Mycological Progress (2024) 23:15
https://doi.org/10.1007/s11557-024-01946-4

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ORIGINAL ARTICLE

A taxonomic study of *Fulvifomes* (*Hymenochaetales*, *Basidiomycota*) in the Federated States of Micronesia and identification of two new species

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Received: 11 July 2023 / Revised: 11 December 2023 / Accepted: 31 December 2023

Abstract
Fulvifomes (*Hymenochaetaceae*) is a genus that was separated from *Phellinus* in the early 2000s based on internal transcribed spacer (ITS) and nuclear large ribosomal subunit (nLSU) analyses. Species recognition in the genus is challenging without molecular analysis due to general absence of discrete morphological characteristics. In this study, we examined *Fulvifomes* diversity in the Federated States of Micronesia (FS Micronesia), primarily found in the tropical and subtropical regions. Seven *Fulvifomes* species were confirmed: two new species, one unrecorded species and four species previously reallocated to *Fulvifomes* from other genera. We provide sequences including data on two protein-coding genes (RNA polymerase II; *RPB2* and translation elongation factor-1 alpha; *TEF1*), nLSU and different conspecific ITS types.

Keywords FS Micronesia · *Hymenochaetaceae* · ITS types · Mangrove · *Phellinus* · Taxonomy

Introduction

The genus *Fulvifomes* Murrill, typified by *F. robiniae* (Murrill) Murrill (Murrill 1914; *Pyropolyporus robiniae* Murrill, basionym), consists of bracket polypores with perennial basidiomes, rough to rimose pileal surfaces, and coloured basidiospores (Murrill 1903, 1904). The taxonomic status of this genus has long been debated. One argument supported *Fulvifomes* as a genus (Kotlaba and Pouzar 1978; Fiasson and Niemelä 1984), whilst the other considered it a synonym (Gilbertson 1980; Ryvarden and Johansen 1980; Ryvarden 1991; Ryvarden 1994) or a subgenus of *Phellinus* Quél. (Dai 1999). This debate was resolved by phylogenetic analyses of the nuclear large ribosomal subunit (nLSU) rDNA, which strongly supported *Fulvifomes* as an independent genus (Wagner and Fischer 2002).

The generic morphological diagnosis of *Fulvifomes* has been redefined based on the phylogenetic analysis of internal transcribed spacer (ITS) and nLSU sequences, which separated *Fulvifomes* species from the others. This revision encompassed characteristics such as annual to perennial growth, sessile, effused-reflexed to substipitate basidiomes with solitary to imbricate pilei, homogeneous or duplex context, a hyphal system ranging from monomitic to dimitic, the absence of hyphoid and hymenial setae, and subglobose to ellipsoid, flattened ventral side, yellowish to brown and slightly thin to thick-walled basidiospores (Zhou 2014; Salvador-Montoya et al. 2018).

Most *Fulvifomes* species are distributed in the tropical and subtropical regions of Asia (Zhou and Zhang 2012; Hattori et al. 2014; Zhou 2014, 2015; Ji et al. 2017), America (Ji et al. 2017; Salvador-Montoya et al. 2018; Zhou et al. 2023), and Africa (Olou et al. 2019). The Federated States of Micronesia (FS Micronesia) is a biodiversity hotspot in the tropical region of the Pacific Ocean. To date, four *Fulvifomes* species have been reported from the FS Micronesia, namely *Aurificaria luteoumbrina* (Romell) D.A. Reid (≡ *Fulvifomes luteoumbrinus* (Romell) Y.C. Dai & Vlasák), *Phellinus fastuosus* (Lév.) S. Ahmad (≡ *Fulvifomes fastuosus* (Lév.) Bondartseva & S. Herrera), *P. mangrovicus* (Imazeki) Imazeki (≡ *Fulvifomes mangrovicus* (Imazeki) T. Hattori), and *P. merrillii* (Murrill) Ryvarden (≡ *Fulvifomes merrilli* (Murrill) Baltazar & Gibertoni) (Zhou 2015; Gilbert et al.

