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Two new species of *Fulvifomes* (Hymenochaetales, Basidiomycota) from America

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Abstract

Two new species of *Fulvifomes* are described from samples collected in America based on morphological characteristic and molecular evidence: *F. centroamericanus* Y.C. Dai, X.H. Ji & Vlasák, **sp. nov.** and *F. krugiodendri* Y.C. Dai, X.H. Ji & Vlasák, **sp. nov.** The former is characterized by perennial and sessile basidiocarps, a concentrically sulcate and cracked pileal surface, a homogeneous context, small pores (7–9 per mm), a dimitic hyphal system, subglobose, yellowish and thick-walled basidiospores 4.3–5 × 4–4.5 µm. Macroscopically it resembles *F. merrillii*, which differs in having larger basidiospores (5–6 × 4–5 µm). *F. centroamericanus* is similar to *F. robiniae* in sharing applanate basidiocarps and subglobose, yellowish and thick-walled basidiospores 3.9–4.5 × 3.7–4.2 µm, whereas *F. robiniae* has larger basidiospores (5–6 × 4.5–5 µm). In nuclear large subunit rDNA (nLSU) and internal transcribed spacer (ITS) based phylogenies, the two new species formed two distinct lineages in the *Fulvifomes* clade.

Key words

Hymenochaetales, polypore, taxonomy, phylogenetic analysis

Introduction

Fulvifomes, typified by *F. robiniae*, was proposed by Murrill (1914) to refer to polypores with perennial and sessile basidiocarps, brown and woody context, colored basidiospores, and lacking setae. Several species were referred to this genus by Murrill (1914, 1915), but *Fulvifomes* has been considered as a synonym of *Phellinus* Quél. by many later mycologists (Ryvarden and Johansen 1980, Gilbertson and Ryvarden 1987, Larsen and Cobb-Poule 1990, Ryvarden 1991, Núñez and Ryvarden 2000). Wagner and Fischer (2002) confirmed *Fulvifomes* as an independent genus in Hymenochaetaceae based on nuclear-encoded large subunit rRNA gene (nLSU) sequences. Larsson et al. (2006) acknowledged that *Fulvifomes* was closely related to a clade hosting *Aurificaria luteoumbrina* (Romell) D.A. Reid and *Inonotus porrectus* Murrill with phylogenetic evidence. Dai (2010) included some species with resupinate basidiocarps and/or hymenial setae into *Fulvifomes* without phylogenetic evidence. Zhou (2014) and Hattori et al. (2014) accepted the circumscription of *Fulvifomes* as a genus by the combination of annual to perennial and effused-reflexed, sessile to substipitate basidiocarps with solitary to imbricate pilei and an homogeneous or duplex context, a monomitic to dimitic hyphal system, lack of hyphoid and hymenial setae, and subglobose to ellipsoid, yellowish to brown, slightly thick to thick-walled basidiospores. Recently, several species were introduced to *Fulvifomes* (Hattori et al. 2014, Zhou 2014 and 2015).

During the study on the hymenochaetaceous fungi from North America (USA and Central America), two species belonging to *Fulvifomes* were found with no existing names available for them. Based on both morphology and phylogenetic analyses, they are described as new to science in the present paper.

Materials and methods

Morphological studies

Specimens studied are deposited in the herbaria of the Institute of Microbiology, Beijing Forestry University (BJFC), National Museum Prague of Czech Republic (PRM), and the private herbarium of J. Vlasák (JV). The microscopic procedure follows He and Li (2011, 2013). Special color terms followed Petersen (1996). Spores were measured from sections cut from the tubes. Five % of measurements were excluded from each end of the range, and were given in parentheses. The following abbreviations were used: **KOH** = 5% potassium hydroxide, **IKI** = Melzer's reagent, **IKI–** = neither amyloid nor dextrinoid, **CB** = Cotton Blue, **CB–** = acyanophilous, **L** = mean spore length (arithmetic average of all spores), **W** = mean spore width (arithmetic average of all spores), **Q** = variation in the ratios of L/W between specimens studied, **n** = number of spores measured from given number of specimens.

Molecular study

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain PCR products from dried specimens. Primer pair LR0R and LR7 (Vilgalys and Hester 1990) was used to amplify nLSU sequences, while ITS region was amplified using primers ITS5 and ITS4 (White et al. 1990). The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 54°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR products were purified and sequenced in Beijing Genomics Institute, China, with the same primers.

Phylogenetic analysis

In this study, thirteen new sequences were generated (Table 1). Other sequences for phylogenetic analysis were downloaded from GenBank which were used in the previous study (Zhou 2015), the nLSU dataset with *Stereum hirsutum* (Willd.) Pers. and *Bondarzewia montana* (Quél.) Singer as the outgroup (Wagner and Fischer 2002) was used to confirm the generic position of the newly sequenced specimens. The ITS dataset was used to further clarify the interspecific relationships of *Fulvifomes* with *Phellinus laevigatus* (P. Karst.) Bourdot & Galzin, and *P. populicola* Niemelä as the outgroup (Wagner and Fischer 2002).

Sequences were aligned with BioEdit (Hall 1999) and ClustalX (Thompson et al. 1997). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps were manually adjusted to optimize the alignment. Sequence alignment was deposited at TreeBase (submission ID 19989; www.treebase.org). Phy-

Table 1. Information for the newly sequences used in this study.

Species	Location	Sample no.	GenBank accessions	
			ITS	nLSU
Fulvifomes centroamericanus	Costa Rica	JV1408/4	–	KX960768
F. centroamericanus	**Guatemala**	**JV0611/III**	**KX960763**	**KX960764**
F. centroamericanus	Guatemala	JV0611/8P	KX960757	–
F. grenadensis	USA	JV1212/2J	KX960756	–
F. grenadensis	Costa Rica	1607/66	KX960758	–
F. krugiodendri	**USA**	**JV0904/1**	**KX960762**	**KX960765**
F. krugiodendri	USA	JV0312/24.10J	KX960760	KX960766
F. krugiodendri	USA	JV1008/21	KX960761	KX960767
Inonotus porrectus	Costa Rica	1412/6J	KX960759	–

Type species are shown in bold.

logenetic analysis was carried out according to previous studies (Zhou 2015). Maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) were employed to perform phylogenetic analysis of the two aligned datasets. The three phylogenetic analysis algorithms generated nearly congruent topologies for each dataset, and, thus, only the topology from the MP analysis is presented along with statistical values from the ML, MP and BI algorithms (simultaneous BS not less than 50 % and BPP not less than 0.8) at the nodes.

Results

The nLSU-based phylogeny (Fig. 1) showed that the newly sequenced specimens fell into the strongly supported *Fulvifomes* clade which, besides species of *Fulvifomes*, also included *Inonotus luteoumbrinus* (Romell) Ryvarden and *I. porrectus* Murrill. Our studied samples, JV1408/4, JV0611/III, and JV0611/8P (as *Fulvifomes centroamericanus*) formed a strongly supported lineage; and JV0904/1, JV0312/24.10-J and JV1008/21 (*Fulvifomes krugiodendri*) formed another supported lineage.

In the phylogeny inferred from the ITS sequences (Fig. 2), four newly sequenced specimens (JV0904/1, JV0312/24.10-J, JV1008/21 as *Fulvifomes krugiodendri* and JV0611/III as *Fulvifomes centroamericanus*) clustered together with all sampled species of *Fulvifomes* and *Inonotus porrectus* in the *Fulvifomes* clade. The two new species formed lineages that had full statistical supports and were separated from other sampled species.

Taxonomy

***Fulvifomes centroamericanus* Y.C. Dai, X.H. Ji & Vlasák, sp. nov.**
MycoBank MB 818638
Figs 3, 4

Holotype. GUATEMALA. Tikal, 4 Nov 2006, leg. J. Kout, on living angiosperm tree, JV0611/III (Holotype in JV, isotype in BJFC).

Etymology. *Centroamericanus* (Lat.): referring to the distribution of species.

Description. Basidiocarps perennial, sessile, broadly attached, solitary, without odor or taste, woody hard, light in weight when dry. Pilei dimidiate, applanate, projecting up to 15 cm, 20 cm wide and 8 cm thick at the base. Pileal surface dark grey, crusted, uncracked; margin cinnamon-buff, obtuse. Pore surface pale yellow, shining; sterile margin distinct, yellowish brown, up to 3 mm wide; pores circular, 8–10 per mm; dissepiments thick, entire. Context yellowish brown, woody hard, up to 5 cm thick. Tubes yellowish brown, woody hard, up to 3 cm thick, tube layers distinctly stratified with intermittent context layers, individual tube layer up to 2 mm thick.

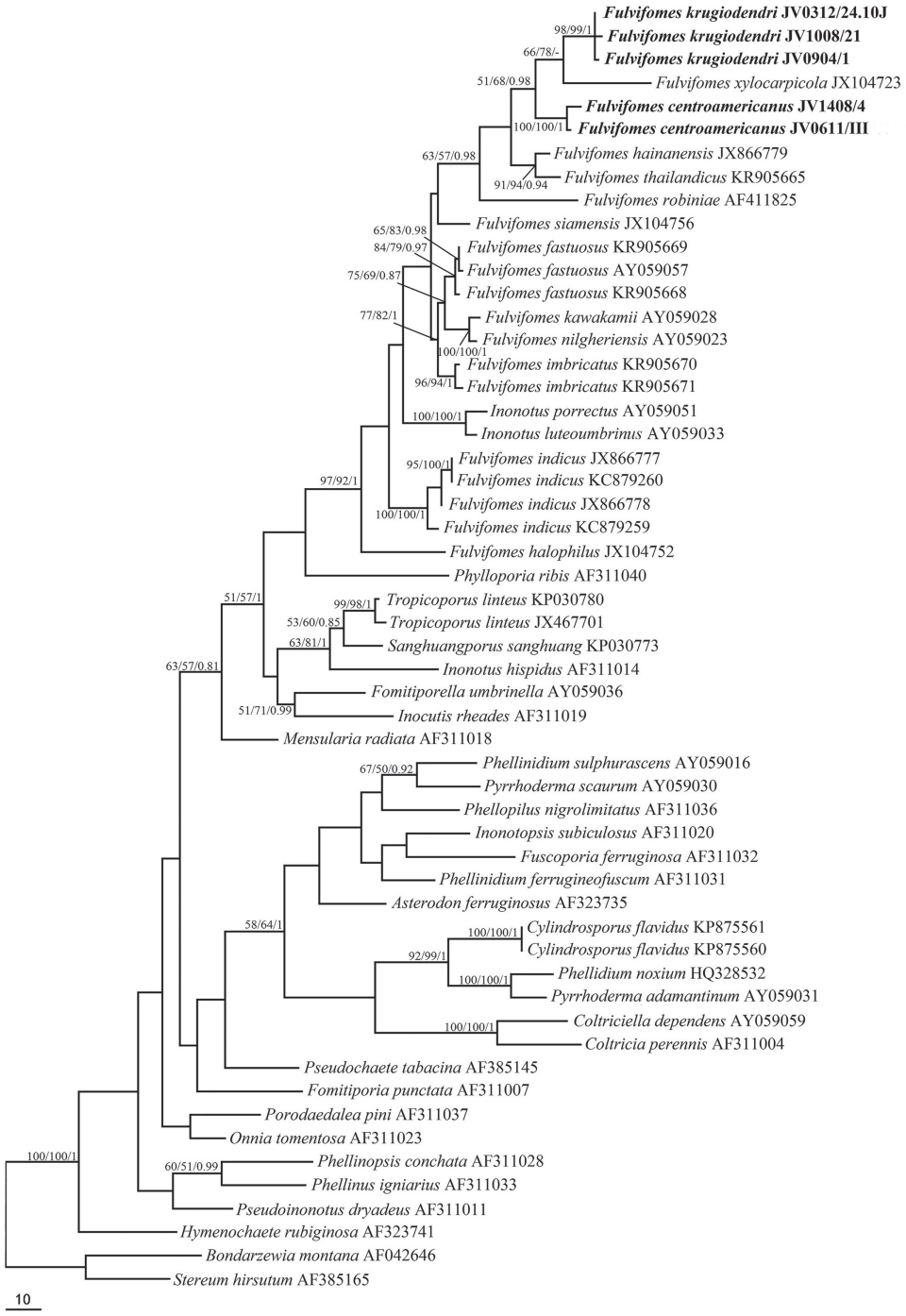

Figure 1. Phylogeny of *Fulvifomes* inferred from nLSU dataset. Topology is from MP tree and statistical values (MP/ML/BI) are indicated for each node that simultaneously received BS from ML and MP not below 50%, and BPP from BI not below 0.8.

