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Four new taxa of *Ilyonectria* and *Thelonectria* (Nectriaceae) revealed by morphology and combined ITS and β-tubulin sequence data

ZHAO-QING ZENG[1,2] & WEN-YING ZHUANG[1*]

[1] *State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, P.R. China*
[2] *University of Chinese Academy of Sciences, Beijing 100049, P.R. China*
[*] *Corresponding author: zhuangwy@im.ac.cn*

Abstract

Thelonectria beijingensis sp. nov. is characterized by a distinct perithecial wall of textura epidermoidea mixed with textura intricata, purple to brown colonies, curved cylindrical macroconidia with rounded ends, ellipsoid to rod-shaped microconidia, and lacking chlamydospores. *Thelonectria yunnanica* sp. nov. is distinguished by a three layered perithecial wall with a palisade of short hyphae that are perpendicular to the outer surface, white colonies, elliptical-fusoid to rod-shaped microconidia with rounded ends and lacking chlamydospores. *Neonectria hubeiensis* and *N. sinensis* are transferred to *Ilyonectria* and *Thelonectria*, respectively. Distinctions between the new taxa and their close relatives are discussed based on morphology and the combined sequence data of nuclear ribosomal DNA ITS1-5.8S-ITS2 and partial β-tubulin gene.

Key words: DNA sequence, morphology, taxonomy

Introduction

The genus *Thelonectria* P. Chaverri & Salgado was established to accommodate species of *Nectria*-like fungi with a distinctive perithecial wall structure and a *Cylindrocarpon*-like asexual state (Chaverri et al. 2011). These species had previously been referred to as the *Nectria mammoidea*-group (Booth 1959, Samuels and Brayford 1990, 1993, 1994, Brayford and Samuels 1993, Rossman et al. 1999). *Thelonectria* is characterized by inconspicuous stromata, superficial, globose, subglobose, or pyriform to elongated and smooth or warty perithecia, a 2- or 3-layered perithecial wall, with prominent papilla and smooth, hyaline, 1-septate ascospores. Members of this genus are mostly found in tropical and subtropical regions on bark of recently killed, dying or diseased trees and rotting roots, and often cause small cankers (Chaverri et al. 2011).

Nine species are currently known in the genus *Thelonectria* (Chaverri et al. 2011), of which five were previously reported from China. *Thelonectria discophora* (as *Nectria discophora* Mont.) was the earliest record of the genus from China (Yunnan Province) (Teng 1936). *Thelonectria coronata* (Penz. & Sacc.) P. Chaverri & Salgado (as *Nectria coronata*) was recently added from Hong Kong (Lu et al. 2000), *T. jungneri* (Henn.) P. Chaverri & Salgado (as *Neonectria jungneri*) and *T. lucida* (as *Neonectria lucida*) were later reported from Taiwan Province (Guu et al. 2007), and *T. veuillotiana* (as *Neonectria veuillotiana*) was found from Hubei Province (Zhuang et al. 2007). In this study, two new species closely related to *T. discophora* from Beijing and Yunnan are described and illustrated based on their distinct morphology and sequence data of the combined nuclear ribosomal DNA ITS1-5.8S-ITS2 (ITS) and partial β-tubulin gene. Morphological distinctions between the new species and their closely related fungi are discussed. In addition, one species of *Neonectria* is transferred to *Thelonectria*, and one species is treated as a member of *Ilyonectria*.

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Material and methods

Material

Specimens were collected from China and deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS), and cultures are kept in the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences. GenBank accession numbers of sequences of ITS region and partial β-tubulin gene of *Neonectria*, *Ilyonectria* and *Thelonectria* used in this study are provided in TABLE 1.

TABLE 1. Materials used in this study.

