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RESEARCH ARTICLE

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Fulvifomes nonggangensis and *F. tubogeneratus* (Hymenochaetales, Basidiomycota): Two New Species from Southern China Based on Morphological and Molecular Evidences

Hai-Fu Zheng[a,b], Fu-Chang Huang[a], Bin Liu[a], Yuan-Yuan Shao[c] and Pei-Sheng Qin[a]

[a]Institute of Applied Microbiology, College of Agriculture, Guangxi University, Nanning, PR China; [b]Guangxi Forest Inventory & Planning Institute, Nanning, PR China; [c]Key Laboratory of Environment Change and Resources Use in Beibu Gulf, Ministry of Education, Nanning Normal University, Nanning, PR China

ABSTRACT

Two new species of *Fulvifomes* are described from specimens collected in rainforests of Nonggang Nature Reserve of southern China, based on morphological characteristics and molecular phylogenetic analysis of the internal transcribed spacer (ITS) and nuclear large subunit ribosomal DNA (nLSU) sequences. *Fulvifomes nonggangensis* sp. nov. is characterized by perennial, sessile and solitary basidiocarps, applanate pileus, small cystidioles of 9.9–15.4 × 2.9–3.5 μm, large pores of 5–6 per mm, a dimitic hyphal system, and broadly ellipsoid basidiospores of 4.3–5.3 × 3.3–4.2 μm. *F. tubogeneratus* sp. nov. is characterized by perennial, sessile, and imbricate basidiocarps, a duplex context, small pores of 7–8 per mm, a dimitic hyphal system, and ovoid to subglobose basidiospores of 5.72 × 5.00 μm.

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1. Introduction

Most species in the family Hymenochaetaceae were of medicinal value, while some were plant pathogens causing a white rot [1–3]. *Fulvifomes* Murrill was established by Murrill in 1914 and typified with *F. robiniae* Murrill to accommodate species with perennial and sessile basidiocarps, sulcate surface, ungulate or applanate pilei and smooth, ferruginous or fulvous spores in the family Hymenochaetaceae [4]. *Fulvifomes* comprises 28 species according to Index Fungorum (http://www.indexfungorum.org/Names/Names.asp, accessed on 2020/10/27). *Fulvifomes* was considered as a synonym of *Phellinus* Quél. for several decades by Ryvarden and other mycologists [5–8], until Wagner and Fischer [9] provided evidence to confirm *Fulvifomes* as an independent generic rank within Hymenochaetaceae based on molecular phylogenetic analyses. Furthermore, the genus *Aurificaria*, represented by *Aurificaria luteoumbrina*, was very close to *Phylloporia* and *Fulvifomes* reflected by the phylogenetic trees. Zhou [10] re-delimited the circumscription of *Fulvifomes* based on phylogenies inferred from nuclear large subunit ribosomal DNA (nLSU) and internal transcribed spacer (ITS) regions. Hattori et al. [11] also provided the key to worldwide species of *Fulvifomes*. Recently, still several new species were included within *Fulvifomes* [12–15].

During the macrofungal diversity survey in southern China, two additional undescribed species of *Fulvifomes* were found and identified as new by morphological characteristics and phylogenetic analysis inferred from the ITS and nLSU regions.

2. Materials and methods

2.1. Morphological studies

Specimens in this study were deposited in the herbarium of Guangxi University (GXU). The method of microscopic procedure followed Dai [2]. Special color definition followed Ridgway [16]. Sections were studied at magnification up to × 1500 using a Nikon Eclipse 80i microscope (Nikon Corporation, Tokyo, Japan). Abbreviations were used in text: IKI: Melzer's reagent; IKI−: negative in Melzer's reagent; KOH: 5% potassium hydroxide; CB: cotton blue; CB+: cyanophilous; CB−: acyanophilous; L: mean spore length (arithmetic average of all spores); W: mean spore width (arithmetic average of all spores); Q: variation in the L/W ratios between the specimens studied; *n*: number of spores measured from given number of specimens.

CONTACT Fu-Chang Huang hfch5685@126.com; Bin Liu liubin@gxu.edu.cn

2.2. DNA extraction, PCR, and sequencing

DNA extraction followed the protocol of conventional cetyl trimethylammonium bromide (CTAB) method. Nuclear ITS and nLSU regions were amplified with primer pairs ITS5/ITS4 [17] and LR0R/LR5 [18]. The PCR products were directly purified and sequenced by Beijing Genomics Institute (BGI; Shenzhen, China). PCR procedure was followed as: initial denaturation at 94 °C for 5 min, followed by 30 cycles at 94 °C for 40 s, 56 °C for 40 s, and 72 °C for 1 min, and a final extension of 72 °C for 10 min.

2.3. Phylogenetic analysis

In this study, eight new sequences were generated and additional sequences were obtained from GenBank are listed in Table 1. The ITS datasets with *Fomitiporella inermis* as outgroup, while the ITS + nLSU datasets with *Oninia tomentosa*, respectively.