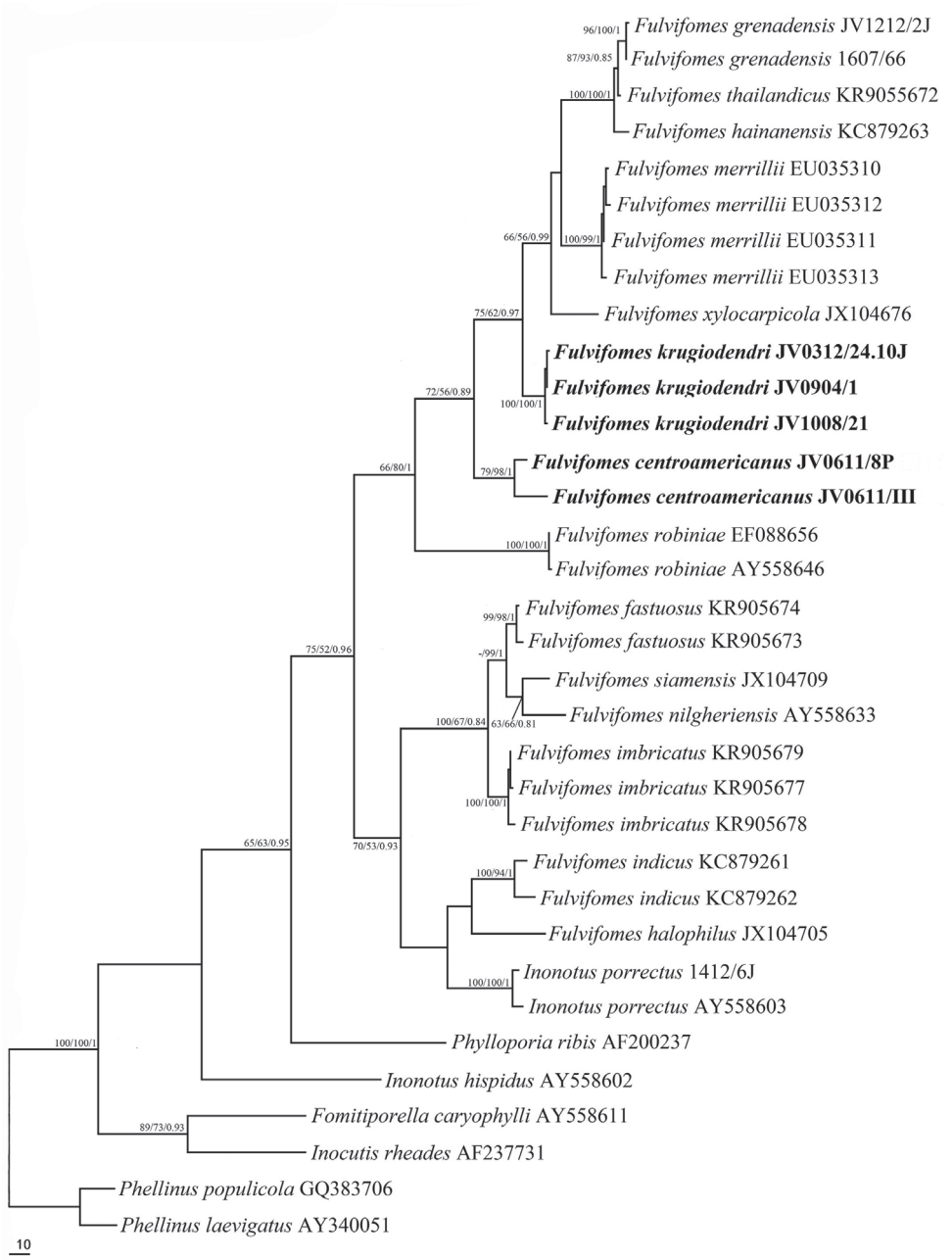

Figure 2. Phylogeny of *Fulvifomes* inferred from ITS dataset. Topology is from MP tree and statistical values (ML/MP/BI) are indicated for each node that simultaneously received BS from ML and MP not below 50%, and BPP from BI not below 0.8.

Figure 3. A basidiocarp of *Fulvifomes centroamericanus*. Scales bar: 10 mm.

Hyphal structure. Hyphal system dimitic; generative hyphae simple septate; skeletal hyphae dominant; tissue darkening but otherwise unchanged in KOH.

Context. Generative hyphae yellowish, slightly thick-walled, unbranched, frequently simple septate, 2–3.5 µm in diam; skeletal hyphae dominant, pale yellow to brown, thick-walled with a wide lumen, unbranched, aseptate, regularly arranged, 3–5 µm in diam.

Tubes. Generative hyphae hyaline to pale yellow, thin- to slightly thick-walled, rarely branched, frequently simple septate, 1.5–3 mm in diam; skeletal hyphae dominant, pale yellow, thick-walled with a wide to narrow lumen, rarely branched, aseptate, interwoven, 2–3 mm in diam. Setae absent; cystidia and cystidioles absent; hymenium collapsed, basidia and basidioles not obserbed.

Spores. Basidiospores subglobose, yellowish brown, thick-walled, smooth, usually collapsed when mature, IKI–, CB–, (3.8–)3.9–4.5(–4.6) × (3.5–)3.7–4.2 µm, L = 4.11 µm, W = 3.92 µm, Q = 1.04–1.05 (n = 60/2).

Additional specimen examined (paratypes). COSTA RICA. Las Pailas Ranger Station, Rincon de la Vieja, July 2016, on living angiosperm tree, JV1607/90 (JV); 1 Aug 2014, on living angiosperm tree, JV1408/4 (JV, BJFC). GUATEMALA. Uaxactún, 2 Nov 2006, leg. J. Kout, on living angiosperm tree, JV0611/8P (JV).

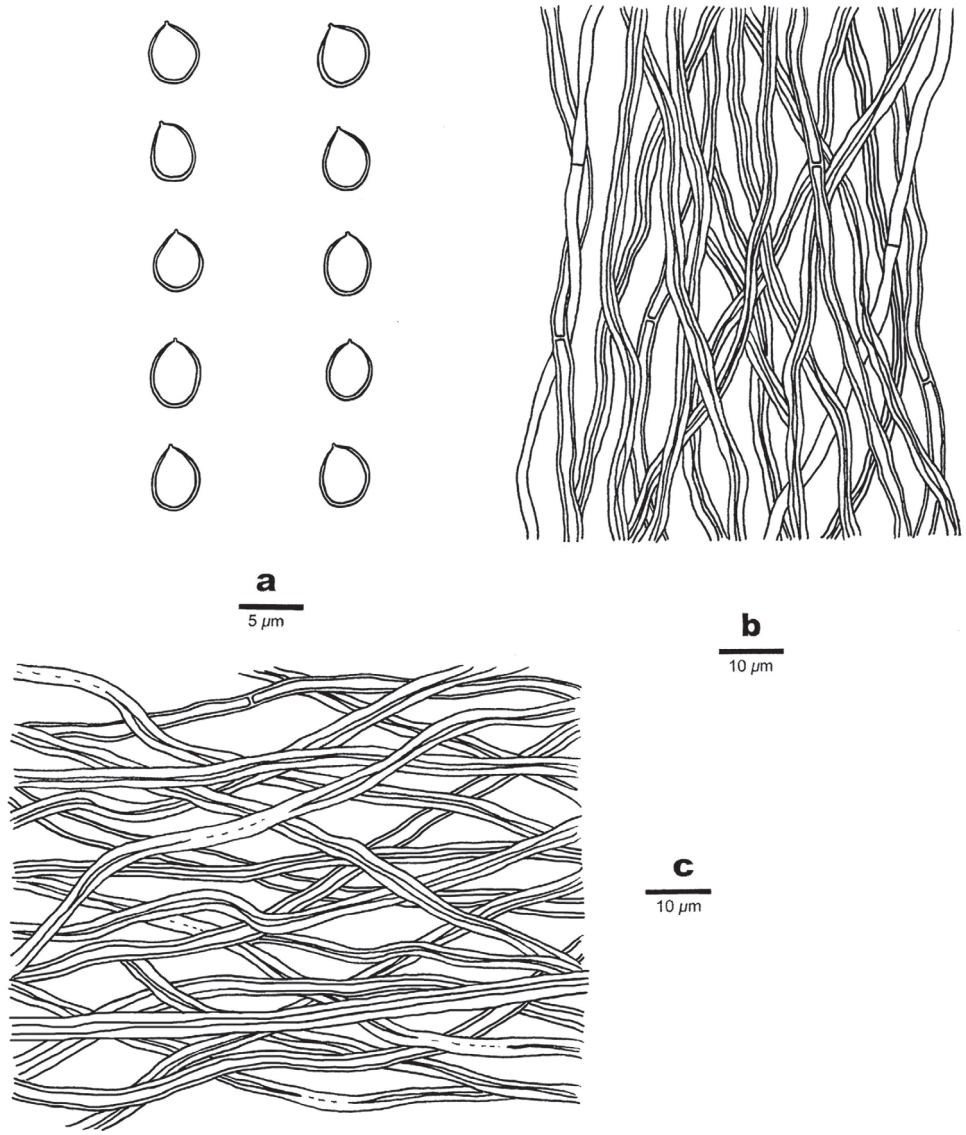

Figure 4. Microscopic structures of *Fulvifomes centroamericanus*. **a** Basidiospores **b** Hyphae from trama **c** Hyphae from context.

***Fulvifomes krugiodendri* Y.C. Dai, X.H. Ji & Vlasák, sp. nov.**
MycoBank MB 818639
Figs 5, 6

Holotype. USA. Florida: Miami, Matheson Hammock, 19 Apr 2009, on living tree of *Krugiodendron ferreum*, *JV0904/1* (Holotype in PRM, isotype in JV and BJFC).

Figure 5. A basidiocarp of *Fulvifomes krugiodendri*. Scales bar: 10 mm.

Etymology. *Krugiodendri* (Lat.): referring to the host tree genus *Krugiodendron*.