Taxon	Geographical origin	Collection number	GenBank accession number	
			ITS	β-tubulin
Ilyonectria coprosmae (Dingley) P. Chaverri & Salgado	New Zealand	CBS 119606	JF735260	JF735373
I. hubeiensis (W.Y. Zhuang, Y. Nong & J. Luo) Z.Q. Zeng & W.Y. Zhuang	Hubei, China	HMAS 98331	FJ560439	FJ860056
I. liriodendri (Halleen, Rego & Crous) P. Chaverri & Salgado	France	CBS 112610	AY677270	AY677244
	Australia	BRIP 54020	JN243768	JN243769
I. radicicola (Gerlach & L. Nilsson) P. Chaverri & Salgado	Venezuela	ATCC 208837	HM364290	HM352856
Neonectria coccinea (Pers.) Rossman & Samuels	Austria	CBS 29181	FJ474075	DQ789874
Neo. confusa J. Luo & W.Y. Zhuang	Hubei, China	HMAS 99197	FJ560437	FJ860054
	Hubei, China	HMAS 99198	JF268760	JF268721
Neo. ditissima (Tul. & C. Tul.) Samuels & Rossman	Ireland	CBS 100316	HM364298	DQ789858
Neo. ditissimopsis P. Zhao, J. Luo & W.Y. Zhuang	Hubei, China	HMAS 98328	JF268762	JF268727
	Hubei, China	HMAS 98329	JF268764	JF268729
Neo. faginata (M.L. Lohman, A.M.J.Watson & Ayers) Castl. & Rossman	USA	CBS 119160	HQ840384	DQ789883
	Canada	CBS 21767	HQ840385	JF268730
Neo. fuckeliana (C. Booth) Castl. & Rossman	Austria	CBS 119200	HQ840387	JF268731
	Scotland	CBS 23929	HQ840386	DQ789871
Neo. major (Wollenw.) Castl. & Rossman	Jiangxi, China	HMAS 183183	JF268766	JF268732
	Jiangxi, China	HMAS 183184	JF268767	JF268733
Neo. microconidia J. Luo, P. Zhao & W.Y. Zhuang	Hubei, China	HMAS 98295	JF268761	JF268722
Neo. ramulariae Wollenw.	England	CBS 15129	AY677291	DQ789863
	unknown	CBS 18236	HM054157	DQ789864
Neo. shennongjiana J. Luo & W.Y. Zhuang	Hubei, China	HMAS 183185	FJ560440	FJ860057
Nectria cinnabarina (Tode) Fr.	Austria	CBS 125150	HM484712	HM484837
N. pseudotrichia Berk. & M.A. Curtis	Guangdong, China	HMAS 183172	GU232860	HM054116
Thelonectria beijingensis Z.Q. Zeng, J. Luo & W.Y. Zhuang	Beijing, China	HMAS 188498	**JQ836656**[a]	**JQ836658**
T. discophora (Mont.) P. Chaverri & Salgado	New Zealand	CBS 125153	HM364294	HM352860
	USA	CBS 125172	HM364296	
	Japan	h172-1		AB237485
	Hubei, China	HMAS 98333	HM054136	HM054131
	Hubei, China	HMAS 98327	HM054140	HM054123
	Hainan, China	HMAS 76856	**JQ836657**	**JQ836659**
	Yunnan, China	HMAS 183191	HM054161	FJ860055
T. lucida (Höhn.) P. Chaverri & Salgado	unknown	I185	GU062255	
	Venezuela	CBS 112455		AY677259
T. sinensis (J. Luo & W.Y. Zhuang) Z.Q. Zeng & W.Y. Zhuang	Hubei, China	HMAS 183186	FJ560441	FJ860058
T. trachosa (Samuels & Brayford) Samuels, P. Chaverri & Salgado	Scotland	CBS 112467	AY677297	AY677258
T. veuillotiana (Sacc. & Roum.) P. Chaverri & Salgado	USA	GJS 91-116	HM054149	HM054126
	Hubei, China	HMAS 99207	HM054146	HM054134
T. westlandica (Dingley) P. Chaverri & Salgado	New Zealand	GJS 83-156	HM484559	HM352868
T. yunnanica Z.Q. Zeng & W.Y. Zhuang	Yunnan, China	HMAS 183564	FJ560438	**JQ836660**

[a] Numbers in boldface indicating the newly submitted sequences.

Morphological observation

The methods used by Luo and Zhuang (2010) were generally followed for morphological studies. Water was

used as the mounting fluid for microscopic examinations and measurements, and photographs were taken from lactophenol cotton blue mounts with a Canon G5 (Tokyo, Japan) digital camera connected to a Zeiss Axioskop 2 plus microscope (Göttingen, Germany). For anatomical studies, longitudinal sections through ascomata were made with a freezing microtome (YD-1508-III, Yidi Medical Appliance Factory, Jinhua, China) at a thickness of 8–10 μm. Measurements of individual structures were based on 30 units, except when otherwise noted. Single-spore isolations were obtained from fresh collections. Characters of colonies were recorded from cultures on potato dextrose agar (PDA, Gams et al. 1998). Microscopic descriptions of the anamorphs were taken from cultures on PDA.

Molecular methods and sequence analysis

DNA was extracted from mycelium harvested from colonies on PDA after 2 weeks following the methods of Wang and Zhuang (2004). Sequences of ITS and the partial β-tubulin gene were amplified with primer pairs ITS5-ITS4 (White et al. 1990) and T1-T222 (O'Donnell and Cigelnik 1997), respectively. The PCR reaction mixture consisted of 2.5 μL 10× PCR buffer, 1.5 μL $MgCl_2$ (25 mM), 1.25 μL each primer (10 μM), 0.5 μL dNTP (10 mM each), 1.25 μL template DNA, 0.25 μL *Taq* polymerase (5 U/μL) and 16.5 μL ddH_2O. Reactions were performed on an ABI 2720 Thermal Cycler (Gene Co. Ltd., California, USA) with cycling conditions consisting of denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C (ITS region) and at 55°C (partial β-tubulin gene) for 30 s and elongation at 72°C for 45 s, with a final extension step at 72°C for 10 min (Zeng and Zhuang 2012). PCR products were purified with the PCR Product Purification Kit (Biocolor BioScience & Technology Co., Shanghai, China) and sequenced with ABI BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, California, USA) on an ABI 3730 XL DNA Sequencer (Gene Co. Ltd., California, USA). Primers ITS5 and ITS4 (for ITS), and T1 and Bt2b (for partial β-tubulin gene) were used for sequencing (White et al. 1990, Glass and Donaldson 1995, O'Donnell and Cigelnik 1997).