Sequence datasets of the ITS and the combinability of ITS + nLSU were aligned with MEGA X version 10.0.5 (Pennsylvania State University, PA, USA) [19] and Clustalx version 1.83 (Information Retrieval, London, England) [20], respectively. Sequence alignment was deposited at TreeBASE (Study Accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S26455) and was executed by paupwin32_4b4a (Sinauer Associates, Sunderland, MA, USA) [21], MrMtgui version 1.0 (http://www.genedrift.org/mtgui.php) and MrModeltest version 2.3 (Uppsala University, Uppsala, Sweden) [22,23] to find the best-fit model for further analysis: MrBayesian analyses were performed by MrBayes version 3.2.2 (University of Rochester, NY, USA) [24] with 5,000,000 generations. Phylogenetic tree of maximum parsimony analyses which performed in PAUP* version 4.0b4a (Sinauer Associates, Sunderland, MA, USA) was generated using tree-bisection reconnection (TBR) branch-swapping algorithm, clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates. Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated. Phylogenetic trees were edited by TreeGraph version 2.3.0-425 beta (BioMed Central, London, England) [25].

Algorithms of two phylogenetic analyses generated nearly congruent topologies for each dataset, only the topology from the MP analysis was presented along with statistical values from the MP and BI algorithms (BS not less than 50% and BPP not less than 0.5) at the nodes.

3. Results

3.1. Phylogenetic analysis

The ITS dataset included sequences from 47 fungal specimens representing 27 taxa. The consensus tree is shown in Figure 1 (TL = 1200, CI = 0.6550, RI = 0.8488, RC = 0.5560, and HI = 0.3450). Best model for the ITS dataset estimated and applied in the Bayesian analysis: HKY + I + G, Lset nst = 2 rates = invgamma, Prset statefreqpr = dirichlet (1,1,1,1). The average standard deviation of split frequencies of Bayesian analysis is 0.004929. Tree topology of the maximum parsimony analysis showed almost same as the tree from Bayesian analysis. The phylogeny based on the ITS dataset (Figure 1) showed that the new species *Fulvifomes tubogeneratus* clustered with a sequence of *F. XHJ-2018h* Dai 9642 up to a higher support (BS = 100, BPP = 1.00), then they clustered with *F. siamensis* to form a small group which evidently fell into the core of *Fulvifomes* clade; new species *F. nonggangensis* clustered with *F. XHJ-2018b* Dai 17470 up to a higher support (BS = 100, BPP = 1.00), then they also clustered with *Inonotus rigidus* up to a higher support (BS = 100, BPP = 1.00) to form another small group but did not fall into the core of *Fulvifomes* clade.

The ITS + nLSU dataset included sequences from 38 fungal specimens representing 20 taxa. The consensus tree is shown in Figure 2 (TL = 1613, CI = 0.6820, RI = 0.8400, RC = 0.5729, and HI = 0.3180). Best model for the ITS + nLSU dataset estimated and applied in the Bayesian analysis: GTR + I + G, Lset nst = 6 rates = invgamma, Prset statefreqpr = dirichlet (1,1,1,1). The average standard deviation of split frequencies of Bayesian analysis is 0.003023. Tree topology of the maximum parsimony analysis showed almost same as the tree from Bayesian analysis.

In the phylogeny inferred from the ITS + nLSU dataset (Figure 2), *F. tubogeneratus* fell into the core of *Fulvifomes* clade, similarly to the phylogeny inferred from the ITS dataset, *F. tubogeneratus* clustered with *F. XHJ-2018h* Dai 9642 and Dai 10809 up to a higher support (BS = 100, BPP = 0.98), and then clustered with *F. siamensis* to form a small group; meanwhile, *F. nonggangensis* clustered with *F. XHJ-2018b* Dai 17203 and Dai 17470 with a higher support (BS = 100, BPP = 1.00), then they also grouped with *F. rhytiphloeus* with high support (BS = 99, BPP = 1.00).

3.2. Taxonomy

Fulvifomes nonggangensis F.C. Huang, H.F. Zheng & Bin Liu, sp. nov. (Figures 3 and 4).

MycoBank: MB835790

Table 1. Information for sequences used in this study.