Section Editor: Yu-Cheng Dai

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Published online: 14 February 2024

2008). However, as various *Fulvifomes* species have been reported in tropical regions, the diversity of *Fulvifomes* species in FS Micronesia is expected to be higher.

In this study, we aimed to examine *Phellinus*-like specimens collected from the FS Micronesia based on ITS and nLSU phylogenetic analyses, and on morphological characteristics. We also generated sequence data for RNA polymerase II (*RPB2*) and translation elongation factor-1 alpha (*TEF1*) protein-coding genes. Three species of *Fulvifomes* were determined, two of which are proposed as new to science. Detailed morphological descriptions are provided for the new taxa. Our findings contribute to the diversity of *Fulvifomes* species in the FS Micronesia.

Material and methods

Morphological studies

Six fruiting bodies were collected from three islands in the FS Micronesia; Chuuk Weno, Kosrae Lelu and Kosrae Tafunsak, in 2016 and 2017 (Fig. 1). Sampling and transport processes were performed with the permission of the FS Micronesia Government. Dried fungal specimens were deposited at the Seoul National University Fungus Collection (SFC). Photographs of the fruiting bodies were captured in the field.

Microscopic examination of the dried specimens was performed by sectioning the tissue into 1 cm × 1 cm pieces. Tissue sections were mounted in 5% KOH on a glass slide and then observed using a Nikon Eclipse 80i microscope (Nikon, Japan). Approximately 20 basidia and basidiospores were measured per specimen using ImageJ software (Collins 2007). The *Q* values of the basidiospores indicate their length-to-width ratios. Colours were described based on 'Methuen Handbook of Colour' (Kornerup and Wanscher 1967). The pore surface and pore size were measured using a Nikon SMZ1500 stereomicroscope (Nikon, Japan). The following abbreviations were used: IKI = Melzer's reagent, IKI − = neither amyloid nor dextrinoid, CB = Cotton Blue, CB − = acyanophilous, L = mean spore length, W = mean spore width, Q = ratios of L/W of specimens studied, and n = number of spores measured from the given number of specimens.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from dried specimens (1 cm × 1 cm of tissue) using the CTAB (Rogers and Bendich 1994) protocol and the AccuPrep® Genomic DNA Extraction Kit (Bioneer, Daejeon, Republic of Korea). Polymerase Chain Reaction (PCR) was performed for four genetic regions. The ITS region was amplified using ITS1F/ITS4 (White et al. 1990) or ITS1/ITS4B primers (Gardes and Bruns 1993). LR0R/LR7 (Vilgalys and Hester 1990) or LR0R/LB-y (Tedersoo et al. 2008) primers were used for nLSU. PCR for the ITS region and nLSU was performed under the following conditions: 5 min initial denaturation at 95 °C, followed by 35 cycles of 40 s at 95 °C, 40 s at 54 °C and 90 s at 72 °C, with a final extension step for 5 min at 72 °C. For the protein-coding genes, *RPB2* region was amplified with primers bRPB2-6F/bRPB2-7.1R (Matheny 2005), under the following conditions:

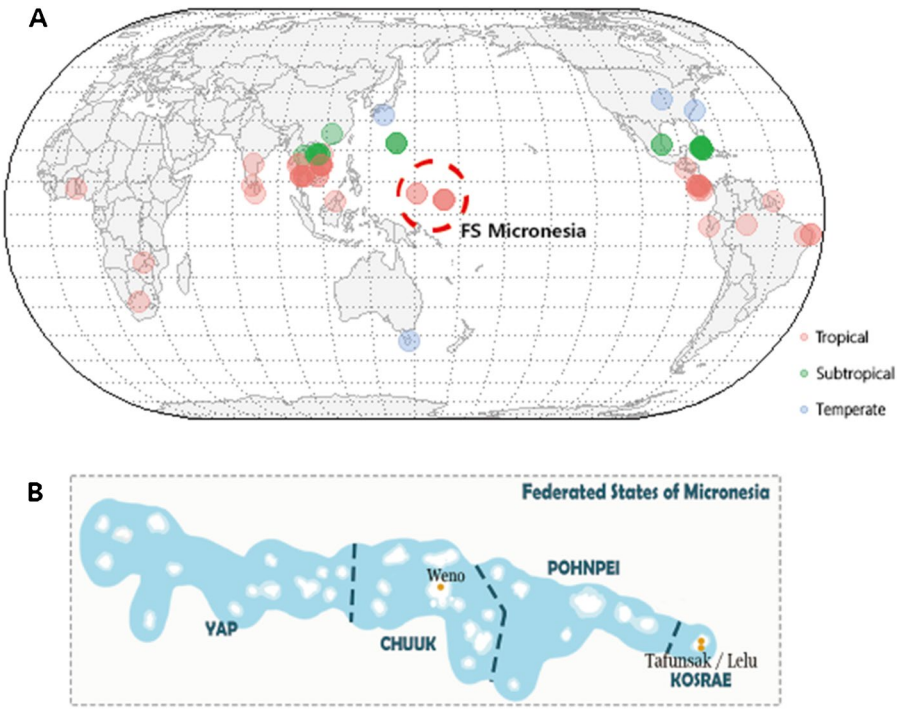

Fig. 1 **A** Geographical map of *Fulvifomes* distribution around the world. Distribution of phylogenetically confirmed *Fulvifomes* species from Table 1 is shown. Red dots indicate the tropical region, green indicates the sub-tropical region, and blue indicates the temperate region. **B** Sampling locations of *Fulvifomes* species in FS Micronesia. Orange dots indicate the locations where specimens were found

initial denaturation at 94 °C for 2 min, 36 cycles of 94 °C for 45 s, 53 °C for 90 s, 72 °C for 90 s, and a final extension at 72 °C for 10 min. The *TEF1* region was amplified with primers EF595F/EF1160R (Kauserud and Schumacher 2001) under the following conditions: initial denaturation at 95 °C for 4 min, 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 7 min.

The amplification of the PCR products was verified by gel electrophoresis using 1% agarose gel with EcoDye DNA staining solution (SolGent Co., Daejeon, Republic of Korea). The products were purified using an ExpinTM PCR Purification Kit (GeneAll Biotechnology, Seoul, Republic of Korea). Sequencing was performed by Macrogen (Seoul, Republic of Korea) using an ABI 3730xL DNA analyser (Life Technologies, Gaithersburg, MD, USA). All sequences were proofread and edited using MEGA version 11 (Kumar et al. 2016) and FinchTV v.1.4. All the newly generated sequences were deposited at GenBank (Table 1).

Multiple peaks were found in the ITS region for three specimens (SFC20170118-26, SFC20170120-06 and SFC20170120-09), which were separated into their respective ITS type through cloning. ITS amplicons were cloned into the pTOP TA V2 blunt end vector (Enzynomics, Korea) and transformed into competent cells (*Escherichia coli*, DH10β). Transformed clones were re-amplified as described by Hattori et al. (2022). Sequencing of each ITS type was performed as above described.

Phylogenetic analysis

Phylogenetic trees were constructed using ITS and nLSU sequences. Newly generated sequences and published reference sequences of *Fulvifomes* from GenBank were collected with GenMine (Seo et al. 2022), and used for phylogenetic analyses. *Inocutis dryophilus* and *Fomitiporella americana* were used as outgroups due to their close phylogenetic relationships with *Fulvifomes* (Guglielmo et al. 2008a, b; Pildain et al. 2018). Sequence data collection, outgroup selection, multiple sequence alignment, trimming, concatenation, model selection and maximum likelihood (ML) tree construction were performed with FunID ver 0.3.4 with 'accurate' preset (https://github.com/Changwanseo/FunID). The final sequence alignments from FunID are in Online Resource 1. Phylogenetic trees were visualised by a modified 'tree_interpretation.py' function of FunID. All processed data are shown in Table 1.

Results

Molecular phylogeny

The final combined dataset (ITS1 + 5.8S + ITS2 + nLSU) included 64 specimens from 40 species. The concatenated sequences comprised 1604 nucleotides, including gaps. The ITS1, 5.8S, ITS2 and nLSU regions comprised 365, 142, 216 and 881 bases, respectively. Protein-coding gene sequences (*RPB2* and *TEF1*) were not included in the phylogenetic analyses because of the lack of reference sequences.