DNA sequences were aligned using ClustalX 1.8 (Thompson et al. 1997), and the alignments were visually adjusted by BioEdit 7.0.5 (Hall 1999) when necessary. A partition homogeneity test was performed with 1000 replicates via PAUP 4.0b10 (Swofford 2002) to evaluate statistical congruence between sequence data of ITS and partial β-tubulin gene regions. *Nectria cinnabarina* and *N. pseudotrichia* were chosen as outgroup taxa. Maximum parsimony (MP) analysis was performed with PAUP 4.0b10 (Swofford 2002) conducted by a heuristic search with the following settings: all characters were equally weighted, gaps were treated as missing characters, starting trees obtained by stepwise addition, addition sequence = random, number of replicates = 1000, number of trees held at each step during stepwise addition = 1, branch-swapping algorithm = tree-bisection-reconnection, steepest descent option not in effect, MulTrees option not in effect, topological constraints not enforced. Clade stability was evaluated by bootstrap method with heuristic search. Other measures were consistency index (CI), retention index (RI) and homoplasy index (HI).

Taxonomy

Ilyonectria hubeiensis (W.Y. Zhuang, Y. Nong & J. Luo) Z.Q. Zeng & W.Y. Zhuang, *comb. nov.*
MycoBank MB564934

Basionym. *Neonectria hubeiensis* Zhuang, Nong & Luo, Fungal Diversity 24: 351. 2007.

Habitat. On fruits of *Rhododendron* sp.

Distribution. China, known only from the type locality.

Description and illustrations. Zhuang et al. (2007).

Notes. The anatomy of perithecia, and uniseptate, hyaline and smooth ascospores of this species suggest that it belongs to the genus *Ilyonectria.* Sequence analysis of the combined ITS and partial β-tubulin gene from type culture confirms its taxonomic position in this genus, which will be discussed later in this paper (Fig. 3).

Thelonectria sinensis (J. Luo & W.Y. Zhuang) Z.Q. Zeng & W.Y. Zhuang, *comb. nov.*
MycoBank MB564935

Basionym. *Neonectria sinensis* Luo & Zhuang, Mycologia 102: 147. 2010.

Habitat. On bark of a coniferous tree.

Distribution. China, known only from the type locality.

Description and illustrations. Luo and Zhuang (2010).

Notes. The perithecial gross morphology and wall structure, and uniseptate, smooth-walled, hyaline ascospores of the fungus indicate its position in *Thelonectria.* The sequence analysis of the ITS and β-tubulin gene from type culture confirms the placement of the fungus, which will be discussed later (FIG. 3).

Thelonectria beijingensis Z.Q. Zeng, J. Luo & W.Y. Zhuang, *sp. nov.*
FIG. 1
MycoBank MB564936

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

Diagnosis. Differing from *Thelonectria sinensis* in having a thinner perithecial wall, relatively large ascospores, and producing microconidia in culture.

Ascomata perithecial, solitary to gregarious up to 10 in a group, with a well-developed stroma that is erumpent through bark, superficial, subglobose to globose, 320–391 μm high, 305–385 μm diam. (n = 10), not collapsing when dry, orange red to red when fresh and red when dry, turning dark red in 3% KOH and orange in lactic acid, surface slightly roughened. *Ascomatal wall* 22–38 μm thick, of two layers: outer layer 14–27 μm thick, of textura epidermoidea mixed with textura intricata in the upper portion, of textura angularis near the base, cells mostly lacking a definite shape, axis of cells near the apical portion somewhat perpendicular to perithecial surface, 5.4–11 × 2.4–4.9 μm, walls 0.8–1.2 μm thick; inner layer 8–11 μm thick, of textura prismatica, cells flattened, 8–19 × 2.2–3.8 μm, walls 0.5–0.8 μm thick. *Asci* subcylindrical, 8-spored, with an apical ring, 82–104 × 5.5–8.5 μm. *Ascospores* fusiform-ellipsoid, uniseptate, not constricted at septum, hyaline, smooth, uniseriate, 13–17 × 4–7 μm.