Description. Basidiocarps perennial, sessile, solitary, without odor or taste, woody hard, light in weight when dry, projecting up to 10 cm, 15 cm wide and 6 cm thick at center. Pileal surface dark grey, crusted, concentrically sulcate with narrow zones, cracked; margin cinnamon-buff, obtuse. Pore surface grayish brown, shining; sterile margin distinct, yellowish brown, up to 3 mm wide; pores circular, 7–9 per mm; dissepiments thick, entire. Context dark brown, woody hard, up to 5 mm thick. Tubes yellowish brown, woody hard, up to 5.5 cm thick, tube layers distinctly stratified with intermittent context layers, individual tube layer up to 3 mm thick.

Hyphal structure. Hyphal system dimitic; generative hyphae simple septate; skeletal hyphae dominant; tissue darkening but otherwise unchanged in KOH.

Context. Generative hyphae pale yellowish, slightly thick-walled, rarely branched, occasionally simple septate, 2–3 µm in diam; skeletal hyphae dominant, yellow to brown, thick-walled with a wide to narrow lumen, unbranched, aseptate, interwoven, 3–4 µm in diam.

Tubes. Generative hyphae hyaline to pale yellow, thin- to slightly thick-walled, rarely branched, frequently simple septate, 1.5–3 µm in diam; skeletal hyphae dominant, pale yellow, thick-walled with a wide to narrow lumen, unbranched, aseptate, loosely interwoven, 2–3 µm in diam. Setae absent; cystidia and cystidioles absent; hymenium collapsed, basidia and basidioles not oberved.

Spores. Basidiospores subglobose, yellowish brown, thick-walled, smooth, some collapsed when mature, IKI–, CB–, (4.0–)4.3–5.0(–5.1) × (3.7–)4.0–4.5(–4.8) µm, L = 4.60 µm, W = 4.21 µm, Q = 1.08–1.09 (n = 60/2).

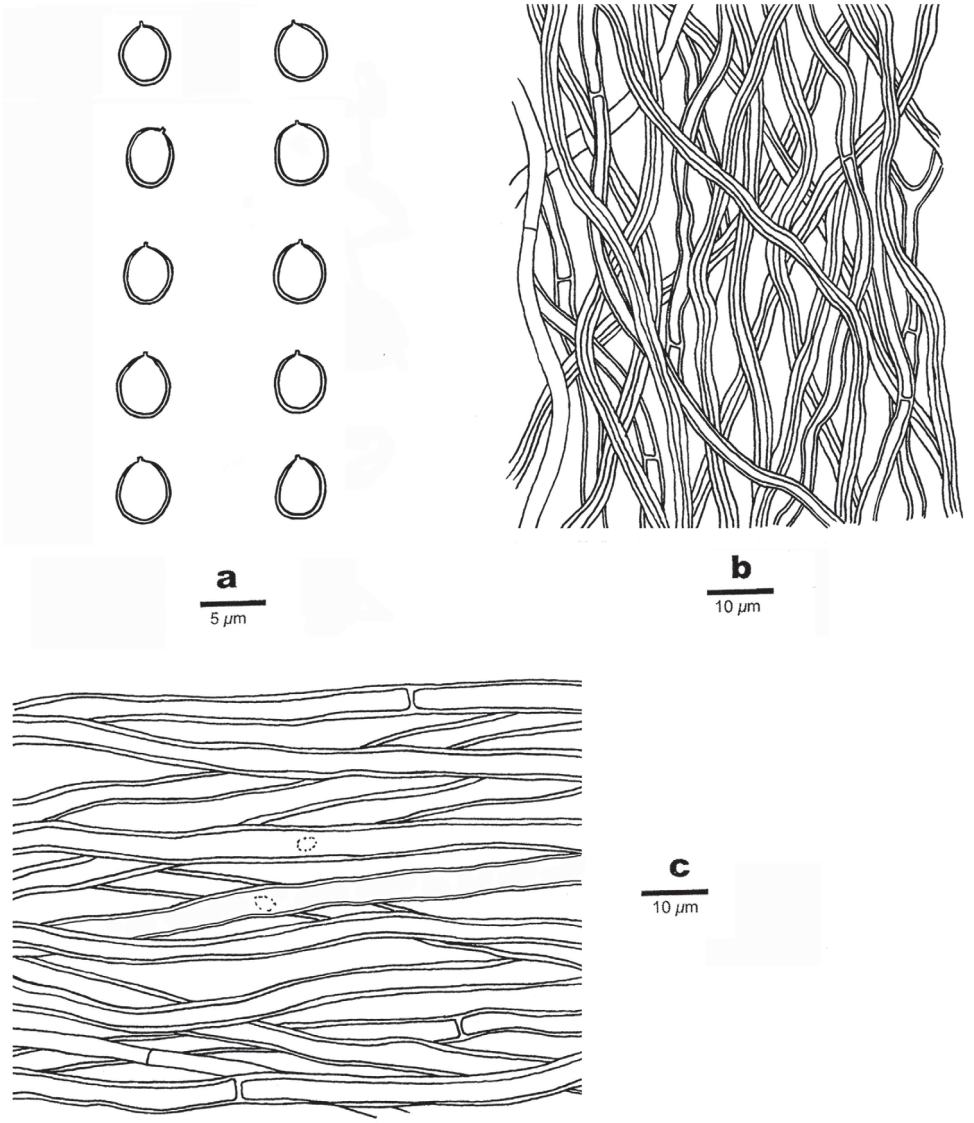

Figure 6. Microscopic structures of *Fulvifomes krugiodendri*. **a**: Basidiospores **b** Hyphae from trama **c** Hyphae from context.

Additional specimens examined (paratypes). USA. Florida: Miami, Matheson Hammock, 24 Dec 2003, on living tree of *Krugiodendron ferreum*, JV0312/24.10-J (JV, BJFC), August 2010, JV1008/21 (JV, BJFC).

Discussion

Fulvifomes krugiodendri and *F. centroamericanus* fit well in *Fulvifomes* (emended by Zhou 2014) with perennial and pileate basidiocarps with a homogeneous, dimitic hyphal system, lack of hyphoid and hymenial setae, and subglobose, yellowish to brown and thick-walled basidiospores. Besides, they formed distinct lineages within the *Fulvifomes* clade in the phylogenies inferred from nLSU and ITS datasets (Figs 1 and 2).

Fulvifomes centroamericanus is mostly similar to *F. robiniae* by sharing applanate basidiocarps and subglobose basidiospores, which are sometimes collapsed on one side (Gilbertson and Ryvarden 1987), and phylogenetically both species are closely related. However, *F. centroamericanus* is distinct by smaller basidiospores (3.9–4.5 × 3.7–4.2 μm), whereas *F. robiniae* has larger basidiospores (5–6 × 4.5–5 μm; Gilbertson and Ryvarden 1987). Moreover, according to Zhou (2015), *F. robiniae* has abundant branched skeletal hyphae in trama and a monomitic hyphal system in the context, which differ from *F. centroamericanus* having unbranched skeletal hyphae in trama and a strictly dimitic system.

Fulvifomes krugiodendri resembles *F. merrillii* (Murrill) Baltazar & Gibertoni by producing perennial and ungulate basidiocarps, concentrically sulcate pileal surface when mature, and an obtuse margin (Ryvarden 2004). However, *F. merrillii* microscopically produces bigger basidiospores (5–6 × 4–5 μm, Ryvarden 2004), while they are 4.3–5.0 × 4.0–4.5 μm in *F. krugiodendri*. Moreover, *F. merrillii* has reddish brown to dull brown and matted to rugose upper surface (Ryvarden 2004) and *F. krugiodendri* has black and crusted upper surface.

Phylogenetically, *Fulvifomes krugiodendri* is closely related to *F. xylocarpicola, F. thailandicus* and *F. hainanensis* (Figs 1 and 2), the latter three species were recently described from Thailand and China (Hattori et al. 2014, Zhou 2015). *F. krugiodendri* and *F. xylocarpicola* resemble each other by similar hyphal structures and basidiospores (4–5.5 × 3.5–4.5 μm in *F. xylocarpicola*; Hattori et al. 2014), but *F. xylocarpicola* differs from *F. krugiodendri* in its uncrust basidiocarps and the bigger pores (4–6 per mm). The morphological characters of *F. krugiodendri* and *F. thailandicus* are different. *F. thailandicus* has broadly attached, dimidiate, applanate basidiocarps, bigger pores (6–7 per mm) and larger basidiospores (4–5.8 × 4.1–4.8 μm, Zhou 2015). *F. krugiodendri* resembles *F. hainanensis* by producing perennial and ungulate basidiocarps, but the latter has duplex context and uncracked pileal surface.

Inonotus porrectus Murrill and *I. luteoumbrinus* (Romell) Ryvarden are nested within the *Fulvifomes* clade (Figs 1 and 2). Data from Sakayaroj et al. (2012) indicated that *I. luteoumbrinus* and *I. porrectus* fell into the *Fulvifomes* clade. However, both species have annual and soft fruiting bodies, a monomitic hyphal structure, and dark brown to black basidiospores, these characters do not correspond to *Fulvifomes,* and for the time being we still keep them in *Inonotus* P. Karst.

Lack of setae and relatively uniform basidiospores (shape, color and size) in most *Fulvifomes* species reduce the number of scorable traits substantially so that the mor-

phological determination is problematic. *Fulvifomes fastuosus* (type from Singapore) and *F. merrillii* (type from Philippines) were described from tropical Asia and their similar kins from Central and South America were simply classified under these old names because of lack of discriminating characters. Nevertheless, our broadly based sequencing of about 50 recent collections from Central America (not shown in the Figs 1 and 2) revealed *F. fastuosus* or *F. merrillii* not existed in Central America but several more or less distinct clades of related but different species which will be published in the coming papers.

Acknowledgments

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Kalbionora palaeotropica, a new genus and species from coastal forests in Southeast Asia and Australia (Malmideaceae, Ascomycota)

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Abstract

A new species and genus, *Kalbionora palaeotropica*, is described for a crustose lichen occurring in coastal forests in Thailand, Vietnam, and northeastern Australia. It is morphologically similar to *Malmidea* and *Eugeniella*, but differing in morphological and chemical characters. The single known species in the new genus contains atranorin, zeorin, the stictic acid chemosyndrome and chlorinated xanthones. Morphologically it is characterized by having asci of the *Catillaria*-type, a yellowish brown colour, a granulose epihymenium, dark brown hypothecium, hyaline, 1–3 transversely septate ascospores. Molecular data strongly support a phylogenetic position in Malmideaceae, sister to a clade including *Malmidea*, *Savoronala* and two species currently placed in *Lecidea* s. lat. (including *L. cyrtidia* and *L. plebeja*).

Key words

Lecanorales, lichens, mangroves, taxonomy, tropical diversity

Introduction

Coastal forests in the tropics, especially mangroves, are species-rich habitats and constitute an important part of tropical biodiversity (Donato et al. 2011; Friess 2016). These forests are comprised of unique plant, fungal, and animal species in the interface between marine, estuarine, and terrestrial ecosystems of the tropical and subtropical regions (Hyde et al. 1998; Rangsiruji et al. 2016; Sethy et al. 2012; Stevens 1979). Despite their importance for tropical biodiversity, mangroves are at great risk, with alarming rates of deforestation, especially in Southeast Asia (Friess et al. 2016; Polidoro et al. 2010; Richards and Friess 2016).