The six newly analysed specimens formed three well-supported clades in the ML analyses (Fig. 2). One specimen (SFC20170120-02) grouped with *Fulvifomes halophilus* with 100% bootstrap value. The other two clades were distinct from any described *Fulvifomes* species (bootstrap = 100). After comparing the morphological descriptions of these specimens with those of *Fulvifomes* species without molecular data (Lowe 1957; Ryvarden and Johansen 1980; Hattori 1999; Dai 2010; Hattori et al. 2014), we proposed them as two new species: *Fulvifomes labyrinthus* sp. nov. and *Fulvifomes rhizophorus* sp. nov. The two new species of *Fulvifomes* were further validated as novel based on their distinguishable morphological characteristics from other known *Fulvifomes* species.

Three ITS types were identified in *Fulvifomes rhizophorus* sp. nov. (SFC20170120-06, SFC20170120-09 and SFC20170118-26). Each specimen had two different types of ITS sequences: Type1 ITS was shared across all three specimens, Type2 ITS was found in SFC20170120-06, and Type3 ITS was found in SFC20170120-09 and SFC20170118-26. Two indels and 13 substitutions were detected (Fig. 3).

Taxonomy

Fulvifomes labyrinthus H. Suh, Y. Cho, D. Kim & Y.W. Lim, sp. nov. (Fig. 4)

MycoBank Number: MB849263

Diagnosis: *Fulvifomes labyrinthus* grows on the trunk of the mangrove tree, *Xylocarpus granatum*, and is characterised by irregular pores.

Holotype: Federated States of Micronesia, Chuuk State, Weno Island; on a rotting trunk of *X. granatum*, 26 January 2016. SFC20160126-30 (dried specimen). GenBank accession numbers: OR168711 (ITS) and OR168721 (nLSU).

Etymology: The epithet 'labyrinthus' referring to its partly irregular pores.

Description: *Basidiome* perennial, pileate, sessile, broadly attached, solitary, woody hard, without odour or taste. *Pileus* dimidiate, triquetrous to subungulate, projecting up to 10 cm, 14 cm wide, 7 cm thick at the base. *Pileal surface* reddish-brown (8E5) to dark-brown (8F5), velutinate, slightly rugose, concentrically sulcate with shallow grooves, irregularly cracked, sometimes covered with moss at the centre. *Pore surface* light brown (7D5) to reddish-gold (6C7); sterile margin distinct, up to 4 mm wide. *Pores* somewhat circular and regular near the sterile

