithecial apex, which displays the typical mammiform knobby flat apex. Results from the phylogenetic

analyses indicate that *T. platycephala* is the most basal species in the genus *Thelonectria*.

Thelonectria rubrococca (Brayford & Samuels) C. Salgado & P. Chaverri, comb. nov.

FIG. 4.8.

Mycobank TBD.

Basionym: *Nectria rubrococca* Brayford & Samuels, Mycologia, 85: 627. 1993.

= *Cylindrocarpon arcuatum* Brayford & Samuels, Mycologia, 85: 627. 1993.

Holotype. FRENCH GUIANA. MONTAGNE DE KAW: route de l'est, ca. 50 km, 04°60'N 52°41'W, on wood, 25 Mar 1986, C. Feuillet, G.J. Samuels (NY GJS 4484, ex-type culture G.J.S. 86-330 = IMI 324475).

Mycelium not visible on host. Perithecia solitary to gregarious, globose to slightly pyriform, (220–)250–450(–500) μm diam, apex acute to broadly rounded, not collapsing when dry, sienna, red in 3% KOH, yellow in lactic acid, scaly or warted, warts up to 60 μm high, paler in color than the rest of the perithecial wall. Cells at the surface of the perithecial wall and warts circular, 10-25 μm diam. Perithecial wall 20–60 μm, comprising three regions, outer with two to three layers of pigmented, angular to circular cells, middle region of two to three layers of pigmented elongated flattened cells, inner regions formed of nonpigmented, compressed flattened cells. Asci cylindrical, (40–)50–88(–100) × (5–)7–9(–11) μm, apex simple with refractive ring, 8-spored. Ascospores ellipsoid, (10.9–)11.3–12.4(–12.9) × (4.5–)4.8–5.7(–6.4) μm (mean 11.9 × 5.3 μm), 1-septate, hyaline, not constricted at the septum, spinulose.

Colonies on PDA 41 mm diam after 15 d at 25 C, aerial mycelium floccose, white, reverse white to salmon, no pigment produced into the media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface, pionnotes sometimes formed close to filter paper or inoculum, forming concentric rings of sporulation on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (12.1–)14.1–19.4(–21.5) × (–3.5)3.7–4.4(–4.7) μm (mean 16.8 × 4 μm), with periclinal thickening and collarette. Macroconidia fusiform with round ends often slightly hooked, 3- septate only: 3-septate (56.7–)59.6–66.2(–69.5) × (5.2–)6–6.8(–7.1) μm (mean 62.9 × 6.4 μm). Microconidia nor chlamydospores produced in culture.

Habitat and distribution. Saprobe on bark of decaying substrates, known from the type locality only.

Notes. *Thelonectria rubrococca* is related to species in the *T. discophora* and *T. lucida* complexes, *T. theobromicola* and *T. trachosa*. Interestingly, this species shares many morphological similarities with species in the genus *Rugonectria*, especially in the surface of the perithecial wall includes warts composed of by circular cells. However *T. rubrococca* does not produce microconidia and only 3-septate macroconidia have been observed in culture.

Thelonectria theobromicola (Booth) C. Salgado & P. Chaverri, comb. nov.

FIG. 4.9.

Mycobank TBD.

Basionym: *Cylindrocarpon theobromicola* Booth, Mycol. Pap. 104:19. 1966

Holotype. PAPUA NEW GUINEA. NEW BRITAIN ISLAND: East New Britain Province, Gazelle District, Keravat, on *Theobroma cacao*, 1965, P.G. Hicks (ex-type culture CBS 218.67/IMI 112161a).

Colonies on PDA 56 mm diam after 15 d at 25 C, aerial mycelium floccose, white with center darker (isabelline), reverse white to isabelline, no pigment produced into media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface, pionnotes sometimes formed close to filter paper on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (11.1–)13.1–18.1(–20.2) × (–3.5)3.8–4.7(–5.6) μm (mean 15.6 × 4.2), with periclinal thickening and collarette. Macroconidia cylindrical or fusiform round ends, 3- septate only: 3-septate (33.5–)36.5–44.6(–48.3) × (5.6–)5.9–7(–7.7) μm (mean 40.5 × 6.5 μm). Microconidia nor chlamydospores produced in culture.

Habitat and distribution. Saprobe on *Theobroma cacao*, known only from the type locality.

Notes. *T. theobromicola* is phylogenetically related to species in the *T. lucida* complex, however, this species has been collected only in the asexual state, and it only produces macroconidia 3-septate in culture conditions. It was found associated with cacao plants, however, in the original description it is not stated if the isolate was causing any king of pathogenesis. Other species in the genus *Ilyonectria* can also be found associated to cacao, yet they can be discriminated based on the morphology of the macroconidia and the presence of unique or several septation patterns.

Thelonectria trachosa (Booth) Samuels, P. Chaverri & C. Salgado, Stud. Mycol. 68: 77. 2011.

FIG. 4.10.

Mycobank MB518573.

Basionym: *Neonectria trachosa* Samuels & Brayford, Mycologia, 96:592. 2004.

Holotype. SCOTLAND. ARGYLLSHIRE: Cowall Peninsula, Argyll Forest Park, ca. 5 km South of Strachur along River Cur, vicinity of Glenbranter Village, Lauder Broadleaves Walk, 50 m, on conifer bark, 12 Apr 1992, D. Brayford, G.J. Samuels (BPI 802661, culture G.J.S. 92-45 = CBS 112467/IMI 352560).

Mycelium not visible on host. Perithecia solitary or gregarious in small groups up to five, superficial, globose, 460–600 μm, nonpapillate, peach to orange, with ostiolar area darker than the rest of perithecium (bay), red in 3% KOH, yellow in lactic acid, not collapsing when dry, smooth or slightly roughened. Perithecial wall 70–100 μm, comprising three regions, outer composed by cells elliptic to circular, middle region of intertwined and branched hyphal elements arranged perpendicular to the perithecial surface, inner composed by elliptic cells intertwined with illumine progressively ticker towards the perithecial apex. Asci cylindrical, (90–)100–140(–160) × (8–)9–11(–12) μm, simple with a conspicuous refractive ring, 8-spored. Ascospores ellipsoid (15.2–)16–19.1(–22.8) × (6.1–)6.8–8.3(–9.6) μm (mean 17.5 × 7.6 μm), 1-septate, spinulose, not constricted at the septum, hyaline. Colonies on PDA 4–7 mm diam after 15 d at 25 C, aerial mycelium floccose, white front and reverse, no pigment produced into media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface, sporulation usually scarse. Phialides borne

apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (14.2–)15.7–22.6(–26) × (–3.5)3.9–4.8(–5.2) μm (mean 19.1 × 4.3), with periclinal thickening and collarette. Macroconidia cylindrical or fusiform round ends, 1–4- septate: 1-septate (10.9–)11.5–18.1(–20.8) × (4.6–)5–6.3(–6.4) μm (mean 14.8 × 5.6 μm), 2-septate (24–)24.3–35.9(–44.4) × (5–)5.2–5.6(–5.7) μm (mean 29.9 × 5.4 μm), 3-septate (28.8–)32.6–51.7(–67) × (4.9–)5.2–6.5(–7.7) μm (mean 42.2 × 5.9 μm), 4-septate (46.9–)50.9–67.8(–68.7) × (4.3–)4.7–5.7(–6.2) μm (mean 59.4 × 5.2 μm). Microconidia produced in culture (6.6–)8.1–11.7(–12.6) × (4.3–)4.5–5.4(–5.7) μm (mean 9.9 × 4.9 μm). No chlamydospores produced in culture.

Habitat and distribution. Saprobe on woody substrates, known from the type locality (Scotland), and New Zealand.

Additional specimen examined. NEW ZEALAND. WESTLAND: vicinity of Greymouth, Paparoa Ra., Croesius Tr., vivinity of Garden Gully, on bark of *Phyllocladus* sp., 3 May 1985, P.R. Johnston, L.M. Kohn, G.J. Samuels (PDD 50060, culture G.J.S. 85-50 = CBS 119608).

Notes. In this study we found that *T. trachosa* has been collected not only in the type locality (Scotland) but also in New Zealand, with probability of being distributed in cold temperate locations. *T. trachosa* is phylogenetically more closely related to species in the *T. discophora* and *T. lucida* complex, however, they have morphological differences, such as the structure of perithecial wall. *T. trachosa* also produces microconidia and macroconidia 1-4-septate usually smaller than the related species. It is also the only species to produce pure white colonies, and to have an

extremely growth rate at temperatures higher than 20 C, with no growth at temperatures higher than 25 C.

Thelonectria westlandica (Dingley) P. Chaverri & C. Salgado, Stud. Mycol. 68: 77. 2011.

FIG. 4.11.

Mycobank MB518576.

Basionym: *Nectria westlandica* Dingley, Trans. Roy. Soc. New Zealand, 79: 201. 1951

≡ *Neonectria westlandica* (Dingley) Samuels & Brayford, Mycologia 96:595. 2004.

Holotype. NEW ZEALAND. SOUTH ISLAND: Westland, Weheka, on *Olearia avicenniaefolia*, Dec 1946, J.M. Dingley (PDD 5129).

Mycelium not visible on host. Perithecia solitary to gregarious in groups up to 15, superficial or with the base partially immersed in substratum, globose, 400–600 μm diam, nonpapillate, saffron to sienna, with blood ostiolar area, red in 3% KOH, yellow in lactic acid, not collapsing when dry. Cells at the surface of the perithecial wall warted, conspicuously circular, 15–40 μm. Perithecial wall 40–60 μm, comprising three regions, outer formed by circular cells including warts, middle region with cells with hyphal characters arranger perpendicular to the surface of the perithecium, inner region with flattened cells becoming progressively more compressed towards the ostiolum. Asci clavate, (98–)110–120(–130) × (15–)17–25(–28) μm, apex with refractive ring, 8-spored. Ascospores ellipsoid (21.7–)23.9–32.2(–34.5) × (9.1–)10.6–12.1(–13.3) μm (mean 28.1 × 11.3 μm), 1-septate, slightly constricted at the septum, smooth, hyaline. Colonies on PDA 15–18 mm diam after

15 d at 25 C, aerial mycelium floccose, white to saffron, colony reverse white to bay, rust pigment produced into media in some isolates. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.3–)13.8–18(–24.3) × (–3.9)4.4–5.1(–6) μm (mean 15.9 × 4.7), with periclinal thickening and collarette. Macroconidia cylindrical or fusiform with round ends, 1–5- septate: 1-septate (20.3–)22.3–29.7(–31.6) × (5.9–)6.1–5.4(–7.3) μm (mean 26 × 6.7 μm), 2-septate (27.8–)28.8–40.7(–46.7) × (6.9–)7.1–7.5(–7.6) μm (mean 34.7 × 7.3 μm), 3-septate (35.4–)44.4–51.7(–58.3) × (5.8–)6.5–7.8(–8.3) μm (mean 48.1 × 7.2 μm), 4-septate (45–)48.2–60.4(–73) × (5.8–)6.6–7.7(–8.2) μm (mean 54.3 × 7.1 μm), 5-septate (47.9–)48.7–58(–63.7) × (6.2–)6.4–7(–7.1) μm (mean 53.4 × 6.7 μm). Microconidia nor chlamydospores produced in culture.

Habitat and distribution. Saprobe found on bark of recently dead or decaying trees, known only from New Zealand.

Additional specimens examined. NEW ZEALAND. WESTLAND: Mount Aspiring Nat. Park, Haast Pass, ca. 70 km East of Haast Junction Cameron Flat, on root, 29 Apr 1985, L.M. Kohn, G.J. Samuels (PDD50055, culture G.J.S. 85-45 = CBS 132330); North of Hari Hari, Lake Ianthe State Forest, on decaying bark of *Dacryocarpus cupressinum*, 21 May 1983, T. Matsushima, A. Rossman, G.J. Samuels (PDD 46336, culture G.J.S. 83-156 = CBS 112464/IMI 326254); Waitakere Ra., Huia, on *Metrosideros robusta* decaying bark, 23 Oct 1980, P.R. Johnston, G.J. Samuels (PDD 41421, culture G.J.S. 80-156A = IMI255610); Masterton, Wairarapa, on *Rosa* sp., G. Laundon (only culture ICMP 10387).

Notes. T. westlandica has a closer phylogenetic relationship with species in the *T. coronata* and *T. veuillotiana* species complex, however, it does not display the typical mammiform morphology of the perithecial apex, and displays an outer layer of warted cells in the perithecial wall. This species has been collected only in the type locality, New Zealand. Colony growth in culture conditions is among the lowest in the genus *Thelonectria*, and together with *T. trachosa* show slow growth in temperatures above 20 C, however, faster in temperatures below 20 C, somewhat representing the prevalent temperatures found in the localities of origin.

New genera

Cinnamonectria C. Salgado & P. Chaverri, gen. nov.

FIG. 4.12.

Mycobank TBD.

Etymology. Refers to the cinnamon color of the perithecia and exudates produced in culture conditions.

Type: *Cinnamonectria cinnamomea.*

Ascoma superficial, globose, (270–)350–550(600) μm, non-papillated, ochreous to umber, with ostiolar area darker, chestnut, perithecial wall warted, asci clavate, 90–100 × 16–20 μm, ascospores ellipsoid to fusiform, 22–38 × 8–12 μm, 1-septate, hyaline, slightly constricted a the septum, surrounded by a wrinkled ochreous sheat. Anamorph cylincrocarpon-like, macroconidia 3-septate. On decaying wood substrates.

Notes. The genus *Cinnamonectria* includes one species that is known primarily on woody substrate. This genus has been collected only in the sexual state and is characterized by having cylindrocarpon-like morphologies with only 3-septate macroconidia produced in culture. The ascomata display a typical cinnamon coloration, which can also be observed in the asexual state. Isolates of *C. cinnamomea* were originally included in the genus *Neonectria*, however, they have little similarity to the type of that genus. Phylogenetic analyses carried out in this study show *Cinnamonectria cinnamomea* forms a monophyletic group related to the genus *Viridispora*.

Cinnamonectria cinnamomea (Brayford & Samuels) C. Salgado & P. Chaverri, comb. nov.

FIG. 4.12.

Mycobank TBD.

Basionym: *Nectria cinnamomea* Brayford & Samuels, Mycologia 85: 617. 1993.

≡ *Neonectria cinnamomea* (Brayford & Samuels) Brayford & Samuels, Mycologia 96: 580. 2004.

≡ *Cylindrocarpon cinnamomeum* Brayford & Samuels, Mycologia 85: 617. 1993.

Perithecia globose, perithecial wall warted, ochreous to umber, ascospores 1-septate, hyaline, slightly constricted at the septum, with ochraceous rugose sheat. Anamorph cylindrocarpon-like with macroconidia 3-septate only. Chlamydospores produced in culture.

Holotype. FRENCH GUIANA. SAUL: circuit Grande Fossé, 03°60'N 53°20'W, 300-350 m, on bark of living liana, 10 Feb 1986, G.J. Samuels (NY GJS3619, ex-type culture G.J.S 86-117 = IMI 325248).

Etymology. Refers to the cinnamon color of the perithecia and exudates produced in culture conditions.

Mycelium visible, white and appressed to the substratum around perithecial aggregates. Perithecia globose, (270–)350–550(600) × (250–)300–560(–600) μm, nonpapillate, ochreous to sienna, with ostiolar area darker than the rest of the perithecium, umber to chestnut, no color change in 3% KOH but sometimes releasing a soluble luteous, pigment, yellow in lactic acid, not collapsing when dry, warted, solitary to cespitose in groups of 4 or more, often forming on bark cracks, superficial on an erumpent white hyphal stroma. Cells at the perithecial wall circular to angular, (5–)7–10(–15) diam, cells in warts loosely connected to the exterior of the perithecial wall and easily dislodged, Perithecial wall including warts 45–90 μm, comprising three intergrading regions, outer including warts and circular to elliptical cells 4–8 μm, middle region elliptical to fusoid 10–20 μm, inner region with cells flattened and compresed 5-10 μm. Asci clavate (80–)90–130(–150) × (13–)16–25(–31) μm, apex simple, 8-spored, ascospores ellipsoidal to fusiform (22.6–)25.5–32(–38) × (8.3–)9.5–11.4(–12.9) μm (mean 28.8 × 10.5 μm), 1-septate, slightly constricted at the septum, smooth, hyaline, surrounded by a 1 μm wide wrinkled hyaline to luteous sheat. Colonies on PDA 30–44 mm diam after 15 d at 25 C, aerial mycelium floccose, white to sienna, producing sienna to cinnamon pigment into media and as exudate droplets over the fungal colony, colony reverse white to sienna. Conidia on SNA forming in

hyaline, slimy droplets in aerial mycelium or on agar surface; pionnotes sometimes formed close to filter paper or inoculum on SNA. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen in the middle (12.2–)13.6–20.6(–28.2) × (–2.7)3.2–4.8(–5.6) μm (mean 17.1 × 3.8 μm), with periclinal thickening and collarette. Macroconidia cylindrical or slightly fusiform, curved and more sharply curved ends, 3- septate: (45–)55.8–74.7(–85.3) × (4.7–)5.66–6.8(–7.9) μm (mean 65.2 × 6.2 μm). Chlamydospores formed in culture (13–)15.1–21.3(–22.4) × (14–)14.3–19.4(–20.8) μm (mean 18.2 × 16.9 μm). No microconidia observed.

Habitat and distribution. On woody substrates of recently dead or decaying plants. Tropical, distributed in Guyana, French Guiana and Northern Brazil.

Additional specimens examined. BRAZIL. BAHIA: Igrapiuna, Michelon Ecological Reserve, on bark, 5 Dec 2010, P. Chaverri et al. (BPI X, culture PC 1222 = CBS 136783). FRENCH GUIANA. Paul Isnard Area, Citron, cs. 150 km S of St. Laurent du Maroni, 04°70'N 53°90'W, on bark of decaying tree, 7-14 March 1986, G.J. Samuels (NY GJS4264, culture G.J.S. 86-300 = IMI 325253); SINNAMARY: Piste St. Elie, on bark, Christian Lechat CLLGUY12050 (BPI X, culture CBS 133756). GUYANA. CUYUNI-MAZARUNI REGION: along Koatse River, ca. 2 km E of Pong River, ca. 5 h walk W of Chimoweing Village, 05°28'N 60°04'W, 550-600 m, on bark of recently dead tree, J. Pipoly, Gharbarran, G.J. Samuels (NY GJS 4928, culture G.J.S. 87-41 = IMI 325256).

Notes. In this study, only specimens from Brazil, French Guiana and Guyana were included, however, there have been reports of the presence of this species in

Colombia, Japan, Puerto Rico, Thailand, Venezuela, and United States. Probably distributed in tropical and subtropical regions. Phylogenetic analyses showed *Viridispora alata* as closely related to *C. cinnamomea*. Even though anamorph morphology of these two genera differs as *V. alata* produces *Penicillifer* anamorphs, the morphology of the teleomorphic (sexual) states is very similar. Both genera have red to brown perithecia in which the outer layer of the perithecial wall contains warts and ascospores green or cinnamon colored.

Macronectria C. Salgado & P. Chaverri, gen. nov.

FIG. 4.15.

Mycobank TBD.

Etymology. Refers to the big size of ascospores and macroconidia species in this genus produce.

Type. *Macronectria jungneri.*

Ascoma superficial, subglobose to pyriform, (370–)400–520(600) μm, constricted bellow the apex to form a wide papilla (150–)180–300(–320), ochreous to umber, with ostiolar area darker, chestnut, perithecial wall smooth , asci clavate, 90–100 × 16–20 μm, ascospores ellipsoid to fusiform, 25–38 × 10–14 μm, 1-septate, hyaline, striated slightly constricted a the septum, surrounded by a narrow sheat when young. Anamorph '*Cylincrocarpon*'-like, macroconidia 5–8-septate. On decaying woody substrates.

Notes. Species in *Macronectria* are among the most common species in the Nectriaceae in the tropics where they can be found on a wide variety of plant organic matter. These species are easily recognized by the perithecial morphology, which

displays the typical mammiform shape. Perithecia are pyriform and elongated with a broad, knobby and often darker papilla. In occasion, the papilla comprises at least ¾ of the diameter of the perithecia. The anamorphic states produces macroconidia 5–8 septate whose size is among the biggest in the group, sometimes reaching 110 μm. Along with the type, 4 additional species are described here.

Macronectria jungneri (Henn.) C. Salgado & P. Chaverri, comb. nov.

FIG. 4.15.

Mycobank TBD.

Basionym: *Nectria jungneri* Henn., Bot. Jahrb, Syst. 22: 75. 1897.

= *Nectria eustoma* Penz. & Sacc., Malpighia 11: 509. 1898 [1897]

= *Nectria leucoloma* Starbäck, Bih. Kongl. Svenska Vetensk.-Akad. Handl. 25: 28. 1899.

= *Nectria cinereopapillata* Henn. & Nyman in Warburg, Monsunia 1: 161. 1900 [1899].

= *Nectria striatospora* Zimm., Centralbl. Bakteriol. II, 7: 105. 1901.

= *Nectria azureostiolata* Doi, Mem. Nat. Sci. Mus. Tokyo 10: 23. 1977.

= *Cylindrocarpon victoriae* Wollenw., Z. Parasintek. (Berlin) 1: 161. 1928.

Holotype. CAMEROON. Bibundi and Zweigen (J.R. Jungner n. 39). (Not examined).

Etymology. Refers to the author of the original species name, J.R. Jungner.

Mycelium visible on host, forming a white mat around perithecia or forming discrete spots. Perithecia gregarious in groups of many, (370–)400–520(–590) μm diam, superficial on an erumpent stroma, subglobose to pyriform, sometimes elongated and flask shaped, constricted below the apex to form a broad papilla 130–300 μm wide,

not collapsing when dry, orange to sienna, with papilla the same color than the perithecium, red in 3% KOH, yellow in lactic acid, surface of the perithecium smooth. Perithecial wall 20–40 μm, of two intergrading regions, outer of intertwined hyphae with cells slightly elliptical, inner region of flattened fusoids cells . Asci clavate, 70–100 × 12–30 μm, apex simple, 8-spored. Ascospores fusiform, sometimes one side flat and the other curved, (25.9–)28.9–32.2(–35.4) × (–7.7)8.9–10.7(–11.8) μm (mean 30.5 × 9.8 μm), 1-septate, not constricted at the septum, hyaline, striated. Colonies on PDA 46–58 mm diam after 15 d at 25 C, aerial mycelium floccose, front and reverse white to salmon, not producing pigment into the media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface, sometimes closer to the inoculum. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (14.3–)15.8–20(–25) × (–3.9)4.2–5.1(–6.2) μm (mean 17.9 × 4.7 μm), with periclinal thickening and collarette. Macroconidia cylindrical or fusiform with round ends, 5–7- septate: 5-septate (66.8–)73.2–88(–100.1) × (9.1–)10.1–11.1(–11.5) μm (mean 80.6 × 10.6 μm), 6-septate (73.4–)77.8–87(–95.4) × (9.1–)10–11.4(–12) μm (mean 82.4 × 10.7 μm), 7-septate (78.6–)83.3–95.5(–102.4) × (9.1–)10.3–11.8(–12.9) μm (mean 89.4 × 11 μm). No microconidia nor chlamydospores produced in culture.

Host and distribution. Saprobe of various woody substrates, as well as other plant organic matter, known from the type locality (Cameroon), Brazil and Costa Rica, probably pantropical.

Additional specimens examined. BRAZIL. BAHIA: Igrapiuna, Michelon Ecological Reserve, on bark, 5 Dec 2010, P. Chaverri et al. (BPI X, culture PC 1219 = CBS

136792). CAMEROON. SOUTHWESTERN PROVINCE: Ndian Division, Mundemba Subdivision, Korup National Park, trail between Mana bridge and Chimpanzee Camp, 05°04'N, 08°51'E, 166m, 11 Dec 2008, G.J. Samuels (BPI 882042, culture G.J.S. 08-233 = CBS 124602). COSTA RICA. LIMON PROVINCE: Parque Nacional Braulio Carrillo, entrance from Calle Zurquí, 10°02'N, 84°01', elev. 1562, on bark of recently killed tree, 13 Mar. 2010, C. Salgado, A.Y. Rossman, G.J. Samuels, Y. Hirooka, C. Herrera, P. Chaverri, (BPI X, culture G.J.S. 10-127); 10°03'N 84°01'W, 1734 m, on bark of undetermined dead tree, 14 March 2010, C. Salgado, C. Herrera, Y. Hirooka, A. Rossman, G.J. Samuels, P. Chaverri (BPI X, culture G.J.S. 10-148 = CBS 136785)

Notes. Isolates in this species produce macroconidia 5-7-septate, and on average has the biggest ascospore size in *Macronectria*.

Macronectria asiatica C. Salgado & J.-R Guu, sp. nov.

FIG. 4.14.

Mycobank TBD.

Similar to *Macronectria jungneri*, ascospores 25 × 9 μm, microconidia produced in culture 8 × 6.6 μm, macroconidia 4–8-septate, distributed in Japan and Taiwan (type locality).

Holotype. TAIWAN. PINGTUNG COUNTY: Machia, Liang-shan waterfall, on bark, 14 Sept 2005, J.-R. Guu (BPI X, culture J.-R. Guu 94091407 = CBS 136795).

Etymology. Refers to the geographic locations this species can be found.

Mycelium visible on host forming discrete spots around fruiting bodies. Perithecia gregarious in groups of many, (340–)370–490(–560) μm diam, superficial on an erumpent stroma, subglobose to pyriform, constricted below the apex to form an

small papilla 50–120 μm wide, not collapsing when dry, peach to orange, with papilla the same color than the perithecium, red in 3% KOH, yellow in lactic acid, surface of the perithecium smooth. Perithecial wall 20–40 μm, of two intergrading regions, outer of intertwined hyphae with cells slightly elliptical, inner region of flattened fusoids cells . Asci clavate, 65–100 × 15–30 μm, apex simple, 8-spored. Ascospores fusiform, sometimes one side flat and the other curved, (19.9–)20.8–23.5(–25) × (–8)8.4–9.5(–10.3) μm (mean 22 × 9 μm), 1-septate, slightly constricted at the septum, hyaline, striated. Colonies on PDA 38–55 mm diam after 15 d at 25 C, aerial mycelium floccose, front and reverse white to luteous, no pigment produced into the media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface, sometimes closer to the inoculum. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.1–)13.9–19.4(–27) × (–3.5)4.1–4.9(–5.2) μm (mean 16.7 × 4.5), with periclinal thickening and collarette. Macroconidia cylindrical or fusiform with round ends, 4–8- septate: 4-septate (52–)54.4–66.5(–69.8) × (7–)7.1–7.9(–8.2) μm (mean 60.4 × 7.5 μm), 5-septate (58.7–)74.5–89(–95.1) × (6.8–)8.1–9.7(–10.8) μm (mean 81.8 × 8.9 μm), 6-septate (75.4–)82.9–93.1(–100.4) × (7.3–)8.6–9.8(–10.6) μm (mean 88 × 9.2 μm), 7-septate (81.3–)87.1–98.4(–104.6) × (7.6–)8.6–9.7(–10.2) μm (mean 92.7 × 9.1 μm), 8-septate (84–)91.3–102.4(–107.3) × (8.3–)8.6–9.7(–10.1) μm (mean 96.9 × 9.2 μm). Microconidia produced (6.6–)7–9(–10.6) × (4.3–)5–8.1(–9) μm (mean 8 × 6.6 μm). No chlamydospores produced in culture.

Host and distribution. Saprobe on *Prunus* x *yedoensis, Swietenia macrophylla* and other woody substrates. Known from Taiwan and Japan.

Additional specimens examined. JAPAN. MIE PREFECTURE: on woody plant, T. Kobayashi (MAFF TPP-h315, culture MAFF 239833); TOKIO: on woody plant, T. Kobayashi (MAFF TPP-h444, culture MAFF 239846); FUKUOKA PREFECTURE: Dazaifushiminnomori, Dazaifu City, on twigs of *Prunus* × *yedoensis*, 01 Apr 2004, Y. Hirooka (TPP-h255) (BPI 882097, culture MAFF 241529). TAIWAN. KAOHSIUNG COUNTY: Meinong, on bark of *Swietenia macrophylla*, 16 Sept 2005, J.-R. Guu (BPI X, culture J.-R. Guu 94091601 = CBS 136794).

Notes. This species can be distinguished due to its restricted locations in Asiatic regions, including Japan and Taiwan, and by the production of microconidia and 4-8-septate macroconidia. Among the species in *Macronectria*, *M. asiatica* has on average the smallest ascospores.

Macronectria magna C. Salgado & C. Lechat, sp. nov.

FIG. 4.15.

Mycobank TBD.

Similar to *Macronectria jungneri*, ascospores 23 × 10 μm, microconidia 9.1 × 4.8 macroconidia 3–8-septate, distributed in French Guiana (type locality) and Guatemala.

Holotype. FRENCH GUIANA. CAMP INSELBERG: Les Nouragues, on *Bauhinia longicupsis*, 2004, C. Lechat CLLGUY12004 (BPI X, ex-type culture CBS 133489)

Etymology. Refers to the big size of macroconidia this species produce.

Mycelium visible on host forming discrete spots around fruiting bodies. Perithecia gregarious in groups of many, (340–)370–490(–560) μm diam, superficial on an erumpent stroma, subglobose to pyriform, constricted below the apex to form an

small papilla 50–120 μm wide, not collapsing when dry, peach to orange, with papilla the same color than the perithecium, red in 3% KOH, yellow in lactic acid, surface of the perithecium smooth. Perithecial wall 20–40 μm, of two intergrading regions, outer of intertwined hyphae with cells slightly elliptical, inner region of flattened fusoids cells. Asci clavate, 65–100 × 15–25 μm, apex simple, 8-spored. Ascospores fusiform, sometimes one side flat and the other curved, (21.9–)22.8–24.5(–25) × (–9)9.5–10.5(–11) μm (mean 23 × 10 μm), 1-septate, slightly constricted at the septum, hyaline, striatedColonies on PDA 38–55 mm diam after 15 d at 25 C, aerial mycelium floccose, white to pale luteous, reverse white to orange, no pigment produced into the media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface, sometimes closer to the inoculum. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (13.8–)14.1–18.4(–25) × (–3.5)4–4.8(–5) μm (mean 16.3 × 4.4), with periclinal thickening and collarette. Macroconidia cylindrical or fusiform with round ends, 3–8- septate: 3-septate (28.2–)29.4–39.3(–41.9) × (5.2–)5.3–6.2(–6.4) μm (mean 34.3 × 5.8 μm), 4-septate (55–)56.6–63.9(–64.9) × (7.5–)7.9–8.8(–9.1) μm (mean 60.2 × 8.3 μm), 5-septate (59.4–)64.8–76.8(–82.8) × (6.6–)8.2–9.5(–10) μm (mean 70.8 × 8.8 μm), 6-septate (67.8–)75–89.2(–97.9) × (8.2–)8.8–9.9(–10.9) μm (mean 82.1 × 9.3 μm), 7-septate (75.2–)81.1–94.4(–110.1) × (8–)8.9–10.1(–11) μm (mean 87.7 × 9.5 μm), 8-septate (95–)96.1–99(–100.1) × (9.9–)10.1–10.7(–10.9) μm (mean 97.5 × 10.4 μm). Microconidia produced (6.6–)8.1–10.7(–11.6) × (4.4–)4.6–5(–5.7) μm (mean 9.9 × 4.9 μm) No chlamydospores were observe in culture.