Recent studies on the diversity of lichen-forming fungi in Thailand have dramatically increased our knowledge of these organisms in Southeast Asia, with numerous new records and new species discovered in a number of different habitats, including coastal forests (Aptroot et al. 2007; Kalb et al. 2012, 2016a, 2016b; Kantvilas et al. 2010; Luangsuphabool et al. 2016a, 2016b; Naksuwankul et al. 2016; Neuwirth et al. 2014, 2016; Papong and Lumbsch 2011; Papong et al. 2014; Pitakpong et al. 2015; Rangsiruji et al. 2016; Sutjaritturakan and Kalb 2015; Buaruang et al. 2017).

During a recent survey of crustose lichens in mangrove habitats of eastern Thailand, the first author collected a species that appeared undescribed and while superficially resembling the common, pantropical *Lecanora caesiorubella*, showed similarities to the genera *Eugeniella* and *Malmidea*, currently placed in Malmideaceae and Pilocarpaceae, respectively (Jaklitsch et al. 2016; Lücking et al. 2016). This species was also collected by Klaus Kalb in Northeastern Australia, who kindly sent us the material. In addition, revision of material of a record of *Dirina paradoxa* from Vietnam (Joshi et al. 2014) turned out to represent this species as well. A new species and genus is described below based on molecular and phenotypical data.

Material and methods

Morphological and chemical analysis

Specimens were studied from the herbaria F, KoLRI, RAMK, and the private herbarium of Klaus Kalb (Neumarkt). Morphological characters were studied using a Leica Wild M 8 dissecting microscope. Observations and measurements of ascospores were made in water at 630× magnification with a Zeiss Axioscope microscope.

Chemical constituents were identified using high-performance thin layer chromatography (HPTLC), implementing standard methods (Arup et al. 1993; Lumbsch 2002).

Molecular methods

Total genomic DNA was extracted from thallus fragments following the manufacturers' instructions using the ZR Fungal/Bacterial DNA Miniprep Kit (Zymo Research

Corp., Irvine, CA). PCR reactions were performed and primers were used as described previously (James et al. 2006; Schmitt et al. 2010). PCR products were sequenced using an ABI PRISM™ 3730 DNA Analyzer (Applied Biosystems). New sequences were assembled and edited using Geneious v8.1.7 (http://www.geneious.com).

RPB2 and nuLSU sequences were aligned to each locus independently in the Miadlikowska et al. (2014) alignment (TreeBase no. 156552) using the '--add' option in the program MAFFT v7 (Katoh and Standley 2013). For the analysis focusing on Malmideaceae, nuLSU and mtSSU sequences were aligned using the 'E-INS-I' alignment algorithm in Mafft v7, with the remaining parameters set to default values. A group I intron in the nuLSU and present in a limited number of nuLSU sequences was not alignable and removed from the data matrix. Ambiguous positions of the mtSSU alignment were removed using Gblocks 0.91b (Castresana 2000). Phylogenetic analyses were performed using RAxML-HPC BlackBox 8.2.6 (Stamatakis 2006) and MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) on the Cipres Science Gateway (http://www.phylo.org; Miller et al. 2010). The model for each locus used in the phylogenetic analysis was estimated using jModelTest v2.1.9 (Darriba et al. 2012; Guindon and Gascuel 2003). In the ML analysis, the GTR+G+I model was used as the substitution model with 1000 pseudoreplicates. The data was partitioned according to the different genes. Two parallel Markov chain Monte Carlo (MCMC) runs were performed each using 8,000,000 generations and sampling every 1,000 steps. A 50% majority rule consensus tree was generated from the combined sampled trees of both runs after discarding the first 25% as burn-in. The tree files were visualized with FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Results and discussion

Taxonomy

***Kalbionora palaeotropica* Sodamuk, Leavitt & Lumbsch, gen. et sp. nov.**
Mycobank # MB820208, MB820209
Figure 1

Type. THAILAND, Trat Province: Muang District, Nhong Sa Nho Subdistrict, the route to Nature Education Center Ban Pak Khlong Nam Chiew, on the bark of *Ceriops tagal* (Perr.) C.B.Rob., 2014, *M. Sodamuk* RAMK-24530 (holotype: RAMK; isotypes: F, S).

Diagnosis. Characterized by having asci of the *Catillaria*-type, yellowish brown, granulose epihymenium, exciple consisting of prosoplectenchymatous cells, dark brown hypothecium, hyaline, 1-3 transversely septate ascospores, and the presence of atranorin, zeorin, and the stictic and arthothelin chemosyndromes.

Etymology. The specific epithet refers to the occurrence of the species in the Paleotropics, whereas the genus is named after our colleague Klaus Kalb who has made

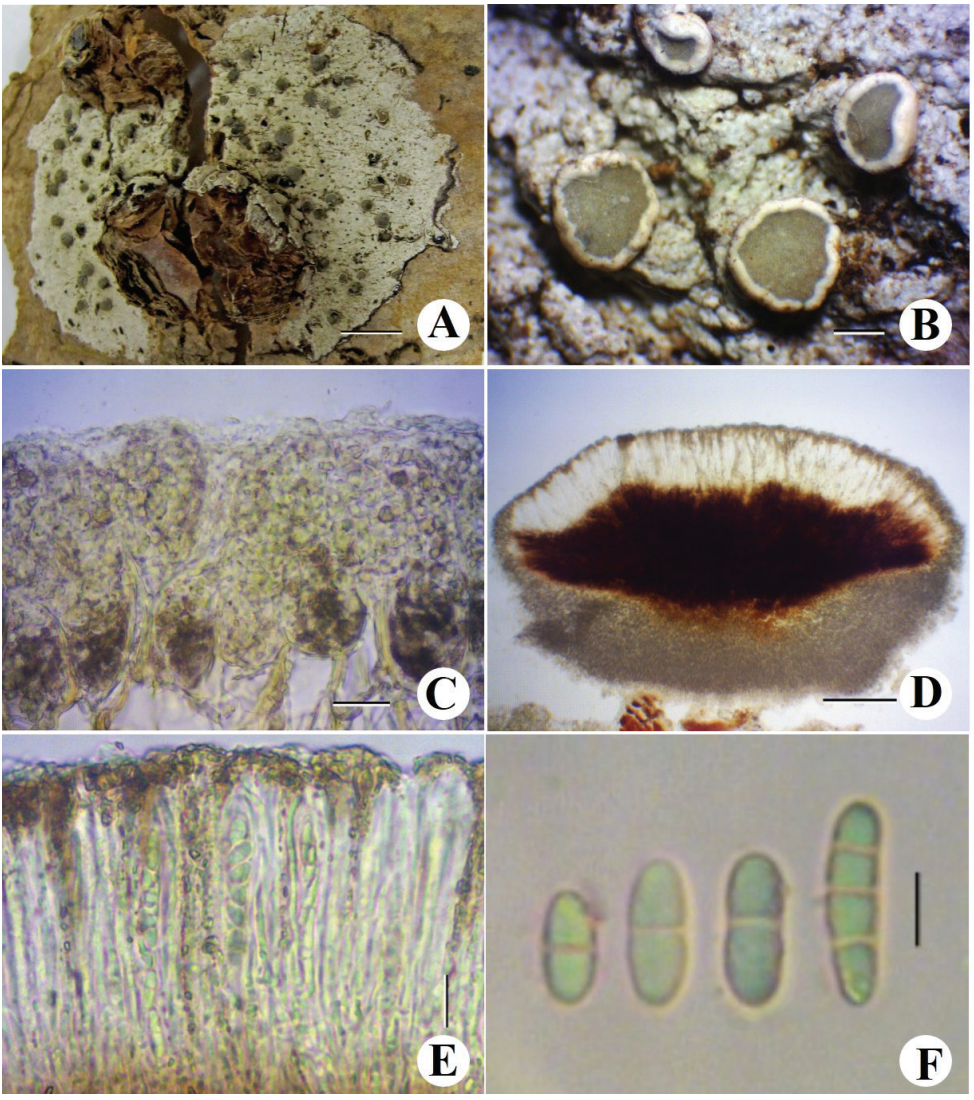

Figure 1. Morphology and anatomy of *Kalbionora palaeotropica*, **A–B** habit **C** cross-section through thallus showing cortex and algal layer **D** cross-section through apothecium showing dark brown hypothecium **E** hymenium, and **F** transversely septate ascospores (holotype). Scale bars: 0.5 cm (**A, B**), 20 μm (**C**), 0.1 mm (**D**), 10 μm (**E**), 5 μm (**F**).

tremendous contributions to our knowledge of tropical lichens and who has been enormously helpful to colleagues in Thailand.

Description. Thallus crustose, corticolous, greenish grey to whitish grey (green fading in herbarium); surface continuous, verruculose, somewhat glossy, prothallus not visible; isidia and soredia absent; corticate, cortex 25–40 μm thick, covered by a

thin, epinecral layer; photobiont chlorococcoid; medulla indistinct, penetrating into the periderm. Ascomata apothecia, simple, dispersed to crowded, disc plane to convex, grayish green to gray, 0.6–1.6 mm diam.; margin white to whitish grey, thick, entire to flexuous; exciple biatorine, prosoplectenchymatous, incrusted with numerous crystals; hymenium clear, amyloid; paraphyses simple to slightly branched, apically not or slightly thickened; epihymenium distinct, yellowish brown, granulose with numerous small brown crystals, rapidly dissolving in KOH, 3–4 µm thick; hypothecium brown to dark brown, 100–140 µm thick; asci cylindrical, tholus uniformly amyloid, corresponding to the *Catillaria*-type of Hafellner (1984); ascospores 8 per ascus, uniseriate, hyaline, thin-walled, non-halonate, ellipsoid, 1–3 transversely septate, non-amyloid; (8.0)8.9–10.4–11.8(16.0) × (2.5)3.2–3.8–4.4(5.5) µm. Pycnidia not found.

Secondary chemistry. Thallus K+ yellowish, C–, P+ yellow; containing atranorin, stictic acid and zeorin as major constituents, and cryptostictic acid, norstictic acid, peristictic acid, and the chlorinated xanthones arthothelin and 6-*O*-methylarthothelin as minor compounds (Australian sample analyzed by J.A. Elix).

Distribution and ecology. The new species was found in coastal forests in eastern Thailand, Vietnam, and northeastern Australia (Queensland), growing on bark. It is known only from a few localities but is expected to be more common and potentially overlooked in mangrove forests of Southeast Asia and Australia.

Notes. Morphologically similar is the genus *Malmidea* – some species have similar ascoma morphology and the ascus in this genus also lacks amyloid structures in the thallus. However, this genus can be easily separated by having non-septate, halonate, thick-walled ascospores, and lacking depsidones. Further, molecular evidence suggests that the genera are only distantly related. Another morphologically similar genus is *Eugeniella* and both *Eugeniella* and the new genus also share similar ascospore septation. However, these taxa readily distinguished by the ascus-type (*Byssoloma*-type in *Eugeniella*), the exciple (composed of moniliform hyphae in *Eugeniella*), and the epihymenium (usually indistinct in *Eugeniella*) (Breuss and Lücking 2015; Cáceres et al. 2013a). The new genus might be confused in the field with the superficially similar, common, pantropical *Lecanora caesiorubella* or has been confused with *Dirina paradoxa*, but is readily distinguished by numerous anatomical characters and a different chemistry.