In culture, colony reaching 22 mm in diam. after 7 days on PDA at 25°C under daylight, surface velvety, aerial mycelium brown to purplish, reverse dark vinaceous. Conidiophores unbranched to sparsely branched, septate, 22–59 × 2.2–3.5 μm, arising from agar surface throughout colony. Microconidia ellipsoid to rod-shaped, straight or slightly curved, hyaline, nonseptate, 5.4–14 × 2.2–4.1 μm. Macroconidia subfusoid to cylindrical, with rounded ends, curved, hyaline, 0–3-septate; 0-septate: 41–51 × 3.2–5.4 μm, 1-septate: 32–46 × 2.7–4 m, 2-septate: 43–51 × 3.2–4 μm, 3-septate: 41–54 × 3.2– 4.9 μm. Chlamydospores absent.

Holotype. CHINA. Beijing, on bark of an unidentified tree, 1 Sept 2010, *L. Cai 7604*, HMAS 188498!, ex type culture HMAS 188566.

Notes. Among the existing species of the genus, *Thelonectria beijingensis* is most similar to *T. sinensis* in cylindrical asci, fusiform-ellipsoid ascospores, and brown to purplish colonies on PDA. The latter fungus differs by having a thicker perithecial wall (32–55 μm thick), relatively small ascospores (10–16 × 3.2–5.8 μm), and not producing microconidia in culture (Luo and Zhuang 2010). It is also similar to *T. discophora* and *T. lucida* in having red perithecia, and cylindrical asci with an apical ring. *Thelonectria discophora* differs in perithecia with smooth surface and seated on a minute stroma, possessing distinct palisade cells in the outermost perithecial wall, with axes of cells perpendicular to perithecial surface; wider asci (72–95 × 7–10 μm); pale brown, spinulose, wider ascospores (12–17 × 5–8 μm); and the absence of microconidia (Brayford et al. 2004). *Thelonectria lucida* differs in having larger perithecia (430–480 μm in diam.) with a smooth instead of slightly roughened surface and thinner walls (20–30 μm thick), a distinct palisade layer of cells in the outer perithecial wall, with axes of cells perpendicular to outer surface, shorter and wider asci (63–72 × 6.5–10 μm), pale brown and spinulose ascospores, 3–8-septate instead of 0–3-septate and larger macroconidia (60–90 × 5–10 μm), white to tan instead of purple to brown colony, and the absence of microconidia (Brayford et al. 2004). Our sequence analysis supports the separation of *T. beijingensis* from its morphologically similar species (FIG. 3).