			GenBank accessions	
Species	Geographic origin	Strain no.	ITS	nLSU
Fulvifomes elaeodendri	South Africa	CMW47808	MH599093	MH599131
Fulvifomes elaeodendri	South Africa	CMW47909	MH599096	MH599132
Fulvifomes fastuosus	Thailand	LWZ 20140801-1	KR905675	–
Fulvifomes fastuosus	Viet Nam	Dai 18292	MH390411	–
Fulvifomes fastuosus	Philippines	CBS 213.36	AY558615	AY059057
Fulvifomes fastuosus	Viet Nam	Dai 18292	MH390411	MH390381
Fulvifomes grenadensis	USA	JV1212 2 J	KX960756	–
Fulvifomes grenadensis	Costa Rica	1607 66	KX960758	–
Fulvifomes grenadensis	Brazil	JRF74	MH048097	MH048087
Fulvifomes grenadensis	Brazil	PH6	MH048096	MH048086
Fulvifomes hainanensis	China	Dai 11573	KC879263	JX866779
Fulvifomes imbricatus	Thailand	IFP LWZ 20140728-16	NR_154003	NG_068762
Fulvifomes imbricatus	Thailand	LWZ 20140729-26	KR905679	KR905671
Fulvifomes imbricatus	Thailand	MRNo309	LC176748	–
Fulvifomes indicus	Zimbabwe	O 25034	KC879262	–
Fulvifomes kawakamii	Brazil	PPT152	MH048095	–
Fulvifomes krugiodendri	USA	JV1008 21	KX960761	KX960767
Fulvifomes krugiodendri	USA	JV0904_1	KX960762	KX960765
Fulvifomes nilgheriensis	Brazil	3028	MH390431	–
Fulvifomes nonggangensis	China	GXU1127	MT571504	MT571502
Fulvifomes nonggangensis	China	GXU2254	MT571503	MT571501
Fulvifomes rhytiphloeus	Brazil	AMO763	MH048091	MH048081
Fulvifomes siamensis	Thailand	STRXG2	JX104708	JX104755
Fulvifomes siamensis	Thailand	KBXG3	JX104706	JX104753
Fulvifomes siamensis	Viet Nam	Dai 18309	MH390434	MH390389
Fulvifomes XHJ-2018b	China	Dai 17203	MH390419	MH390397
Fulvifomes XHJ-2018b	China	Dai 17470	MH390418	MH390395
Fulvifomes XHJ-2018e	USA	JV 0904/65	MH390422	–
Fulvifomes XHJ-2018e	USA	JV 0312/23.1	MH390423	–
Fulvifomes XHJ-2018e	USA	JV 0904/76	MH390424	–
Fulvifomes XHJ-2018h	China	Dai 9642	MH390429	MH390379
Fulvifomes XHJ-2018h	China	Dai 10809	MH390428	MH390378
Fulvifomes XHJ-2018i	USA	JV 0904/68	MH390408	–
Fulvifomes XHJ-2018i	USA	JV 1109/77	MH390409	–
Fulvifomes XHJ-2018l	China	Dai 17911	MH390405	–
Fulvifomes XHJ-2018l	China	Dai 17917	MH390406	–
Fulvifomes squamosus	Peru	CS385	MF479268	MF479265
Fulvifomes squamosus	Peru	CS456	MF479267	MF479266
Fulvifomes thailandicus	Thailand	IFP LWZ 20140731-1	NR154002	–
Fulvifomes thailandicus	Thailand	LWZ 20140731-1	KR905672	KR905665
Fulvifomes tubogeneratus	China	GXU2468	MT580805	MT580800
Fulvifomes tubogeneratus	China	GXU2478	MT580806	MT580801
Fulvifomes yoroui	Benin	OAB0097	MN017126	MN017120
Fomitiporella inermis	USA	JV 1109/19 A	KX181304	–
Fomitiporella inermis	USA	JV 1009/56	KX181306.	KX181347
Fomitiporella inermis	USA	JV 0509/57 K	KX181305	KX181346
Fomitiporia tsugina	USA	TOL2-1	KC551821	KC551843
Fomitiporia tsugina	USA	TOL2-3	KC551823	KC551845
Inonotus porrectus		CAW-30	HQ589219	–
Inonotus porrectus		CAW-31	HQ589220	–
Inonotus rigidus	China	Cui 8588	KX674579	–
Inonotus rigidus	China	Cui 8465	KX674580	–
Onnia tomentosa	Canada	Bud-551-C-1	JX110072	JX110116
Phellinus merrillii		PM950703-1 clone 2	EU035311	–
Phellinus merrillii		PM950703-1 clone 3	EU035312	–
Phellinus robiniae	USA	CBS 211.36	AY558646	AY059038
Phellinus robiniae	USA	CFMR:2693	KX065961	KX065995
Phellinus robiniae	USA	CFMR:2735	KX065962	KX065996
Phellinus piptadeniae	Brazil	MF008	KP412289	–
Phellinus piptadeniae	Brazil	MF027	KP412291	–
Phellinus piptadeniae	Brazil	MF034	KP412295	KP412276
Phellinus piptadeniae	Brazil	MF036	KP412297	KP412277
Phellinus piptadeniae	Brazil	MF038	KP412299	KP412278
Phylloporia ephedrae	Turkmenistan	13690	MH151184	–
Phylloporia gutta	China	Cui6945	MH151182	–
Phylloporia gutta	China	Dai16070	MH151183	–
Tropicoporus boehmeriae	Thailand	LWZ 20140729-10	KT223640	–
Tropicoporus boehmeriae	Thailand	LWZ 20140729-13	KT223641	–

Etymology: *nonggangensis* (Lat.): referring to the locality of the type specimen.

Type: China, Guangxi Autonomous Region, Chongzuo, Longzhou County, Nonggang Nature Reserve, on living trunks of angiosperm tree, September 19 2012, GXU1127 (Holotype in GXU).

rDNA sequences ex holotype: MT571504 (ITS), MT571502 (nLSU).