Specimens examined. Australia, Queensland: Daintree National Park, Cape Tribulation, c. 63km N of Mossman, in a dense tropical, coastal rainforest, dominated by *Pandanus* sp., 2008, *K. Kalb 37355* (hb. Kalb). Thailand, Trat Province: Muang District, Nhong Sa Nho Subdistrict, the route to Nature Education Center Ban Pak Khlong Nam Chiew, on the bark of *Ceriops tagal* (Perr.) C.B.Rob., 2011, *M. Sodamuk*, RAMK—24241, 24242 & 25036 (RAMK); ibid., 2014, *M. Sodamuk*, RAMK—24531, 24532 & 24533 (RAMK); ibid., *Excoecaria agallocha* L., 2011, *M. Sodamuk*, RAMK—25035 (RAMK). Vietnam, Dak Lak Province: Buon Ma Thuot City, Museum, 19 Feb 2013, *Oh & Thanh*, VN130046 (KoLRI).

Phylogenetic analysis

Sequences of *RPB2* and nuLSU rDNA were generated (Genbank nos. KY926780-KY926790) from the type specimen of the new species and added to an alignment used by Miadlikowska et al. with over 1300 representatives in Lecanoromycetes (downloaded from https://treebase.org – study no. 156552; Miadlikowska et al. 2014). In a second analysis focusing on Malmideaceae, we aligned nuLSU and mtSSU sequences from three specimens of the new species with all Malmideaceae sequences used in Ertz et al. (2013). Based on the phylogenetic relationship of the new species to other taxa within Lecanoromycetes inferred in this study and published results from Ertz et al. (2013), we selected two species in the genus *Frutidella* and *Miriquidica garovaglii* as outgroups to assess relationships within Malmideaceae.

In our phylogenetic analysis assessing the relationship of *Kalbionora palaeotropica* within the Lecanoromycetes (Suppl. material 1), the type specimen did not cluster with Pilocarpaceae but in Malmideaceae as circumscribed by Ertz et al. (2013). Hence we performed a second analysis focusing on Malmideaceae. In the resulting tree (Fig. 2), the three specimens representing the new species clustered together in a strongly supported monophyletic group, supporting our re-identification of the Vietnamese material [recorded as *Dirina paradoxa* (Joshi et al. 2014)] as belonging to our new species. The new species, which is below described as *Kalbionora palaeotropica,* formed a strongly supported sister-group relationship to a clade including *Malmidea*, *Savoronala*, *Lecidea plebeja*, and *L. cyrtidia.*

In Malmideaceae, *Lecidea plebeja* and *L. cyrtidia* are temperate species occurring in North America and/or Europe and are poorly known. The morphology and distribution of the saxicolous *L. cyrtidia* has been discussed in the literature (Coppins and Muhr 1997; Hertel 1969), and it was suggested that it is closely related to the lignicolous *L. plebeja*, based on shared traits, such as an indistinct thallus, ascus-type, paraphyses with brown apical caps, ascospores of similar dimensions, and similar hypothecium and excipulum. Currently, these two species are poorly understood and additional sampling is necessary to evaluate the relationship of these two taxa. The genus *Savoronala* was recently described to accommodate a single species from coastal *Erica* heathland in Madagascar (Ertz et al. 2013), from which ascomata are unknown. This genus is morphologically characterized by having small, placodioid thalli, sporodochia at the apices of stipes, and brown conidia dispersed with an algal cell. It contains zeorin and usnic acid. The genus *Malmidea* was recently described (Kalb et al. 2011) to accommodate the bulk of corticolous and foliicolous, crustose tropical lichens previously included in the large, polyphyletic genus *Lecidea*, but differing in numerous characters, including the ascus-type (Hafellner 1984). Species in the genus were previously placed in the distantly related, now monotypic genus *Malcolmiella* and includes about 50 species with a thallus usually composed of goniocysts, usually paraplectenchymatous excipulum, prosoplectenchymatous hypothecium, and an ascus of the *Catillaria*-type, i.e. a tholus with no tubular structures to observe (Breuss and Lücking 2015; Cáceres et al. 2012, 2013b; Kalb et al. 2011, 2012). Species in

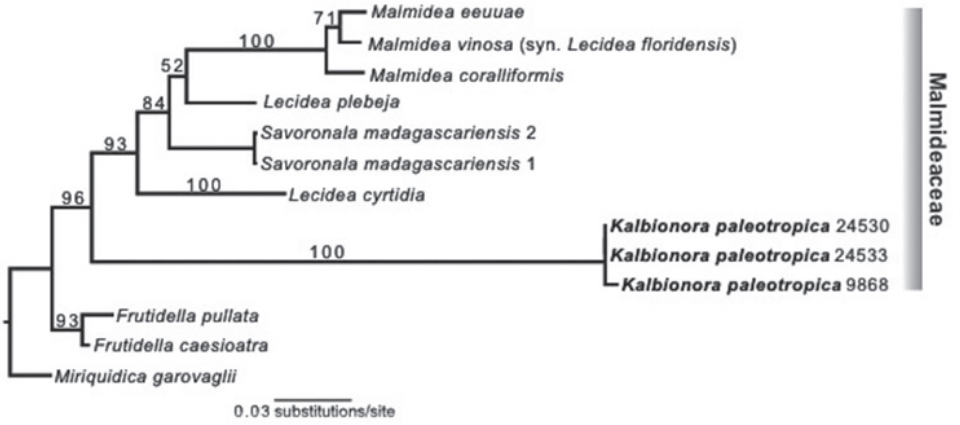

Figure 2. Phylogenetic tree depicting the relationship of *Kalbionora palaeotropica* in Malmideaceae based on mtSSU and nuLSU rDNA sequences. Bootstrap support values above 50% are displayed at nodes.

Malmidea often contain atranorin, sometimes in addition anthrachinones or biphenyls. *Kalbionora palaeotropica* differs morphologically by having a thallus not composed of goniocysts, transversely septate ascospores, and a different chemistry. Molecular data (Fig. 2) support that it is distinct from *Malmidea* and hence a new genus is described here to accommodate this new species.

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Supplementary material 1

Phylogenetic tree depicting phylogenetic relationships of Kalbionora palaeotropica based on RPB2 and nuLSU rDNA sequences

Authors: Mattika Sodamuk, Kansri Boonpragob, Pachara Mongkolsuk, Anders Tehler, Steven D. Leavitt, H. Thorsten Lumbsch

Data type: molecular data

Explanation note: Bootstrap support values above 50% are displayed at nodes.

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RESEARCH ARTICLE

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Ganoderma sichuanense (Ganodermataceae, Polyporales) new to Thailand

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Abstract

Ganoderma sichuanense (Ganodermataceae) is a medicinal mushroom originally described from China and previously confused with *G. lucidum*. It has been widely used as traditional medicine in Asia since it has potential nutritional and therapeutic values. We collected 8 specimens of *Ganoderma* species from Thailand and show that they represent the first record of *G. sichuanense* for Thailand. In this paper, we describe our specimens of *Ganoderma sichuanense* based on fresh basidiomes, and provide line drawings and photographs. The data from macro- and microscopic features are consistent with the characteristics of the species. Analysis of ITS sequence data indicates that the Thai collections cluster in same species clade as the epitype of *G. sichuanense*.

Key words

Ganoderma lingzhi, *Ganoderma lucidum*, Phylogeny, Taxonomy

Introduction

The genus *Ganoderma* was established by Karsten (1881) based on *Ganoderma lucidum* (Curtis) P. Karst. The genus *Ganoderma* includes the subgenera *Ganoderma* (which in turn includes sections *Ganoderma* and *Phaenema*), *Elfvingia*, and *Trachyderma* (Zhao and Zhang 2000). Many members of this genus are found in subtropical and tropical regions and appear to thrive in hot and humid conditions (Pilotti et al. 2004). *Ganoderma* species grow as facultative parasites of trees but can also live as saprobes on rotting stumps and roots (Turner 1981, Pilotti 2005). Basidiomes are commonly in the form of a bracket (Pilotti et al. 2004). Bioactive compounds from *Ganoderma* show a huge structural and chemical diversity (Deepalakshmi and Mirunalini 2011). These bioactive constituents are reported to be responsible for anti-cancer, anti-inflammatory, anti-tumor, anti-oxidant, immunomodulatory, immunodeficiency, anti-diabetic, anti-viral, anti-bacterial, anti-fungal, anti-hypertensive, anti-atherosclerotic, anti-aging, anti-androgenic, hepatoprotective, radical scavenging properties, neuroprotection, sleep promotion, cholesterol synthesis inhibition, preventing hypoglycemia, inhibition of lipid peroxidation/oxidative DNA damage, maintenance of gut health, prevention of obesity, and stimulation of probiotics (Paterson 2006, De Silva et al. 2012a, b, De Silva et al. 2013, Bishop et al. 2015, Hapuarachchi et al. 2016a, 2016b). The traditional taxonomy of *Ganoderma* is based on morphological traits, and the genus was divided into two distinct groups, the laccate (*G. lucidum* complex) and the non–laccate (*G. applanatum* complex) groups, which correspond to the subgenera *Ganoderma* and *Elfvingia*, respectively (Zheng et al. 2007). There are 437 epithets listed in Index Fungorum (2017) for *Ganoderma*, of which 414 are accepted by Species Fungorum in May, 2017) (http://www.speciesfungorum.org/Names/Names.asp).

"Lingzhi" is the Chinese name mainly referring to *G. lucidum* (Curtis) P. Karst, which has been widely used in China for medicinal purposes for over two millennia (Sliva 2006). However, this species was originally described from Europe (Ryvarden and Gilbertson 1993). Patouillard (1907) reported *G. lucidum* from China for the first time and Teng (1934) described collections of *G. lucidum* from different regions in China. Liu (1974) compiled a monograph of traditional Chinese medicinal fungi, and he reported *G. lucidum* in his book. Since then, *G. lucidum* was accepted as the scientific binomial of "Lingzhi" in many reports on Chinese edible and medicinal mushrooms (Ying et al. 1987, Mao 1998, Dai et al. 2009). Moncalvo et al. (1995) mentioned that *G. lucidum sensu stricto* was distributed in northern and southern Europe, and probably extended to China. However, their further studies confirmed that the species named *G. lucidum* from both Europe and mainland China was not conspecific based on analyses of ITS and 25S ribosomal DNA sequences. Later, other authors (Pegler and Yao 1996, Smith and Sivasithamparam 2000, Hong and Jung 2004) have confirmed the same idea.