Habitat and distribution. Saprobe on *Bauhinia longicupsis* and other plant organic matter, known from the type locality and Guatemala, probably distributed in the Neotropics.

Additional specimens examined. GUATEMALA. On *Theobroma cacao* fruit, Oct 1959, W. Gerlach (culture only CBS 213.59).

Notes. This species is similar to *T. neotropicalis*, however, *T. magna* produces microconidia in culture. See notes for *M. neotropicalis*.

Macronectria neotropicalis C. Salgado & P. Chaverri, sp. nov.

FIG. 4.16.

Mycobank TBD.

Similar to *Macronectria jungneri*, ascospores 26.2 × 9.7 μm, macroconidia 5–7-septate, chlamydospores produced 10 × 15 μm diam, distributed in Brazil (type locality) and Costa Rica.

Holotype. BRAZIL. BAHIA: Igrapiuna, Michelin Ecological Reserve, on bark, 5 Dec 2010, P. Chaverri et al. (BPI X, culture PC 1213 = CBS 136791).

Etymology. Refers to the Neotropics, were this species can be found.

Mycelium not visible on host. Perithecia gregarious in groups of many, (350–)375–495(–540) μm diam, superficial on an erumpent stroma, subglobose to pyriform, constricted below the apex to form an broad papilla 150–250 μm wide, not collapsing when dry, orange to sienna, with papilla the same color than the perithecium, red in 3% KOH, yellow in lactic acid, surface of the perithecium smooth. Perithecial wall 25–50 μm, of two intergrading regions, outer of intertwined hyphae with cells slightly elliptical, inner region of flattened fusoids cells. Asci clavate, 55–100 × 15–30 μm,

apex simple, 8-spored. Ascospores fusiform, sometimes one side flat and the other curved, (21.4–)23.2–29.2(–31.4) × (–8.1)9–10.3(–11) μm (mean 26.2 × 9.7 μm), 1-septate, slightly constricted at the septum, hyaline, striated. Colonies on PDA 43–47 mm diam after 15 d at 25 C, aerial mycelium floccose, white to luteous, reverse white to cinnamon, no pigment released into the media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface, sometimes closer to the inoculum. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (12.8–)13.1–20(–25) × (–3.5)4–5(–6) μm (mean 16.5 × 4.5), with periclinal thickening and collarette. Macroconidia cylindrical or fusiform with round ends, 5–7- septate: 5-septate (70.9–)74.7–80.6(–84.9) × (8–)8.6–9.7(–10.3) μm (mean 77.7 × 9.2 μm), 6-septate (72.9–)78–84.1(–89.8) × (8.3–)8.7–9.9(–10.4) μm (mean 81 × 9.3 μm), 7-septate (79.6–)83.1–89.5(–90) × (9.3–)9.4–10.3(–10.6) μm (mean 86.3 × 9.9 μm). No microconidia nor chlamydospores produced in culture.

Habitat and distribution. Saprobe on woody substrates, know from the type locality (Brazil) and Costa Rica, possible Neotropical.

Additional specimens examined. COSTA RICA. LIMON PROVINCE: Parque Nacional Braulio Carrillo, entrance from Calle Zurquí, 10°02'N, 84°01', elev. 1562, on bark of recently killed tree, 13 Mar. 2010, C. Salgado, A.Y. Rossman, G.J. Samuels, Y. Hirooka, C. Herrera, P. Chaverri, (BPI X, culture G.J.S. 10-125 = CBS 136790).

Notes. Two species in *Macronectria* are found to have a Neotropical distribution, *M. magna* and *M. neotropicalis*, however, the latter produces 3-8-septate macroconidia, compared to *M. magna* having 5-7-septate macroconidia.

Macronectria shennongjiana (J. Luo & W.Y. Zhuang) C. Salgado & P. Chaverri, comb. nov.

Mycobank TBD.

Basionym: Neonectria shennongjiana J. Luo & W.Y. Zhuang, Mycologia, 102: 145. 2010.

Holotype. CHINA. HUBEI: Shennongjia, 1700 m, on twigs of a dicotyledonous tree, 17 Sept 2003, X.M. Zhang Z153 (HMAS 183185, ex-type culture HMAS 173254.

Habitat and distribution. Saprobe on woody substrates, known from the type locality only.

Descriptions and illustrations. See Luo and Zhuang (2010).

Notes. The morphology of the asexual state of *Macronectria shennongjiana* is similar to that of "*Nectria" lugdunensis* (=*Heliscus lugdunensis*), specifically the presence of clove-shaped conidia. Further work is needed to confirm if these two names are synonyms, or if the correspond to different species. In this study, we found *M. shennongjiana* to be related to species in *Macronectria*.

Macronectria venezolana C. Salgado & P. Chaverri, sp. nov.

FIG. 4.17.

Mycobank TBD.

Similar to *Macronectria jungneri*, ascospores 29.3 × 11 μm, macroconidia 5–7-septate. Only known from Venezuela (type locality).

Holotype. VENEZUELA. ARAGUA STATE: ca. 10 km, above Maracay, on the Maracay-Choroni road, Parque Nacional Henry Pittier, on unidentified wood, 12 Jul

1971, K.P. Dumont, J.H. Haines, G.J. Samuels (NY Dumont-VE 1980, ex-type culture C.T.R. 71-244 = CBS 122576).

Etymology. Refers to the localities this species can be found.

Mycelium not visible on host. Perithecia gregarious in groups of many, (330–)375–480(–530) μm diam, superficial on an erumpent stroma, subglobose to pyriform or flask shaped, constricted below the apex to form an broad papilla 170–280 μm wide, not collapsing when dry, orange to sienna, with papilla the same color than the perithecium, red in 3% KOH, yellow in lactic acid, surface of the perithecium smooth. Perithecial wall 25–50 μm, of two intergrading regions, outer of intertwined hyphae with cells slightly elliptical, inner region of flattened fusoids cells. Asci clavate, 53–100 × 15–30 μm, apex simple, 8-spored. Ascospores fusiform, sometimes one side flat and the other curved, (24.1–)26.7–31.8(–33) × (–10.6)11–12.2(–13.2) μm (mean 29.3 × 11.6 μm), 1-septate, slightly constricted at the septum, hyaline, striated. Colonies on PDA 40–48 mm diam after 15 d at 25 C, aerial mycelium floccose, white to luteous, reverse white to saffron, no pigment released into the media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface, sometimes closer to the inoculum. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (10.8–)11.1–20(–27) × (–3.5)4–6(–7) μm (mean 15.5 × 5), with periclinal thickening and collarette. Macroconidia cylindrical or fusiform with round ends, 5–7-septate: 5-septate (73.1–)76.8–85.5(–90.7) × (8.3–)8.9–10.1(–10.6) μm (mean 81.2 × 9.5 μm), 6-septate (74.6–)80.7–88.6(–90.2) × (8.2–)8.9–10.3(–11) μm (mean 84.6 ×

9.6 μm), 7-septate (82.3–)84.9–91(–94.3) × (9–)9.5–10.8(–11.5) μm (mean 88 × 10.2 μm). No microconidia nor chlamydospores produced in culture.

Habitat and distribution. Saprobe on woody substrates, found only in Venezuela.

Additional specimens examined. VENEZUELA. BOLIVAR STATE: The Gran Sabana National Park, 1139 m, on wood of unidentified dead tree, 29 Jun 2009, C. Salgado, Y. Hirooka (BPI X, culture G.J.S. 09-1343 = CBS 136786).

Notes. Isolates produce macroconidia 5-7-septate in culture. This species can only be found in Venezuela, being this geographic restriction what can be used to differentiate this species with others in the group also producing macroconidia 5-7-septate in culture.

Tumenectria C. Salgado & Rossman, gen. nov.

FIG. 4.18.

Mycobank TBD.

Etymology. Refers to the round knobby aspect of the perithecial apex, often looking like a protuberance or a bump.

Type. *Tumenectria laetidisca* (Rossman) C. Salgado & Rossman

Ascomata solitary or several arising from same point, stroma absent, easily detached from substratum, globose, 370–580 × 300–415 μm, orange to scarlet, red in 3% KOH, yellow in lactic acid, perithecial wall smooth, asci narrowly clavate, ascospores fusiform 3-septate, hyaline. Anamorph cylindrocarpon-like, 3–6-septate. Found on wood and culms of bamboo in Jamaica.

Notes. The genus *Tumenectria* includes one species that is found primarily on woody substrate of bamboos. This genus has been collected only in the teleomorphic state,

and it is characterized by producing phragmo-ascospores (3-septate) and by having '*Cylindrocarpon*'-like anamorphs with only 3–6-septate macroconidia produced in culture. Ascomata displays typical morphology of species in the genus *Thelonectria* due to the presence of a dark and prominent papilla. Isolates in *Tumenectria laetidisca* were originally included in the genus *Nectria*, however, they have little similarity to the type of that genus. Phylogenetic analyses carried out in this study show it forms a monophyletic group sister to the genera *Campylocarpon* and *Rugonectria*.

Tumenectria laetidisca (Rossman) C. Salgado & Rossman, comb. nov.

FIG. 4.18.

Mycobank TBD.

Basyonym: *Nectria laetidisca* Rossman, Mycol. Pap. 150:36. 1983.

Ascome solitary, globose, 370–580 × 300–415 μm, , perithecial wall smooth, apex broad mammiform-like, asci narrowly clavate, ascospores fusiform 3-septate, hyaline. Anamorph '*Cylindrocarpon*'-like, 3–6-septate. Found on wood and culms of bamboo in Jamaica.

Holotype. JAMAICA. ST. ANDREW PARISH: Chesterville Youth Camp, on bark of bamboo, 8 Jan 1971, R.P. Korf (NY CUP-MJ 768, ex-type culture not available).

Etymology. Refers to the mammiform shape of the apex.

Mycelium not visible on host, perithecia solitary or gregarious arising from the same point, superficial without stroma, perithecia orange to sienna, red in 3% KOH, yellow in lactic acid, 370–580 × 300–415 μm, with broadly rounded to flattened papilla, perithecial wall smooth, with two regions, outer 20-35 μm wide with angular cells,

inner region 5–8 μm wide with elongated cells. Asci unitunicate, apex simple, 100–120 × 17–30 μm, clavate, 8-spored. Ascospores fusiform with round ends, (54.1–)56.6–63.3(–69) × (7.9–)8.2–9.2(–9.9) μm (mean 60 × 8.7 μm), 3-septate, hyaline, smooth regularly multiseriated. Colonies on PDA 28–31 mm diam after 15 d at 25 C, aerial mycelium floccose, white to buff, reverse white to buff, with no pigment diffusing to the media. Conidia on SNA forming in hyaline, slimy droplets in aerial mycelium or on agar surface. Phialides borne apically on irregularly branching clusters of cells or directly from hyphae, cylindrical or slightly swollen (20–)25.5–31.2(–36) × (–3.6)4–5(–5.6) μm (mean 27.5 × 4.5 μm), with periclinal thickening and with slight constriction at base of collarette. Macroconidia cylindrical to slightly fusiform, wider at the center, with round ends, 3–6- septate: 3-septate (48.6–)50.2–60.4(–77.1) × (7.4–)8.1–9.5(–10.4) μm (mean 55.3 × 8.8 μm), 4-septate (49.8–)54–61.7(–66.4) × (8–)8.5–9.8(–10.9) μm (mean 57.8 × 9.1 μm), 5-septate (54.9–)57.6–67(–71.1) × (7.7–)8.3–9.5(–10) μm (mean 62.3 × 8.9, 6-septate (61.7–)61.9–70.5(–71.1) × (9.1–)9.4–9.7(–10) μm (mean 62.1 × 9.5 μm). No microconidia nor chlamydospores produced in culture.

Habitat and distribution. On wood and culms of bamboo, distributed in Jamaica (type locality) and Japan. Possibly widely distributed in the tropics.

Additional specimens examined. JAMAICA. ST. ANDREW PARISH: trail between Holywell and source of Wagwater River, on bamboo, 11 Jan 1971, R.P. Korf et al. (NY CUP-MJ 770, culture C.T.R. 71-14 = CBS 101909). JAPAN. NAGANO PREFECTURE: Ueda, Bessho Onsen forest park, temple area near Ueda city, ca 50 m

behind Bessho Shrine, on bark of recently dead bamboo stem, 15 Aug 1997, H.-J. Schroers (CBS H.J.S. 203, culture CBS 100284).

Notes. *Tumenectria laetidisca* was first thought to belong to the genus *Thelonectria*, based on the morphology of the perithecial wall, the presence of a broad papilla and the '*Cylindrocarpon*'-like anamorph. However, it is the only producing phragmoascospores and cylindrocarpon-like anamorphs. This species is rarely collected, possibly due to its host specificity, as it seems to only occur on bamboo species. The original description of the species includes an isolate from Venezuela, which displays ascospores consistently 5-septate. It is possible it constitutes a different species within the genus, also happening on bamboo species.

Discussion

Generic concepts

In this study, we revised the generic concept of *Thelonectria* sensu Chaverri et al (2011) and the taxonomic position of species with morphological resemblance to those of *Thelonectria*. Based on the results obtained in this study and those from previous studies (Chaverri et al. 2011, Salgado-Salazar et al. 2012, 2013, Salgado-Salazar et al. *submitted*) it was determined that the criteria used in the past for generic differentiation were insufficient to reflect accurately the degree of diversity shown by these species. The application of additional criteria has resulted in the grouping of these highly divergent species under a few morphological genera. With the use of genetically sensitive characters, such as DNA, we have reached a more compatible system of taxonomy that reflects a phylogenetic classification. Here, we propose a redefinition of the genus *Thelonectria* that excludes *T. jungneri* and *T. viridispora*,

and establish three new genera to accommodate *T. jungneri* and the diversity of species having cylindrocarpon-like anamorphs that have not been included in previous taxonomic studies, such as '*Nectria' laetidisca* and '*Neonectria' cinnamomea*. These new groups show high bootstrap and posterior probability values supporting their distinction. Even though the use of molecular characters has improved the field of fungal taxonomy, the existence of extensive morphological convergence still poses a challenge, as the accurate classification of species can only be achieved using molecular phylogenetic approaches. As the formal description of taxonomic novelties requires diagnostic characters as part of the description, these characters may still be ambiguous. This issue is widely recognized among fungal taxonomists, and, in recent years, the incorporation of molecular sequences to define taxa has been proposed as valid (Hawksworth et al. 2011, Jones et al. 2011).

Species concepts

Sequence-based methods to delimit species are central in fungal taxonomy. In spite of diverse species recognition criteria (Sites and Marshall 2003, de Queiroz 2007), the dominant fungal operational species criterion is the Genealogical Concordance Phylogenetic Species Recognition (GCPSR, Taylor et al 2000). This recognition criterion is based on the Phylogenetic Species Concept (PSC) defined by Cracraft (1983), however, it incorporates a method to account for the presence of genealogical discordance, or gene tree/species tree incongruence (Taylor et al. 2000, Taylor et al. 2006). This criterion has been proven to be immensely useful in fungi, including members of the family Nectriaceae, in which many of the species lack discrete morphological characters useful for species delimitation, allowing the definition of a

higher number of putative species than is possible with a morphological species concept (Hibbet et al. 1995, Hirooka et al. 2011, Salgado-Salazar et al. 2012, 2013). Nowadays, a taxonomic revision of morphologically cryptic species like these begins with a phylogenetic analysis aimed at sorting individual isolates into discrete groups, and ends with the recognition of variable phenotypic characters that correlate with the phylogenetic species (Taylor et al. 2006). In this study, and in previous ones by Salgado-Salazar et al (2012, 2013, *submitted*), overlapping variability of the quantifiable morphological features, such as size of ascospores, macro- and microconidia, was observed, however, in most of the cases average values proved to be separate species. Only when this careful observation of morphology is combined with data about geographic origin and hosts specificity, when available, is it possible to delineate and diagnose species. Despite the difficulty of defining and characterizing species in *Thelonectria* and related genera without prior genetic grouping, we have identified significant differences among species, especially those forming species complexes. Within the genus *Macronectria*, species deliminatation posed a special difficulty because many of the morphological characters have ambiguous limits. Four out of the 19 isolates formed single isolate lineages, highlighting the need of an increase in the number of representatives samples for this group to increase support values for otherwise distinct species. The resolution of the species relationships of the taxa considered in this study improved greatly as methods were used for masking regions where the alignment is uncertain. This is particularly evident in the genus *Macronectria*, in which the internal resolution for the species is improved compared with the one obtained using the original alignment. Despite

losing information during masking using less stringent settings (Gbocks-2), there is an overall increase in the phylogenetic signal as the same species relationships were recovered compared to the analyses of the original alignment although with slightly lower branch support (see FIG 4.1 AND S4.1). In this case analyses of alignments including divergent and problematic regions recover the true species structure of the group, while a better resolution of intraspecific relationships is improved with the use of masking approaches (Talavera and Castresana 2007, Wu et al. 2012).

Morphological stasis, evolution and taxonomy

The discussion of the phylogenetic patterns observed in this study is largely based on the results of the combined analyses of sequence data. The results showed that the cylindrocarpon-like conidial and mammiform perithecial morphology are symplesiomorphic characters among these species, and have remained relatively unchanged from their ancestral form. In the kingdom Fungi, it is suggested that genetic isolation precedes reproductive isolation and both of these precede morphological divergence (Taylor et al. 2006). This explains the highly divergent species with similar morphologies like those encountered in this study. Little is known about the adaptive significance of retained ancestral characters in microorganisms, however, one must not exclude the possibility that the cylindrocarpon-like conidial and mammiform perithecial morphology may have initially been retained in these species because they confer a selective advantage. In the phylogenetic tree we observe that the cylindrocarpon-like morphology precedes the appearance of the mammiform perithecial morphology and that only one loss of

the cylindrocarpon-like morphology and three of the mammiform perithecial morphology have occurred along the diversification of species in the group.

The challenge of the biogeographic distribution

The presence of species that seemingly group individuals from geographically distant locations represents an interesting observation because it challenges the paradigms of isolation by distance (Meirmans 2012). Since no definitive explanation for the causes behind this phenomenon is available, a set of equally plausible hypotheses are explored. One of them deals with the possibility of either intentional or unintentional human or animal movement of plant material together with the associated saprobic species from one geographic place to another. Even though this hypothesis can explain these observations, it is difficult to prove without questioning, as the human movement of plant material without associated endangered, invasive or pathogenic species occurs mostly undetected. A second hypothesis explaining these results relies on the similarity of environments where individuals of a species are found. For example, the species *T. discophora* groups individuals coming from two distant locations, the Cowal Peninsula in Scotland and Llanquihue Province in Chile. These two regions exhibit similar climatic and environmental conditions, i.e. both are in temperate regions, with hard winters, high precipitation and mild summers. According to this, it is possible that the environmental homogeneity either drove individuals to show genetic and genotypic convergence, such that they coalesce in a monophyletic group because they have evolved similar genetic information, or this constitutes a case of biogeographic dispersal of evolutionarily conserved ecotypes. Even though the first possibility might constitute a scenario of genetic convergence

per se, it is difficult to prove this for microorganisms (Christin et al. 2010). The second case of biogeographic dispersal of evolutionarily conserved ecotypes seems more plausible as it has been proven in animals (Moen et al. 2013). Further studies addressing the roles of both evolutionary conservatism and convergence in species traits of fungi are needed, in order to understand similarity in traits over large spacial and temporal scales. Lastly, it is possible that this geographic distribution results from the fairly incomplete picture we have of the range and distribution of many fungi. Incomplete fungal inventories together with the incomplete knowledge of fungal ecology and biogeographic distribution have contributed to the observation of species with individuals from vastly different geographic locations (Bass and Richards 2011). Increased and intensive sampling throughout the potential species range could reveal either a continuous distribution, indicative of a cosmopolitan distribution, or would allow the observation of discontinuities in the geographic or ecological range of the species distribution (Mora et al. 2011).

Conclusions

The purpose of this study was to assess the monophyly of the genus *Thelonectria* as presently recognized and to determine the phylogenetic relationships of this genus with those species having morphological similarities but of uncertain phylogenetic placement. Our results support partially the previous classification of *Thelonectria* species, however, since one of the more common and easily recognized species in the group, *T. jungneri*, does not form a monophyletic group with the rest of the species, the generic limits have to be redefined. The differences in the phylogenetic inference produced by the analyses of the original alignment and alignments in which

uncertainties were removed or minimized by automated, reproducible methods (Gblocks) resulted in an overall increase in support and resolution of lineages. As taxonomists have more tools for identifying evolutionary independent genetic lineages within morphologically similar species, the ability to understand patterns and timing of species diversification will increase (Singhal and Moritz 2013). Even as many challenging questions have yet to be answered, with this study we have uncovered exceptionally high levels of undescribed cryptic diversity, increasing the species richness in *Thelonectria* from 22 to 39 species, and added three more genera to the diverse family Nectriaceae.

Key to *Thelonectria* species and new genera described

1. Perithecia with or without mammiform apex; ascospores 1-septate……..…2

1. Perithecia with mammiform apex, ascospores 3-septate……*Tumenectria laetidisca*

2. Perithecia with mammiform apex ……. ……..……...3

2. Perithecia without mammiform apex……..……..15

3. Perithecia globose with a flattened knobby apex and a fringe of saccate cells……*T. coronata* complex (See Chapter 1)

3. Perithecia globose with flattened knobby apex without a fringe of saccate cells…..4

4. Perithecia globose with a flattened knobby apex encompassing at least ¾ of perithecial diameter, surface of perithecial wall rough but not conspicuously warted; ascospores 15–23 × 6–9 μm, spinulose……*T. veuillotiana* complex (See Chapter 1)

4. Perithecia globose with a flattened or rounded knobby apex, constricted at the septum encompassing at least ¾ of perithecial diameter, surface of perithecial wall smooth; ascospores 26–34 × 9–13 μm, striated or spinulose……...5

5. Perithecia globose with a flattened knobby apex, not constricted at the base encompassing up to 1/3 of perithecial diameter; ascospores spinulose, not striated……6

5. Perithecia globose with a flattened knobby apex and constricted at the base encompassing at least ¾ of perithecial diameter; ascospores striated……10

6. Ascospores averaging 9–15 × 6–9 μm; colonies in culture purple with purple pigment produced in the media…… *T. discophora* complex (See Chapter 3)

6. Ascospores averaging 15–23 × 8–10 μm; colonies in culture white, not producing purple pigment……7

7. Ascospores average length and width up to 14 × 6 μm; 3-septate macroconidia present……*T. lucida*

7. Ascospores average length and width more than 14 × 6 μm; 3-septate macroconidia present or not……..8

8. Ascospores average 20.8 × 9.4 μm, macroconidia 4–7-septate, no microconidia or macroconidia 1–3-septate present…….*T. elata*

8. Ascospores averaging 19–22 × 8.4–10.4, microconidia present, macroconidia 1–7 septate present or not……9

9. Ascospores averaging 19.3 × 8.3 μm, microconidia present, macroconidia 1–7-septate present……*T. papillata*

9. Ascospores averaging 22 × 10.4 μm, microconidia absent, macroconidia 5–7-septate only, known only from Puerto Rico……*T. gibba*

10. Perithecia globose-subglobose, without prominent papilla, ascospores 12–16 × 3–4.8 μm, macroconidia clove-shaped…….……...*Macronectria shennongjiana*

10. Perithecia globose to pyriform, with broad, round papilla……..…….11

11. Perithecia globose to pyriform, with broad, round papilla, ascospores averaging 30.5 × 9.8 μm, no microconidia produced, macroconidia 5–7-septate……..*Macronectria jungneri*

11. Perithecia globose to pyriform, with broad and round papilla, ascospores averaging less than 30.5 × 9.8 μm, microconidia present, macroconidia 3, 4, 8-septate present…….12

12. Ascospores aver. 25 × 9 μm, microconidia present 8 × 6.6 μm, macroconidia 4–8 septate, only known from Asia (Japan-Taiwan)…….*Macronectria asiatica*

12. Microconidia present, not from Asian regions……13

13. Ascospores averaging 23 × 10 μm, microconidia present 9.1 × 4.8 μm, macroconidia 4–8-septate present, only known from Neotropical regions (French Guiana and Guatemala)……..*Macronectria magna*

13. Microconidia absent, also from Neotropical regions……14

14. Ascospores aver. 22.6 × 9.7 μm, macroconidia 5–7-septate only, chlamydospores present, from Brazil-Costa Rica……..*Macronectria neotropicalis*

14. Ascospores aver. 29.3 × 11 μm, macroconidia 5–7-septate, only known from Venezuela……..*Macronectria venezolana*

15. Only known from the asexual state, chlamydospores produced, sometimes found associated with plant roots, macroconidia 1–6-septate……...*T. olida*

15. Only known from the asexual state, no chlamydospores produced, unique macroconidia septation observed……...16

16. Asexual state only known, associated with *Theobroma cacao* plants, only macroconidia 3-septate produced, with slightly hooked ends……..*T. theobromicola*

16. Only macroconidia 3-septate produced, known from asexual and sexual states…….17

17. Perithecia globose to pyriform, surface of the perithecial wall looking scaly, with warts of circular cells 10–25 μm diam, macroconidia 3-septate produced only……..*T. rubrococca*

17. Perithecia globose to pyriform, surface of the perithecial wall looking scaly, with or without warts, macroconidia 1–5 septate……..18

18. Cells at the surface of perithecial wall warted, circular 15–40 μm diam, macroconidia 1–5-septate, only known from New Zealand…….*T. westlandica*

18. Cells at the surface of perithecial wall warted or smooth, circular 15–40 μm diam, macroconidia 1–4 or 3-septate only…….19

19. Surface of perithecial wall smooth or slightly roughened, without warts, ascospores aver. 17.5 × 7.6 μm diam, macroconidia 1–4-septate, known from the type locality (Scotland) and New Zealand……..…….*T. trachosa*

19. Perithecia pale brown to brick colored, with ostiolar area dark brown or nearly black, ascospores averaging 28.8 × 10.5 μm, cultures producing a characteristic

cinnamon colored pigment, macroconidia with sharply curved ends, 3-septate only……...*Cinnamonectria cinnamomea*

Notes. *Thelonectria platycephala* shares many morphological similarities with species in the *T. veuillotiana* complex. *Thelonectria platycephala* is only known from the type locality in Jamaica while none of the species in the *T. veuillotiana* complex are found in that locality. *Thelonectria mamma* can be segregated from other species in the *T. discophora* complex by the lack of the typical purple colony of the majority of representatives of *T. discophora*, and produces microconidia and macroconidia 1-6-septate. Macroconidia having 1-6 septae were not produced by any species in the *T. discophora* complex.