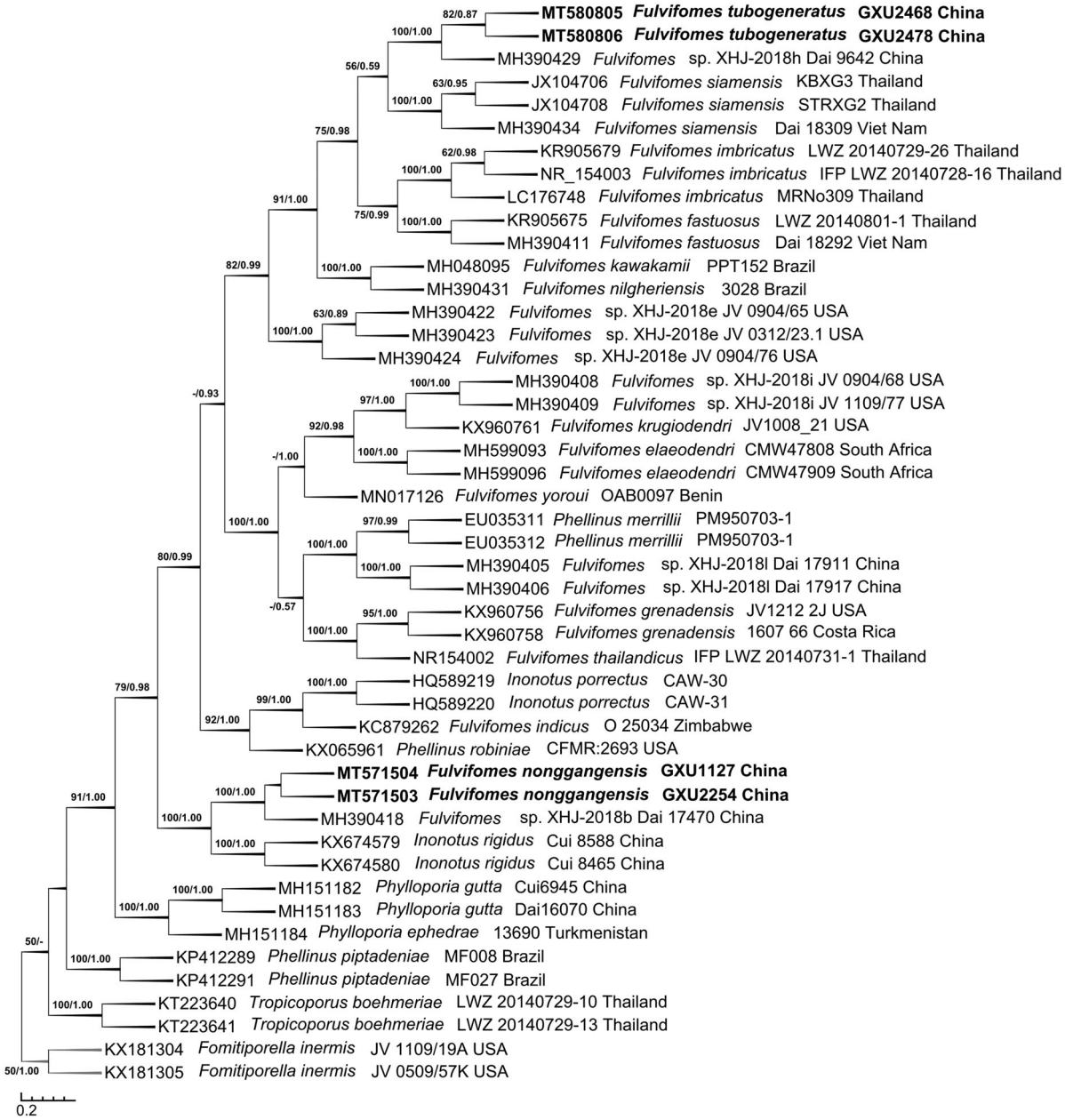

Figure 1. Phylogenetic tree was generated using maximum parsimony analyses based on ITS sequences. Bootstrap values (before the/) higher than 50% and Bayesian posterior probabilities (after the/) more than 0.50 are indicated along the branches.

Description: Basidiocarps perennial, sessile, solitary, occasionally smaller pileus fused along adjacent margins with other, broadly attached on living trunk, and old fruit body also found on dead trunk, without odor or taste, woody hard. Pileus applanate, of old fruit body (4–5 years) projecting up to 11.1 cm, 14.1 cm wide, and 6.4 cm thick at base. Pileal surface capucine orange, orange to amber brown when fresh, capucine yellow to orange at the actively growing part, orange to antique brown, argus brown when dry, densely tomentose, up to 0.1 cm thick, rough, nodulose, separated by a dense black line tissue from context, concentrically sulcate indistinct to distinct, part of tomentum becoming thinner, nodulose less, and sulcate zone more distinct with age, margin capucine yellow to orange when fresh and capucine orange to raw sienna when dry, obtuse. Pilei of old basidiocarps (4–5 years) argus brown, raw umber to almost black, concentrically sulcate zone distinct, margin narrow, and each year re-expanding from the position beneath the margin forming by previous year, making peripheral part usually looked like slowly descending ladders, and the periphery also radially cracked. Context up to 2.8 cm thick, apricot yellow to orange citrine, woody hard, occasionally a few black line tissues randomly distributed in context. Context of old basidiocarps (4–5 years) up to 1.5 cm thick, carob brown to chestnut brown, and its tomentum becoming a thin layer. Tube layers capucine orange, orange to argus brown, woody hard, not

Figure 2. Phylogenetic tree was generated using maximum parsimony analyses based on combined ITS + nLSU sequences. Bootstrap values (before the/) higher than 50% and Bayesian posterior probabilities (after the/) more than 0.50 are indicated along the branches.

stratified, or indistinct, up to 4.9 cm thick. Pores surface shining, raw sienna to antique brown when fresh, orange, argus brown to medal bronze when dry, sterile margin absent or narrow. Pores surface of old basidiocarps (4–5 years) orange citrine to medal bronze; sterile margin narrow to 2.8 mm width. Pores circular to angular, 5–6 per mm; dissepiments thin to thick, entire or some lacerate on margin.

Hyphal system dimitic, generative hyphae simple septate, tissue darkening in KOH, unchanged in Melzer's reagent.

Generative hyphae from tomentum hyaline, thin-walled, frequently branched with simple septate, 1.6–3.8 µm in diam. Skeletal hyphae from tomentum yellow to brown, thick-walled with a wide to narrow lumen, occasionally branched, simple septate often in part of hyphae with wide lumen, 4.6–14.1 µm in diam.