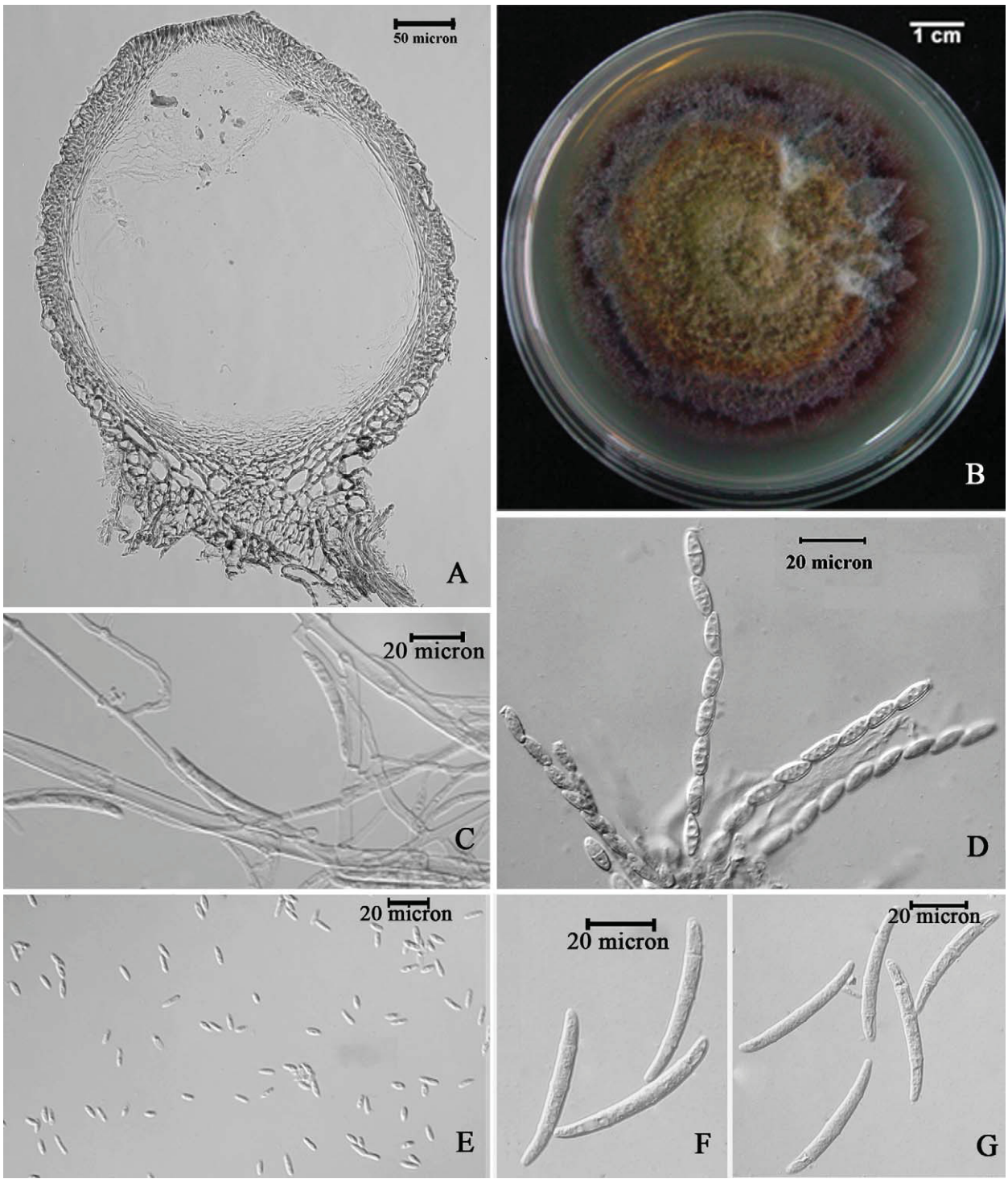

Figure 1. *Thelonectria beijingensis* (holotype). A. Median section of an ascoma; B. Colony on PDA; C. Conidiophores and macroconidia; D Asci with ascospores; E. Microconidia; F, G. Macroconidia.

Thelonectria yunnanica Z.Q. Zeng & W.Y. Zhuang, *sp. nov.*
Fig. 2

MycoBank MB564937

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

Diagnosis. Differing from *Thelonectria discophora* in having a thicker perithecial wall, longer cells in the

palisade layer, larger asci, wider ascospores, white instead of violet to dark violet colony, and presence of microconidia in culture.

Ascomata perithecial, solitary or gregarious up to 20 in a group, superficial or with base partially immersed in substratum on a minute stroma, subglobose, 350–380 µm high, 450–490 µm diam. (n = 10), not collapsing when dry, red when fresh and brick-red when dry, ostioles blackened with age, slightly papillate, turning dark red in 3% KOH and orange red to orange in lactic acid, surface smooth to slightly roughened. *Ascomatal wall* 49–71 µm thick, of three layers: outer layer 18–46 µm thick, cells elongated, axis of cells perpendicular to perithecial surface forming a palisade layer, cells 19–24 × 2.4–3.2 µm; middle layer 10–16 µm thick, of texture intricata, hyphae 3–4 µm; inner layer 5.4–10 µm thick, of textura prismatica to textura porrecta, cells rectangular with the long axis parallel to perithecial surface, 8–16 × 4–5.5 µm. *Asci* cylindrical, 8-spored, with an apical ring, 87–120 × 8.2–9.6 µm. *Ascospores* ellipsoid, uniseptate, not constricted at septum, slightly yellowish, spinulose, uniseriate with ends overlapping, 13–17 × 6–7.9 µm.

Colony reaching 32 mm in diam. after 7 days on PDA at 25°C under daylight, surface velvety, aerial mycelium white. Conidiophores unbranched to sparsely branched, septate, (28–)30–77(–80) × (1.2–)1.5–3.0(–3.2) µm, arising from agar surface. Microconidia elliptical-fusoid to rod-shaped, straight or slightly curved, rounded at the distal end, hyaline, 0–1-septate, (8–)8.2–16(–16.5) × (3–)3.2–4.5(–4.8) µm. Macroconidia subfusoid to cylindrical, curved, hyaline, (3–)4–7(–9)-septate; (59–)60–93(–93.5) ´ (5.5–)6–8.8(–9) mm. Chlamydospores absent.

Holotype. CHINA. Yunnan, Baoshan, on bark of an unidentified tree, 15 Oct 2003, *W.P. Wu W7122*, HMAS 183564!, ex type culture HMAS 188567.

Notes. Among the existing species of the genus, *Thelonectria yunnanica* is most similar to *T. discophora* in perithecial shape, size and color, structure of the perithecial outer wall, cylindrical asci with an apical ring, and spinulose ascospores. However, *T. discophora* differs in its thinner perithecial wall (30–50 µm thick) of two layers instead of 3-layered wall, shorter cells in the palisade layer (2–2.5 µm long), smaller asci (72–95 × 7–10 µm), narrower ascospores (12–17 × 5–8 µm), sparse or absence of microconidia, and violet to dark violet colony instead of white (Brayford et al. 2004, Guu et al. 2007, Hirooka and Kobayashi 2007).