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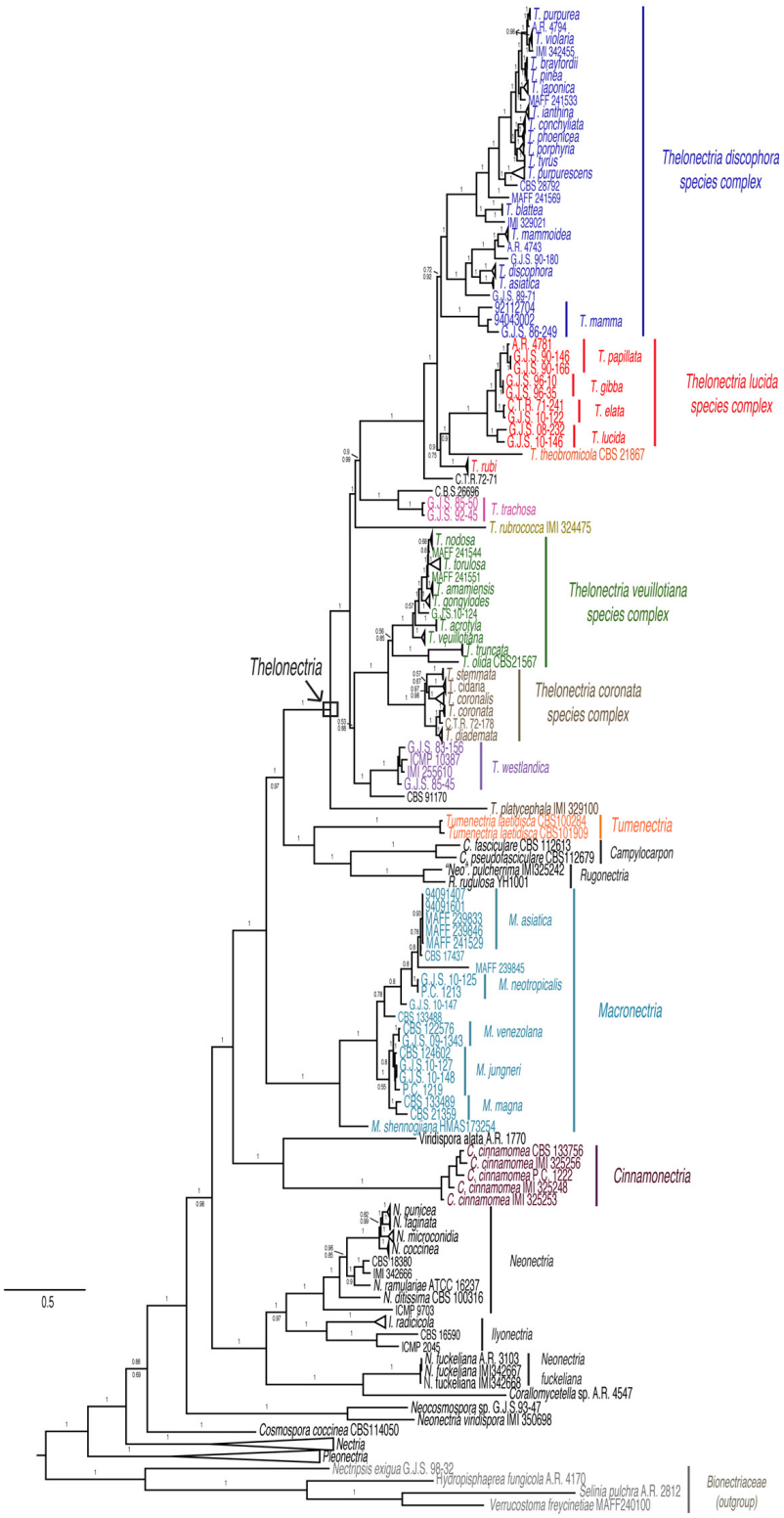
T. purpurea A.R. 4784
T. violaria IMI 342455
T. brayfordii
T. pinea
T. japonica MAFF 241533
T. ianthina
T. conchyllata
T. phoenicea
T. porphyria
T. tyrus
T. purpurescens CBS 28792
MAFF 241569
T. blattea IMI 329021
T. mammoidea A.R. 4743
G.J.S. 90-180
T. discophora
T. asiatica
G.J.S. 89-71
92112704
94043002
G.J.S. 86-249
T. mamma
Thelonectria discophora species complex
A.R. 4781
G.J.S. 90-146
G.J.S. 90-166
T. papillata
G.J.S. 96-10
G.J.S. 96-12
T. gibba
G.J.S. 96-35
G.J.S. 71-241
T. elata
G.J.S. 10-122
G.J.S. 08-232
G.J.S. 10-146
T. lucida
T. theobromicola CBS 21867
Thelonectria lucida species complex
T. rubi
C.T.R.72-71
C.B.S.26696
G.J.S. 85-51
G.J.S. 92-45
T. trachosa
T. rubrococca IMI 324475
T. nodosa MAFF 241544
T. torulosa MAFF 241551
T. amamiensis
T. gongyloides
G.J.S.10-124
T. acrotyla
T. veuillotiana
T. truncata
T. olida CBS21567
Thelonectria veuillotiana species complex
Thelonectria
T. stemmata
T. cidaria
T. coronalis
T. coronata
C.T.R. 72-178
T. diademata
Thelonectria coronata species complex
G.J.S. 88-156
ICMP 10387
IMI 255610
G.J.S. 85-45
CBS 91170
T. westlandica
T. platycephala IMI 329100
Tumenectria laetiosca CBS100284
Tumenectria laetiosca CBS101909
Tumenectria
C. fasciculare CBS 112613
C. pseudolasciculare CBS112679
Campylocarpon
"Neo." pulcherrima IMI325242
R. rugulosa YH1001
Rugonectria
94091407
94091601
MAFF 239833
MAFF 239845
MAFF 241529
CBS 17437
M. asiatica
MAFF 239845
G.J.S. 10-125
P.C. 1213
G.J.S. 10-147
M. neotropicalis
CBS 133488
CBS 122576
G.J.S. 09-1343
M. venezolana
CBS 124802
G.J.S. 10-127
G.J.S. 10-148
P.C. 1219
M. jungneri
CBS 133489
CBS 21359
M. magna
M. shennongjiana HMAS179254
Macronectria
Viridispora alata A.R. 1770
C. cinnamomea CBS 133756
C. cinnamomea IMI 322256
C. cinnamomea P.C. 1222
C. cinnamomea IMI 325248
C. cinnamomea IMI 325253
Cinnamonectria
N. punicea
N. faginata
N. microconidia
N. coccinea
CBS 18380
IMI 342666
N. ramulariae ATCC 16237
N. ditissima CBS 100316
ICMP 9703
Neonectria
0.5
I. radicicola
CBS 16590
ICMP 2045
Ilyonectria
N. fuckeliana A.R. 3103
N. fuckeliana IMI342667
N. fuckeliana IMI342668
Neonectria fuckeliana
Corallomycetella sp. A.R. 4547
Neocosmospora sp. G.J.S. 99-47
Neonectria viridispora IMI 350698
Cosmospora coccinea CBS114050
Nectria
Pleonectria
Nectripsis exigua G.J.S. 98-32
Hydropisphaerea fungicola A.R. 4170
Selinia pulchra A.R. 2812
Verrucostoma freycinetiae MAFF240100
Bionectriaceae (outgroup)

FIGURE 4.1. Majority rule Bayesian phylogram showing relationships among species in *Thelonectria* and related species with *Cylindrocarpon*-like anamorphs based on the concatenated analysis of eight loci. Number above branches indicate Bayesian posterior probabilities for analysis of data set after cleaning alignment of problematic regions with Gblocks setting 2. Number below branches indicate Bayesian posterior probabilities for analysis of original data set. Bionectriaceae was used as outgroup.

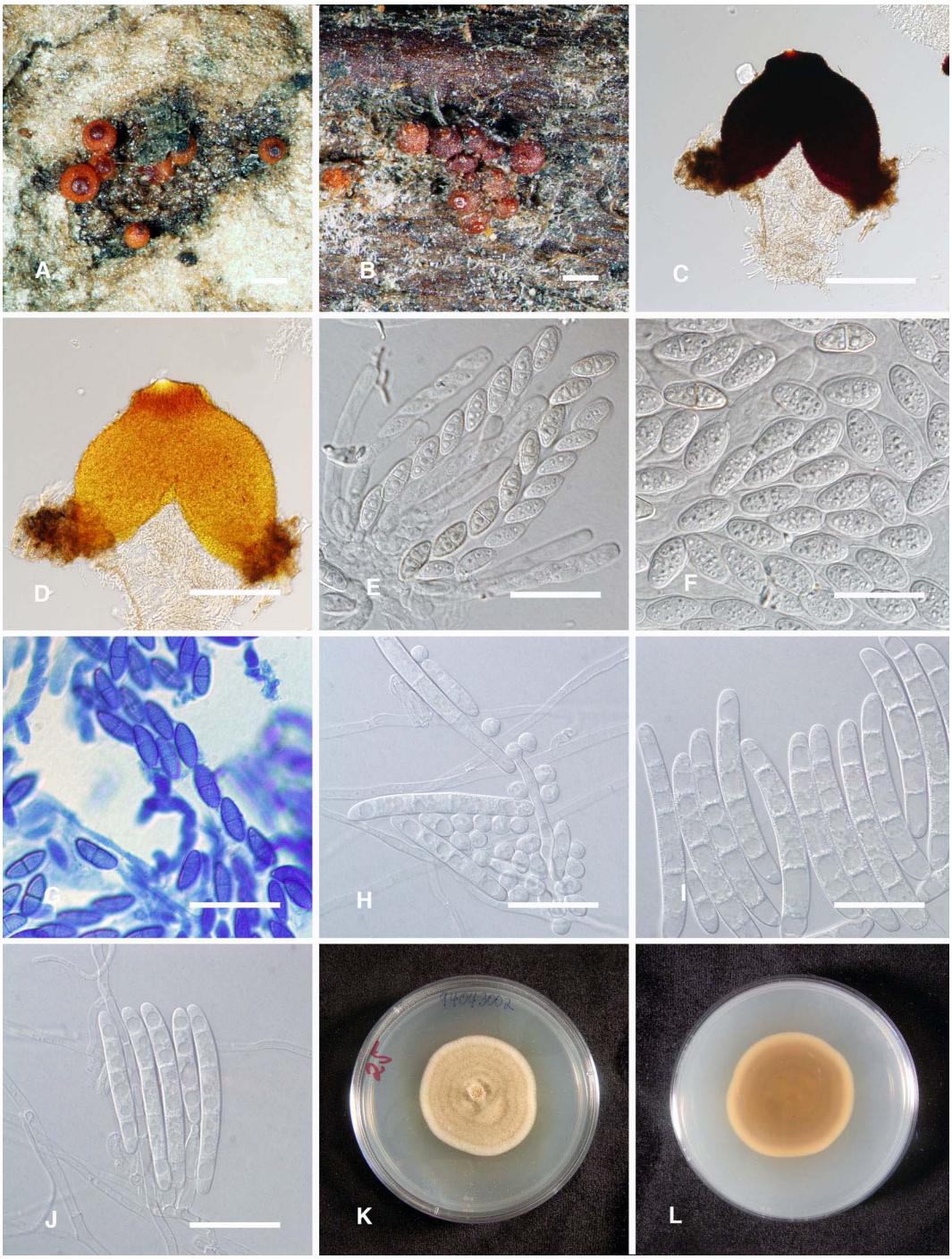

FIGURE 4.2. *Thelonectria mamma*. A. Perithecia (94043002 = BPI X). B. Perithecia (92112704 = BPI X). C. Squash mount of perithecia with 3% KOH, note the perithecial apex (94043002 = BPI X). D. Squash mount of perithecia after lactic acid (94043002 = BPI X). E-G. Asci and ascospores in 3% KOH and cotton blue

(92112704 = BPI X). H. Conidiophores, macroconidia and microconidia on SNA (94043002 = CBS 136787). I-J. Macroconidia on SNA (94043002 = CBS 136787). K. Colony on PDA (94043002 = CBS 136787). L. Colony reverse on PDA (94043002 = CBS 136787). Bars: A-B = 500 µm; C-D = 200 µm; E-J = 50 µm.

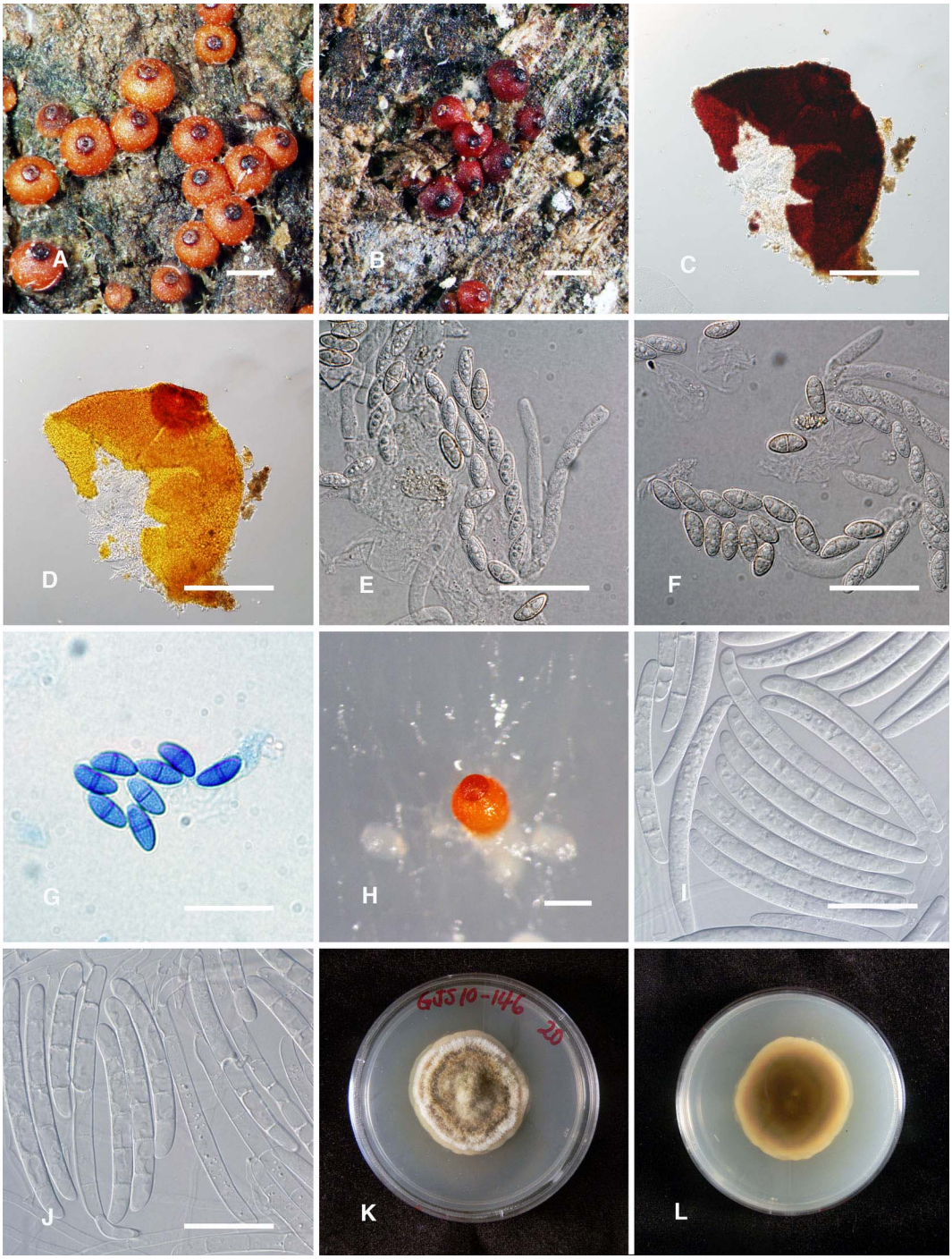

FIGURE 4.3. *Thelonectria lucida* s. str. A. Perithecia (G.J.S. 10-146 = BPI X). B. Perithecia (G.J.S. 08-232 = BPI 882041). C. Squash mount of perithecia with 3% KOH, note the perithecial apex (G.J.S. 10-146 = BPI X). D. Squash mount of perithecia after lactic acid (G.J.S. 10-146 = BPI X). E-G. Asci and ascospores in 3% KOH and cotton blue. H. Perithecia growing on SNA (G.J.S. 10-146 = CBS 136788). I-J. Macroconidia on SNA (G.J.S. 10-146 = CBS 136788). K. Colony on PDA (G.J.S. 10-146 = CBS 136788). L. Colony reverse on PDA (G.J.S. 10-146 = CBS 136788). Bars: A-B = 500 μm; C-D = 200 μm; E-J = 50 μm.

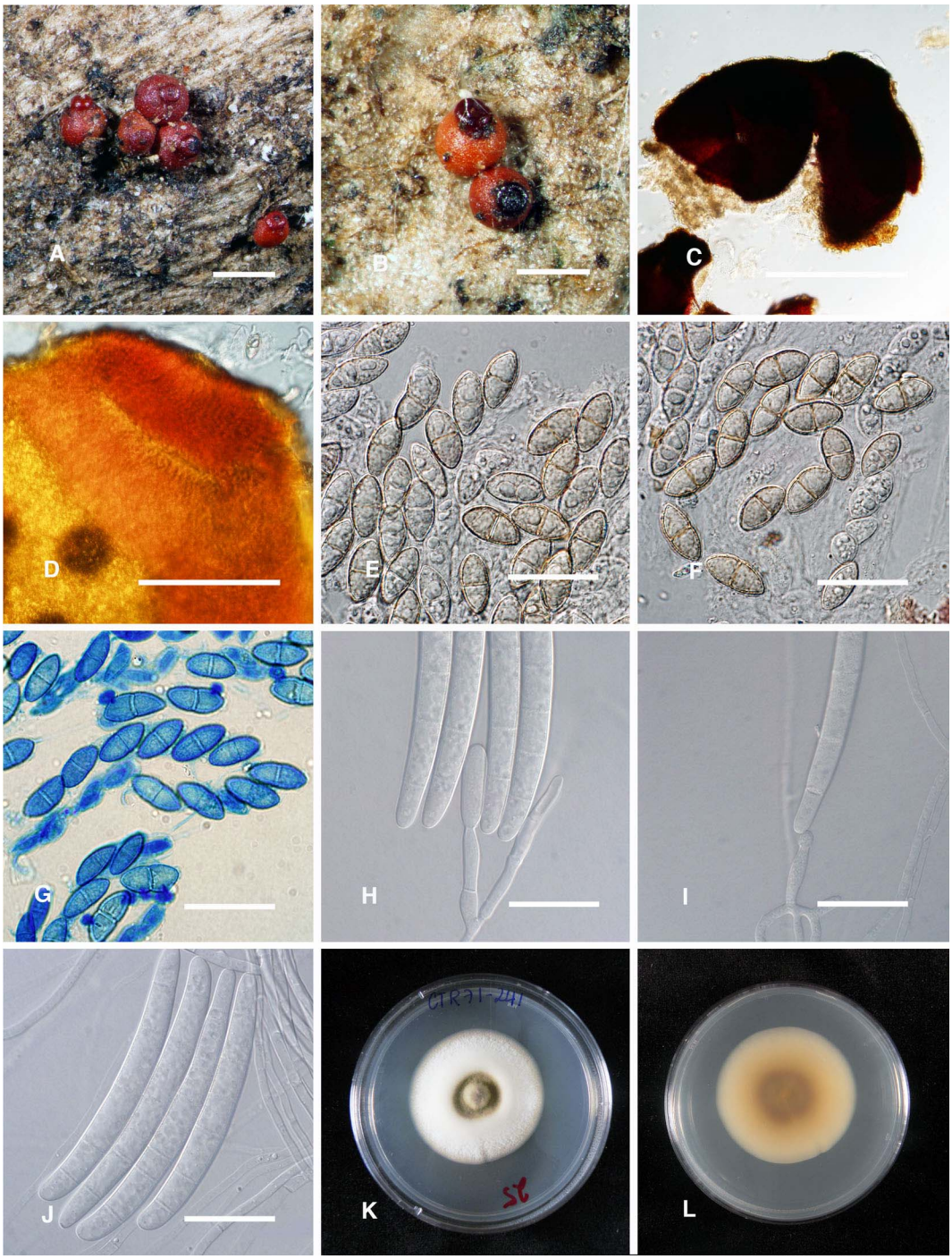

FIGURE 4.4. *Thelonectria elata*. A. Perithecia (C.T.R. 71-241 = NY KPD-VE 1702). B. Perithecia (G.J.S. 10-122 = BPI X). C. Squash mount of perithecia with 3% KOH, note the perithecial apex (G.J.S. 10-122 = BPI X). D. Squash mount of perithecia after lactic acid (G.J.S. 10-122 = BPI X). E-G. Asci and ascospores in 3% KOH and

cotton blue (G.J.S. 10-122 = BPI X). H-I. Conidiophores (C.T.R. 71-241 = CBS 112454). J. Macroconidia (C.T.R. 71-241 = CBS 112454). K. Colony on PDA (C.T.R. 71-241 = CBS 112454). L. Colony reverse on PDA (C.T.R. 71-241 = CBS 112454). Bars: A-B = 500 μm; C = 200 μm; D = 100 μm; E-J = 50 μm.

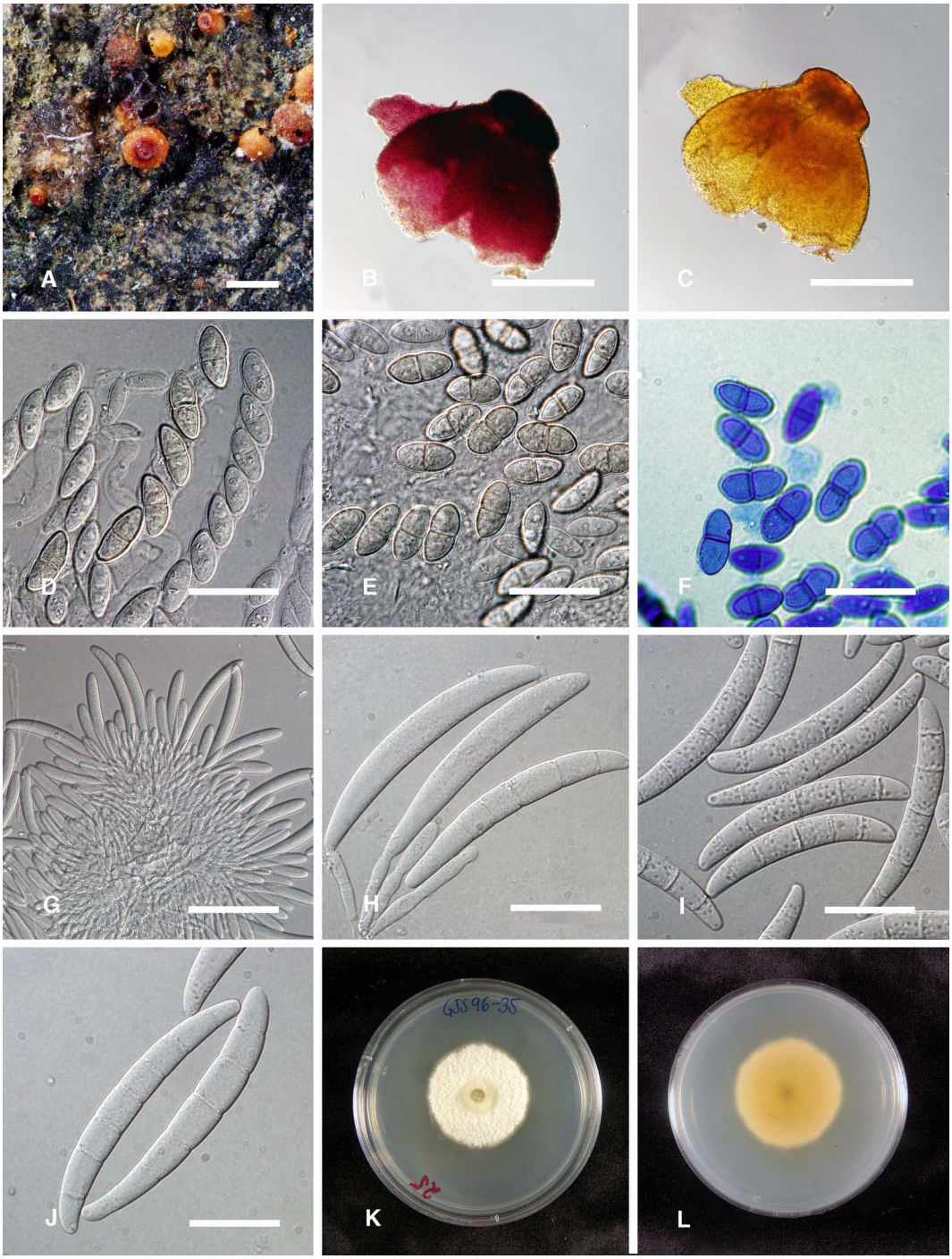

FIGURE 4.5. *Thelonectria gibba*. A. Perithecia (G.J.S. 96-10 = BPI 744634). B. Squash mount of perithecia with 3% KOH, note the perithecial apex (G.J.S. 96-10 = BPI 744634). C. Squash mount of perithecia after lactic acid (G.J.S. 96-10 = BPI 744634). D-F. Asci and ascospores with 3% KOH and cotton blue (G.J.S. 96-35 = BPI 745543). G. Pionnotes on SNA (G.J.S. 96-10 = IMI 370944). H. Conidiophores on SNA (G.J.S. 96-10 = IMI 370944). I-J. Macroconidia on SNA (G.J.S. 96-10 = IMI 370944). K. Colony on PDA (G.J.S. 96-35 = CBS 112456). L. Colony reverse on PDA (G.J.S. 96-35 = CBS 112456). Bars: A = 500 μm; B-C = 200 μm; D-J = 50 μm.

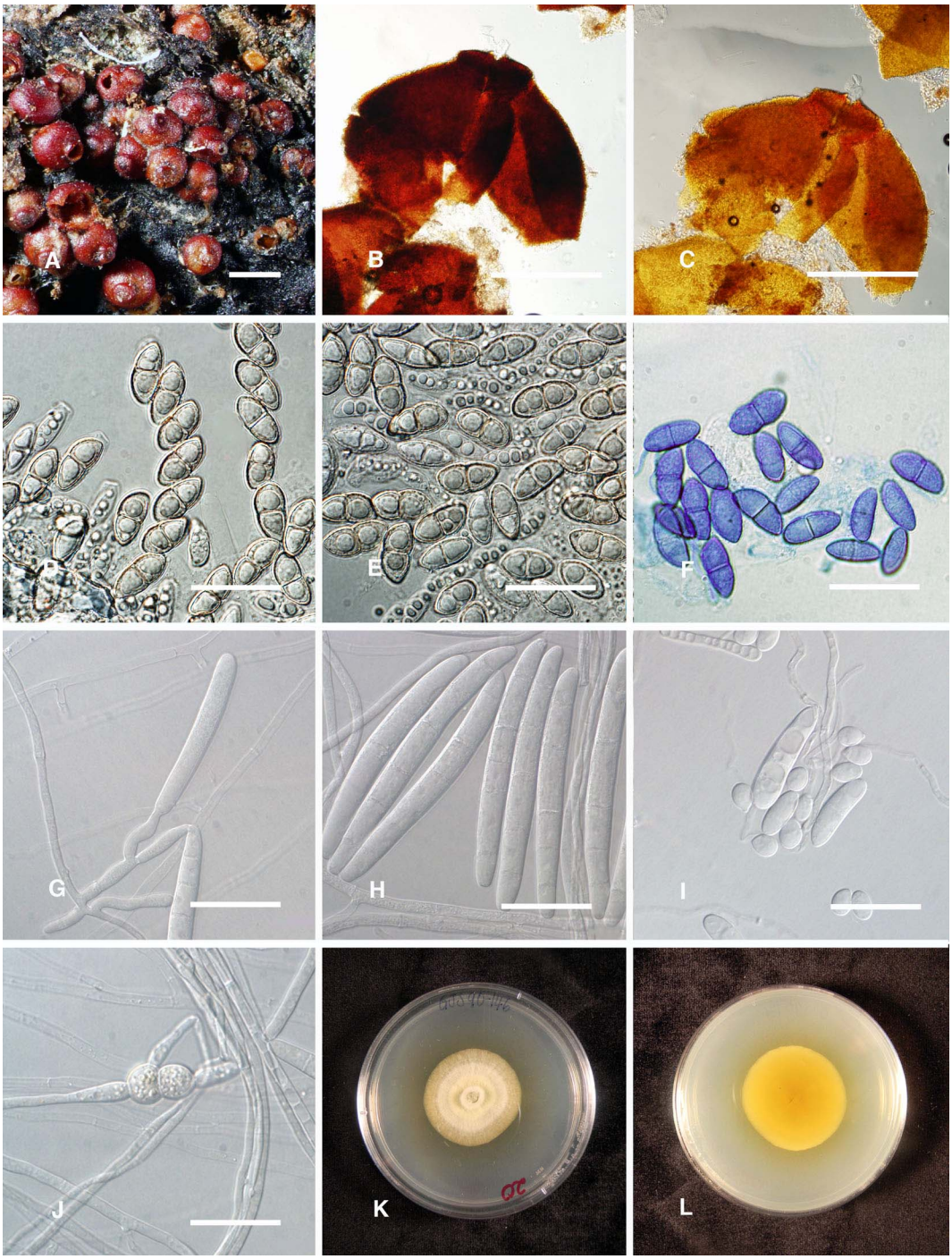

FIGURE 4.6. *Thelonectria papillata*. A. Perithecia (G.J.S. 90-146 = BPI 842126). B. Squash mount of perithecia with 3% KOH, note the perithecial apex (A.R. 4781 = BPI X). C. Squash mount of perithecia after lactic acid (A.R. 4781 = BPI X). D-F. Asci and ascospores in 3% KOH and cotton blue (A.R. 4781 = BPI X). G-H.

Conidiophores and macroconidia on SNA (A.R. 4781 = CBS 134136). I. Microconidia and macroconidia 1-septate on SNA (A.R. 4781 = CBS 134136). J. Chlamydospores on SNA (G.J.S. 90-146 = CBS 134032). K. Colony on PDA (G.J.S. 90-146 = CBS 134032). L. Colony reverse on PDA (G.J.S. 90-146 = CBS 134032). Bars: A = 500 μm; B-C = 200 μm; D-J = 50 μm.

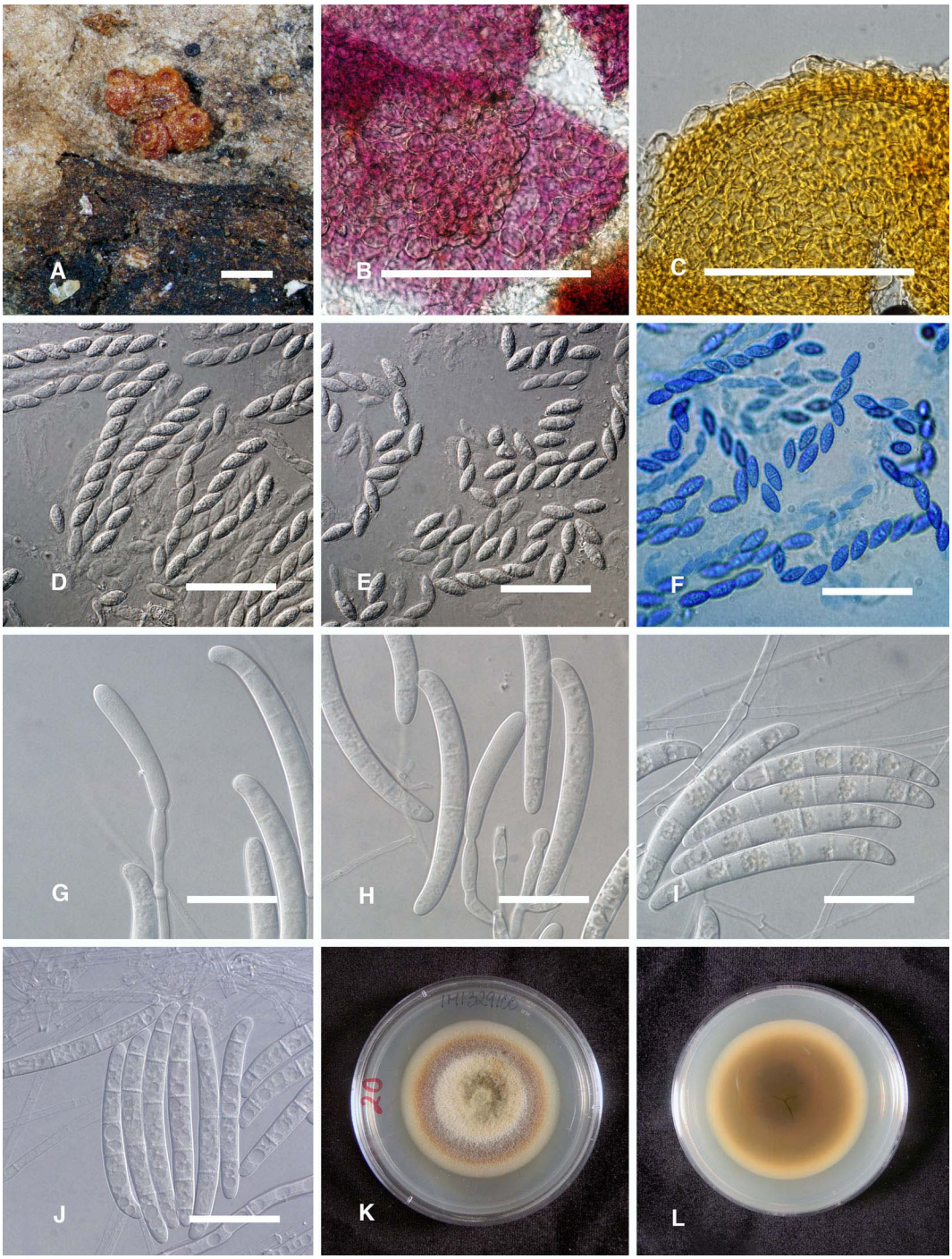

FIGURE 4.7. *Thelonectria platycephala*. A. Perithecia (C.T.R. 71-25 = CUP-MJ 790). B. Squash mount of perithecial wall in 3% KOH (C.T.R. 71-25 = CUP-MJ 790). C. Squash mount of perithecial wall after lactic acid (C.T.R. 71-25 = CUP-MJ 790). D-F. Asci and ascospores in 3% KOH and cotton blue (C.T.R. 71-25 = CUP-MJ 790).