Context generative hyphae hyaline to pale yellow, thin- to slightly thick-walled, frequently branched, simple septate, 2.6–5.2 µm in diam; context skeletal hyphae dominant, orange to brown, occasionally branched, thick-walled with a wide to narrow lumen, simple septate often in part of hyphae with wide lumen, 3.6–10.6 µm in diam.

Tramal generative hyphae hyaline to pale yellow, thin- to slightly thick-walled, simple septate, frequently branched, 2.4–3.7 µm in diam; tramal

Figure 3. Basidiocarps of *Fulvifomes nonggangensis*. A: pileal surface of a mature basidiocarp; B: tube surface of a mature basidiocarp; C: a young basidiocarp; D: two aged basidiocarps. Scales bar: 1 cm.

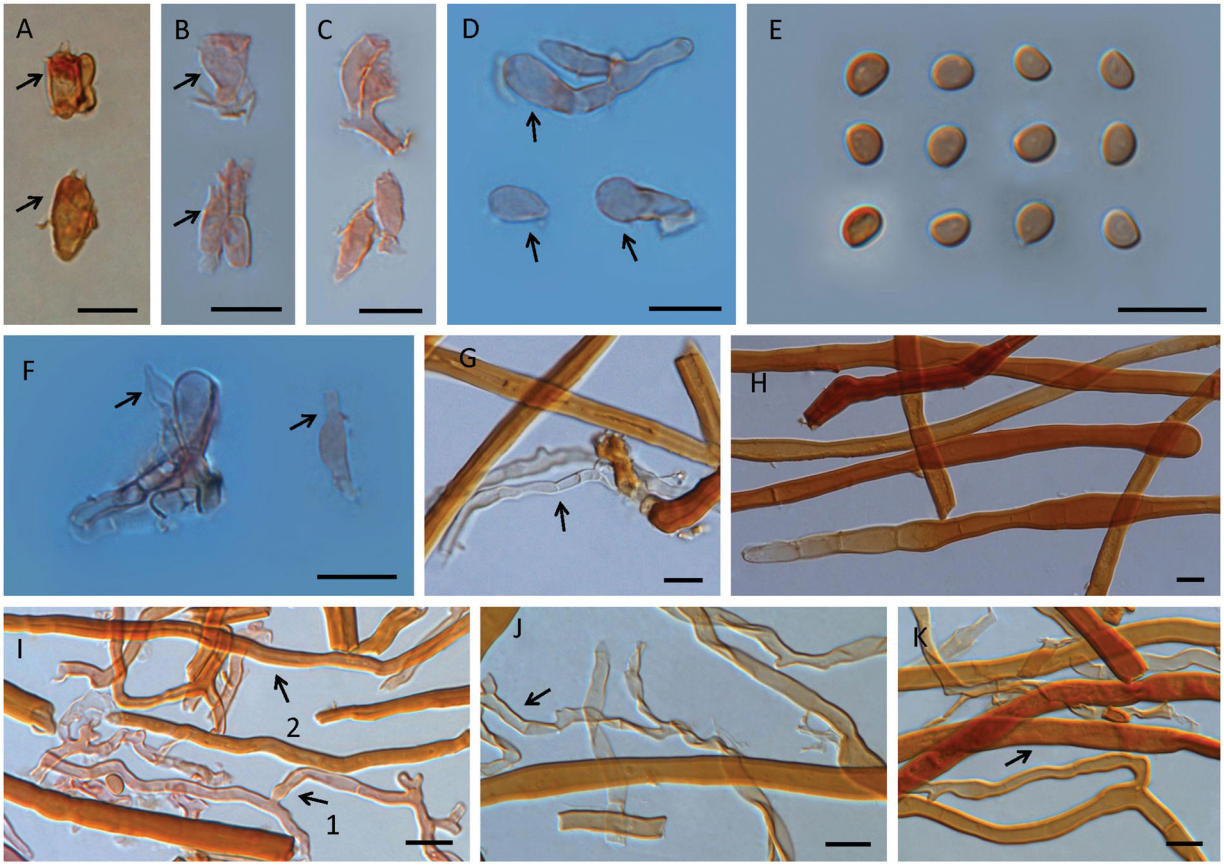

Figure 4. Microscopic structures of *Fulvifomes nonggangensis*. Scales bar: 10 μm. A, B, C: basidia; D: basidioles; E: basidiospores; F: cystidioles (arrow pointed); G: generative hyphae from tomentum (arrow pointed); H: skeletal hyphae from tomentum; I: 1, tramal generative hyphae, 2, tramal skeletal hyphae; J: generative hyphae from context (arrow pointed); K: skeletal hyphae from context (arrow pointed).

Figure 5. Basidiocarps of *Fulvifomes tubogeneratus*. A: basidiocarps imbricate; B: new fruit bodies generated from tube surface. Scales bar: 1 cm.

skeletal hyphae orange to brown, dominant, rare branched, thick-walled with a wide to narrow lumen, simple septate occasionally in part of hyphae with wide lumen, 3.1–8.2 µm in diam.

Hymenial setae lacking; cystidioles present, fusoid, hyaline, thin-walled, 9.9–15.4 × 2.9–3.5 µm; basidia clavate to barrel-shaped, hyaline, with basal simple septum and four sterigmata, 7.9–17.4 × 2.8–6.8 µm; basidioles clavate, barrel to elliptical shape, 8.3–18.8 × 3–6.8 µm.