It is also similar to *T. trachosa* and *T. viridispora* in the 3-layered perithecial wall. *Thelonectria trachosa* differs in having larger ascospores (17.5–20.5 × 6–9 µm vs. 13–17 × 6–7.9 µm) and the absence of microconidia (Brayford et al. 2004). *Thelonectria viridispora* differs in having smaller perithecia (300–375 µm vs. 450–490 µm in diam.) and the absence of microconidia (Brayford et al. 2004).

Results

Combined ITS and β-tubulin sequence data have been shown to be reliable in separating species and genera of Nectriaceae (Zhao et al. 2011) and were therefore used in this study. Partition homogeneity test (P = 0.01) indicated that the individual partitions were not highly incongruent (Cunningham 1997), and thus ITS and partial β-tubulin sequences were combined for sequence analysis. The combined datasets of 37 sequences include 1100 characters, of which 365 were parsimony-informative, 112 were variable and parsimony-uninformative, and 623 were constant. The MP analysis resulted in 36 most parsimonious trees. A strict consensus tree is shown in FIG. 3.

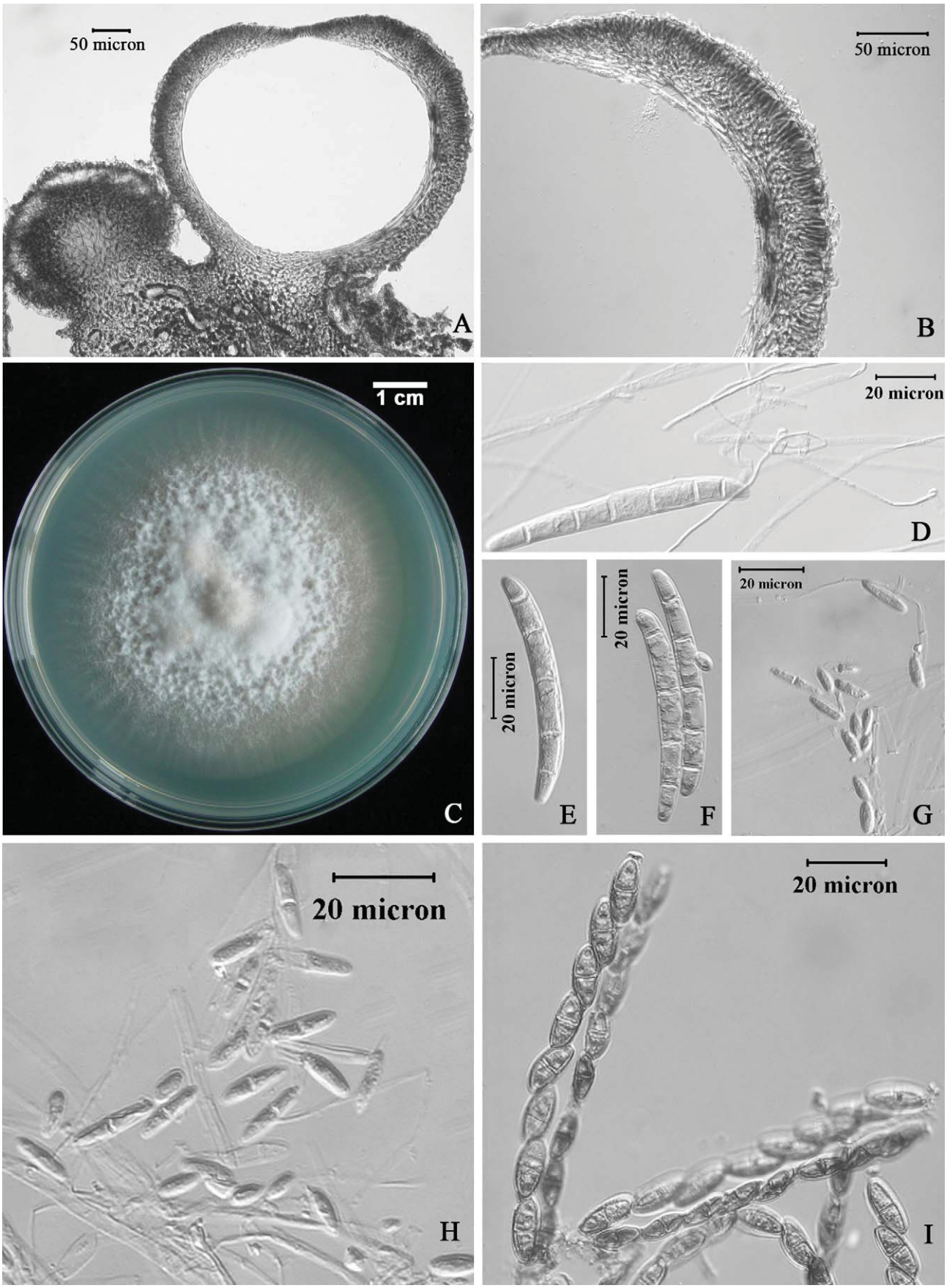

Figure 2. *Thelonectria yunnanica* (holotype). A. Median section of an ascoma; B. Structure of lateral perithecial wall; C. Colony on PDA; D–F. Macroconidia; G, H. Conidiophores and microconidia; I. Asci with ascospores.