Hawksworth (2005) suggested to conserve the name *G. lucidum* for an Asian type and introduce a new name for the European species. Later, it was found that *G. lucidum* from tropical Asia is not conspecific with *G. lucidum sensu stricto*, and not even

conspecific with the real "Lingzhi" distributed in East Asia, and was named *G. multipileum* Ding Hou, (Wang et al. 2009). Cao et al. (2012) named the medicinal species *G. lucidum* from China as *G. lingzhi*. Among the Chinese *Ganoderma* species, *G. flexipes* Pat, *G. multipileum* D. Hou, *G. sichuanense* J.D. Zhao and X.Q. Zhang, *G. tropicum* (Jungh.) Bres. and *G. tsugae* Murrill are the most similar species to *G. lingzhi*. However, the validity of the separation of *G. lingzhi* and *G. sichuanense* has been debated recently. Wang et al. (2012) proposed that '*G. lucidum*' for Chinese species is incorrect and this should be corrected to *Ganoderma sichuanense.* Furthermore, Cao et al. (2012) proposed the name *G. lingzhi* for "Lingzhi" species which has an eastern Asian distribution based on strong morphology and molecular evidence. Yao et al. (2013) proposed *G. lingzhi and G. sichuanense* as synonyms based on morphological data from an epitype of *G. sichuanense*. However, Zhou et al. (2015) again challenged this opinion, with *G. lingzhi* and *G. sichuanense* being an independent and taxonomically valid species by stressing that species types depends on their ecological environments. Richter et al. (2015) stated that the new taxon *G. lingzhi* is taxonomically superfluous because the rules of fungal nomenclature require that the oldest valid name of any given taxon should be given preference. In 2016, Mark Stadler annotated this record in Mycobank (http://www.mycobank.org/). Now *G. lingzhi* is regarded as a later synonym of *G. sichuanense* in Species Fungorum (http://www.indexfungorum.org/names/names.asp) and Mycobank. Despite all this taxonomic work, the Chinese "Lingzhi" has continuously been referred to as *G. lucidum* in monographs of Ganodermataceae in China (Hapuarachchi et al. 2015).

The aim of this study is to report and illustrate the new findings of this medicinal species in Thailand and further, to improve the understanding of species delimitation in the genus *Ganoderma*.

Materials and methods

Sample collection

Eight *Ganoderma* specimens growing up from soil were collected in a single site in Mae On District, Chiang Mai Province, northern Thailand (18°52.02'N, 99°18.18'E) during the rainy season between June 2015 and September 2015.

Macroscopic and microscopic characterization

Macro-morphological characters were described based on fresh material, and on the photographs provided here. Colour codes (e.g. 3A3) are from Kornerup and Wanscher (1978). Specimens were dried and placed separately in plastic bags. Material was deposited at Mae Fah Luang University herbarium (MFLU), Chiang Rai, Thailand. Living cultures were not obtained in this study. For micro-morphological examina-

tion, basidiomes were examined under a stereo dissecting microscope (Motic SMZ 168 series) and sections were cut with a razor blade, mounted in 5% KOH, and then observed, measured, and illustrated under a compound microscope (Nikon ECLIPSE 80i) equipped with a camera (Canon 600D). Measurements were made using Tarosoft (R) Image Frame Work v. 0.9.7. At least 20 basidiospores were measured from each mature specimen except for very scanty materials. The basidiospore size was measured both with and without the myxosporium based on those with collapsed apex, but only spore sizes with myxosporium were used for comparisons. The cuticle sections were taken from the mature pileus portion and mounted in Melzer's reagent for observations. In the description of the basidiospores: *n* indicates the number of spores which were measured; L_m is the mean spore length over a population of spores; W_m the mean spore width over a population of spores; Q the length/width ratio *(L/W)* of a spore in side view; and Q_m the average Q of all spores measured. The Facesoffungi number is provided as explained in Jayasiri et al. (2015).

DNA Extraction, PCR and sequencing

Dried samples of basidiome were used to extract genomic DNA. Genomic DNA was extracted using an EZgene Fungal gDNA Kit (Biomiga, CA, USA) according to the manufacturer instructions. DNA concentrations were estimated visually in agarose gel by comparing band intensity with a DNA ladder 1Kb (Invitrogen Biotech). The nuclear ribosomal internal transcribed spacer (ITS) was amplified using primers ITS5 and ITS4 (White et al. 1990). Reaction mixtures (20 µl) contained 1 µl template DNA (ca. 10 ng), 10 µl distilled water, and 1 µl (10 µM) of each primer (ITS5/ITS4) and 7 µl 2× BenchTop Taq Master Mix (Biomigas). Amplification conditions were 35 cycles of 95 °C for 30 s, 59 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. Amplified PCR products were verified by 1% agarose gel electrophoresis stained with ethidium bromide in 1x TBE. The PCR products were sequenced by Invitrogen Biotechnology (Beijing).

Sequence alignment and phylogenetic analysis

Other sequences used in the analyses (Table 1) were obtained from GenBank based on ITS BLAST searches in GenBank (Benson et al. 2017) and recently published data. Sequences that had possibly been contaminated by fungi or other unnamed species (such as those with aff. in the species name) were discarded, ambiguous regions were excluded and gaps were treated as missing data in the analysis (Nilsson et al. 2012). 110 strains representing 40 species of Ganodermataceae from Asia, America and Europe were retrieved and those retrieved sequences and the newly generated sequences were aligned with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html; Katoh and Standley 2013). The resulting alignment was improved manually when necessary

Table 1. Sequences used in the phylogenetic analysis.

Species	Voucher /strain	Origin	5.8 ITS	Reference
Ganoderma adspersum	ITA 39	Unknown	EF060011	GenBank
Ganoderma adspersum	PF263	Italy	JN176908	GenBank
Ganoderma applanatum	GA165	Unknown	DQ425009	GenBank
Ganoderma applanatum	K(M)120829	UK	AY884179	GenBank
Ganoderma annulare	KCTC 16803	Unknown	JQ520160	Park et al. 2012
Ganoderma atrum	7	Unknown	JQ886403	GenBank
Ganoderma australe	HMAS86595	England	AY884184	GenBank
Ganoderma australe	GDGM25831	China	JX195200	Genbank
Ganoderma boninense	WD2085 (FFPRI)	Japan	KJ143906	Zhou et al. 2015
Ganoderma boninense	WD2028 (FFPRI)	Japan	KJ143905	Zhou et al. 2015
Ganoderma carnosum	K(M) 109415	UK	AY884175	GenBank
Ganoderma cupreum	HMAS130804	Australia	JX840345	GenBank
Ganoderma curtisii	CBS 100131	NC, USA	JQ781848	Zhou et al. 2015
Ganoderma curtisii	CBS 100132	NC, USA	KJ143967	Zhou et al. 2015
Ganoderma destructans	CMW43672	South Africa	KR183858	Coetzee et al. 2015
Ganoderma destructans	CMW43671	South Africa	KR183857	Coetzee et al. 2015
Ganoderma flexipes	Wei5200 (IFP)		JN383978	Cao and Yuan 2013
Ganoderma flexipes	Wei5494 (IFP)	Hainan, China	JN383979	Cao and Yuan, 2013
Ganoderma fornicatum	TN23	India	FJ655476	GenBank
Ganoderma fornicatum	KR20	India	FJ655474	GenBank
Ganoderma fornicatum	BCRC35374	Taiwan	JX840349	Wang et al. 2014
Ganoderma fornicatum	TNM-F0010592	China	JX84034	Wang et al. 2014
Ganoderma fornicatum	TNM-F0009926	China	JX840348	Wang et al. 2014
Ganoderma fulvellum	xsd08051	Unknown	FJ478088	GenBank
Ganoderma gibbosum	XSD-34	Unknown	EU273513	GenBank
Ganoderma hoehnelianum	Dai 12096	China	KU219989	GenBank
Ganoderma hoehnelianum	Dai 11995	China	KU219988	GenBank
Ganoderma leucocontextum	GDGM44303	China	KJ027607	Li et al. 2014
Ganoderma leucocontextum	TL-2013	China	KF011548	Li et al. 2014
Ganoderma lipsiense	FIN 131R610	Unknown	EF060004	GenBank
Ganoderma lipsiense	NOR74/67/5	Unknown	EF060002	GenBank
Ganoderma lingzhi	HKAS76642 (Iso type)	Yunnan, China	KC222318	Yang and Feng 2013
Ganoderma lingzhi	Dai12574 (IFP)	Liaoning, China	KJ143908	Cao et al. 2012
Ganoderma lingzhi	HSD06B	Taihang mountains, China	KC511557	GenBank
Ganoderma lingzhi	Dai3583	China	JQ781868	Cao et al. 2012
Ganoderma lingzhi	Dai12374	China	JQ781867	Cao et al. 2012
Ganoderma lingzhi	Li245	China	JQ781863	Cao et al. 2012
Ganoderma lingzhi	Dai12426	China	JQ781870	Cao et al. 2012
Ganoderma lingzhi	Cui6982	China	JQ781862	Cao et al. 2012
Ganoderma lingzhi	Cui4018	China	JQ781856	Cao et al. 2012
Ganoderma lobatum	JV 0402/24	Unknown	KF605677	GenBank
Ganoderma lobatum	JV 1212/10J	Unknown	KF605676	GenBank
Ganoderma lucidum	Dai11593 (IFP)	Finland	JQ781852	Cao et al 2012
Ganoderma lucidum	K175217	UK	KJ143911	Zhou et al. 2015

Species	Voucher /strain	Origin	5.8 ITS	Reference
Ganoderma lucidum	MT2610 (BRNM)	Czech Republic	KJ143912	Zhou et al. 2015
Ganoderma lucidum	Dai2272 (IFP)	Sweden	JQ781851	Cao et al. 2012
Ganoderma lucidum	HKAS76455	Yunnan, China(Cultivated)	KC222320	Yang and Feng 2013
Ganoderma lucidum	HKAS76643	Yunnan, China	KC222323	Yang and Feng 2013
Ganoderma lucidum	HKAS71088	Yunnan, China	KC222321	Yang and Feng 2013
Ganoderma lucidum	OE–234	India	AY636059	GenBank
Ganoderma lucidum	GICN04	Italy	AM906058	Guglielmo et al. 2008
Ganoderma lucidum	XZ-G-B	Unknown	HQ235632	GenBank
Ganoderma lucidum	CSAAS0801	Unknown	FJ940919	GenBank
Ganoderma lucidum	XZ-G-A1	Unknown	HQ235630	GenBank
Ganoderma lucidum	CSAAS0801	Unknown	FJ940919	GenBank
Ganoderma lucidum	GIT 099	Italy	AM269773	GenBank
Ganoderma mastoporum	CMU-HM1	Thailand	JN643730	GenBank
Ganoderma mastoporum	TNM-F0018838	China	JX840350	Wang et al. 2012
Ganoderma mastoporum	Gma-1	Unknown	GU213486	GenBank
Ganoderma multipileum	HMAS242384	Sichuan Province, China	JF915409	Wang et al. 2012
Ganoderma multipileum	CWN04670	Taiwan, China	KJ143913	Wang et al. 2012
Ganoderma multipileum	Dai9447	Hainan, China	KJ143914	Wang et al. 2012
Ganoderma multipileum	DYU	Taiwan, China	KJ868083	GenBank
Ganoderma multiplicatum	URM83346	Brazil	JX310823	GenBank
Ganoderma neojaponicum	ASI 7032	Unknown	JQ520193	Park et al. 2012
Ganoderma orbiforme	BCC22324	Thailand	JX997990	Isaka et al. 2013
Ganoderma oregonense	CBS 265.88	OR, USA	JQ781875	Zhou et al. 2015
Ganoderma oregonense	CBS 266.88	OR, USA	JQ781876	Zhou et al. 2015
Ganoderma oerstidii	GO138	Argentina	DQ425011	GenBank
Ganoderma parvulum	URM83343	Brazil	JQ618246	GenBank
Ganoderma parvulum	URM80765	Brazil	JX310822	GenBank
Ganoderma pfeifferi	CBS 747.84	Netherlands	JQ520198	Park et al. 2012
Ganoderma pfeifferi	K(M)120818	UK	AY884185	GenBank
Ganoderma pfeifferi	874 (CAS–IM)	Czech Republic	AM906059	Guglielmo et al. 2008
Ganoderma philippi	E7098	Indonesia, Sumatra islands	AJ536662	GenBank
Ganoderma philippi	E7425	Malaysia, Selangor	AJ608713	GenBank
Ganoderma ramosissium	xsd08085	Unknown	FJ478127	GenBank
Ganoderma ramosissium	xsd08032	Unknown	EU918700	GenBank
Ganoderma resinaceum	BR 4150 (Rivoire)	France	KJ143915	Zhou et al. 2015
Ganoderma resinaceum	Gre4	Italy (Modena)	KJ509598	GenBank
Ganoderma resinaceum	CBS 194.76	Netherlands	KJ143916	Zhou et al. 2015
Ganoderma sichuanense	MFU 16-2667	Thailand	KY244061	This study
Ganoderma sichuanense	MFU 16-2668	Thailand	KY244062	This study
Ganoderma sichuanense	MFU 16-2669	Thailand	KY244063	This study
Ganoderma sichuanense	MFU 16-2709	Thailand	KY244068	This study
Ganoderma sichuanense	MFU 16-2670	Thailand	KY404119	This study
Ganoderma sichuanense	MFU 16-2671	Thailand	KY244064	This study
Ganoderma sichuanense	MFU 16-2672	Thailand	KY244065	This study