G-H. Conidiophores on SNA (C.T.R. 71-25 = IMI 329100). I-J. Macroconidia on SNA (C.T.R. 71-25 = IMI 329100). K. Colony on PDA (C.T.R. 71-25 = CUP-MJ 790). L. Colony reverse on PDA (C.T.R. 71-25 = CUP-MJ 790). Bars: A = 500 μm; B-C = 100 μm; D-J = 50 μm.

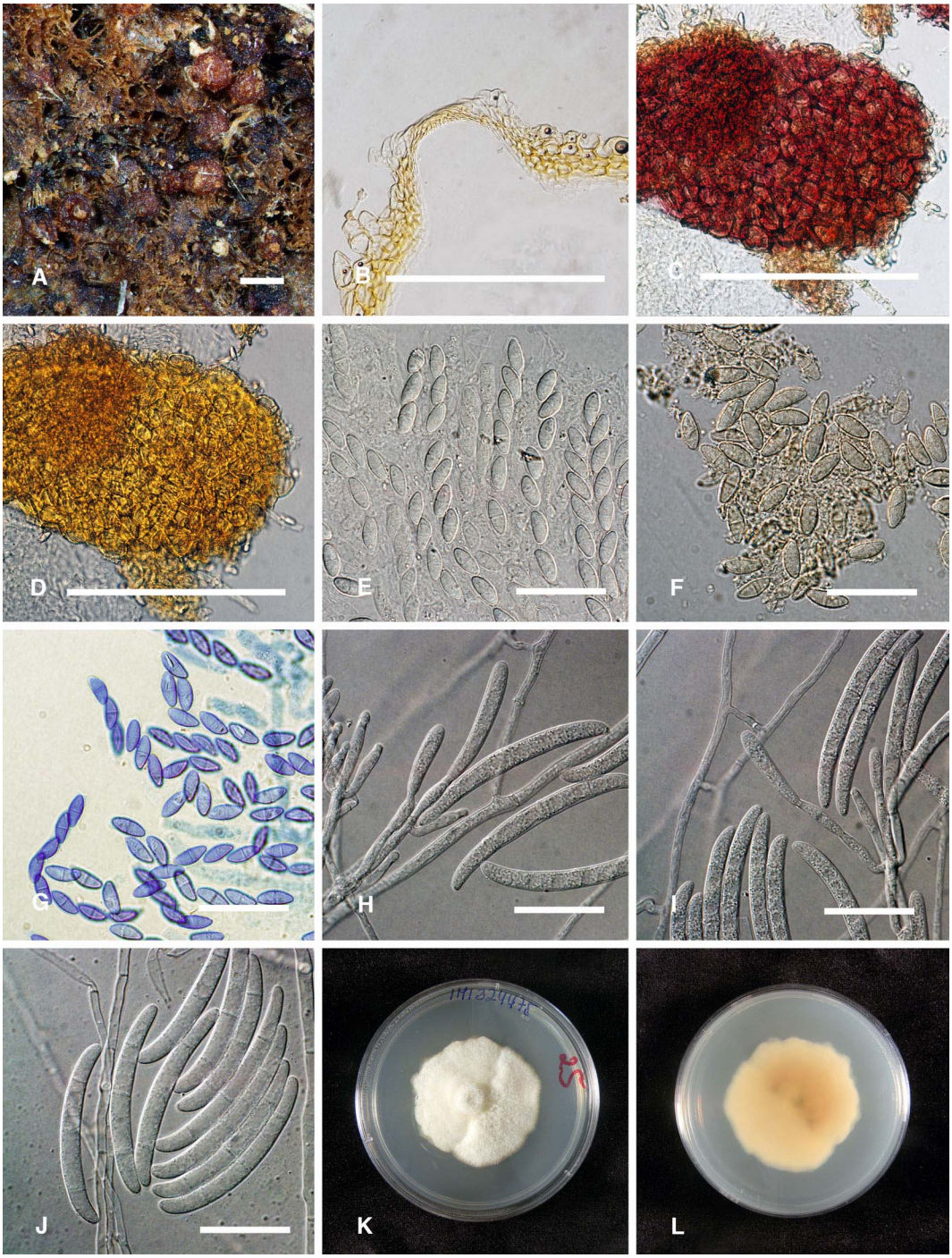

FIGURE 4.8. *Thelonectria rubrococca*. A. Perithecia (G.J.S. 83-330 = NY GJS4484). B Longitudinal sections of perithecia (G.J.S. 83-330 = NY GJS4484). C. Squash mount of perithecial wall with warts in 3% KOH (G.J.S. 83-330 = NY GJS4484). D. Squash mount of perithecial wall with warts after lactic acid (G.J.S. 83-330 = NY GJS4484). E-G. Asci and ascospores in 3% KOH and cotton blue (G.J.S. 83-330 = NY GJS4484). H-J. Conidiophores and macroconidia on SNA (G.J.S. 83-330 = IMI 324475). K. Colony on PDA (G.J.S. 83-330 = IMI 324475). L. Colony reverse on PDA (G.J.S. 83-330 = IMI 324475). Bars: A = 500 μm; B = 200 μm; C-D = 100 μm; E-J = 50 μm.

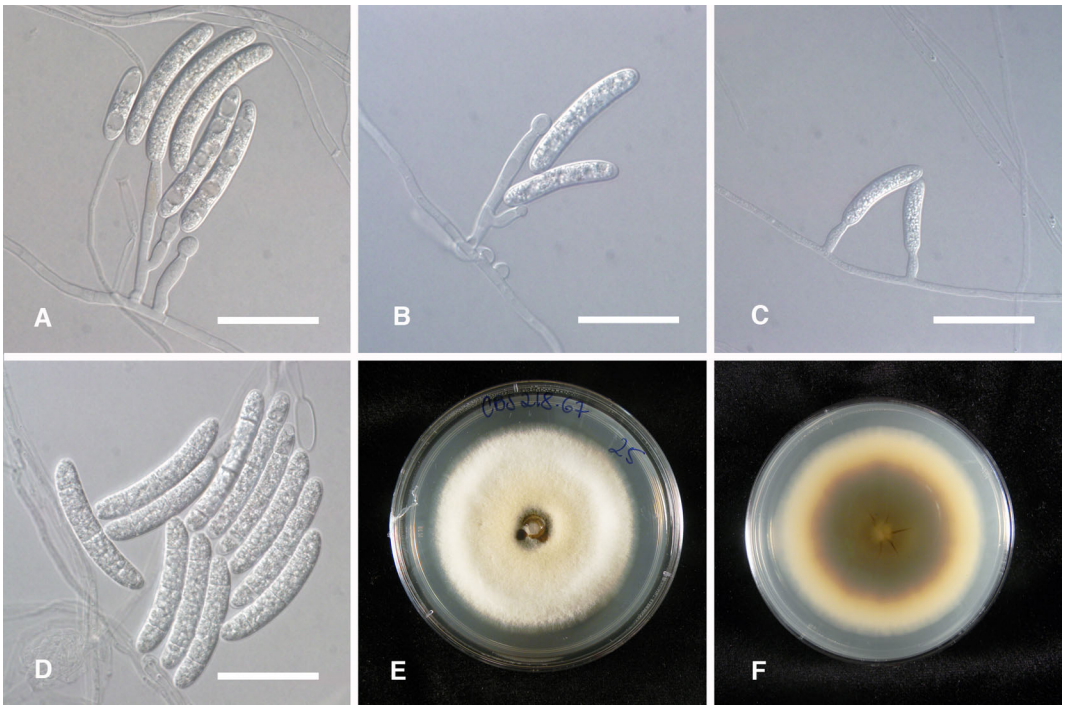

FIGURE 4.9. *Thelonectria theobromicola*. A-D Conidiophores and macroconidia on SNA (CBS 21867). E. Colony on PDA (CBS 21867). F. Colony reverse on PDA (CBS 21867). Bars: A-D = 50 μm.

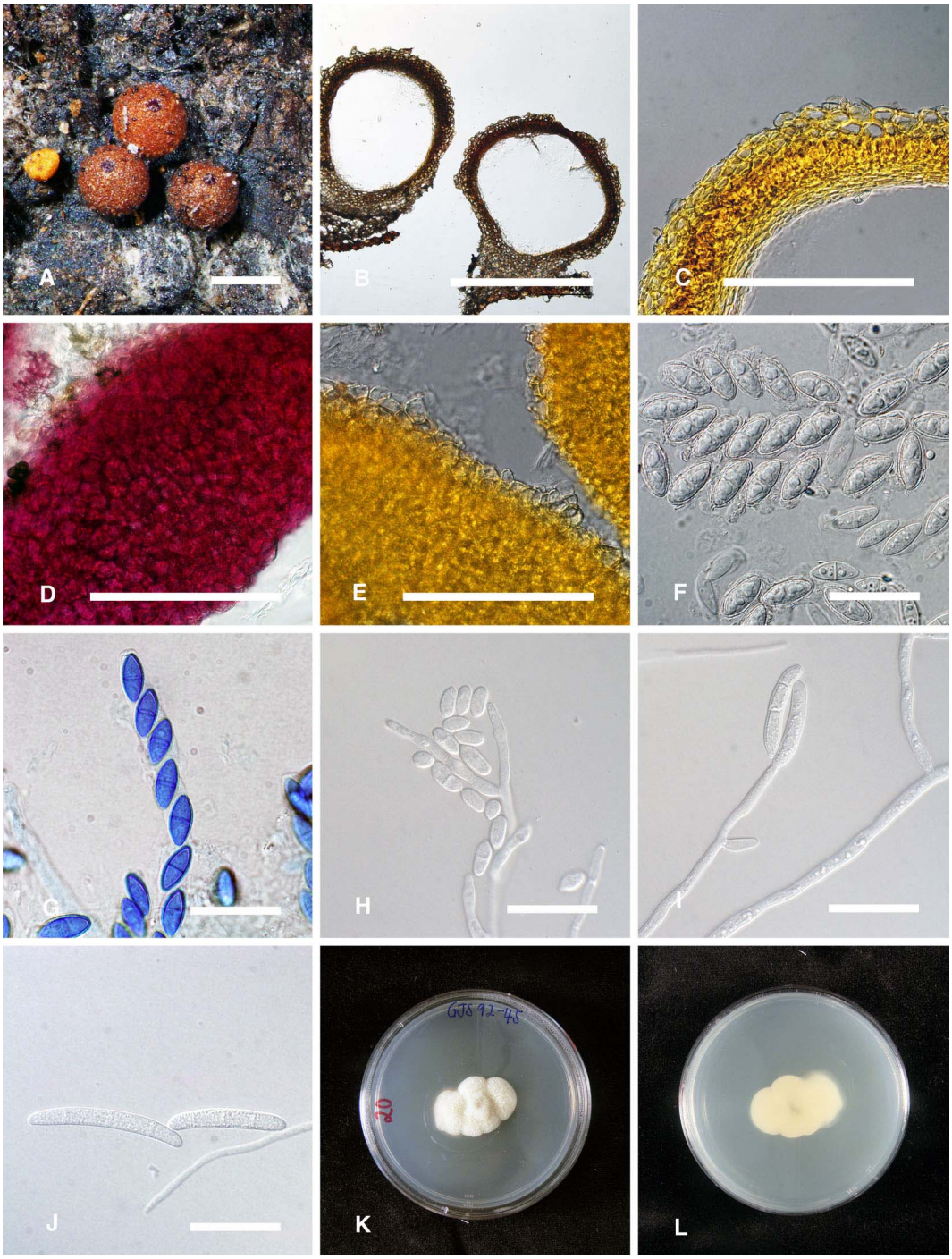

FIGURE 4.10. *Thelonectria trachosa*. A. Perithecia (G.J.S. 92-45 = BPI 802661). B-C. Longitudinal sections of perithecia (G.J.S. 92-45 = BPI 802661). D. Squash mount of perithecial wall with 3% KOH (G.J.S. 92-45 = BPI 802661). E. Squash mount of perithecial wall after lactic acid (G.J.S. 92-45 = BPI 802661). F-G. Asci and

ascospores in 3% KOH and cotton blue (G.J.S. 92-45 = BPI 802661). H. Conidiophores and microconidia on SNA (G.J.S. 85-50 = CBS 119608). I-J. Conidiophores and macroconidia on SNA (G.J.S. 85-50 = CBS 119608). K. Colony on PDA (G.J.S. 92-45 = CBS 112467). L. Colony reverse on PDA (G.J.S. 92-45 = CBS 112467). Bars: A-B = 500 μm; C-D = 200 μm; E-J = 50 μm.

FIGURE 4.11. *Thelonectria westlandica*. A. Perithecia (G.J.S. 85-45 = PDD 50055). B. Perithecia (G.J.S. 83-156 = PDD 43336). C. Squash mount of perithecia with 3% KOH (G.J.S. 83-156 = PDD 43336). D. Squash mount of perithecia after lactic acid (G.J.S. 83-156 = PDD 43336). E. Asci in 3% KOH (G.J.S. 85-45 = PDD 50055). F-

G. Ascospores in KOH and cotton blue (G.J.S. 83-156 = PDD 43336). H-I. Conidiophores and macroconidia (ICMP 10387). J. Macroconidia 1-3-septate (ICMP 10387). K. Colony in PDA (ICMP 10387). L. Colony reverse on PDA (ICMP 10387). Bars: A-B = 500 µm; C = 200 µm; D-E = 100 µm, F-J = 50 µm.

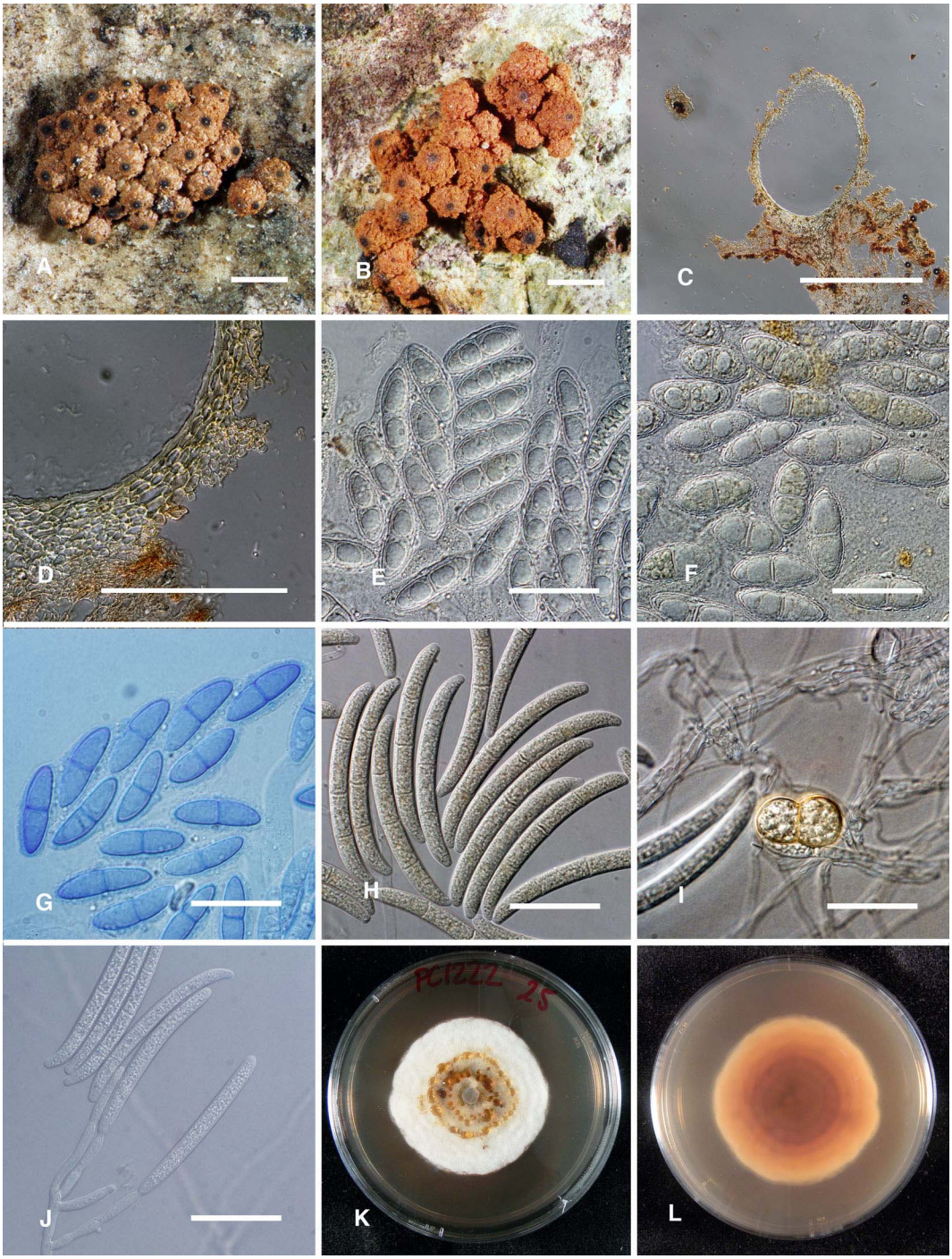

FIGURE 4.12. *Cinnamonectria cinnamomea*. A. Perithecia (G.J.S. 86-300 = NY GJS4264). B. Perithecia (PC 1222 = BPI X). C-D. Longitudinal section of perithecia (G.J.S. 86-117 = NY GJS3619). E-G. Asci and ascospores in 3% KOH and cotton blue (PC 1222 = BPI X). H. Macroconidia on SNA (CLLGUY12050 = CBS 133756). I. Chlamydospores on SNA (CLLGUY12050 = CBS 133756). J. Conidiophores on SNA (G.J.S. 86-300 = IMI 325253). K. Colony on PDA (PC 1222 = CBS 136783). L. Colony reverse on PDA (PC 1222 = CBS 136783). Bars: A-C = 500 μm; D = 100 μm; E-J = 50 μm.

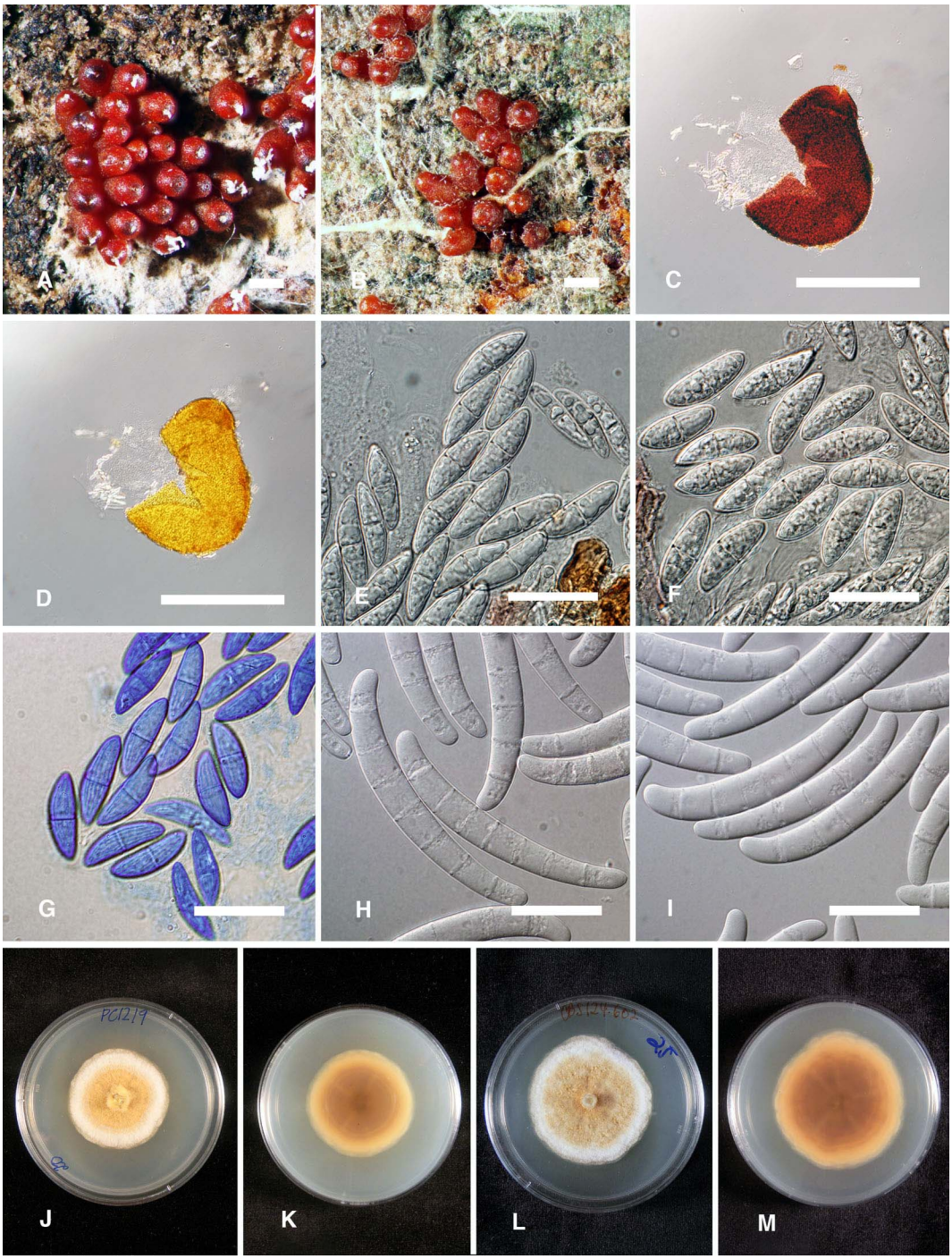

FIGURE 4.13. *Macronectria jungneri* s. str. A. Perithecia (G.J.S. 10-148 = BPI X). B. Perithecia (PC 1219 = BPI X). C. Squash mount of perithecia with 3% KOH (G.J.S. 10-127 = BPI X). D. Squash mount of perithecia with after lactic acid (G.J.S. 10-127

= BPI X). E. Asci in 3% KOH (PC 1219 = BPI X). F-G. Asci and ascospores in 3% KOH and cotton blue (G.J.S. 10-148 = BPI X). H-I. Macroconidia on SNA (PC 1219 = CBS 136792). J. Colony on PDA (PC 1219 = CBS136792). K. Colony reverse on PDA (PC 1219 = CBS 136792). L. Colony on PDA (G.J.S. 08-233 = CBS 124602). M. Colony reverse on PDA (G.J.S. 08-233 = CBS 124602). A-D = 500 μm; E-I = 50 μm.

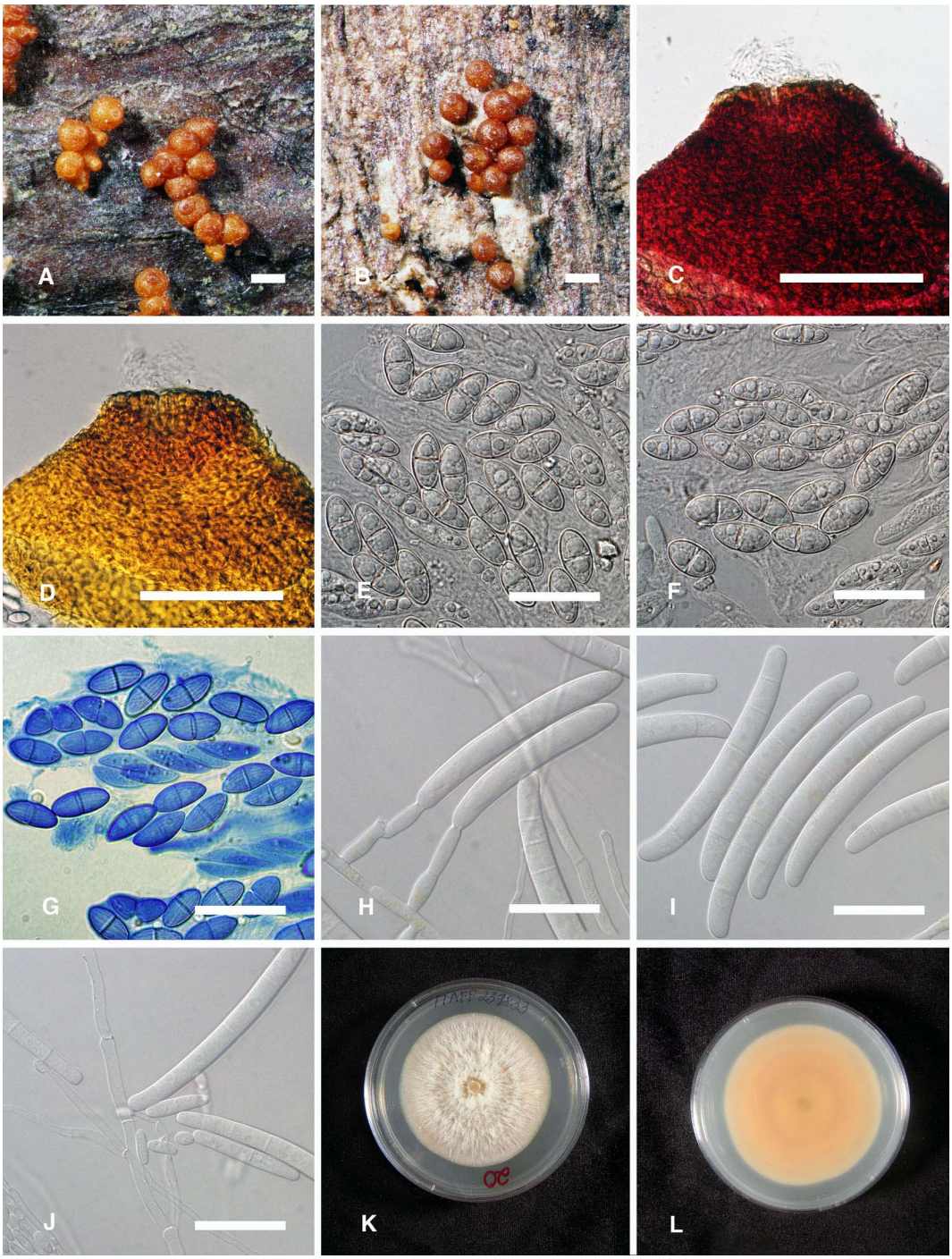

FIGURE 4.14. *Macronectria asiatica*. A. Perithecia (94091407 = BPI X). B. Perithecia (94091601 = BPI X). C. Squash mount of perithecia with 3% KOH (94091601 = BPI X). D. Squash mount of perithecia after lactic acid (94091601 = BPI X). E-G. Asci

and ascospore in 3% KOH and cotton blue (94091601 = BPI X). H. Conidiophores on SNA (MAFF 239833). I-J. Macroconidia and microconidia on SNA (MAFF 239833). K. Colony on PDA (MAFF 239833). L. Colony reverse on PDA (MAFF 239833 A-B = 500 μm; C-D = 100 μm; E-J = 50 μm.

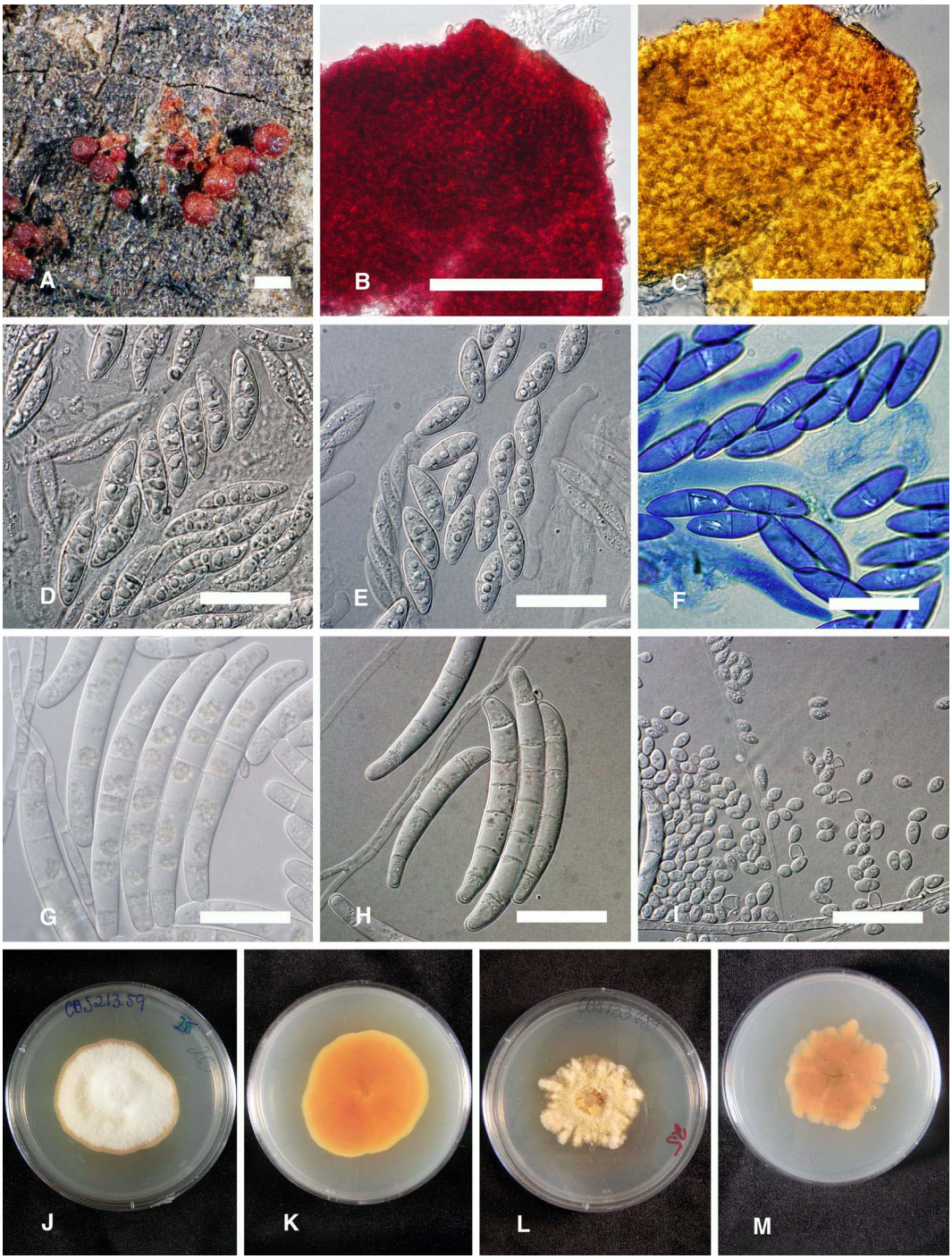

FIGURE 4.15. *Macronectria magna*. A. Perithecia (CLLGUY12004 = BPI X). B. Squash mount of perithecia with 3% KOH (CLLGUY12004 = BPI X). C. Squash mount of perithecia after lactic acid (CLLGUY12004 = BPI X). D-F. Asci and

ascospores in 3% KOH and cotton blue (CLLGUY12004 = BPI X). G. Macroconidia on SNA (CBS 21359). H. Macroconidia on SNA (CLLGUY12004 = CBS 133489). I. Microconidia on SNA (CLLGUY12004 = CBS 133489). J. Colony on PDA (CBS 21359). K. Colony reverse on PDA (CBS 21359). L. Colony on PDA (CLLGUY12004 = CBS 133489). M. Colony reverse on PDA (CLLGUY12004 = CBS 133489). Bars: A = 500 μm; B-C = 100 μm; D-I = 50 μm.