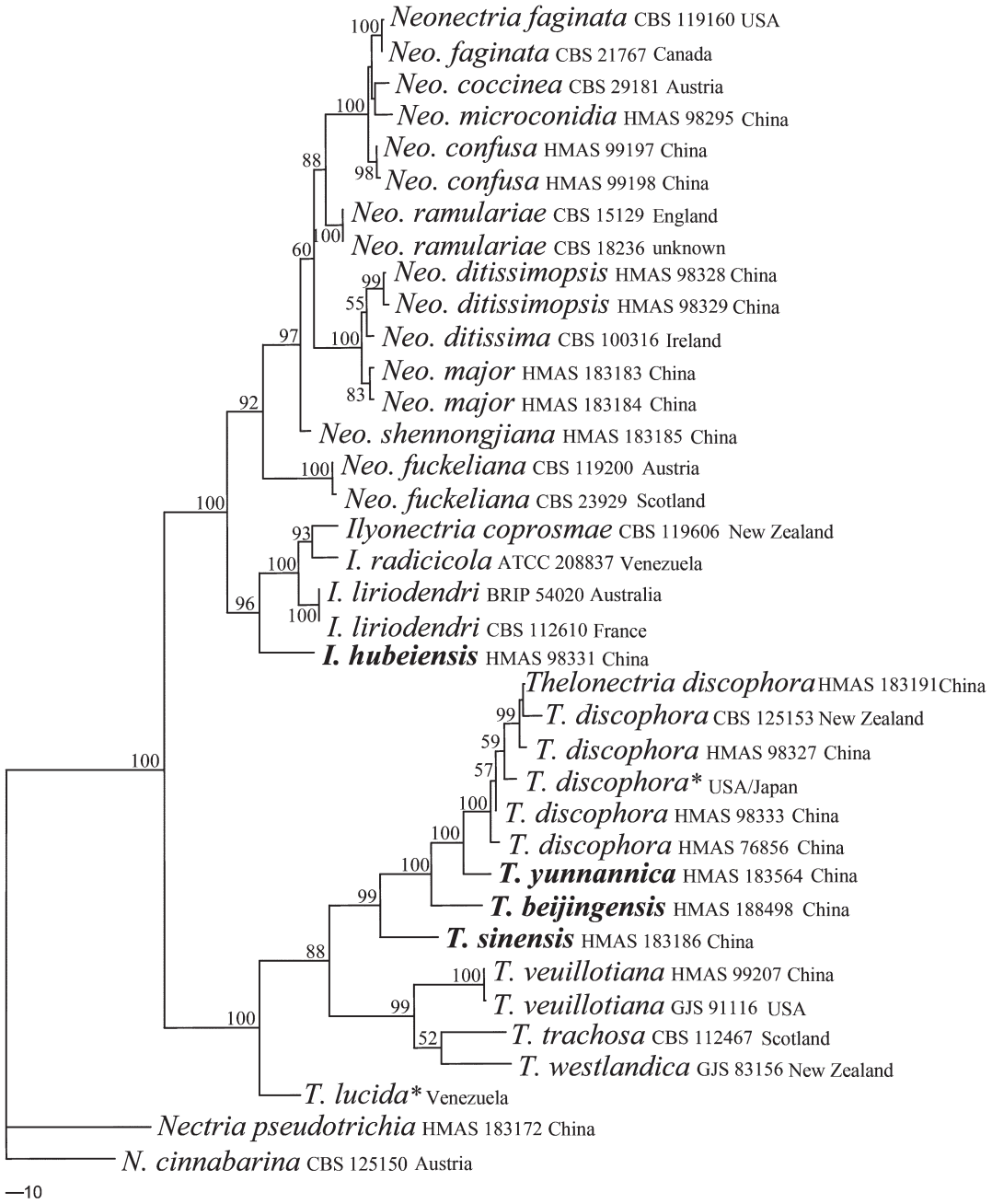

FIGURE 3. One of thirty-six equally most parsimonious trees inferred from combined ITS and β-tubulin partial sequences. Abbreviations: *Neo. = Neonectria*, *I. = Ilyonectria*, *T. = Thelonectria*, *N. = Nectria.* Tree length = 1169, CI = 0.6296, HI = 0.3704, CI excluding uninformative characters = 0.5856, HI excluding uninformative characters = 0.4144, RI = 0.8426, Rescaled consistency index = 0.5305. Bootstrap values ≥50% from 1000 replicates are noted above internodes. Asterisks indicate sequences of ITS and β-tubulin gene were from different strains as shown in TABLE 1.