Species	Voucher /strain	Origin	5.8 ITS	Reference
Ganoderma sichuanense	MFU 16-2673	Thailand	KY244066	This study
Ganoderma sichuanense	CGMCC5.2175 (epitype)	Sichuan, China	KC662402	Yao et al. 2013
Ganoderma sichuanense	Cui 7691 (BJFC)	Guangdong, China	JQ781878	Zhou et al. 2015
Ganoderma sinense	GS175	Unknown	DQ425014	GenBank
Ganoderma sinense	GS92	Unknown	DQ424982	GenBank
Ganoderma subresinosum	T162	Unknown	KJ654376	GenBank
Ganoderma subresinosum	7-SU-3-C-70(M)-B	Indonesia	KJ654472	GenBank
Ganoderma subresinosum	3C-29	Indonesia	KJ654406	GenBank
Ganoderma subresinosum	5-D-3-D-26	Indonesia	KJ654467	GenBank
Ganoderma tornatum	NPG1	Malaysia	KJ767488	GenBank
Ganoderma tropicum	BCRC37122 (TNM)	Taiwan, China	EU021457	Wang et al. 2009
Ganoderma tropicum	He 1232	China	KF495000	GenBank
Ganoderma tropicum	Dai9724	China	JQ781879	Cao et al. 2012
Ganoderma tropicum	AP17	India	FJ491960	GenBank
Ganoderma tsugae	Dai3937 (IFP)	China	JQ781853	Cao et al. 2012
Ganoderma tsugae	12751b (BJFC)	USA(CT)	KJ143919	Zhou et al. 2015
Ganoderma tsugae	AFTOL-ID771	Unknown	DQ206985	Matheny et al. 2007
Ganoderma tsugae	Yuan5649	China	JQ781854	Cao et al. 2012
Ganoderma tsugae	Dai12760	USA	KJ143920	Zhou et al. 2015
Ganoderma valesiacum	CBS 428.84	USA	JQ520218	Park et al. 2012
Ganoderma zonatum	FL02 (TNM)	USA(FL)	KJ143921	Zhou et al. 2015
Ganoderma zonatum	FL03 (TNM)	USA(FL)	KJ143922	Zhou et al. 2015
Tomophagus colossus	TC-02 (TNM)	Vietnam	KJ143923	Zhou et al. 2015

using BioEdit v. 7.0.5.2 (Hall 1999). The Maximum Likelihood (ML) analyses were performed using RAxML-HPC2 (Stamatakis 2014) on the CIPRES Science Gateway V. 3.3 (Miller and Blair 2009), with default settings except that the number of bootstrap replicates was set to 1,000. A partitioned model analysis was performed with ITS1+ITS2 and 5.8S. For Bayesian analysis (BY), the GTR+I+G model of nucleotide evolution was selected with the help of MrModeltest 2.2 (Nylander 2004) as the best-fit model and posterior probabilities (PP) (Rannala and Yang 1996) were determined by Markov Chain Monte Carlo sampling (BMCMC) using MrBayes v3.1.2 (Ronquist et al. 2012). BY analyses were conducted with six simultaneous Markov chains and trees were summarized every 100th generation. The analyses were stopped after 5,000,000 generations when the average standard deviation of split frequencies was below 0.01. The convergence of the runs was checked using TRACER v1.6 (Rambaut et al. 2013). The first 25% of the resulting trees were discarded as burn-in, and PP were calculated from the remaining sampled trees. In both ML and BY analyses, *Tomophagus colossus* was selected as the outgroup. ML bootstrap values and BY posterior probabilities greater than or equal to 70% and 0.95, respectively, were considered as significant support. The phylogenetic tree was visualized with FigTree version 1.4.0 (Rambaut 2012) available at http://tree.bio.ed.ac.uk/software/figtree/.

Results

Phylogeny

The tree topologies obtained from ML and BY were identical. Therefore, only the ML tree is shown (Fig. 1). Six major clades were identified in *Ganoderma* (Fig. 1). Our eight collections of *Ganoderma sichuanenese* from Thailand clustered with all *G. sichuanenese* sequences, including the epitype, in a well-supported clade (BS=98%; BPP=1.0).

Taxonomy

***Ganoderma sichuanense* J.D. Zhao & X.Q. Zhang, Acta Mycologica Sinica 2:159. 1983.**
Facesoffungi number. FoF 02721

Ganoderma lucidum sensu S.C. Teng, Sinensia 5: 198. 1934. **Misapplied name.**
Ganoderma lingzhi Sheng H. Wu, Y. Cao & Y.C. Dai, in Cao, Wu & Dai, Fungal Diversity 56, 1: 54, 2012. **Synonymy.**

Description. *Basidiome* annual to perennial, with distinctly contracted base to stipitate, corky, becoming hard corky to woody hard when dry. *Pileus* 4.5–8 cm, up to 0.5 cm thick at the base, dimidiate, subreniform; upper surface when young pale yellow (3A3) to light orange (5A5), becoming brownish orange (7C8) when old, strongly laccate to partly laccate, distinctly concentrically sulcate, distinctly radially rugose. *Spore deposit* usually pale orange; margin abruptly paler, pale yellow, slightly lobate. *Context* duplex, not completely homogeneous in color, greyish orange (5B3) corky; generative hyphae (1.1–1.3 µm diam, colorless, thin-walled; binding hyphae (2.1–3.1) µm in diam., branched, with clamp-connections, skeleton hyphae (3.05–3.1) µm in diam. thick walled, sometimes branched, reddish brown in KOH, dextrinoid. *Pore surface* pale yellow when young, becoming brownish orange (6C4) when old; tubes up to 0.2 cm long in total, pale brown or smoky brown, without context layer between tube layers; pores sub circular. *Basidiospores* with a dark brown eusporium bearing thick echinulae, overlaid by a hyaline myxosporium, (8.2)8.3–9.8(10.2) × (5.6)5.7–6.8(7.3) µm (with myxosporium), (5.3)6.2–7.5(7.7) × (4.2)4.2–5.3(5.7) µm (without myxosporium), ellipsoid, Q_m = 1.37 (n = 20). L_m = 9.09 µm, W_m = 6.27µm, *Cutis* 4–12 mm thick, pale brown streaks the cutis, a closely-packed palisade, yellowish brown, clavate terminal elements, about 15–30 µm long. *Stipe* flattened or sub cylindrical to cylindrical, lateral to horizontally lateral or eccentric, (6–9) × (1.5 along stipe) cm, dark brown (8F5).

Material examined. THAILAND, Chiang Mai Province, Mae On District, (18°52.02'N, 99°18.18'E), eight specimens (MFU 16-2667, MFU 16-2668, MFU 16-2669, MFU 16-2670, MFU 16-2671, MFU 16-2672, MFU 16-2673, MFU 16-2709).

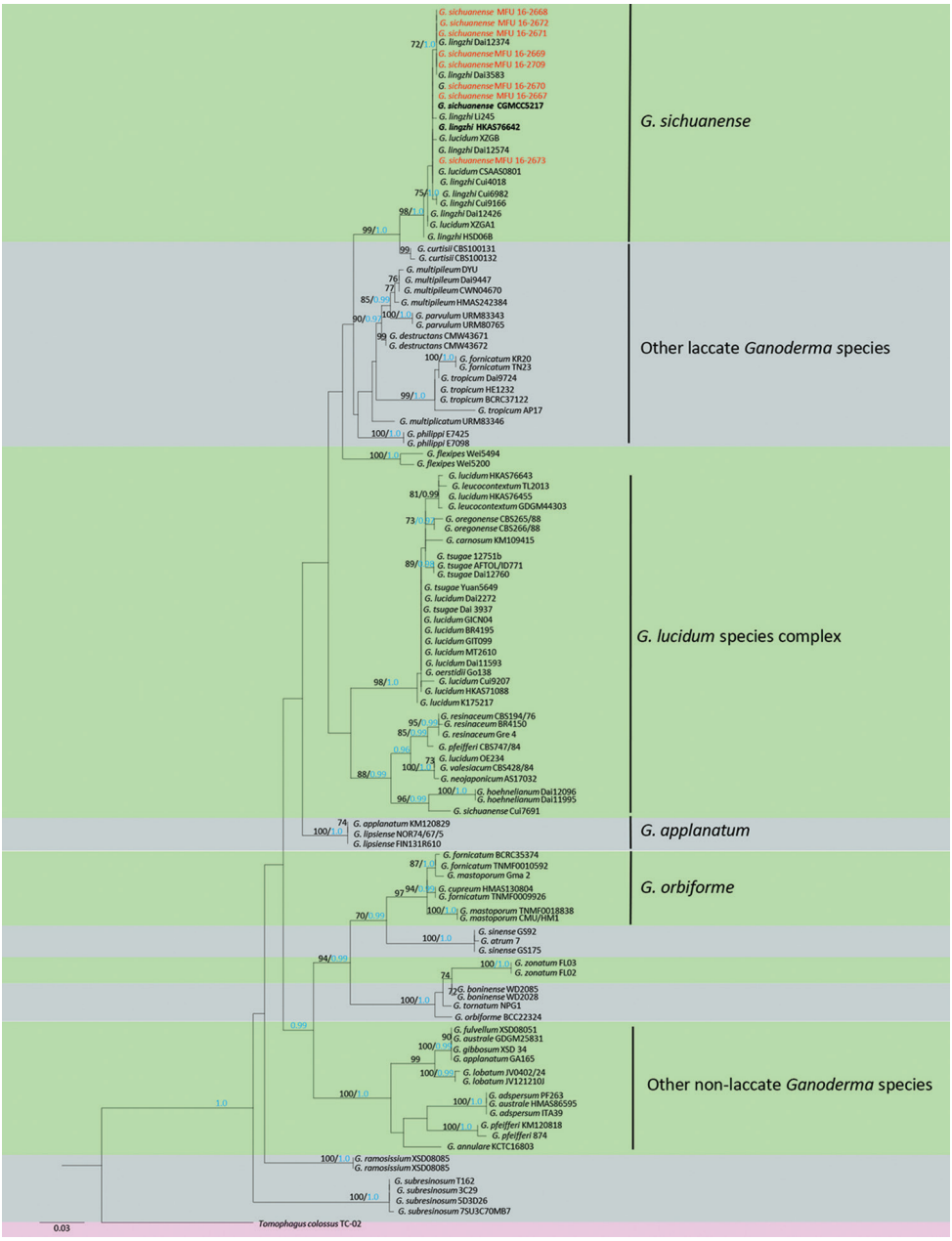

Figure 1. Phylogram generated by maximum likelihood analysis of 5.8S-ITS rDNA sequences. Bootstrap support values for maximum likelihood (in black) greater than 70% and Posterior Probabilities (PP) from Bayesian Inference (in blue) ≥0.95 are given above branches. The tree was rooted with *Tomophagus colossus*. The strain numbers are mentioned after the species names. Specimens of the newly recorded species are indicated in red and type specimens are indicated in black bold. (Treebase ID 20740)

Habitat. Rotten wood, in dry dipterocarp forest and in upper mixed deciduous forest and growing up from soil.