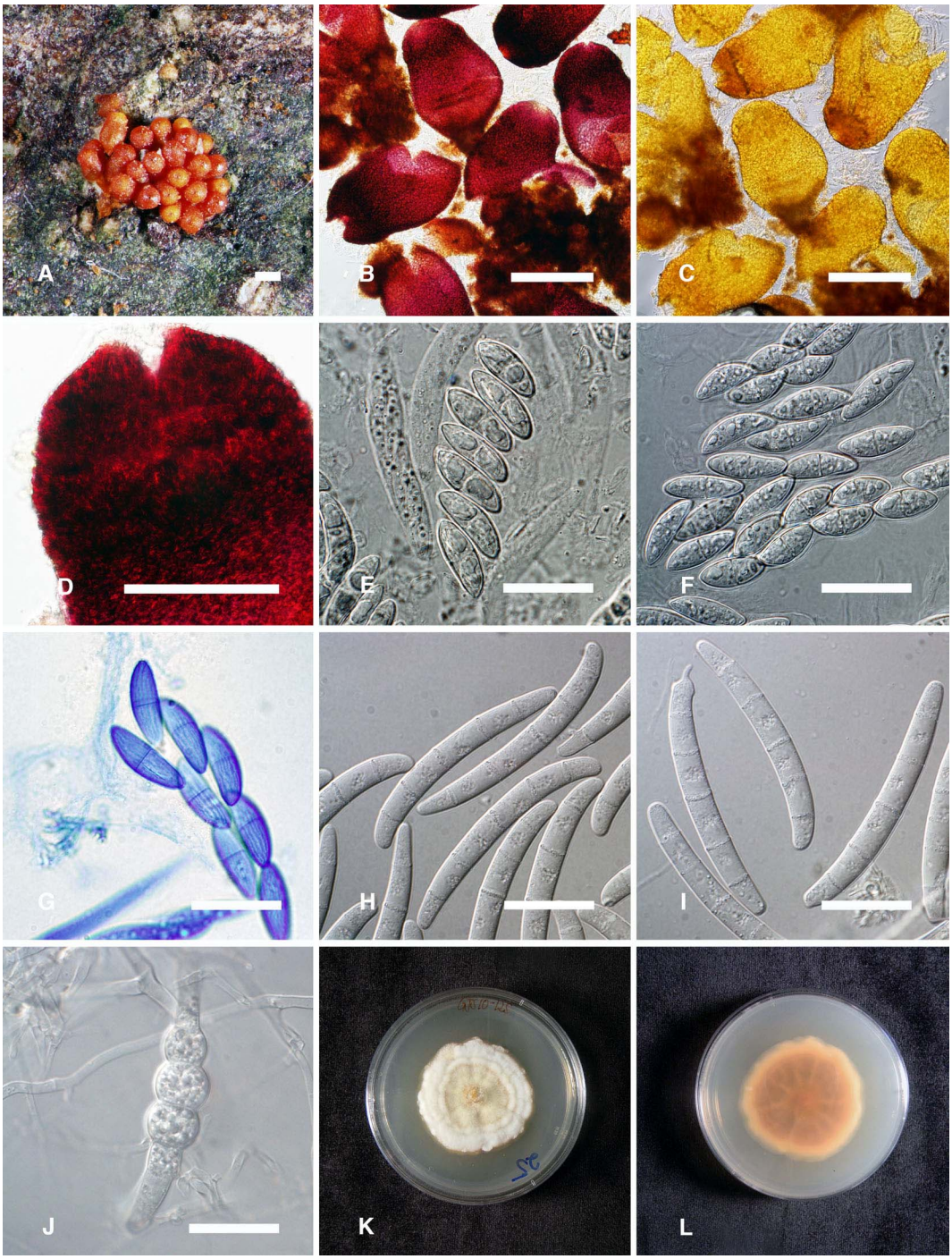

FIGURE 4.16. *Macronectria neotropicalis*. A. Perithecia (PC 1213 = BPI X). B. Squash mount of perithecia with 3% KOH. Note the big and round perithecial apex (PC 1213 = BPI X). C. Squash mount of perithecia after lactic acid (PC 1213 = BPI X). D. Squash mount of perithecia with detail on the apex (PC 1213 = BPI X). E-J.

Asci and ascospores with 3% KOH and cotton blue (PC 1213 = BPI X). H-I. Macroconidia on SNA (G.J.S. 10-125 = CBS 136790). J. Chlamydospores on SNA (G.J.S. 10-125 = CBS X). K. Colony on PDA (G.J.S. 10-125 = CBS 136790). L. Colony reverse on PDA (G.J.S. 10-125 = CBS 136790). Bars: A-C = 500 μm; D = 100 μm; E-J = 50 μm.

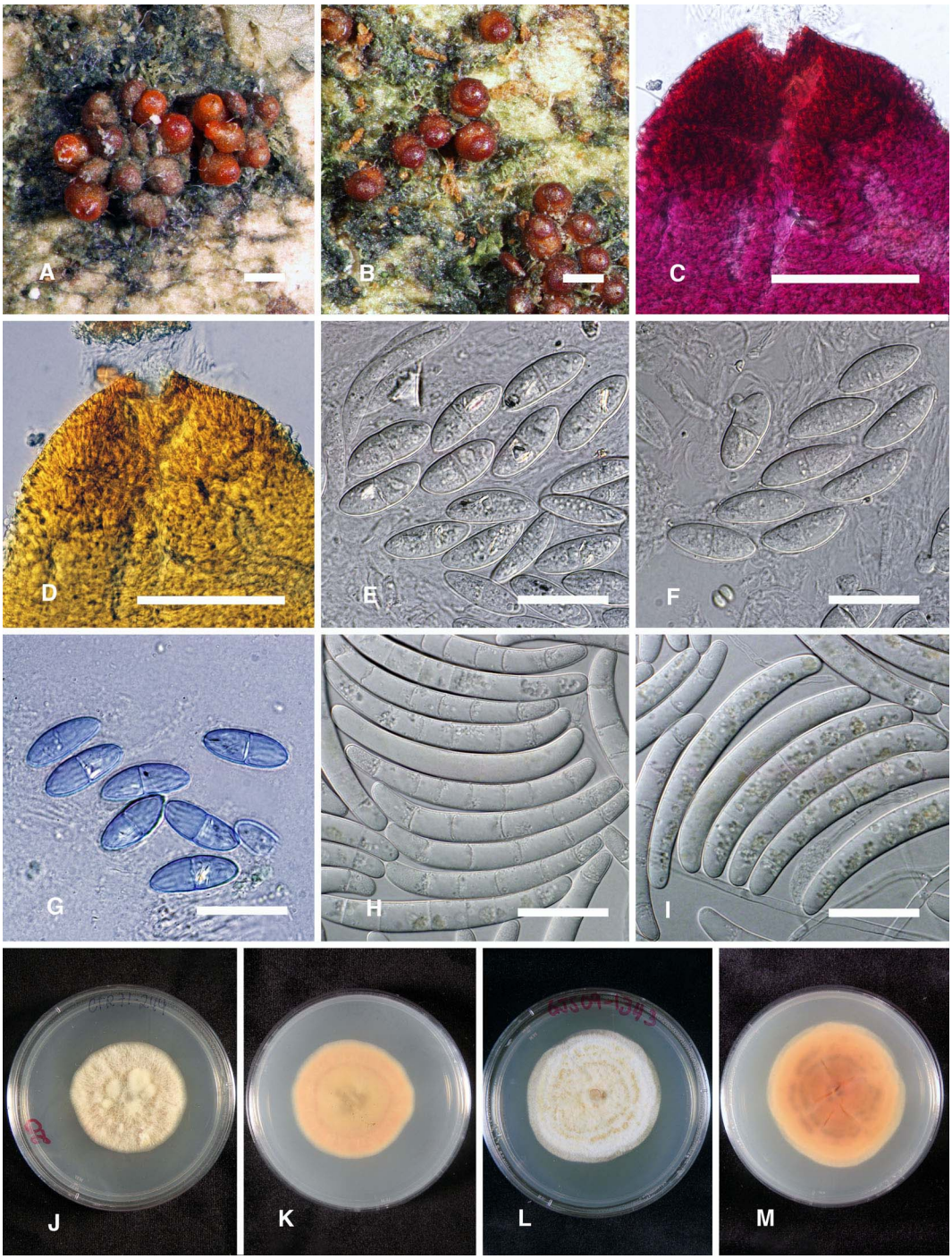

FIGURE 4.17. *Macronectria venezolana*. A-B. Perithecia (C.T.R. 71-244 = NY KPD-VE 1980). C. Squash mount of perithecia with detail on apex section in 3% KOH (C.T.R. 71-244 = NY KPD-VE 1980). D. Squash mount of perithecia with detail on apex section after lactic acid (C.T.R. 71-244 = NY KPD-VE 1980). E-G. Ascospores

in 3% KOH and cotton blue (C.T.R. 71-244 = NY KPD-VE 1980). H-I. Macroconidia on SNA (G.J.S. 09-1343 = CBS X). J. Colony on PDA (C.T.R. 71-244 = CBS 122576). K. Colony reverse on PDA (C.T.R. 71-244 = CBS 122576). L. Colony on PDA (G.J.S. 09-1343 = CBS 136786). M. Colony reverse on PDA (G.J.S. 09-1343 = CBS 136786). Bars: A-B = 500 μm; C=D = 100 μm; E-I = 50 μm.

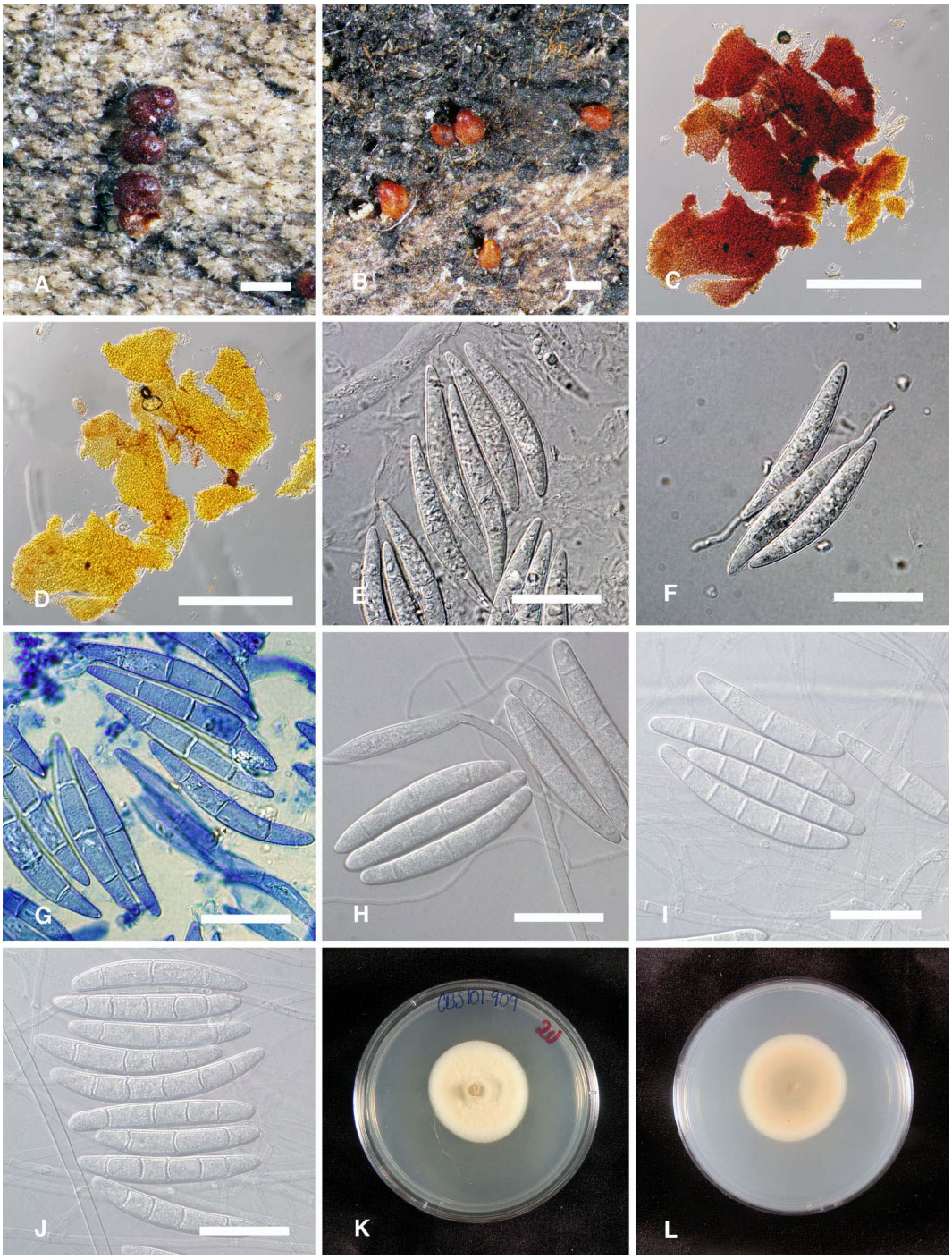

FIGURE 4.18. *Tumenectria laetidisca.* A. *T. laetidisca* perithecia (C.T.R. 71-14 = CUP-MJ 770). B. Perithecia in CUP-MJ 768. C. Squash mount of perithecia with 3% KOH (CUP-MJ 768). D. Squash mount of perithecia after lactic acid (CUP-MJ 768).

E-G. Ascospores in 3% KOH and stained with cotton blue (CUP-MJ 768). H-J. Conidiophores and macroconidia on SNA (CBS 100284). K. Colony on PDA (C.T.R. 71-14 = CBS 101909). L. Colony reverse on PDA (C.T.R. 71-14 = CBS 101909). Bars: A-B = 500 μm; C-D = 200 μm; E-J = 50 μm.

TABLE 4.1. Taxa used in this study, including information about the origin of the fungal material, collection codes and GenBank accession numbers.

Strain	Code	Origin	GenBank accession numbers							
			act	ITS	LSU	*rpb1*	*rpb2*	*SSU*	*tef1*	*tub*
Thelonectria acrotyla	G.J.S 90-171 (=CBS 123766)	Venezuela	JQ365047	JQ403368	JQ403329	JQ403407	KJ022559	KJ022181	JQ394751	JQ394720
Thelonectria acrotyla	IMI 345086	Venezuela	KJ022238	KJ021971	KJ022026	KJ022407	KJ022590	KJ022211	KJ022347	KJ022293
T. amamiensis	MAFF239820	Japan (Okinawa)	JQ365055	JQ403338	JQ403376	JQ403413	KJ022595	KJ022216	KJ022349	JQ394720
T. amamiensis	MAFF239819	Japan (Kagoshima)	JQ365054	JQ403337	JQ403375	KJ022408	KJ022594	KJ022215	KJ022348	JQ394727
Thelonectria asiatica	G.J.S. 88-84 (=IMI 348190)	China	KC121403	KC153741	KC121467	KC153934	KJ022549	KJ022171	KC153870	KC153806
Thelonectria asiatica	MAFF 241576	Japan	KC121436	KC153774	KC121500	KC153967	KJ022613	KJ022234	KC153903	KC153839
Thelonectria blattea	CBS 14277	Netherlands	KC121382	KC153720	KC121446	KC153913	KJ022489	KJ022110	KC153849	KC153785
Thelonectria blattea	CBS 95268	Germany	KC121387	KC153725	KC121451	KC153918	KJ022501	KJ022123	KC153854	KC153790
Thelonectria brayfordii	CBS 118612	New Zealand	KC121381	KC153719	KC121445	KC153912	KJ022483	KJ022104	KC153848	KC153784
Thelonectria brayfordii	ICMP 14105	New Zealand	KC121420	KC153758	KC121484	KC153951	KJ022578	KJ022200	KC153887	KC153823
Thelonectria brayfordii	IMI 384045	New Zealand	KC121424	KC153762	KC121488	KC153955	KJ022213	KJ022592	KC153891	KC153827
T. cidaria	GJS10-135 (=CBS 132323)	Costa Rica	JQ365036	JQ403356	JQ403316	JQ403393	KJ022530	KJ022152	KJ022350	JQ394714
T. cidaria	GJS10-136 (=CBS 132324)	Costa Rica	KJ022239	KJ021972	JQ403324	JQ403401	KJ022531	KJ022153	KJ022351	JQ394715
T. cidaria	CTR71-79 (=IMI 325844)	Jamaica	JQ365043	KJ021973	KJ022027	JQ403402	KJ022508	KJ022130	JQ394741	JQ394707
Thelonectria conchyliata	CBS 26636	Germany	KC121385	KC153723	KC121449	KC153916	KJ022118	KJ022496	KC153852	KC153788
Thelonectria conchyliata	C.T.R. 72-90	Venezuela	KC121390	KC153728	KC121454	KC153921	KJ022512	KJ022134	KC153857	KC153793

conchyliata	(=IMI 325855)									
Thelonectria conchyliata	G.J.S. 87-49 (=CBS 112461)	Guyana	KC121402	KC153740	KC121466	KC153933	KJ022548	KJ022170	KC153869	KC153805
Thelonectria conchyliata	G.J.S. 89-57 (=CBS 112459)	Guyana	KC121404	KC153742	KC121468	KC153935	KJ022551	KJ022173	KC153871	KC153807
Thelonectria conchyliata	G.J.S. 89-60	Guyana	KC121405	KC153743	KC121469	KC153936	KJ022552	KJ022174	KC153872	KC153808
Thelonectria conchyliata	G.J.S. 89-65 (=CBS 123970)	Guyana	KC121406	KC153744	KC121470	KC153937	KJ022553	KJ022175	KC153873	KC153809
Thelonectria conchyliata	G.J.S. 90-212 (=CBS 134028)	Venezuela	KC121412	KC153750	KC121476	KC153943	KJ022561	KJ022183	KC153879	KC153815
Thelonectria conchyliata	G.J.S 96-22 (=IMI 370946)	Puerto Rico	KC121417	KC153755	KC121481	KC153948	KJ022573	KJ022195	KC153884	KC153820
Thelonectria coronalis	93082102 (=CBS 132337)	Taiwan (Taipei County)	KJ022240	JQ403380	JQ403343	JQ403418	KJ022459	KJ022080	JQ394761	JQ394732
Thelonectria coronalis	94043006 (=CBS 132338)	Taiwan (Taipei County)	KJ022241	JQ403381	JQ403344	JQ403419	KJ022462	KJ022083	KJ022352	JQ394733
Thelonectria coronata	G.J.S. 85-207 (=IMI325241)	Indonesia	JQ365044	JQ403365	JQ403326	JQ403404	KJ022542	KJ022164	JQ394749	JQ394717
Thelonectria coronata	92100902 (=CBS 132334)	Taiwan (Taipei County)	JQ365059	KJ021974	JQ403342	JQ403417	KJ022456	KJ022077	JQ394760	JQ394731
Thelonectria coronata	G.J.S. 10-108 (=CBS 132322)	Costa Rica	JQ365040	JQ403360	JQ403320	JQ403397	KJ022521	KJ022143	JQ394746	JQ394711
Thelonectria cf. coronata	C.T.R. 72-178 (=CBS 132335)	Venezuela	JQ365036	JQ403356	JQ403316	JQ403393	KJ022509	KJ022131	JQ394742	JQ394708
Thelonectria diademata	A.R. 4765 (=CBS 132331)	Argentina (Tucuman)	JQ365029	JQ403348	JQ403308	JQ403384	KJ022474	KJ022095	JQ394736	JQ394700
Thelonectria diademata	A.R. 4787A (=CBS 132332)	Argentina (Tucuman)	JQ365032	JQ403351	JQ403311	JQ403387	KJ022478	KJ022099	JQ394738	JQ394703
Thelonectria diademata	C.T.R. 71-52 (CBS 132333)	Jamaica	KJ022242	JQ403354	JQ403314	JQ403391	KJ022507	KJ022129	JQ394740	JQ394706
Thelonectria diademata	G.J.S. 10-137 (=CBS 132321)	Costa Rica	KJ022243	JQ403364	JQ403325	JQ403403	KJ022532	KJ022154	JQ394748	JQ394716
Thelonectria discophora	A.R 4742 (=CBS 134034)	Chile	KC121376	KC153714	KC121440	KC153907	KJ022471	KJ022092	KC153843	KC153779
Thelonectria discophora	G.J.S. 92-48 (=CBS 134031)	Scotland	KC121415	KC153753	KC121479	KC153946	KJ022571	KJ022193	KC153882	KC153818
Thelonectria cf.	A.R 4743	Argentina	KF569827	KF569837	KF569846	KF569874	KJ022472	KJ022093	KF569855	KF569864

discophora	(=CBS 136793)									
Thelonectria cf. discophora	A.R. 4794 (=CBS 134037)	Argentina	KC121379	KC153717	KC121443	KC153910	KJ022479	KJ022100	KC153846	KC153782
Thelonectria cf. discophora	CBS 28792	Brazil	KC121386	KC153724	KC121450	KC153917	KJ022498	KJ022120	KC153853	KC153789
Thelonectria cf. discophora	G.J.S. 89-71 (= CBS 134026)	Guyana	KC121407	KC153745	KC121471	KC153938	KJ022554	KJ022176	KC153874	KC153810
Thelonectria cf. discophora	G.J.S. 90-180 (=CBS 134027)	Venezuela	KC121411	KC153749	KC121475	KC153942	KJ022560	KJ022182	KC153878	KC153814
Thelonectria cf. discophora	IMI 329021	New Zealand	KC121422	KC153760	KC121486	KC153953	KJ022587	KJ022209	KC153889	KC153825
Thelonectria cf. discophora	IMI 342455	Kenya	KC121423	KC153761	KC121487	KC153954	KJ022589	KJ022210	KC153890	KC153826
Thelonectria cf. discophora	MAFF 241533	Japan	KC121429	KC153767	KC121493	KC153960	KJ022604	KJ022225	KC153896	KC153832
Thelonectria cf. discophora	MAFF 241569	Japan	KC121435	KC153773	KC121499	KC153966	KJ022612	KJ022233	KC153902	KC153838
Thelonectria gongylodes	GJS89-131 (=IMI 336160)	USA, NC	JQ365053	JQ403374	JQ403336	JQ403412	KJ022550	KJ022172	JQ394756	JQ394726
Thelonectria gongylodes	GJS90-48 (=CBS 125118)	USA, NC	HM352888	JQ403369	JQ403330	HM364338.1	KJ022563	KJ022185	HM364357.1	HM352870.1
Thelonectria gongylodes	GJS90-50 (=IMI 343571)	USA, NC	JQ365048	JQ403370	JQ403331	JQ403408	KJ022564	KJ022186	JQ394752	JQ394721
Thelonectria gongylodes	GJS04-171 (=CBS 124611)	USA, TN	JQ365038	JQ403358	JQ403318	JQ403395	KJ022514	KJ022136	JQ394744	JQ394710
Thelonectria ianthina	92122107 (=CBS 134038)	Taiwan	KC121373	KC153711	KC121437	KC153904	KJ022458	KJ022079	KC153840	KC153775
Thelonectria ianthina	G.J.S. 10-118 (=CBS 134023)	Costa Rica	KC121393	KC153731	KC121457	KC153924	KJ022523	KJ022145	KC153860	KC153796
Thelonectria japonica	MAFF 241524	Japan	KC121428	KC153766	KC121492	KC153959	KJ022602	KJ022223	KC153895	KC153831
Thelonectria japonica	MAFF 241543	Japan	KC121431	KC153769	KC121495	KC153962	KJ022606	KJ022227	KC153898	KC153834
Thelonectria japonica	MAFF 241554	Japan	KC121432	KC153770	KC121496	KC153963	KJ022609	KJ022230	KC153899	KC153835
Thelonectria japonica	MAFF241563	Japan	KC121433	KC153771	KC121497	KC153964	KJ022610	KJ022231	KC153900	KC153836
Thelonectria	94091407	Taiwan	KJ022244	KJ021975	KJ022028	KJ022409	KJ022463	KJ022084	KJ022353	KJ022294

jungneri	(=CBS 136795)									
Thelonectria jungneri	94091601 (=CBS 136794)	Taiwan	KJ022245	KJ021976	KJ022029	KJ022410	KJ022464	KJ022085	KJ022354	KJ022295
Thelonectria jungneri	CBS 133488	French Guiana	KJ022246	KJ021977	KJ022030	KJ022413	KJ022486	KJ022107	KJ022355	KJ022296
Thelonectria jungneri	CBS 133489	French Guiana	KJ022247	KJ021978	KJ022031	KJ022414	KJ022487	KJ022108	KJ022356	KJ022297
Thelonectria jungneri	CBS 17437	Germany	KJ022249	KJ021980	KJ022033	KJ022416	KJ022490	KJ022112	KJ022359	KJ022299
Thelonectria jungneri	CBS 21359	Guatemala	KJ022250	KJ021981	KJ022034	KJ022417	KJ022493	KJ022115	KJ022361	KJ022300
Thelonectria jungneri	C.T.R. 71-244 (=CBS 122576)	Venezuela	KJ022251	KJ021984	KJ022035	KJ022411	KJ022484	KJ022105	KJ022363	KJ022301
Thelonectria jungneri	G.J.S. 08-233 (=CBS 124602)	Cameroon	KJ022252	KJ021985	KJ022036	KJ022412	KJ022485	KJ022106	KJ022364	KJ022302
Thelonectria jungneri	G.J.S. 09-1343 (=CBS 136786)	Venezuela	KJ022253	KJ021986	KJ022037	KJ022418	KJ022518	KJ022140	KJ022365	KJ022303
Thelonectria jungneri	G.J.S. 10-125 (=CBS 136790)	Costa Rica	KJ022254	KJ021987	KJ022039	KJ022419	KJ022526	KJ022148	KJ022366	KJ022304
Thelonectria jungneri	G.J.S. 10-127	Costa Rica	KJ022255	KJ021988	KJ022040	KJ022420	KJ022527	KJ022149	KJ022367	KJ022305
Thelonectria jungneri	G.J.S. 10-147 (=CBS 136789)	Costa Rica	KJ022256	KJ021990	KJ022041	KJ022421	KJ022535	KJ022157	KJ022368	KJ022306
Thelonectria jungneri	G.J.S. 10-148 (=CBS 136785)	Costa Rica	KJ022257	KJ021991	KJ022042	KJ022422	KJ022536	KJ022158	KJ022369	KJ022307
Thelonectria jungneri	MAFF 239833	Japan	KJ022258	KJ021992	KJ022043	KJ022423	KJ022596	KJ022217	KJ022370	KJ022308
Thelonectria jungneri	MAFF 239845	Japan	KJ022259	KJ021993	KJ022044	KJ022424	KJ022597	KJ022218	KJ022371	KJ022309
Thelonectria jungneri	MAFF 239846	Japan	KJ022260	KJ021994	KJ022045	KJ022425	KJ022598	KJ022219	KJ022372	KJ022310
Thelonectria jungneri	MAFF 241529	Japan	KJ022261	KJ021995	KJ022046	KJ022426	KJ022603	KJ022224	KJ022373	KJ022311
Thelonectria jungneri	P.C. 1213 (=CBS 136791)	Brazil	KJ022262	KJ021996	KJ022047	KJ022427	KJ022614	KJ022235	KJ022374	KJ022312
Thelonectria jungneri	P.C. 1219 (=CBS 136792)	Brazil	KJ022263	KJ021997	KJ022048	KJ022428	KJ022615	KJ022236	KJ022375	KJ022313
Thelonectria lucida	92112704	Taiwan	KF569828	KF569838	KF569847	KF569875	KJ022457	KJ022078	KF569856	KF569865