Three clades are recognized in the phylogenetic tree, which represent respectively the genera *Neonectria*, *Ilyonectria* and *Thelonectria*, in agreement with Chaverri et al. (2011). Species of *Neonectria* and *Ilyonectria* grouped together with 100% bootstrap support (FIG. 3). The *Neonectria* clade constitutes ten species with 92% bootstrap support. In the clade of *Ilyonectria*, four taxa are represented. *Ilyonectria hubeiensis* turns out to be closely related to *I. coprosmae*, *I. liriodendri* and *I. radicicola* (the type species of the genus) receiving a high bootstrap value (96%). In the *Thelonectria* clade, the eight investigated species formed a monophyletic group (100% bootstrap support), in which *T. beijingensis*, *T. sinensis* and *T. yunnanica* were closely related to *T. discophora* (the type species of the genus).

Discussion

Thelonectria is originally circumscribed as perithecia superficial, sometimes seated on an immersed inconspicuous stroma, smooth or sometimes warty, globose, subglobose, or pyriform to elongated, 300–600 µm diam., most species with prominent and darkened papilla; perithecial walls of 2 or 3 regions, 20–50(–100) µm thick; ascospores mostly smooth, rarely spinulose or striate, hyaline, becoming brownish at maturity, generally 1-septate; anamorphs *Cylindrocarpon*-like; microconidia rare, sometimes seen on nature substrates; chlamydospores rare, abundant in one species; macroconidia curved with rounded ends (Chaverri et al. 2011). The two new species, *T. beijingensis* and *T. yunnanica*, fit well the above mentioned generic concept, and are characterized by their production of microconidia in culture.

In the *Thelonectria* clade, three subclades were recognized among the taxa tested (FIG. 3). One contains *T. discophora* and its relatives, *T. beijingensis*, *T. sinensis* and *T. yunnananica*; the second include *T. trachosa*, *T. veuillotiana* and *T. westlandica*; and the third at the bottom of the tree consists of a single species *T. lucida*. This result is coincident with an important taxonomic criterion, i.e. the perithecial wall structure. Members of the first subclade are common in the presence of a palisade layer containing club-shaped cells, sometimes mixed with the angular ones, in the outer most layer of perithecial wall. Taxa of the second subclade are of more or less isodiametric-angular to nearly spherical cells in the outer perithecial walls. *Thelonectria lucida*, the representative of the third subclade, is with the perithecial wall of textura epidermoidea.

Collections of *T. discophora* and its allies formed a highly supported subclade (100% bootstrap value). Three strains of *T. discophora* derived from Hubei and Yunnan provinces of China and New Zealand constituted a well-supported terminal branch (99% bootstrap value). They were further clustered with other collections from China and USA/Japan with low bootstrap supports (59% and 57%, respectively). The variation within *T. discophora* seems not correlate to geographical locations. The phenomenon of sequence divergence (1–14 bp) among collections of this species agrees with other studies in which *T. discophora* is treated as a heterogeneous species (Booth 1959, 1966).

Key to the known species of *Thelonectria*

1. Teleomorph unknown ..... *T. olida* (Chaverri et al. 2011)
- Teleomorph occurring on nature substrate 2
2. Ascospores averaging ≤20 µm long 3
- Ascospores averaging >20 µm long 11
3. Perithecial apex with a fringe of saccate cells ..... *T. coronata* (Brayford and Samuels 1993)
- Perithecial apex not as above 4
4. Perithecial wall three-layered 5
- Perithecial wall two-layered 7
5. Microconidia present, (8–)8.2–16(–16.5) × (3–)3.2–4.5(–4.8) µm ..... *T. yunnanica* (this paper)
- Microconidia absent 6
6. Perithecia smooth to slightly roughened ..... *T. trachosa* (Brayford et al. 2004)
- Perithecia strongly warty ..... *T. viridispora* (Brayford et al. 2004)
7. Ascospores averaging ≥7 µm wide ..... *T. veuillotiana* (Brayford and Samuels 1993)
- Ascospores averaging <7 µm wide 8
8. Perithecia smooth, surface shining 9
- Perithecia rough or conspicuously warty 10
9. Perithecial wall 30–50 µm; colonies purple on PDA ..... *T. discophora* (Brayford et al. 2004)
- Perithecial wall 20–30 µm; colonies white to tan on PDA..... *T. lucida* (Brayford et al. 2004)
10. Perithecial wall 22–38 µm thick; ascospores smooth; microconidia present .....*T. beijingensis* (this paper)
- Perithecial wall 32–55 µm thick; ascospores spinulose; microconidia absent..... *T. sinensis* (Luo and Zhuang 2010)
11. Perithecia smooth, surface shining; ascospores (15–)22–29(–36) × (5–)8.5–10.2 µm ..... *T. jungneri* (Brayford et al. 2004)
- Perithecia rough to warty, not shining if nearly smooth; ascospores (24–)25–34(–37) × (7–)8.5–11.5(–13) µm ..... *T. westlandica* (Brayford et al. 2004)

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