Distribution. Tropical and temperate regions of China; Thailand (this study).

Discussion

Ganoderma strains used in this study were clustered in six major clades (*G. applanatum*, *G. sichuanense*, other laccate and non laccate *Ganoderma*, *G. lucidum* species complex, *G. orbiforme* and *Ganoderma* species). Sequences obtained from the eight Thai collections clustered in the well-supported *G. sichuanense* group. The Thai specimens are closely related to the originally described Chinese *G. lingzhi* taxa (Cui 9166, Cui 6982 and Dai 12426) and the epitype of *G. sichuanense* (CGMCC5.2175), forming a monophyletic group with *G. sichuanense* from China with 98% bootstrap support. However, one of the strains of *G. sichuanense* (Cui7691) of which we retrieved a sequence from GenBank clustered in the *G. lucidum* species complex. This strain was most likely wrongly identified. The specimens have been collected at some geographical distance (min. 100 m between two collection points), which makes it unlikely that all come from the same mycelium. Nevertheless it is interesting to note that all new isolates cluster together and could not be segregated based on our phylogenetic analyses (Fig. 1). Given the phylogenetic results obtained herein where our new collections are found in a clade with *G. sichuanense* – including the type specimen – we believe that it would taxonomically more appropriate to establish them as new records of *G. sichuanense*. Furthermore, the deep nodes are not supported well in the tree, but this does not affect the final conclusions of the study. However, to obtain a better view of the evolution of the genus, a phylogeny with more genes, and in particular single-copy nuclear genes such as tef1 or rpb2 would be recommended.

Ganoderma sichuanense was originally described from the Sichuan Province in 1983 and was diagnosed as having a distinctly radially rugose pileus, with a verrucose or tuberculose upper surface; pore surface yellowish when young, becoming brown or black when bruised; and small spores (Fig. 2) distinguished from other *Ganoderma* species (Zhao and Zhang 2000). The size range of basidiospores was described as (7.4–9.5 × 5–7) µm cum myxosp., in the original description (Zhao et al. 1983). Later, this range was updated to (7.8–10.4 × 5.2–6.4) µm cum myxosp. (Zhao et al. 1989, Zhao and Zhang 2000) and (9–11.5 × 6.5–8) µm cum myxosp. (Wang et al. 2012). In this study basidiospores were (8.2)8.3–9.8(10.2) × (5.6)5.7–6.8(7.3) µm cum myxosp., which lies within the range given by the original authors and is not distinct from those of basidiospores found in other specimens. Cao et al. (2012) stated that *G. sichuanense* differs from *G. lingzhi* in its sessile basidiocarps and smaller basidiospores (7.4–9.2 × 5–6.6) µm (Fig. 3). Furthermore, they revealed that the original description was a mixture of *G. sichuanense* and *G. weberianum* especially with the small spores and smooth

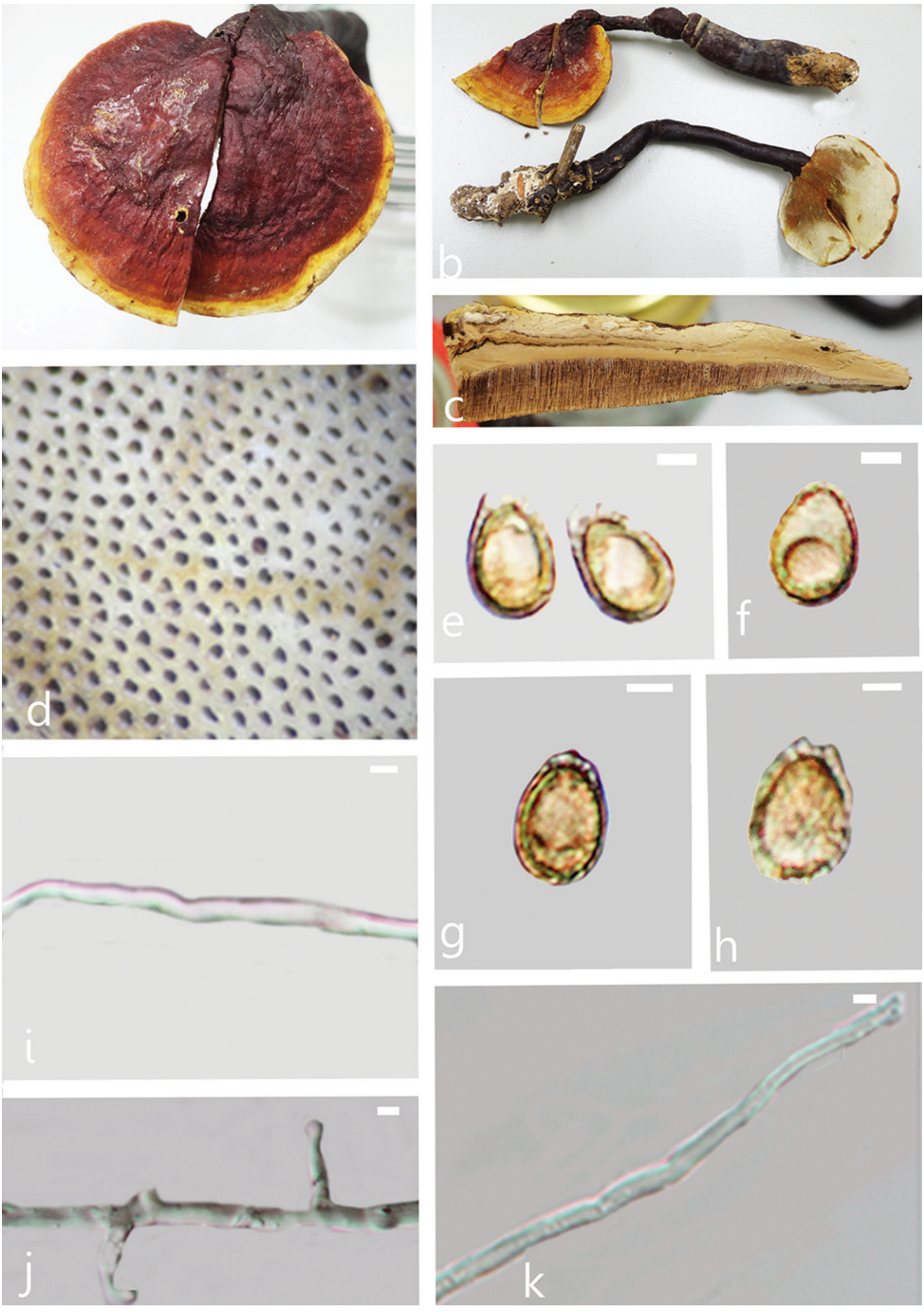

Figure 2. *Ganoderma sichuanense* (MFU 16-2668). **A** upper surface **B** lower surface **C** cut side of pileus **D** pore surface **E–H** spore **I** generative hyphae **J** binding hyphae **K** skeleton hyphae. Scale bars: 10 μm.

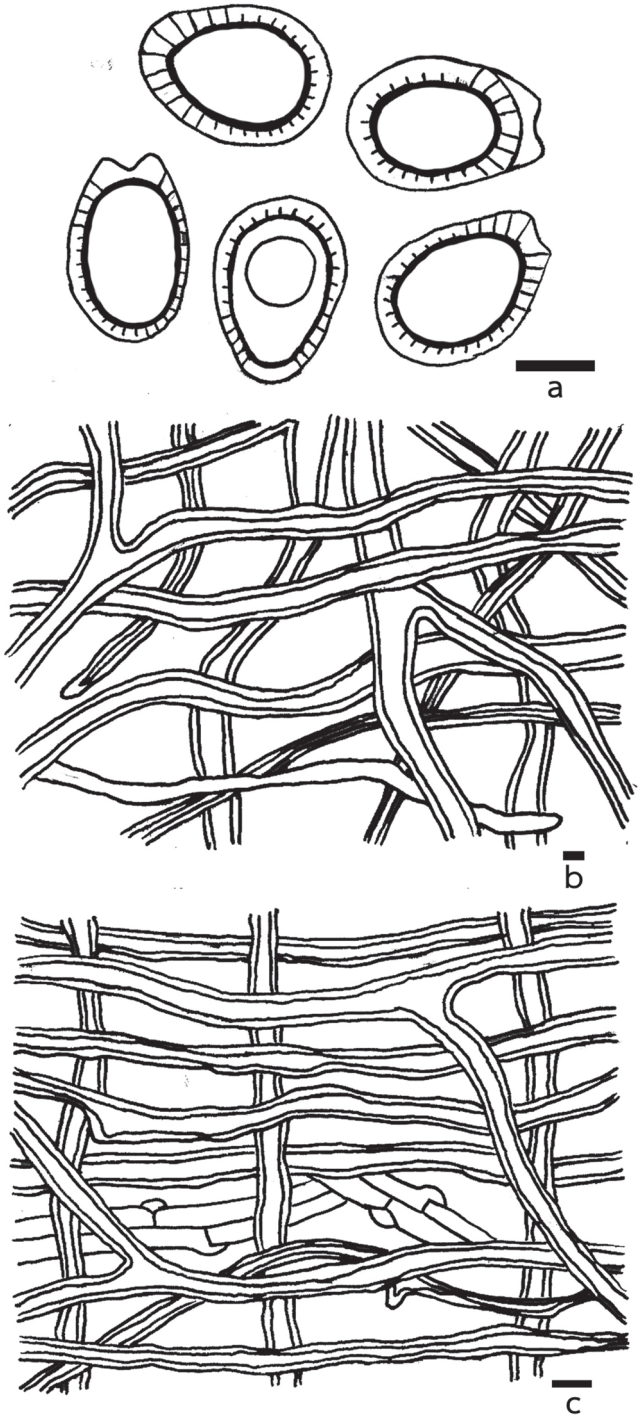

Figure 3. Microscopic structures of *Ganoderma sichuanense* (MFU 16-2668) **A** basidiospores **B** hyphae from trama **C** hyphae from context. Scale bars: 5 μm.

or slightly echinulate eusporium. *Ganoderma curtisii*, originally described from North America (Moncalvo and Ryvarden 1997) is a sister taxon to *G. sichuanense* in the phylogenetic estimate. *Ganoderma flexipes, G. multipileum* and *G. tropicum* are also closely related with *G. sichuanense* and are reported from China.

Conclusion

Macroscopic, microscopic, and molecular data all confirm that the collections from Thailand belong to *G. sichuanense*. This is the first discovery of the species in Thailand. The study of more collections of this species is needed to better estimate the variability of this taxon.

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