Basidiospores broadly ellipsoid, brown, slightly thick-walled, smooth, IKI−, CB−, or weak reaction (4.2−)4.3 − 5.3(−5.5) × (3.1−)3.3 − 4.2 µm, $L = 4.93$ µm, $W = 3.73$ µm, $Q = 1.32$ ($n = 62$).

Habitat: growing on living angiosperm trunks.

Additional specimens examined: China, Guangxi, Chongzuo, Nonggang Nature Reserve, on living angiosperm trunks, June 20 2012, GXU0501, GXU0766, and GXU1102; on dead angiosperm trunks, November 18 2018, GXU2254.

Note: Differs from other species by basidiocarps perennial, sessile, solitary, pileus applanate, the periphery of pilei radially cracked on old fruiting body. Pores circular to angular, 5–6 per mm. Hyphal system dimitic, setae absent, cystidioles fusoid, 9.9–15.4 × 2.9–3.5 µm, basidiospores broadly ellipsoid, 4.93 × 3.73 µm on average.

Fulvifomes tubogeneratus F.C. Huang, H.F. Zheng & Bin Liu, sp. nov. (Figures 5 and 6).

MycoBank: MB835791

Etymology: *tubogeneratus* (Lat.): referring to new fruit body generated from tubes surface.

Type: China, Guangxi Autonomous Region, Chongzuo, Longzhou County, Nonggang Nature Reserve, on dead trunks of angiosperm tree, November 19 2018, GXU2468 (Holotype in GXU).

rDNA sequences ex holotype: MT580805 (ITS), MT580800 (nLSU).

Description: Basidiocarps perennial, sessile, broadly attached on dead drunk, frequently new fruit bodies generated on surface of tubes, occasionally on pileal surface, imbricate. Pileus semicircular to subcircular, applanate, up to 10.2 × 7.4 and 4.1 cm thick, pileus surface distinctly concentrically sulcate, velutinate, brussels brown to medal bronze, margin entire, acute or dull, cadmium yellow to raw sienna, and claret brown when age. Context wood hard, yellow ocher, buckthorn brown to cinnamon brown, duplex, separated by a black line, the upper context up to 0.6 cm, lower context up to 3.1 cm, black lines also frequently distributing in context, tubes, and between context and tube with age. Pore surface shining, rood's brown to burnt umber, pores not appearing on too young fruit body, but gradually increasing with mature, and some places of surface no pores differentiation and development even fruit body becoming old, and remaining with velutinate, these places capucine yellow, orange to cadmium yellow when young, brussels brown when old, sterile margin with the same color, up to 2.2 cm (no pores differentiation area) even old, pores angular or circular, 7–8 per mm; dissepiments most thin, and parts thick, entire, or some lacerate on margin. Tubes concolorous with pore surface, woody hard, up to 0.8 cm long, not stratified when young, but indistinct stratified with age, rood's brown to chocolate.

Hyphal system dimitic; generative hyphae simple septate; tissue darkening in KOH, unchanged in Melzer's reagent.

Upper context generative hyphae frequently branched, simple septate, hyaline to pale yellow, thin to slightly thick-walled with a wide lumen, 1.7–3.3 µm in diam; skeletal hyphae dominant, unbranched, capucine orange to orange, mostly thick-walled with a narrow to wide lumen and septate, and some still with a part of solid, 3–6 µm in diam. Lower context generative hyphae hyaline to pale yellow, frequently branched, thin to slightly thick-walled with a wide lumen, simple septate,