Thelonectria lucida	94043002 (=CBS 136787)	Taiwan	KF569829	KF569839	KF569848	KF569876	KJ022461	KJ022082	KF569857	KF569866
Thelonectria lucida	A.R. 4781 (=CBS 134036)	Argentina	KC121378	KC153716	KC121442	KC153909	KJ022477	KJ022098	KC153845	KC153781
Thelonectria lucida	CBS 18380	Unknown	KJ022264	KJ022000	KJ022050	KJ022430	KJ022492	KJ022114	KJ022360	KJ022314
Thelonectria lucida	C.T.R. 71-241 (=CBS 112454)	Venezuela	KJ022265	KJ021999	KJ022051	KJ022431	KJ022504	KJ022126	KJ022377	KJ022315
Thelonectria lucida	C.T.R. 72-71 (=CBS 112455)	Venezuela	KJ022266	KJ022001	KJ022052	KJ022432	KJ022511	KJ022133	KJ022378	KJ022316
Thelonectria lucida	G.J.S. 08-232	Cameroon	KJ022267	KJ022002	KJ022053	KJ022433	KJ022516	KJ022138	KJ022379	KJ022317
Thelonectria lucida	G.J.S. 86-249 (=IMI 325261)	French Guiana	KF569830	KF569840	KF569849	KF569877	KJ022546	KJ022168	KF569858	KF569867
Thelonectria lucida	G.J.S. 10-122 (=CBS 136784)	Costa Rica	KJ022268	KJ022003	KJ022054	KJ022434	KJ022524	KJ022146	KJ022380	KJ022318
Thelonectria lucida	G.J.S. 10-146 (=CBS 136788)	Costa Rica	KJ022269	KJ021989	KJ022055	KJ022435	KJ022534	KJ022156	KJ022381	KJ022319
Thelonectria lucida	G.J.S. 90-146 (= CBS 134032)	Venezuela	KC121408	KC153746	KC121472	KC153939	KJ022556	KJ022178	KC153875	KC153811
Thelonectria lucida	G.J.S. 90-166 (=CBS 126099)	Venezuela	KC121410	KC153748	KC121474	KC153941	KJ022558	KJ022180	KC153877	KC153813
Thelonectria lucida	G.J.S. 96-10 (=IMI 370944)	Puerto Rico	KC121416	KC153754	KC121480	KC153947	KJ022572	KJ022194	KC153883	KC153819
Thelonectria lucida	G.J.S. 96-35 (=CBS 112456)	Puerto Rico	KC121419	KC153757	KC121483	KC153950	KJ022575	KJ022197	KC153886	KC153822
Thelonectria lucida	ICMP 2045	New Zealand	KJ022270	KJ022004	KJ022056	KJ022436			KJ022382	KJ022320
Thelonectria lucida	ICMP 9703	New Zealand	KJ022271	KJ022005	KJ022057	KJ022437	KJ022580	KJ022202	KJ022383	KJ022321
Thelonectria mammoidea	CBS 32881	Switzearland	KF569826	KF569836	KF569845	KF569873	KJ022499	KJ022121	KF569854	KF569863
Thelonectria mammoidea	G.J.S. 83-188 (=IMI 326256)	New Zealand	KC121396	KC153734	KC121460	KC153927	KJ022538	KJ022160	KC153863	KC153799
Thelonectria mammoidea	G.J.S. 83-206 (=IMI 326258)	New Zealand	KC121397	KC153735	KC121461	KC153928	KJ022539	KJ022161	KC153864	KC153800
Thelonectria	G.J.S. 85-27	New Zealand	KC121400	KC153738	KC121464	KC153931	KJ022543	KJ022165	KC153867	KC153803

mammoidea	(=CBS 112457)									
Thelonectria mammoidea	G.J.S. 92-34 (=CBS 134030)	Scotland	KC121414	KC153752	KC121478	KC153945	KJ022569	KJ022191	KC153881	KC153817
Thelonectria mammoidea	ICMP 5287	New Zealand	KC121421	KC153759	KC121485	KC153952	KJ022579	KJ022201	KC153888	KC153824
Thelonectria mammoidea	IMI 69361	England	KC121425	KC153763	KC121489	KC153956	KJ022593	KJ022214	KC153892	KC153828
Thelonectria nodosa	A.R. 4500 (=CBS 124742)	United States	JQ365028	JQ403306	JQ403346	JQ403383	KJ022469	KJ022090	JQ394735	JQ394699
Thelonectria nodosa	A.R. 4505 (=CBS 125173)	United States	HM352862.1	JQ403307	JQ403347	HM364328.1	KJ022470	KJ022091	HM364348	HM352862
Thelonectria nodosa	G.J.S. 04-155 (=CBS 132327)	United States	JQ365037	JQ403317	JQ403357	JQ403394	KJ022513	KJ022135	JQ394743	JQ394709
Thelonectria nodosa	G.J.S. 90-66 (=CBS 124352)	United States	JQ365049	JQ403332	JQ403371	JQ403409	KJ022565	KJ022187	JQ394753	JQ394722
Thelonectria nodosa	G.J.S. 91-105 (=IMI 351445	United States	JQ365050	JQ403333	JQ403372	JQ403410	KJ022566	KJ022188	JQ394754	JQ394723
Thelonectria nodosa	G.J.S. 91-116 (=CBS 124740)	United States	JQ365051	JQ403334	JQ403373	JQ403411	KJ022567	KJ022189	HM054126.1	JQ394724
Thelonectria olida	CBS 21567	Unknown	HM352884	KJ021982	KJ022058	HM364334				
Thelonectria phoenicea	94031007 (=CBS 134039)	Taiwan	KC121374	KC153712	KC121438	KC153905	KJ022460	KJ022081	KC153841	KC153776
Thelonectria phoenicea	G.J.S. 09-509	Australia	KC121392	KC153730	KC121456	KC153923	KJ022520	KJ022142	KC153859	KC153795
Thelonectria phoenicea	G.J.S. 85-179 (=IMI 329113)	Indonesia	KC121398	KC153736	KC121462	KC153929	KJ022540	KJ022162	KC153865	KC153801
Thelonectria phoenicea	G.J.S. 85-187 (=ATCC76478)	Indonesia	KC121399	KC153737	KC121463	KC153930	KJ022541	KJ022163	KC153866	KC153802
Thelonectria pinea	A.R. 4321 (=CBS 134033)	New Zealand	KC121375	KC153713	KC121439	KC153906	KJ022466	KJ022087	KC153842	KC153777
Thelonectria pinea	A.R. 4324 (=CBS 125153)	New Zealand	HM352875	HM364294	HM364307	HM364326	KJ022467	KJ022088	HM364345	HM352860
Thelonectria porphyria	MAFF 241515	Japan	KC121426	KC153764	KC121490	KC153957	KJ022599	KJ022220	KC153893	KC153829
Thelonectria porphyria	MAFF 241517	Japan	KC121427	KC153765	KC121491	KC153958	KJ022600	KJ022221	KC153894	KC153830
Thelonectria	MAFF 241539	Japan	KC121430	KC153768	KC121494	KC153961	KJ022605	KJ022226	KC153897	KC153833

porphyria										
Thelonectria purpurea	C.T.R. 71-281 (=CBS 112458)	Venezuela	KC121388	KC153726	KC121452	KC153919	KJ022506	KJ022128	KC153855	KC153791
Thelonectria purpurea	G.J.S. 10-131 (=CBS 134024)	Costa Rica	KC121394	KC153732	KC121458	KC153925	KJ022529	KJ022151	KC153861	KC153797
Thelonectria purpurea	G.J.S. 10-145 (=CBS 134025)	Costa Rica	KC121395	KC153733	KC121459	KC153926	KJ022533	KJ022155	KC153862	KC153798
Thelonectria purpurea	G.J.S. 90-155 (=CBS 123966)	Venezuela	KC121409	KC153747	KC121473	KC153940	KJ022557	KJ022179	KC153876	KC153812
Thelonectria purpurescens	G.J.S. 09-1327 (=CBS 134022)	Venezuela	KC121391	KC153729	KC121455	KC153922	KJ022517	KJ022139	KC153858	KC153794
Thelonectria purpurescens	G.J.S. 96-23 (=IMI 370947)	Puerto Rico	KC121418	KC153756	KC121482	KC153949	KJ022574	KJ022196	KC153885	KC153821
Thelonectria purpurescens	MAFF 241564	Japan	KC121434	KC153772	KC121498	KC153965	KJ022611	KJ022232	KC153901	KC153837
Thelonectria rubi	CBS 11312	Switzerland	KC121380	KC153718	KC121444	KC153911	KJ022482	KJ022103	KC153847	KC153783
Thelonectria rubi	CBS 17727	Netherlands	KC121383	KC153721	KC121447	KC153914	KJ022491	KJ022113	KC153850	KC153786
Thelonectria rubi	CBS 24129	Scotland	KC121384	KC153722	KC121448	KC153915	KJ022495	KJ022117	KC153851	KC153787
Thelonectria stemmata	C.T.R. 71-19 (=CBS 112468)	Jamaica	JQ365033	JQ403312	JQ403352	JQ403388	KJ022502	KJ022124	JQ394739	JQ394704
Thelonectria stemmata	C.T.R. 71-21 (=CBS 132336)	Jamaica	JQ365034	JQ403313	JQ403353	JQ403389	KJ022503	KJ022125	KJ022384	JQ394705
Thelonectria stemmata	C.T.R. 71-27 (=IMI 325840)	Jamaica	JQ365052	KJ022006	KJ022060	JQ403390	KJ022505	KJ022127	KJ022385	KJ022322
Thelonectria stemmata	G.J.S. 10-117 (=CBS 132325)	Costa Rica	KJ022272	JQ403321	JQ403361	JQ403398	KJ022522	KJ022144	KJ022386	JQ394712
Thelonectria stemmata	G.J.S. 10-130 (=CBS 132326)	Costa Rica	JQ365042	JQ403323	JQ403363	JQ403400	KJ022528	KJ022150	KJ022387	KJ022323
Thelonectria stemmata	G.J.S. 90-141 (=CBS 125117)	Venezuela	JQ365046	JQ403328	JQ403367	JQ403406	KJ022555	KJ022177	KJ022388	JQ394719
Thelonectria torulosa	A.R. 4764 (=CBS 132399)	Argentina	JQ365030	JQ403309	JQ403349	JQ403385	KJ022473	KJ022094	KJ022389	JQ394701
Thelonectria torulosa	A.R. 4768A (=CBS 132340)	Argentina	JQ365031	JQ403310	JQ403350	JQ403386	KJ022476	KJ022097	JQ394737	JQ394702
Thelonectria	G.J.S. 85-50	New Zealand	KF569831	KF569841	KF569850	KF569878	KJ022545	KJ022167	KF569859	KF569868

trachosa	(=CBS 119608)									
Thelonectria trachosa	G.J.S. 92-45 (=CBS 112467)	Scotland	KF569832	KF529842	KF569851	KF569879	KJ022570	KJ022192	KF569860	KF569869
Thelonectria truncata	G.J.S 04-357 (=CBS 132239	United States	JQ365039	JQ403319	JQ403359	JQ403396	KJ022515	KJ022137	JQ394745	KJ022324
Thelonectria truncata	MAFF 241521	Japan	JQ365056	JQ403339	JQ403377	JQ403414	KJ022601	KJ022222	JQ394757	KJ022325
Thelonectria tyrus	A.R. 4499 (=CBS 125172)	United States	HM352877	HM364296	HM364309	HM364327	KJ022468	KJ022089	HM364347	HM364327
Thelonectria tyrus	G.J.S. 90-46 (=CBS 134029)	United Stated	KC121413	KC153751	KC121477	KC153944	KJ022562	KJ022184	KC153880	KC153816
Thelonectria veuillotiana	A.R. 1751 (=CBS 132341)	Azores Island	KJ022273	JQ403305	JQ403345	JQ403382	KJ022465	KJ022086	JQ394734	JQ394698
Thelonectria veuillotiana	G.J.S. 92-24 (=CBS 124114)	France	GQ505980	JQ403335	GQ506005	GQ506034	KJ022568	KJ022190	JQ394755	JQ394725
Thelonectria cf. veuillotiana	G.J.S. 09-407 (=CBS 136782)	Cameroon	KJ022274	KJ022007	KJ022038	KJ022438	KJ022519	KJ022141	KJ022390	KJ022326
Thelonectria cf. veuillotiana	G.J.S. 10-124 (=CBS 132328)	Costa Rica	JQ365057	JQ403340	JQ403378	JQ403415	KJ022525	KJ022147	JQ394758	JQ394729
Thelonectria cf. veuillotiana	MAFF 241544	Japan	JQ365057	JQ403340	JQ403378	JQ403415	KJ022607	KJ022228	JQ394758	JQ394729
Thelonectria cf. veuillotiana	MAFF241551	Japan	JQ365058	JQ403341	JQ403379	JQ403416	KJ022608	KJ022229	JQ394759	JQ394730
Thelonectria violaria	A.R. 4766 (=CBS 134035)	Argentina	KC121377	KC153715	KC121441	KC153908	KJ022475	KJ022096	KC153844	KC153780
Thelonectria violaria	C.T.R. 72-188 (=CBS 134040)	Venezuela	KC121389	KC153727	KC121453	KC153920	KJ022510	KJ022132	KC153856	KC153792
Thelonectria westlandica	G.J.S 83-156 (=CBS 112464)	New Zealand	KF569835	JQ403305	JQ403345	JQ403382	KJ022537	KJ022159	JQ394734	JQ394698
Thelonectria westlandica	G.J.S 85-45 (=CBS 132330)	New Zealand	JQ365056	JQ403339	JQ403377	JQ403414	KJ022544	KJ022166	JQ394757	KF569872
Thelonectria westlandica	ICMP 10387	New Zealand	KF569833	KF569843	KF569852	KF569880	KJ022577	KJ022199	KF569861	KF569870
Thelonectria westlandica	IMI 255610	New Zealand	KF569834	KF569844	KF569853	KF569881	KJ022581	KJ022203	KF569862	KF569871
Campylocarpon fasciculare	CBS 112613	South Africa	HM352881		HM364313	HM364331				KJ022327
Campylocarpon	CBS 112679	South Africa	HM352882		HM364314	HM364332				KJ022328

pseudofasciculare										
Cylindrocarpon arcuatum	IMI 324475	French Guiana	KJ022275	KJ022008	KJ022061	KJ022439	KJ022582	KJ022204	KJ022391	KJ022329
Cylindrocapon candidulum	CBS 26696	Netherlands	KJ022276	KJ022013	KJ022062	KJ022440	KJ022497	KJ022119	KJ022396	KJ022330
Cylindrocarpon candidulum	CBS 91170	Germany	KJ022277	KJ022014	KJ022063	KJ022441	KJ022500	KJ022122	KJ022397	KJ022331
Cylindrocarpon permirum	IMI 329100	Jamaica		KJ022015	KJ022064	KJ022442	KJ022588		KJ022398	KJ022332
Cylindrocarpon theobromicola	CBS 16590	Brazil	KJ022278			KJ022443		KJ022111	KJ022358	KJ022333
Cylindrocarpon theobromicola	CBS 21867	Papua New Guinea	KJ022279	KJ021983	KJ022059	KJ022444	KJ022494	KJ022116	KJ022362	KJ022334
Cosmospora coccinea	A.R. 2741 (=CBS 114050)	Germany	GQ505967	HM484537	GQ505990	GQ506020			HM484515	HM484589
Corallomycetella sp.	A.R. 4547 (=CBS 123826)	French Guiana	JF832440	JF832594	JF832679	JF832763			JF832517	JF832838
Hydropisphaera fungicola	A.R. 4170 (=CBS 122304)	United States	GQ505970	HM484863	GQ505995	GQ506025			HM484845	HM484877
Ilyonectria radicicola	A.R. 2553 (=ATCC208837)	Venezuela	HM352871	HM364290	HM364341	HM364325			HM364341	HM352856
Ilyonectria radicicola	G.J.S. 10-132	Costa Rica	KJ022280	KJ022016	KJ022065				KJ022399	KJ022335
Nectria antartica	A.R. 2767 (=CBS 115033	United States	HM484501	HM484556	HM484560	HM484575			HM484516	HM484601
Nectria cinnabarina	A.R. 4477 (=CBS 125165)	France	HM484503	HM484548	HM484562	HM484577			HM484527	HM484606
Nectria laetidisca	CBS 100284	Japan	KJ022281	KJ022017	KJ022066	KJ022445	KJ022480	KJ022101	KJ022400	KJ022336
Nectria laetidisca	C.T.R. 71-14 (=CBS 101909)	Jamaica	KJ022282	KJ022018	KJ022067	KJ022446	KJ022481	KJ022102	KJ022401	KJ022337
Nectria pseudotrichia	CBS 55184	Japan	GQ505976	HM484554	GQ506000	GQ506030			HM484532	HM484602
Nectriopsis exigua	G.J.S. 98-32 (=CBS 126110)	Puerto Rico	GQ505979	HM484865	GQ505986	GQ506014			HM484852	HM484883
Neocosmospora sp.	G.J.S 93-47 (=CBS 125113)		HM484839	HM484862	HM484870	HM484873			HM484850	HM484880
Neonectria sp.	IMI 342666	Switzerland	KJ022283	KJ022019	KJ022068	KJ022447			KJ022402	KJ022338

Neonectria sp.	IMI 342667	Switzerland	KJ022284	KJ022020	KJ022069	KJ022448			KJ022403	KJ022339
Neonectria sp.	IMI 342668	Switzerland	KJ022285	KJ022021	KJ022070	KJ022449			KJ022404	KJ022340
Neonectria cinammomea	CBS 133756	French Guiana	KJ022248	KJ021979	KJ022032	KJ022415	KJ022488	KJ022109	KJ022357	KJ022298
Neonectria cinammomea	G.J.S. 86-117 (=IMI 325248)	French Guiana	KJ022286	KJ022010	KJ022072	KJ022451	KJ022584	KJ022206	KJ022393	KJ022341
Neonectria cinammomea	G.J.S. 86-300 IMI 325253	French Guiana	KJ022287	KJ022011	KJ022073	KJ022452	KJ022585	KJ022207	KJ022394	KJ022342
Neonectria cinammomea	G.J.S. 87-41 (=IMI 325256)	Guyana	KJ022288	KJ022012	KJ022074	KJ022453	KJ022586	KJ022208	KJ022395	KJ022343
Neonectria cinammomea	P.C. 1222 (=CBS 136783)	Brazil	KJ022289	KJ021998	KJ022049	KJ022429	KJ022616	KJ022237	KJ022376	KJ022344
Neonectria coccinea	A.R. 3700 (=CBS 119156)	Slovakia	KC660421	KJ022022	KC660579	KC660671			KC660437	KC660726
Neonectria coccinea	A.R. 3705 (=CBS 119150)	Slovakia	KC660504	KC660504	KC660577	KC660665			KC660440	KC660717
Neonectria coccinea	G.J.S. 92-33 (=CBS 134254)	Scotland	KC660418		KC660581	KC660673			KC660467	KC660722
Neonectria ditissima	CBS 100316	Ireland	HM352880	HM364298	HM364311	HM364330			HM364350	HM352864
Neonectria faginata	A.R. 4097 (=CBS 118938)	United States	KC660411	KC660508	KC660614	KC660654			KC660446	KC660739
Neonectria faginata	A.R. 4151 (=CBS 119162)	United States	KC660410	KC660512	KC660572	KC660659			KC60450	KC660737
Neonectria faginata	A.R. 4153 (=CBS 119163)	United States	KC660405	KC660514	KC660554	KC660656			KC660452	KC660732
Neonectria fuckeliana	A.R. 3103 (=CBS 125133)	Austria	HM352872	HM364291	HM446654				HM364342	HM352857
Neonectria microconidia	HMAS98294	Taiwan	KC660402	KC660530	KC660587	KC660680			JF268739	JF268724
Neonectria microconidia	MAFF 241518	Japan	KC660396	KC660534	KC660582	KC660687			KC660476	KC660756
Neonectria microconidia	MAFF 241570	Japan	KC660395	KC660549	KC660608	KC660683			KC660494	KC660751
Neonectria	MAFF 241572	Japan	KC660401	KC660550	KC660576	KC660689			KC660495	KC660755

microconidia										
Neonectria pulcherrima	G.J.S 85-219 (=IMI 325242)	Indonesia	KJ022290	KJ022009	KJ022071	KJ022450	KJ022583	KJ022205	KJ022392	KJ022345
Neonectria punicea	A.R. 3454 (=CBS 119525)	Slovakia	KC660360	KC660497	KC660592	KC660622			KC660432	KC660696
Neonectria punicea	CLL10040 (=CBS 134248)	France	KC660366	KC660523	KC660556	KC660625			KC660462	KC660703
Neonectria punicea	G.J.S. 98-133 (=CBS 134255)	France	KC660364	KC660529	KC660598	KC660624			KC660469	KC660701
Neonectria ramulariae	ATCC16327		HM352879	HM364297	HM364310	HM364329			HM364349	HM352863
Neonectria shennonjgiana	HMAS 173254	Taiwan	KJ022291	KJ022024	KJ022076	KJ022455	KJ022576	KJ022198	KJ022406	KJ022346
Neonectria viridispora	IMI 350698	Ecuador	KJ022292	KJ022025	KJ022075	KJ022454	KJ022591	KJ022212	KJ022405	
Pleonectria aurigera	A.R. 3717 (=CBS 109874)	France	HM484511	HM484551	HM484573	HM484586			HM484521	HM484600
Pleonectria balsamea	A.R. 2798 (=CBS 125132)	United States	JF832453	JF832598	JF832719	JF832800			JF832556	JF832846
Pleonectria berolensis	A.R. 2776 (=CBS 126112)	Austria	HM484510	HM484543	HM484568	HM484583			HM484517	HM484594
Pleonectria cucurbitula	CBS 30175	France	JF832461	JF862621	JF832720	JF832808			JF832563	JF832854
Pleonectria okinawensis	MAFF 241410	Japan	JF832451	JF832674	JF832751	JF832827			JF832585	JF832878
Rugonectria rugulosa	Y.H. 10-01 (=CBS 129158)	United States	JF832515	JF832661	JF832761	JF832836			JF832545	JF832545
Selinia pulchra	A.R. 2812		GQ505982	HM484859	GQ505992	GQ506022			HM484841	HM484884
Verrucostoma freycinetiae	MAFF 240100	Japan	GQ505984	HM484866	GQ506013	GQ506018			HM484853	HM484885
Viridispora alata	A.R. 1770 (=CBS 125123)	Madeira Island	GQ505985	JF832678	GQ505989	GQ506019			JF832592	JF832912

TABLE 4.2. List of molecular markers and substitution models for the eight loci used in this study.

Locus	Primer	Substitution model	%GC	Primers	Reference
act	TRIACT1-TRIACT2	GTR+G	55.9	F 5'TGGCACCACACCTTCTACAATGA3' R 5'TCCTCCGCTTATTGATATGC3'	Samuels *et al*. 2006
ITS	ITS5-ITS4	GTR+I+G	55.7	F 5'GGAAGTAAAAGTCGTAACAAGG3' R 5'TCCTCCGCTTATTGATATGC3'	White *et al*. 1990
LSU	LROR-LR5	GTR+I+G	53.2	F 5'ACCCGCTGAACTTAAGC3' R 5'TCCTGAGGGAAACTTCG3'	Vilgalys*
tef	EF728-EF1567	TIM3+I+G	54.9	F 5'CATCGAGAAGTTCGAGAAGG3' R 5'ACHGTRCCRATACCACCRAT3'	Carbone & Kohn, 1999
tub	T1-T2	HKY+I+G	56	F 5'AACATGCGTGAGATTGTAAGT3' R 5'TAGTGACCCTTGGCCCAGTTG3'	O'Donnell & Cigelnik, 1997
rpb1	RPB1a-RPB1c	GTR+G	52.7	F 5'CAYCCWGGYTTYATCAAGAA3' R 5'CCNGCDATNTCRTTRTCCATRTA3'	Castlebury *et al*. 2004
rpb2	RPB2-7cf-RPB2-11aR	TrN+I+G	52.3	F 5'ATGGGYAARCAAGCYATGGG3' R 5'GCRTGGATCTTRTCRTCSACC3'	Liu et al. 1999
SSU	NS1-SR7	SYM+I	45.5	F 5'GTAGTCATATGCTTGTCTC3' R 5'GTTCAACTACGAGCTTTTTAA3'	Vilgalys *

* http://www.botany.duke.edu/fungi/mycolab

TABLE 4.3. Summary of parameters and statistics before and after alignments were cleaned from problematic alignment regions using Gblocks

Locus	Original alignment length	Variable sites (%)	Parsimony informative sites (%)	After Gblocks 1[a] length	Variable sites (%)	Parsimony informative sites (%)	After Gblocks 2[b] length	Variable sites (%)	Parsimony informative sites (%)
act	581	5	26.3	571	5	26.6	572	5	26.7
ITS	610	7.8	50	278	5	24.4	431	5.1	42.2
LSU	841	5.4	18	811	5.4	17.3	824	5.5	18
tef	938	6	48.5	169	6.5	23	793	4.8	45.9
tub	652	7.2	49.8	282	4.9	27.6	476	3.5	54.6
rpb1	700	5.8	55.7	515	4	52.6	623	3.6	58.7
rpb2	856	2.9	35.6	856	2.9	35.6	856	2.9	35.6
SSU	521	2.8	5.3	521	2.8	5.3	521	2.8	5.3

[a] Gblocks under stringent parameters: Minimum number of sequences for a conserved position 102, Minimum number of sequences for a flank position 172, Maximum number of contiguous non-conserved positions 8, Minimum length of a block 10, Allowed gap positions none.
[b] Gblocks under less stringent parameters: Minimum number of sequences for a conserved position 102, Minimum number of sequences for a flank position 102, Maximum number of contiguous nonconserved positions 10, Minimum length of a block 5, Allowed gap positions half.

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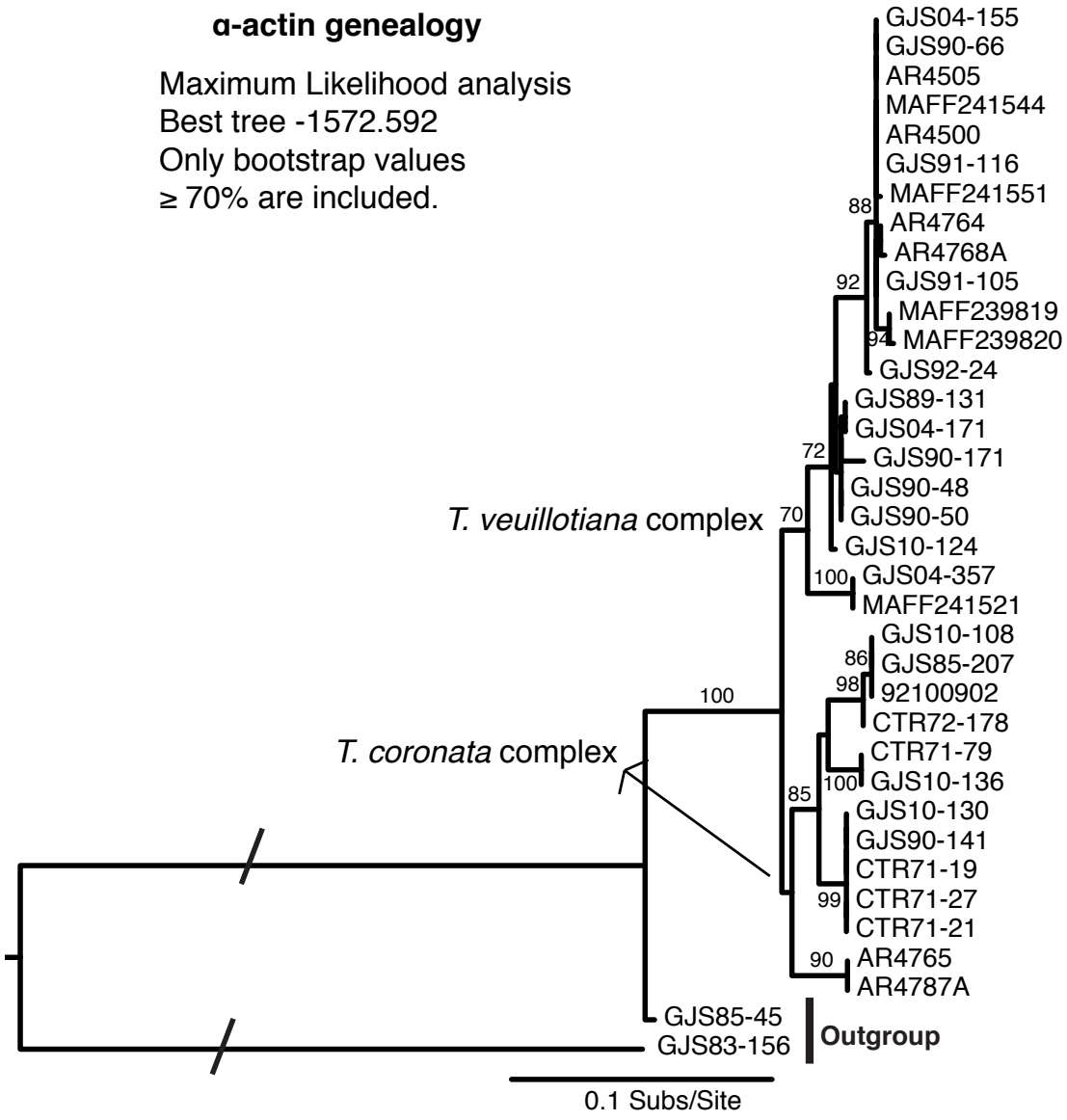
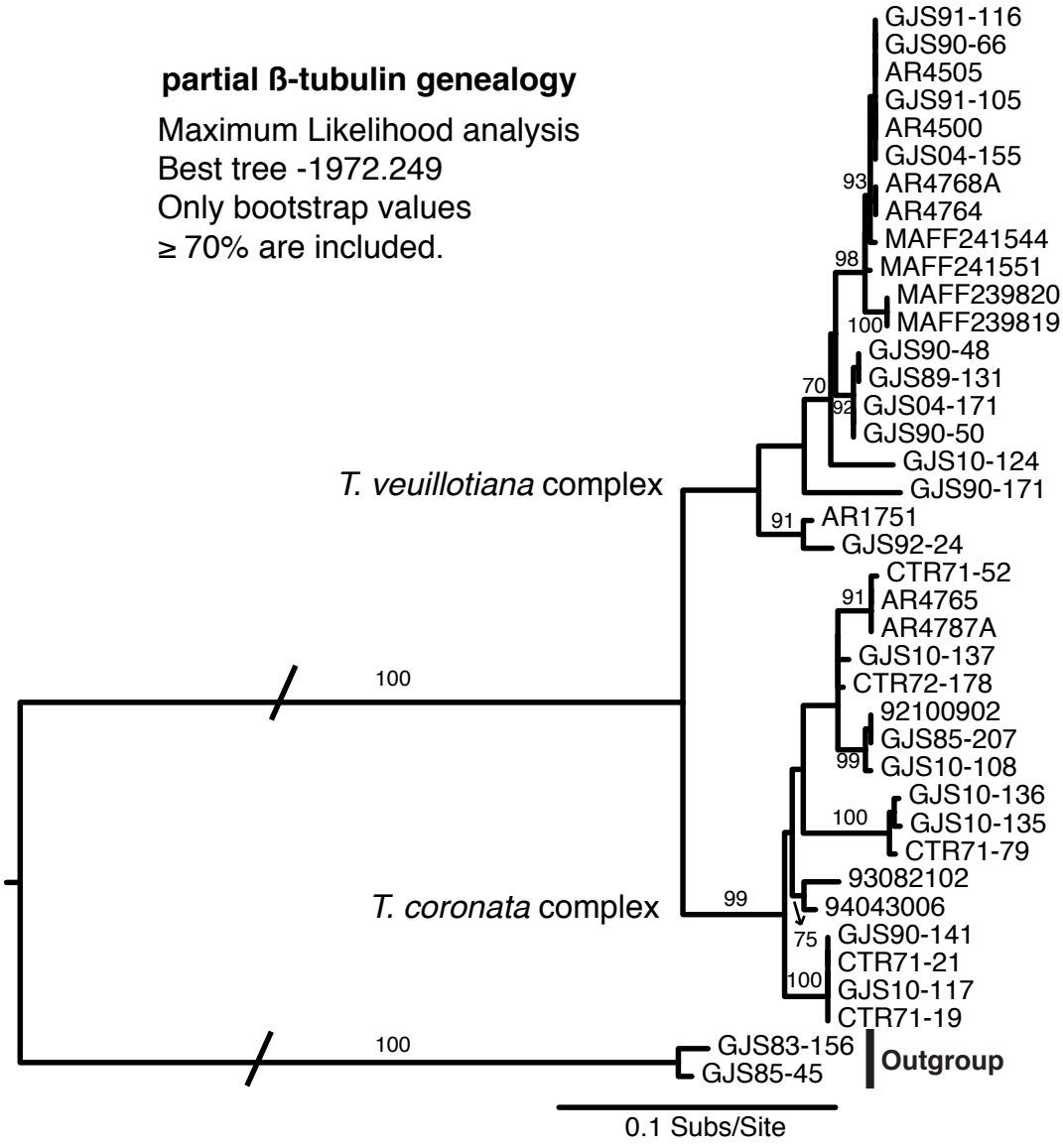
Appendices

Figures called as supplementary.

FIGURE S1. The following six figures correspond to single gene genealogies recalled in Chapter 1. Trees are Maximum Likelihood phylograms. For clade identity see Figure 1.1.

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Best tree -1972.249
Only bootstrap values
≥ 70% are included.
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GJS91-105
AR4500
GJS04-155
AR4768A
AR4764
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MAFF241551
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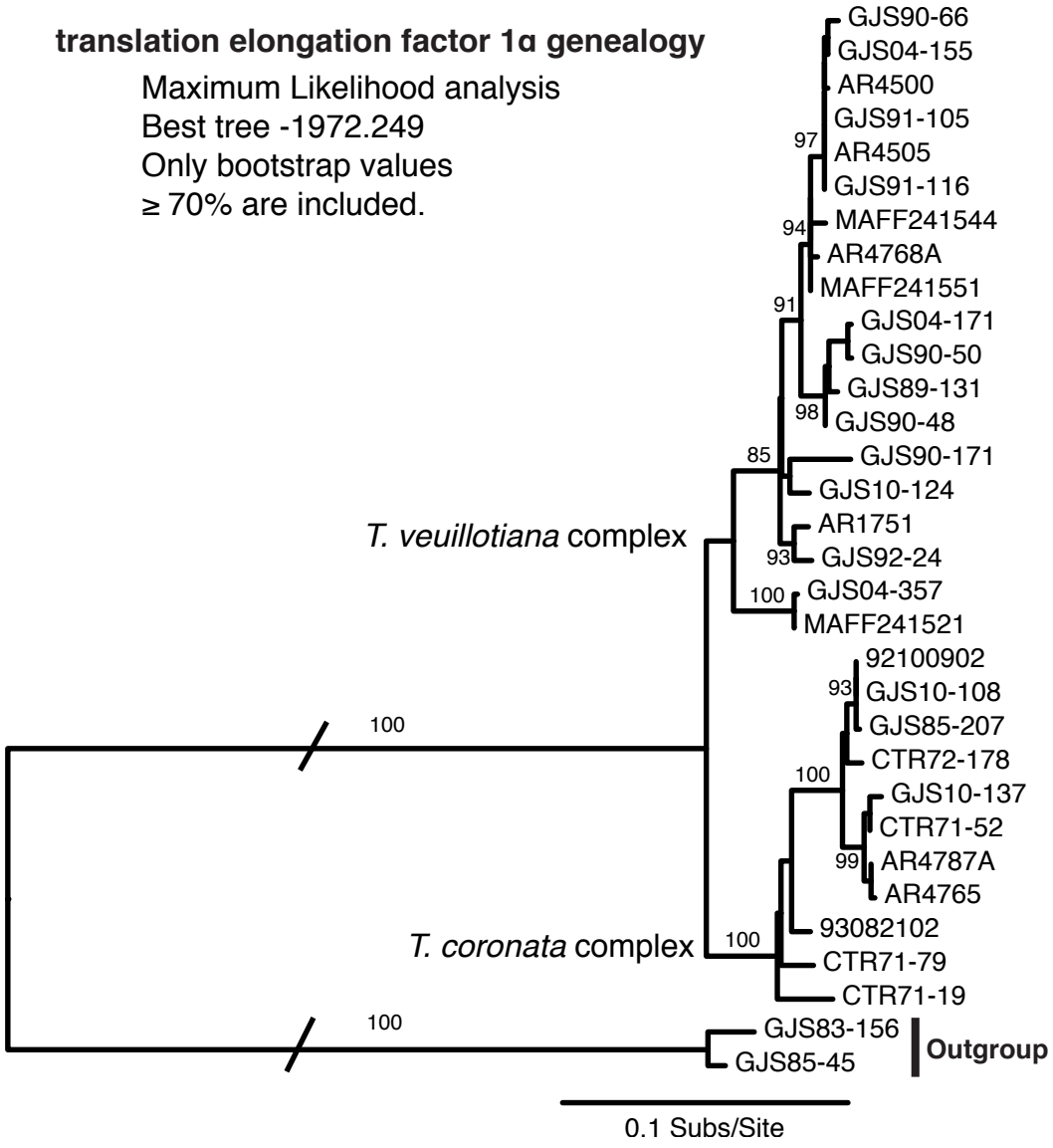
GJS90-48
GJS89-131
GJS04-171
GJS90-50
GJS10-124
GJS90-171
AR1751
GJS92-24
CTR71-52
AR4765
AR4787A
GJS10-137
CTR72-178
92100902
GJS85-207
GJS10-108
GJS10-136
GJS10-135
CTR71-79
93082102
94043006
GJS90-141
CTR71-21
GJS10-117
CTR71-19
GJS83-156
GJS85-45
Outgroup
T. veuillotiana complex
T. coronata complex
93
98
100
70
92
91
91
99
100
100
99
75
100
100
100
0.1 Subs/Site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Maximum Likelihood analysis
Best tree -1972.249
Only bootstrap values
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AR4505
GJS91-116
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MAFF241551
91
GJS04-171
GJS90-50
GJS89-131
98
GJS90-48
85
GJS90-171
GJS10-124
T. veuillotiana complex
AR1751
93
GJS92-24
100
GJS04-357
MAFF241521
92100902
93
GJS10-108
GJS85-207
100
CTR72-178
100
GJS10-137
CTR71-52
99
AR4787A
AR4765
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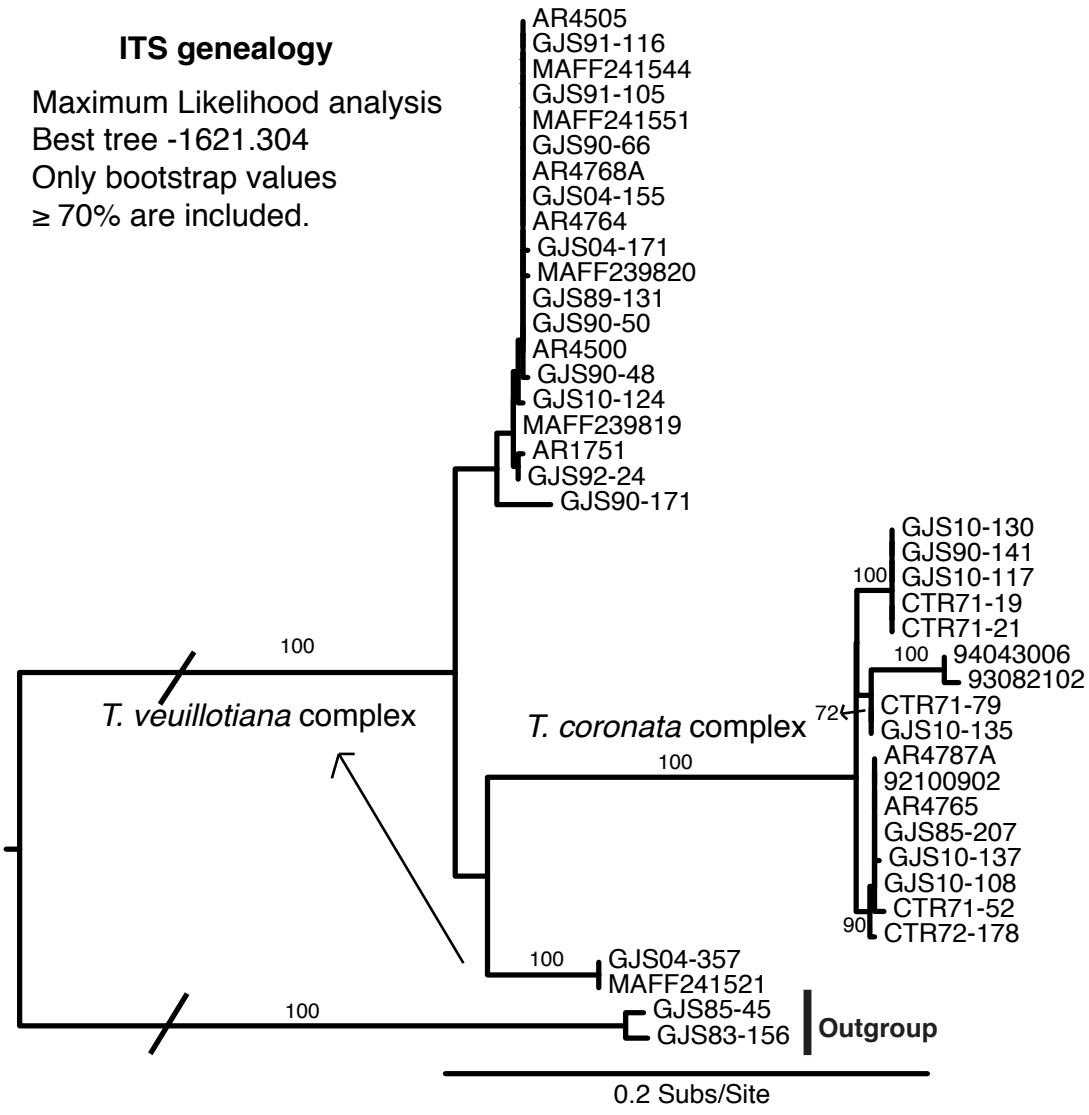
GJS85-45
Outgroup
0.1 Subs/Site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Only bootstrap values
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AR4764
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GJS89-131
GJS90-50
AR4500
GJS90-48
GJS10-124
MAFF239819
AR1751
GJS92-24
GJS90-171
GJS10-130
GJS90-141
GJS10-117
CTR71-19
CTR71-21
94043006
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GJS10-135
AR4787A
92100902
AR4765
GJS85-207
GJS10-137
GJS10-108
CTR71-52
CTR72-178
GJS04-357
MAFF241521
GJS85-45
GJS83-156
Outgroup
100
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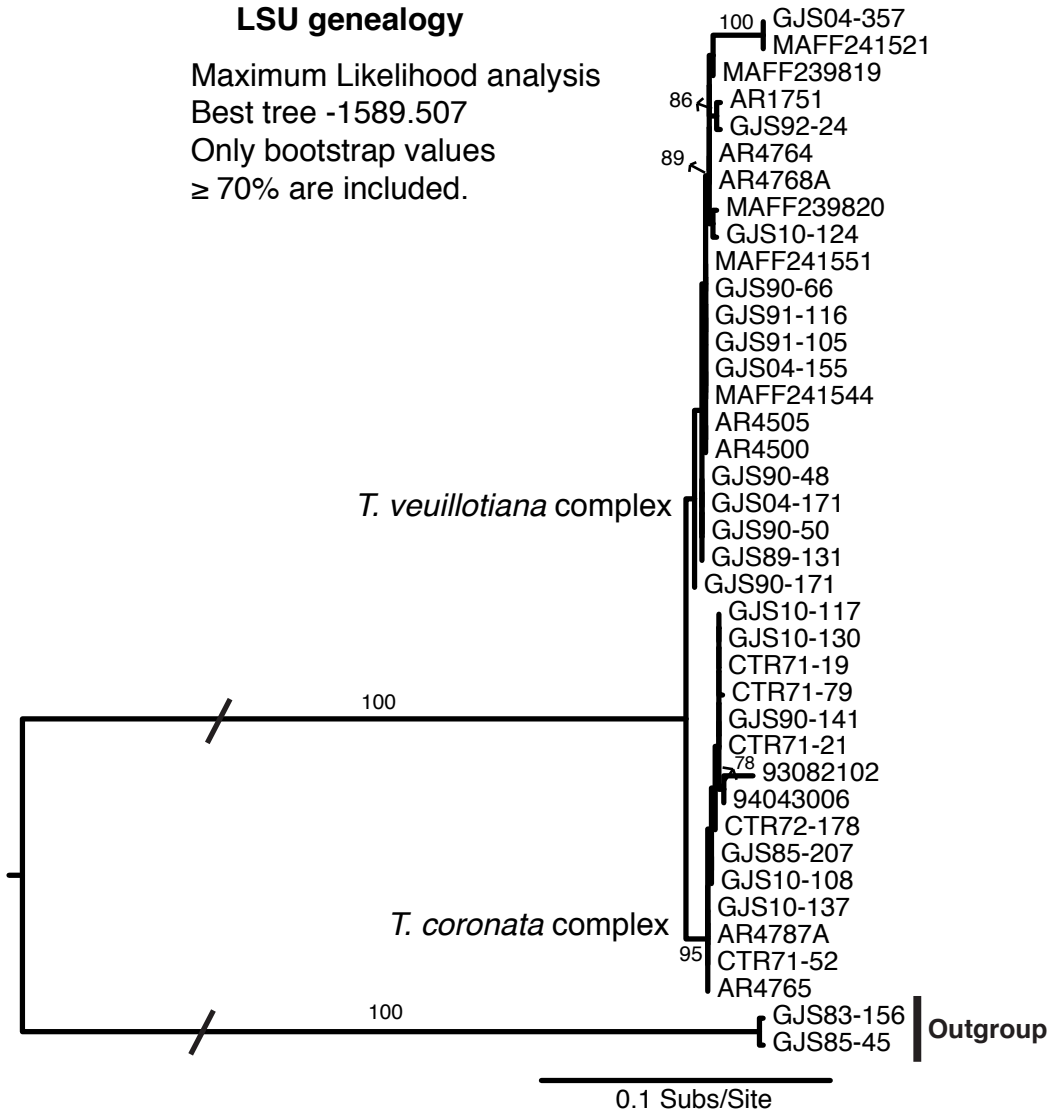
100
72
100
100
90
100
T. veuillotiana complex
T. coronata complex
0.2 Subs/Site


LSU genealogy
Maximum Likelihood analysis
Best tree -1589.507
Only bootstrap values
≥ 70% are included.
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GJS92-24
89
AR4764
AR4768A
MAFF239820
GJS10-124
MAFF241551
GJS90-66
GJS91-116
GJS91-105
GJS04-155
MAFF241544
AR4505
AR4500
GJS90-48
T. veuillotiana complex
GJS04-171
GJS90-50
GJS89-131
GJS90-171
GJS10-117
GJS10-130
CTR71-19
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CTR71-79
GJS90-141
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78
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94043006
CTR72-178
GJS85-207
GJS10-108
GJS10-137
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AR4765
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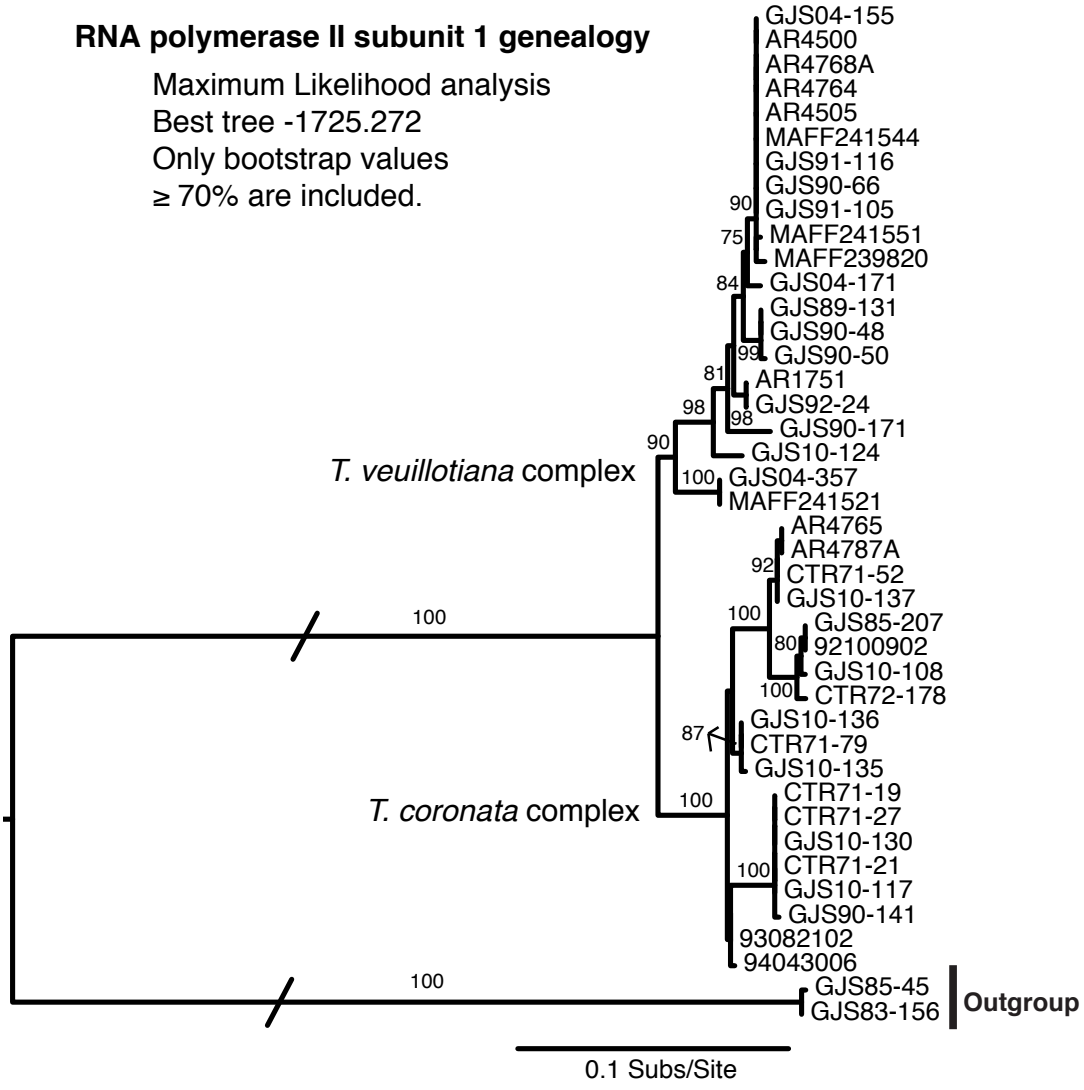
GJS83-156
GJS85-45
Outgroup
0.1 Subs/Site


RNA polymerase II subunit 1 genealogy
Maximum Likelihood analysis
Best tree -1725.272
Only bootstrap values
≥ 70% are included.
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AR4768A
AR4764
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GJS90-66
GJS91-105
MAFF241551
MAFF239820
GJS04-171
GJS89-131
GJS90-48
GJS90-50
AR1751
GJS92-24
GJS90-171
GJS10-124
GJS04-357
MAFF241521
AR4765
AR4787A
CTR71-52
GJS10-137
GJS85-207
92100902
GJS10-108
CTR72-178
GJS10-136
CTR71-79
GJS10-135
CTR71-19
CTR71-27
GJS10-130
CTR71-21
GJS10-117
GJS90-141
93082102
94043006
GJS85-45
GJS83-156
Outgroup
T. veuillotiana complex
T. coronata complex
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92
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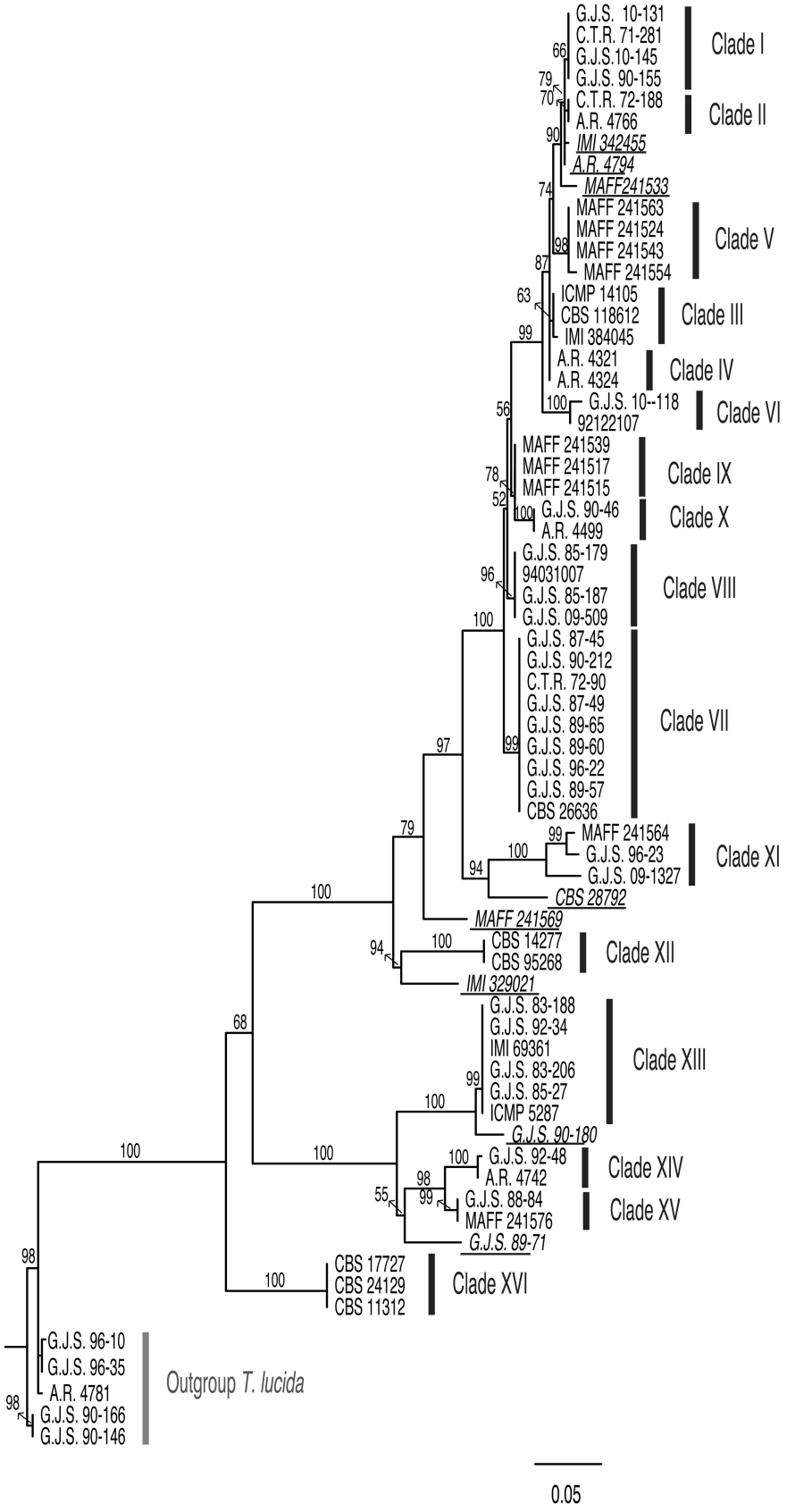
0.1 Subs/Site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MAFF 241543
MAFF 241554
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G.J.S. 09-1327
Clade XI
CBS 28792
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CBS 14277
CBS 95268
Clade XII
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G.J.S. 92-34
IMI 69361
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ICMP 5287
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CBS 24129
CBS 11312
Clade XVI
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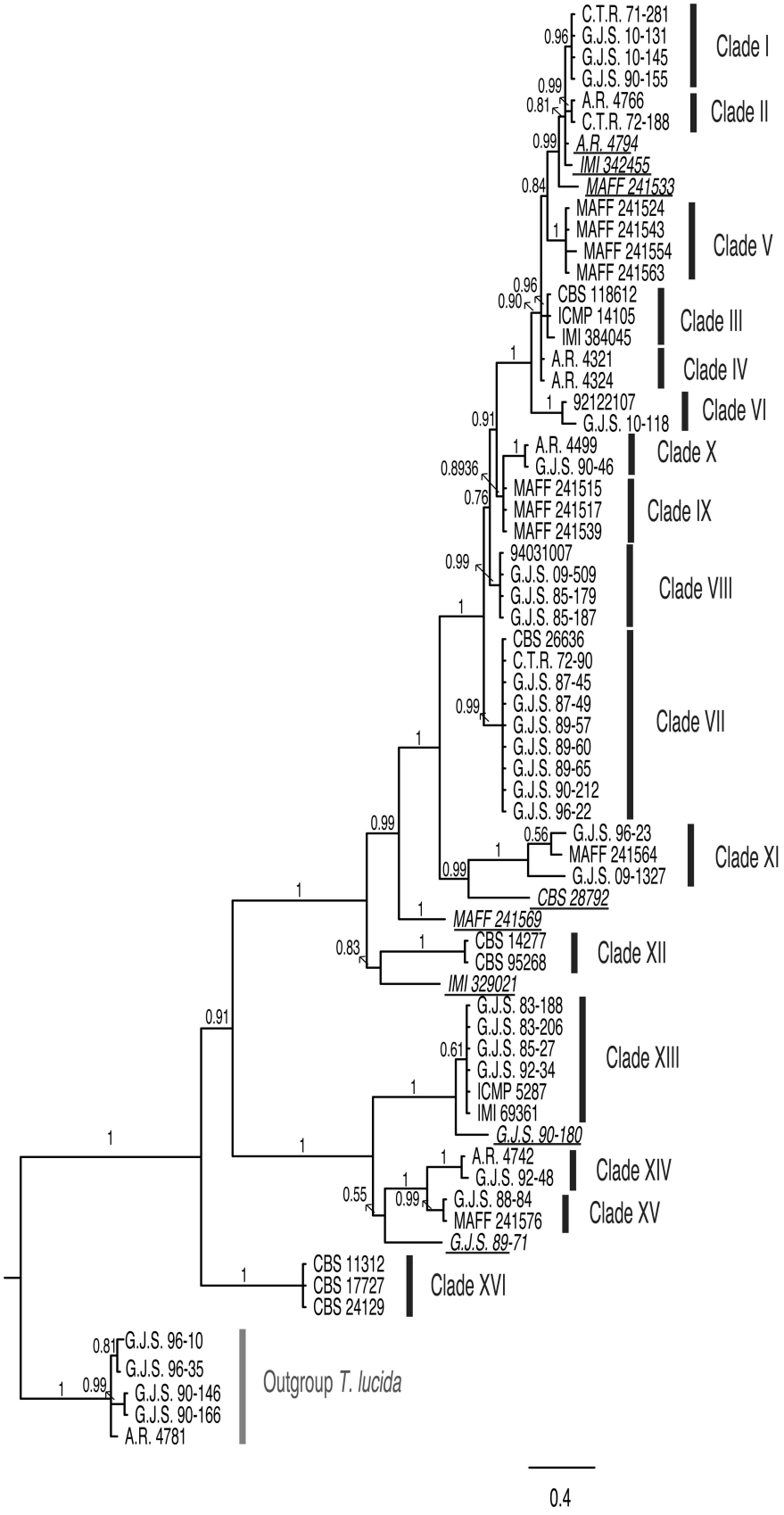
G.J.S. 96-35
A.R. 4781
G.J.S. 90-166
G.J.S. 90-146
Outgroup T. lucida
0.05