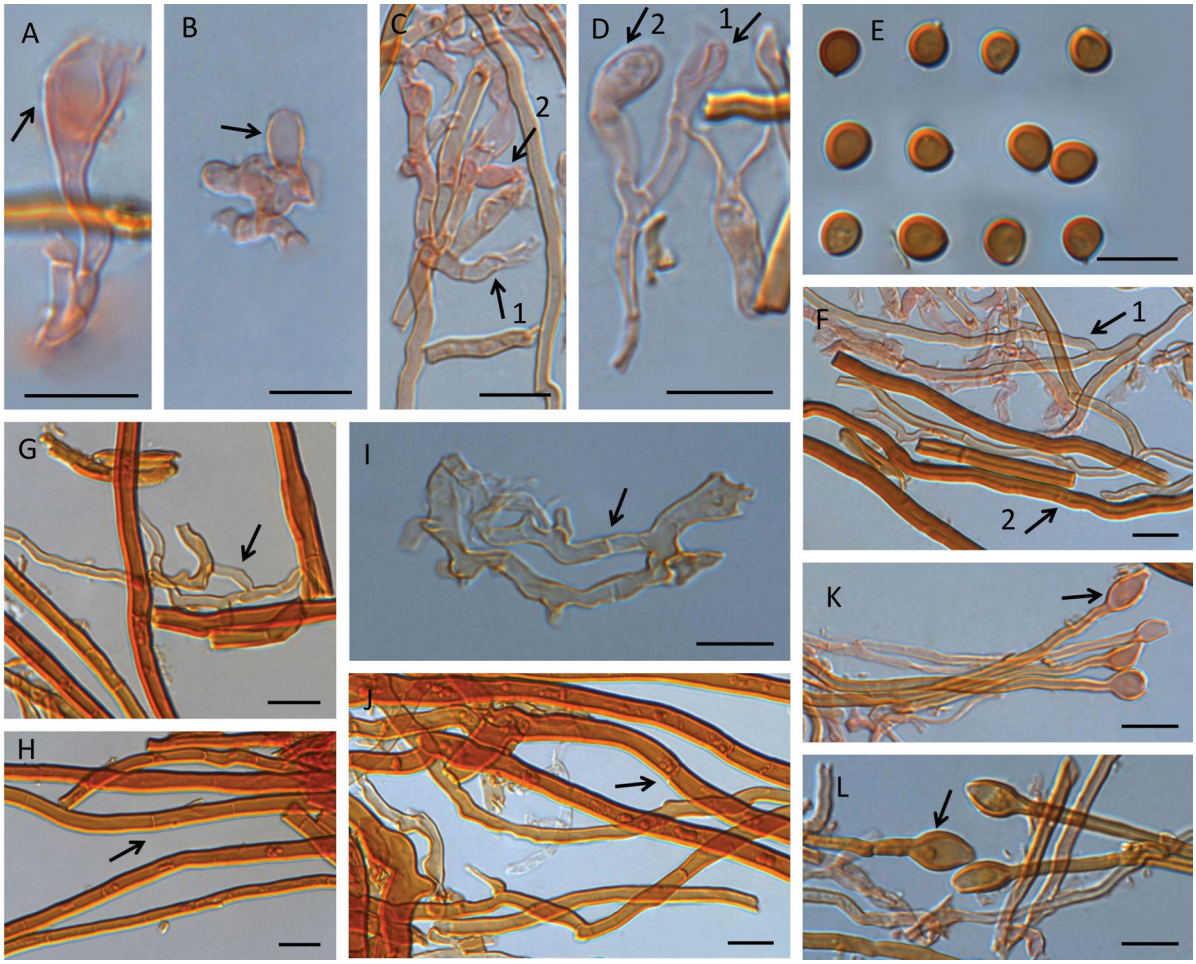

Figure 6. Microscopic structures of *Fulvifomes tubogeneratus*. Scales bar: 10 μm. A: basidia; B: basidioles; C, D: 1, basidia, 2, basidioles; E: basidiospores; F: 1, tramal generative hyphae, 2, tramal skeletal hyphae; G: generative hyphae from upper context (arrow pointed); H: skeletal hyphae from upper context (arrow pointed); I: generative hyphae from lower context; J: skeletal hyphae from lower context (arrow pointed); K, L: hyphoid setae swollen at apex from tramal.

1.9–6.1 μm in diam; skeletal hyphae frequent, occasionally branched, capucine orange to orange, thick-walled with a narrow to wide lumen, a few septate, 3.6–7.2 μm in diam.

Tramal generative hyphae frequent, hyaline to pale yellow, thin to slightly thick-walled, frequently branched, septate, 1.7–3.8 μm in diam; skeletal hyphae frequent, thick-walled with wide to narrow lumen, or solid, occasionally branched, rare septate, pale yellow to orange, 2.5–4.7 μm in diam.

Hymenial setae absent; hyphoid setae pale yellow to orange, frequent swollen at apex, and subglobose to fusiform, thick-walled, 7–19.8 × 4–7.2 μm; basidia clavate to barrel-shaped, flask-shaped, with four sterigmata, and a simple septum at the base, 9.9–31.4 × 3.1–7.6 μm; basidioles clavate to barrel-shaped, subglobose, 7.5–27.5 × 2.8–8 μm; cystidia and cystidioles absent.

Basidiospores ovoid to subglobose, capucine yellow to mars yellow, slightly thick-walled, smooth, IKI−, CB−, (5−)5.2 − 6.2(−6.4) × (4.1−) 4.5 − 5.7(−5.8) μm, $L = 5.72$ μm, $W = 5.00$ μm, $Q = 1.14$ ($n = 60$).

Habitat: growing on dead trunks of angiosperm trees.

Additional specimens examined: China, Guangxi, Chongzuo, Nonggang Nature Reserve, on dead angiosperm trunks, November 20 2018, GXU2478.

Note: Differs from other species by basidiocarps perennial, sessile, imbricate, new fruit bodies frequently generated from surface of tubes, context duplex, pores angular or circular, 7–8 per mm. Hyphal system dimitic, hyphoid setae present, basidiospores ovoid to subglobose, 5.72 × 5.00 μm on average.

4. Discussion

Morphology and DNA sequence analysis confirmed that the unique of the two new species *F. nonggangensis* and *F. tubogeneratus*. Species in the genus *Fulvifomes* are somewhat heterogeneous in certain characters, such as the presence or absence of setae and spores being cyanophilous (CB+) or acyanophilous (CB–) [2]. However, the basidiospores of two

new species in this investigation were both CB–, and hymenial setae absent as well.

Compared with other species, especially querying keys built by Dai [2] and Hattori et al. [11], *F. indicus* (Massee) L.W. Zhou, *F. merrillii* (Murrill) Baltazar & Gibertoni, and *F. squamosus* Salvador-Montoya & Drechsler-Santos are similar to *F. nonggangensis* and *F. tubogeneratus* in morphology, all of their basidocarps pileate, applanate, or ungulate to applanate, tube layers not stratified to indistinct, hymenial setae absent. Nevertheless, each of them has some specific characteristics different from two new species.