FIGURE S2.1. Maximum likelihood phylogram showing relationships among isolates of *T. discophora*-like species based on the *rpb1* loci. ML bootstrap is indicated on top of each branch. No values below branches indicate branch was not recovered/supported.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A.R. 4324
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Clade IX
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G.J.S. 85-187
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Clade VII
G.J.S. 96-23
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Clade XI
CBS 28792
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CBS 95268
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G.J.S. 92-48
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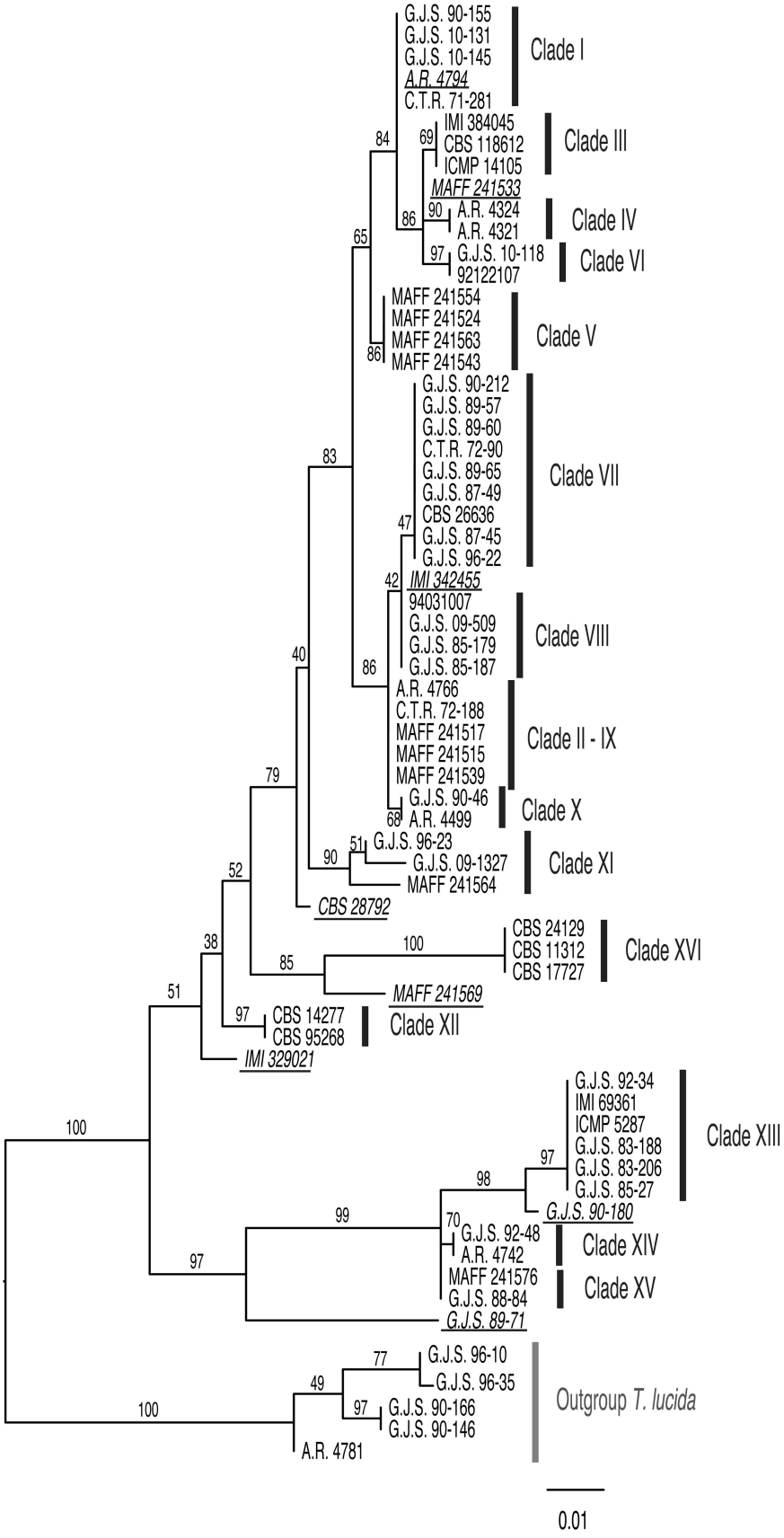
Clade XV
G.J.S. 89-71
CBS 11312
CBS 17727
CBS 24129
Clade XVI
G.J.S. 96-10
G.J.S. 96-35
G.J.S. 90-146
G.J.S. 90-166
A.R. 4781
Outgroup T. lucida
0.4

FIGURE S2.2. Bayesian phylogram showing relationships among isolates of *T. discophora*-like species based on the *rpb1* loci. Bayesian posterior probabilities are indicated on top of each branch. No values below branches indicate branch was not recovered/supported.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ICMP 14105
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A.R. 4324
A.R. 4321
Clade IV
G.J.S. 10-118
92122107
Clade VI
MAFF 241554
MAFF 241524
MAFF 241563
MAFF 241543
Clade V
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G.J.S. 89-57
G.J.S. 89-60
C.T.R. 72-90
G.J.S. 89-65
G.J.S. 87-49
CBS 26636
G.J.S. 87-45
G.J.S. 96-22
Clade VII
IMI 342455
94031007
G.J.S. 09-509
G.J.S. 85-179
G.J.S. 85-187
Clade VIII
A.R. 4766
C.T.R. 72-188
MAFF 241517
MAFF 241515
MAFF 241539
Clade II - IX
G.J.S. 90-46
A.R. 4499
Clade X
G.J.S. 96-23
G.J.S. 09-1327
MAFF 241564
Clade XI
CBS 28792
CBS 24129
CBS 11312
CBS 17727
Clade XVI
MAFF 241569
CBS 14277
CBS 95268
Clade XII
IMI 329021
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IMI 69361
ICMP 5287
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G.J.S. 83-206
G.J.S. 85-27
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G.J.S. 92-48
A.R. 4742
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MAFF 241576
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Clade XV
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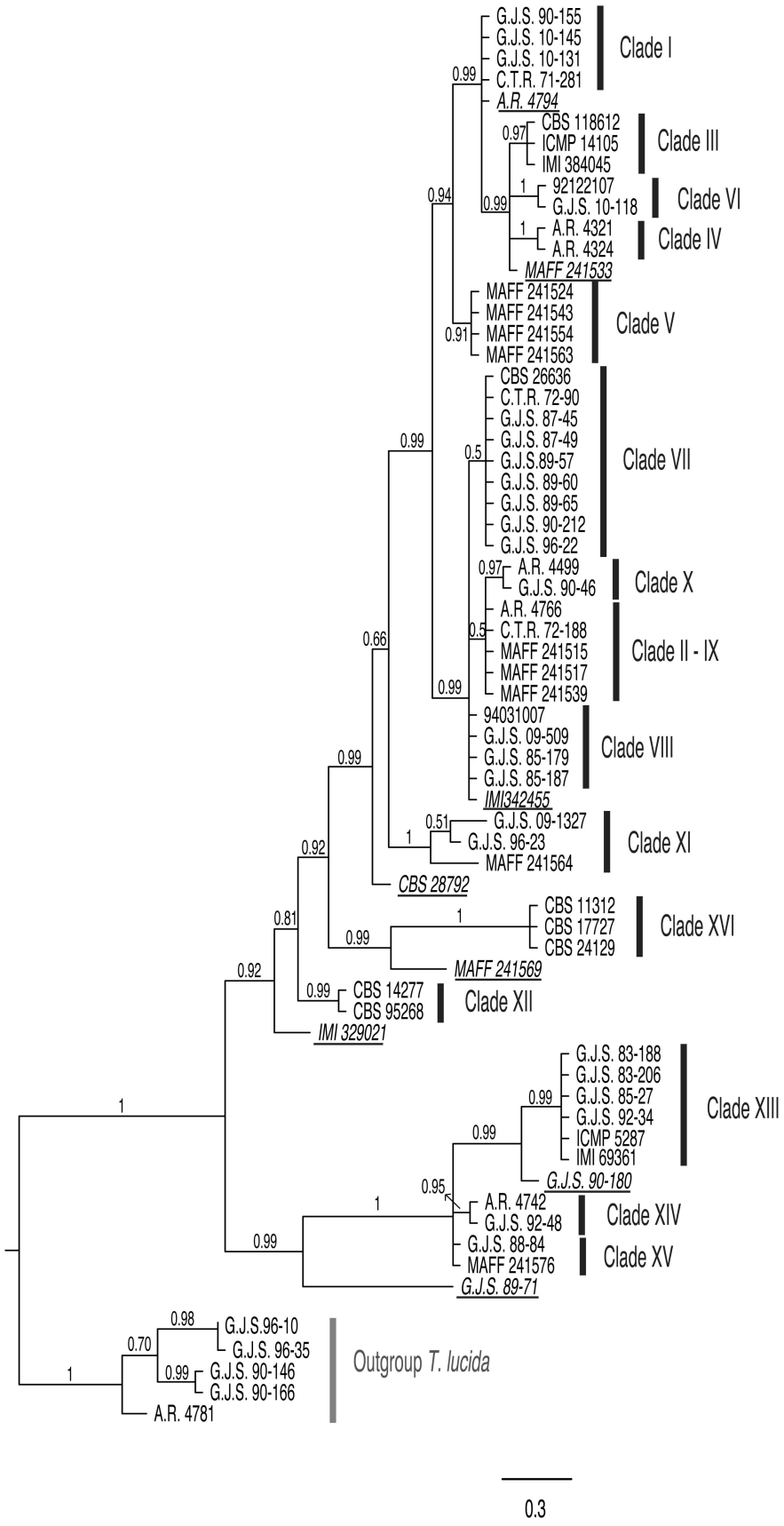
G.J.S. 96-10
G.J.S. 96-35
G.J.S. 90-166
G.J.S. 90-146
A.R. 4781
Outgroup T. lucida
0.01

Figure S2.3. Maximum likelihood phylogram showing relationships among isolates of *T. discophora*-like species based on the ITS loci. ML bootstrap is indicated on top of each branch. No values below branches indicate branch was not recovered/supported.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92122107
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A.R. 4321
A.R. 4324
MAFF 241533
MAFF 241524
MAFF 241543
MAFF 241554
MAFF 241563
CBS 26636
C.T.R. 72-90
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G.J.S. 90-46
A.R. 4766
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G.J.S. 85-187
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CBS 11312
CBS 17727
CBS 24129
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CBS 95268
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G.J.S. 92-34
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A.R. 4742
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MAFF 241576
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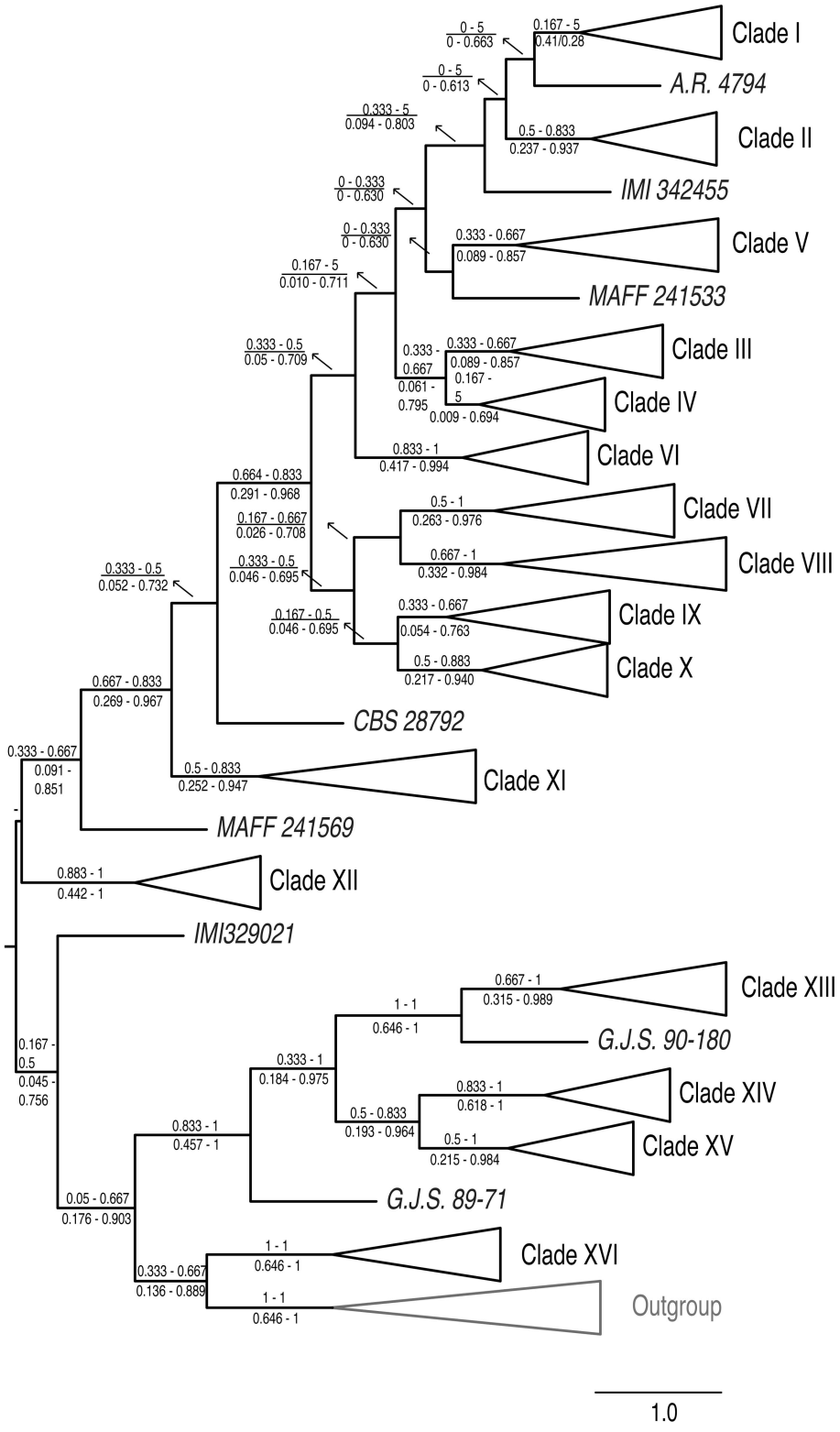
G.J.S.96-10
G.J.S. 96-35
G.J.S. 90-146
G.J.S. 90-166
A.R. 4781
Clade I
Clade III
Clade VI
Clade IV
Clade V
Clade VII
Clade X
Clade II - IX
Clade VIII
Clade XI
Clade XVI
Clade XII
Clade XIII
Clade XIV
Clade XV
Outgroup T. lucida
0.3

FIGURE S2.4. Bayesian phylogram showing relationships among isolates of *T. discophora*-like species based on the ITS loci. Bayesian posterior probabilities indicated on top of each branch. No values below branches indicate branch was not recovered/supported.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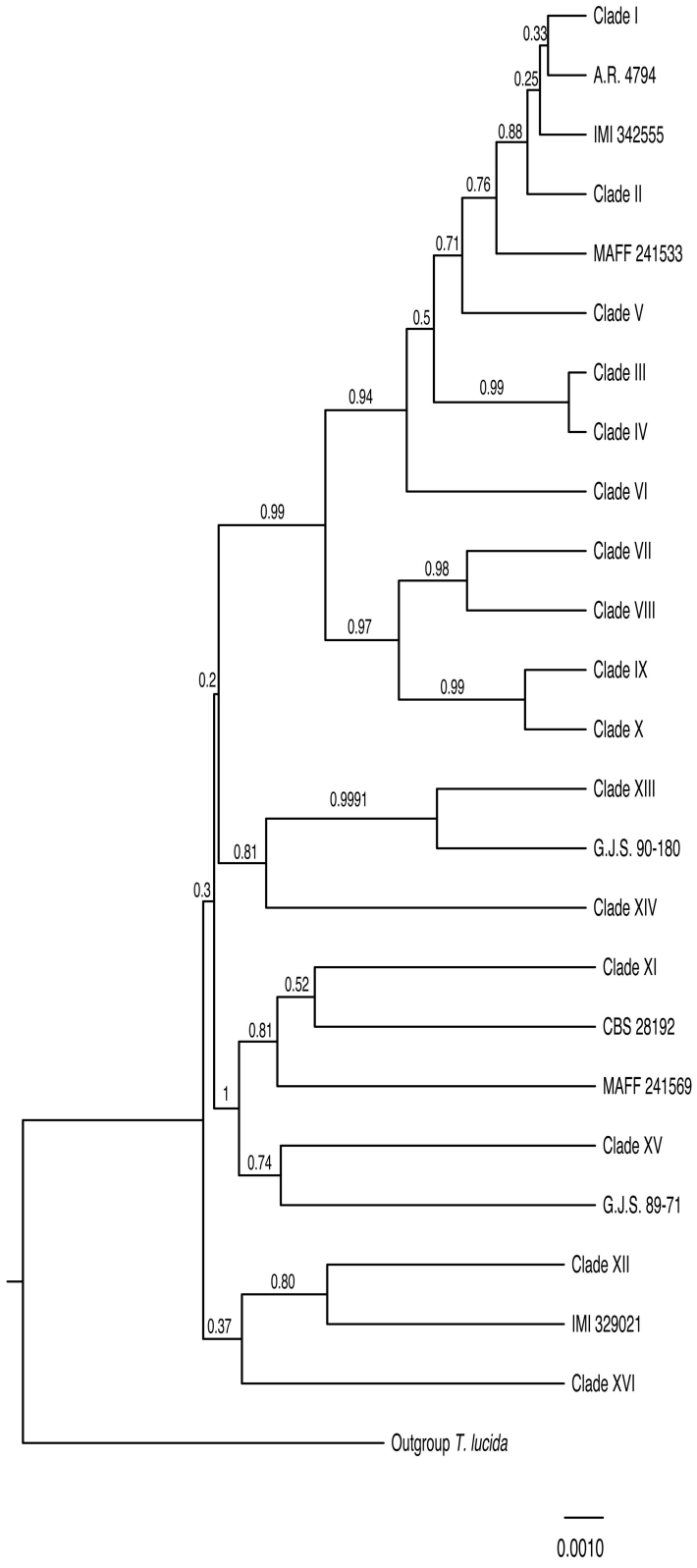
Clade X
CBS 28792
Clade XI
MAFF 241569
Clade XII
IMI329021
Clade XIII
G.J.S. 90-180
Clade XIV
Clade XV
G.J.S. 89-71
Clade XVI
Outgroup
1.0

FIGURE S2.5. Primary concordance tree estimated by BCA analysis, values above branches indicate sample-wide 95% CI and below branches indicate genome-wide 95% CI.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Clade VII
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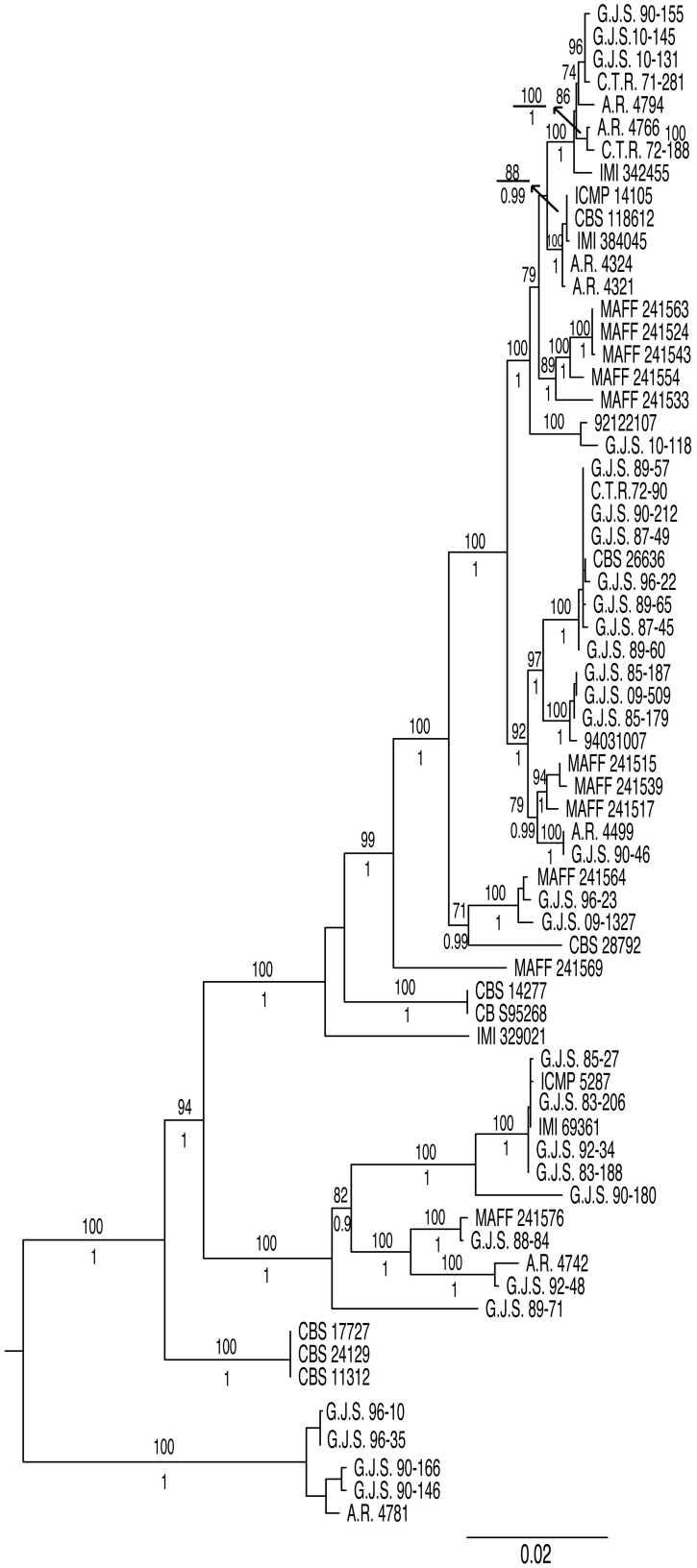
Clade IX
Clade X
Clade XIII
G.J.S. 90-180
Clade XIV
Clade XI
CBS 28192
MAFF 241569
Clade XV
G.J.S. 89-71
Clade XII
IMI 329021
Clade XVI
Outgroup T. lucida
0.33
0.25
0.88
0.76
0.71
0.5
0.99
0.94
0.99
0.98
0.97
0.99
0.2
0.9991
0.81
0.3
0.52
0.81
1
0.74
0.80
0.37
0.0010

FIGURE S2.6. Maximum clade credibility tree from concatenated analyses in *BEAST excluding ITS loci. This tree represents the posterior sample with the maximum sum of clade posterior probabilities at the internal nodes. Branch lengths equal to expected substitutions per site in concatenated data set. Posterior probabilities of each clade are shown above branches.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G.J.S. 10-131
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A.R. 4794
A.R. 4766
C.T.R. 72-188
IMI 342455
ICMP 14105
CBS 118612
IMI 384045
A.R. 4324
A.R. 4321
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MAFF 241524
MAFF 241543
MAFF 241554
MAFF 241533
92122107
G.J.S. 10-118
G.J.S. 89-57
C.T.R.72-90
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G.J.S. 87-49
CBS 26636
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G.J.S. 89-60
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G.J.S. 85-179
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MAFF 241539
MAFF 241517
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G.J.S. 96-23
G.J.S. 09-1327
CBS 28792
MAFF 241569
CBS 14277
CB S95268
IMI 329021
G.J.S. 85-27
ICMP 5287
G.J.S. 83-206
IMI 69361
G.J.S. 92-34
G.J.S. 83-188
G.J.S. 90-180
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A.R. 4742
G.J.S. 92-48
G.J.S. 89-71
CBS 17727
CBS 24129
CBS 11312
G.J.S. 96-10
G.J.S. 96-35
G.J.S. 90-166
G.J.S. 90-146
A.R. 4781
0.02

FIGURE S2.7. Bayesian phylogram showing relationships among isolates of *T. discophora*-like species when excluding ITS loci. This tree represents the posterior sample with the maximum sum of clade posterior probabilities at the internal nodes. Branch lengths equal to expected substitutions per site in concatenated data set. ML bootstrap support are shown above branches. BI posterior probabilities are shown below branches. No values above or below branches indicate branch was not recovered/supported.

FIGURE S3.1. Majority rule Bayesian phylogram showing relationships among isolates of *T. discophora*-like and *T. beijingensis* and *T. yunnanica* species based on the concatenated analysis of ITS and *tub* loci. Thick branches indicate Bayesian

posterior probabilities >0.95 and ML bootstrap >70%. No thick branches indicate branch was not recovered/supported.

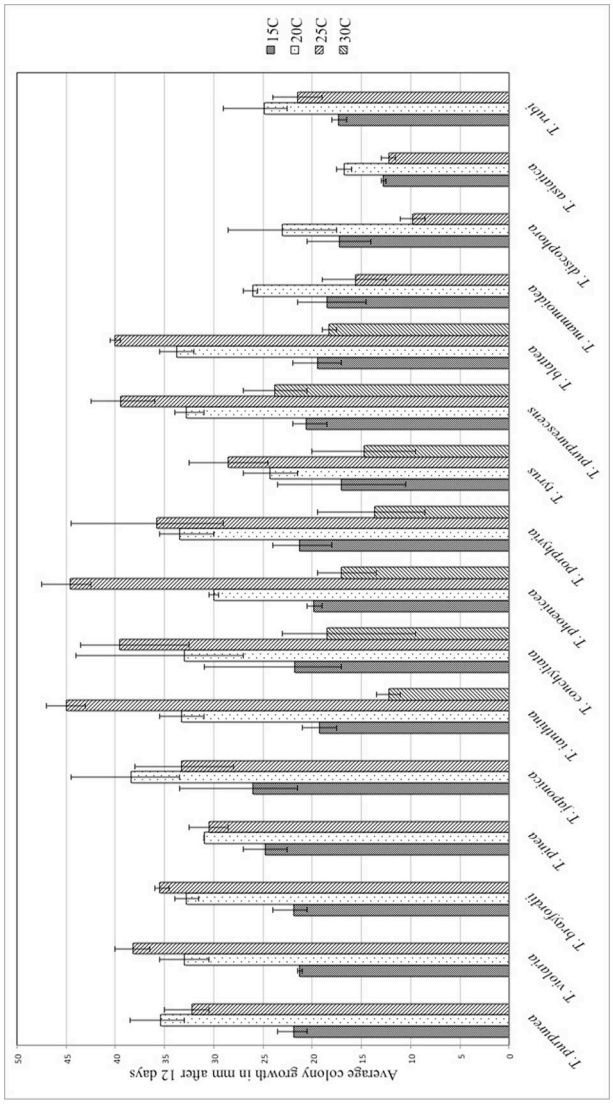

FIGURE S3.2. Average colony growth of *T. discophora* species complex under different temperatures. Bar indicates 95% confidence interval.

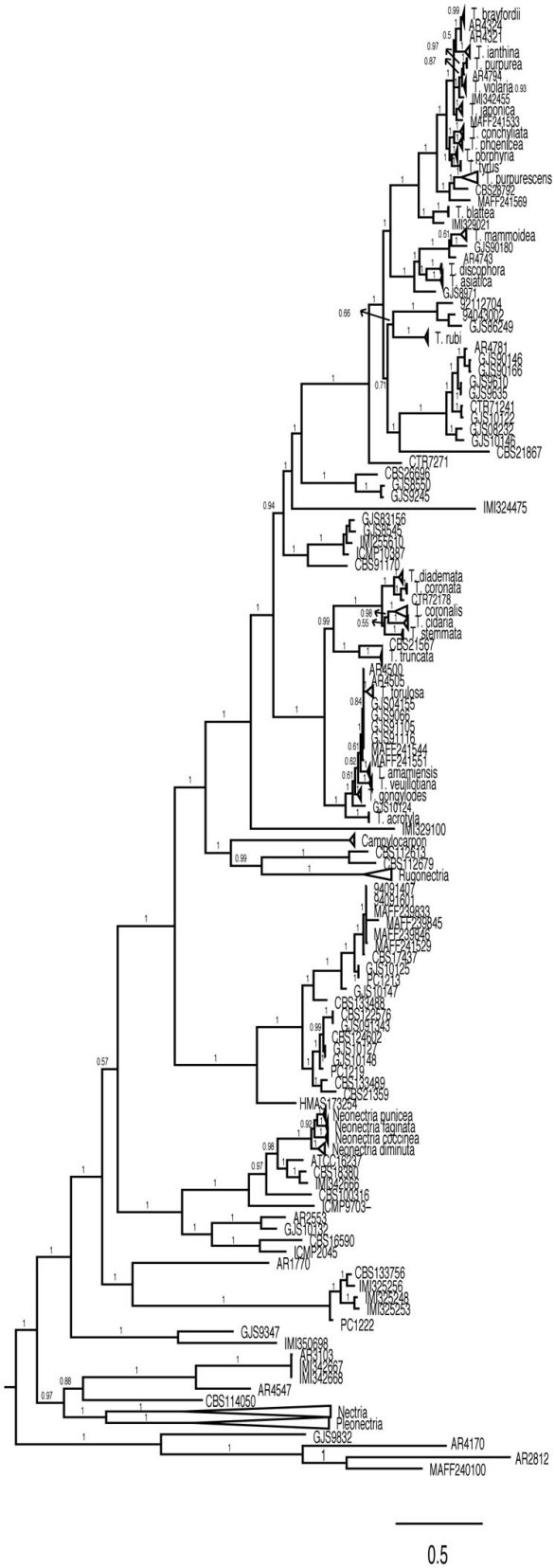
T. brayfordii
AR4324
AR4321
T. ianthina
T. purpurea
AR4794
T. violaria
IMI342455
T. japonica
MAFF241533
T. conchyliata
T. phoenicea
T. porphyria
lyxus
T. purpurescens
CBS2752
MAFF241569
T. blattea
IMI329021
T. mammoidea
GJS90180
AR4749
T. discophora
T. asiatica
GJS9837
92112704
94043002
GJS86249
T. rubi
AR4781
GJS90146
GJS90166
GJS9610
GJS9635
CTR71241
GJS10122
GJS10222
GJS10146
CBS21867
CTR7271
CBS29696
GJS9850
GJS9245
IMI324475
GJS83156
GJS8545
IMI255610
ICMP10387
CBS91170
T. diademata
T. coronata
CTR7278
T. coronalis
T. cidaria
T. stemmata
CBS21567
T. truncata
AR4500
AR4505
T. torulosa
GJS04155
GJS9068
GJS81175
GJS81178
MAFF241544
MAFF241551
T. amamiensis
T. veuillotiana
T. gongylodes
GJS10124
T. acrotia
IMI329100
Campylocarpon
CBS112613
CBS112679
Rugonectria
94091407
94091601
MAFF239833
MAFF239845
MAFF239846
MAFF241529
CBS17437
GJS10125
PC1213
GJS10147
CBS133489
CBS122576
GJS10143
CBS124602
GJS10127
GJS10148
PC1219
CBS133489
CBS21359
HMAS179254
Neonectria punicea
Neonectria faginata
Neonectria coccinea
Neonectria diminuta
ATCC18927
CBS18280
IMI342666
CBS100316
ICMP9703
AR2553
GJS10136
CBS516590
ICMP2045
AR1770
CBS133756
IMI325256
IMI325248
IMI325253
PC1222
GJS9347
IMI350698
AR3703
IMI342667
IMI342668
AR4547
CBS114050
Nectria
Pleonectria
GJS9832
AR4170
AR2812
MAFF240100
0.5

FIGURE S4.1. Majority rule Bayesian phylogram showing relationships among species in *Thelonectria* and related species with *Cylindrocarpon*-like anamorphs based on the concatenated analysis of eight loci. Support above branches indicate Bayesian posterior probabilities for analysis of data set after cleaning alignment of problematic regions with Gblocks setting 1. Number below branches indicate Bayesian posterior probabilities for analysis of original data set. Bionectriaceae was used as outgroup. For taxon reference see FIGURE 4.1.