F. indicus is distinguished from two new species by basidiocarps annual [10], sessile or substipitate with a contracted base, hyphal system monomitic, pores the largest (4–5 per mm). Furthermore, basidiospores is larger (5.4–6.5 × 4.7–5.5 µm) than that of *F. nonggangensis*.

According to the data of Dai [2], *F. merrillii* differs from *F. nonggangensis* by pilei subungulate to applanate, pores smaller (7 − 8 per mm), tube layers indistinct, cystidioles larger (18–22 × 4.5–6 µm), basidiospores subglobose; and differs from *F. tubogeneratus* by basidiocarps subungulate to applanate, solitary, cystidioles present, and hyphoid setae absent and basidiospores smaller (4.4–5.4 × 3.7–4.7 µm).

F. squamosus [13] is distinguishable from two new species by having squamose pilear surface with long scales, hyphal system monomitic in the context, basidiospores with the ventral side flattened, and without cystidioles and hyphoid setae.

Similar to previous phylogenetic studies on *Fulvifomes* [12,14,15], the core of *Fulvifomes* in phylogenetic trees was mainly divided into two clades, one including *F. fastuosus*, *F. imbricatus*, *F. siamensis et al.*, another including *F. elaeodendri*, *F. krugiodendri*, *F. squamosus*, *F. thailandicus*, and *F. yoroui et al.*

F. nonggangensis is closely related to *F. XHJ-2018b*, *F. rhytiphloeus* (Mont.) Camp.-Sant. & Robledo, and *I. rigidus* B.K. Cui & Y.C. Dai, meanwhile, *F. tubogeneratus* closely related to *F. XHJ-2018h* and *F. siamensis* T. Hatt., Sakay. & E.B.G. Jones (Figures 1 and 2), isolates of *F. XHJ-2018b* and *F. XHJ-2018h* both came from China but were not formally described yet.

F. rhytiphloeus was proposed by Campos-Santana et al. [26] as a new complex species, and it resembles *F. nonggangensis* by basidiocarps pileate, applanate, solitary, with a distinct black line below pileus surface, setae absent. But its tubes were mostly distinctly stratified, pores smaller, 7–9 per mm, context fibrous and easily fragmented, cystidioles absent, and spores subglobose.

I. rigidus [27] differed from *F. nonggangensis* in its basidiocarps annual, resupinate, pores smaller, 8–9 per mm, hyphal system monomitic, cystidioles absent, and slightly smaller basidiospores (3.9–4.5 × 2.9–3.7 µm). But it also fitted in *Fulvifomes* with hymenial setae absent, ellipsoid, yellowish brown and thick-walled basidiospores, whether it is necessary to transferred *I. rigidus* to *Fulvifomes* which still needed more evidences.

F. siamensis was proposed by Hattori et al. [11], and resembled *F. tubogeneratus* by basidiocarps perennial, context woody hard, pores 7–8 per mm, setae absent. But it differed from the latter by without a distinct black line, and hyphal system monomitic in context, hyphoid setae absent. Moreover, basidiocarps of *F. tubogeneratus* imbricate, and new fruit bodies often generated from tube surface.

Key to species of *Fulvifomes* from China [2,10,28]

1. Hymenial setae absent 2
1. Hymenial setae present 11
2. Basidiocarps resupinate 3
2. Basidiocarps pileate, effused-reflexed 4
3. Basidiospores larger, 4.3–5.1 × 3.4–4.2 µm, cystidioles present ..... *F. inermis*
3. Basidiospores smaller, 3.1-4.2 × 2.6-3.1 µm, cystidioles absent ..... *F. membranaceus*
4. Basidiocarps effused-reflexed 5
4. Basidiocarps pileate 6
5. Tube layer distinct, basidiospores ellipsoid, 4.7–5.8 × 3.7–4.6 µm ..... *F. macgregorii*
5. Tube layer not stratified, basidiospores oblong-ellipsoid, 4.2–5.1 × 3–3.5 µm ..... *F. collinus* 6. Basidiocarps annual, hyphal system monomitic . *F. indicus*
6. Basidiocarps perennial, hyphal system dimitic 7
7. Tube layer distinct 8
7. Tube layer not stratified to indistinct 9
8. Pores 3–4 per mm, spores ellipsoid, chlamydospores absent ..... *F. hainanensis*
8. Pores 6–7 per mm, spores subglobose, chlamydospores present ..... *F. durissimus*
9. Hyphoid setae present, cystidioles absent *F. tubogeneratus*
9. Hyphoid setae absent, cystidioles present 10
10. Pores 5–6 per mm, spores broadly ellipsoid, 4.3 − 5.3 × 3.3 − 4.2 µm ..... *F. nonggangensis*
10. Pores 7–8 per mm, spores subglobose, 4.4–5.4 × 3.7–4.7 µm ..... *F. merrillii*
11. Basidiocarps effused-reflexed to pileate, pores 6–7 per mm ..... *F. johnsonianus*
11. Basidiocarps resupinate, pores 8–11 per mm... 12
12. Basidiospores 2–2.5 µm long, tube layers distinct ..... *F. minisporus*
12. Basidiospores 2.3–4.1 µm long, tube layers not stratified to indistinct 13

13. Basidiocarps annual, pore surface cracked when dry, spores CB(−).....*F. glaucescens*
13. Basidiocarps perennial, pore surface not cracked when dry, spores CB(+).....*F. cesatii*

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ORCID

Fu-Chang Huang http://orcid.org/0000-0002-2648-9751
Bin Liu http://orcid.org/0000-0001-9345-4674

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