Table 1 List of *Fulvifomes* specimens and GenBank accessions

Species	Accession	Country	GenBank accession number				Reference
			ITS	nLSU	*TEF1*	*RPB2*	
Fulvifomes acaciae	JV 0312/23.4-J	USA	OP828594	OP828596			Zhou et al. (2023)
	JV 2203/71	Costa Rica	OP828595	OP828597			Zhou et al. (2023)
Fulvifomes azonatus	Dai 17470	China	MH390418	NG_088067			Wu et al. (2022)
Fulvifomes boninensis	FFPRI 421009	Japan	LC315786	LC315777			Hattori et al. (2022)
Fulvifomes caligoporus	Dai 17660	China	MH390421	MH390391			Wu et al. (2022)
Fulvifomes centroamericanus	JV 1408/4	Costa Rica		KX960768			Ji et al. (2017)
	JV 0611/III	Guatemala	KX960763	KX960764			Ji et al. (2017)
Fulvifomes costaricensis	JV 1607/103	Costa Rica	MH390414	MH390386			Wu et al. (2022)
Fulvifomes dracaenicola	Dai 22097	China	MW559800	MW559805			Wu et al. (2022)
Fulvifomes elaeodendri	CMW47909	South Africa	MH599096	MH599132			Tchoumi et al. (2020)
Fulvifomes fastuosus	UOC DAMIA D27b	Sri Lanka	KJ206286				Ediriweera et al. (2014)
	LWZ 20140731–13	Thailand	KR905674	KR905668			Zhou (2015)
	Dai 18292	Vietnam	MH390411	MH390381			Wu et al. (2022)
Fulvifomes floridanus	JV 0904_76	USA	MH390424	MH390388			Wu et al. (2022)
Fulvifomes grenadensis	JV 1212_2J	USA	KX960756				Ji et al. (2017)
	JV 1607_66	Costa Rica	KX960758				Ji et al. (2017)
	PH6	Brazil	MH048096	MH048086			Jayawardena et al. (2019)
Fulvifomes hainanensis	Dai 11573	China	KC879263	JX866779			Olou et al. (2019)
Fulvifomes halophilus	XG 4	Thailand	JX104705	JX104752			Sakayaroj et al. (2012)
	JV 1502/4	Borneo	MH390427	MH390392			Wu et al. (2022)
	SFC20170120-02	FS Micronesia	OR168710	OR168720	OR215498		This study
Fulvifomes imazekii	FFPRI 421007	Japan	LC315788	LC315779			Hattori et al. (2022)
Fulvifomes imbricatus	MRNo309	Thailand	LC176748				Zhou (2015)
	LWZ 20140728–16	Thailand	KR905677	KR905670			Zhou (2015)
Fulvifomes indicus	Yuan 5932	China	KC879261	JX866777			Zhou (2014)
	O 25034	Zimbabwe	KC879262	KC879259			Zhou (2014)
Fulvifomes jouzaii	JV 1504/16	Costa Rica	MH390425	MH390400			Wu et al. (2022)
	JV 1504_39	Costa Rica	MH390426				Wu et al. (2022)
Fulvifomes kawakamii	PPT152	Brazil	MH048095	MH048085			Zhou (2015)
	CBS 428.86	USA		AY059028			Wagner and Ryvarden (2002)
Fulvifomes krugiodendri	JV 0904/1	USA	KX960762	KX960765			Ji et al. (2017)
Fulvifomes labyrinthus	SFC20160126-30	FS Micronesia	OR168711	OR168721	OR215495	OR196705	This study
	SFC20160126-34	FS Micronesia	OR168712	OR168722			This study
Fulvifomes lloydii	Dai 10809	China	MH390428	MH390378			Wu et al. (2022)
Fulvifomes luteoumbrinus	CBS 296.56	USA	AY558603	AY059051			Zhou (2015)
	JV 1412/6 J	Costa Rica	KX960759				Ji et al. (2017)
Fulvifomes mangroviensis	KSM-MP12a	Tamil Nadu	OM897221	OM897222			Tan et al. (2022)
Fulvifomes merrillii	Kout-6	Thailand	MH390416	MH390383			Wu et al. (2022)
	Dai 12094	China	MH390415	MH390382			Wu et al. (2022)
Fulvifomes nakasoneae	JV 1109/62	USA	MH390407	MH390376			Wu et al. (2022)
Fulvifomes nilgheriensis	CBS 209.36	USA	AY558633	AY059023			Wagner and Ryvarden (2002)
	URM 3028	Brazil	MH390431	MH390384			Wu et al. (2022)
Fulvifomes nonggangensis	GXU1127	China	MT571504	MT571502			Zheng et al. (2021)

Table 1 (continued)

Species	Accession	Country	GenBank accession number				Reference
			ITS	nLSU	*TEF1*	*RPB2*	
Fulvifomes rhizophorus	SFC20170118-26	FS Micronesia	OR168715	OR168723	OR215496		This study
	SFC20170120-06	FS Micronesia	OR168713	OR168724	OR215497	OR196706	This study
	SFC20170120-09	FS Micronesia	OR168714	OR168725		OR196707	This study
Fulvifomes rhytiphloeus	JV 1704/71	Costa Rica	MZ506738	MZ505207			Wu et al. (2022)
	JV 1808/76	French Guiana	MZ506739	MZ505208			Wu et al. (2022)
Fulvifomes rigidus	Dai 17496	China	MH390432	MH390398			Wu et al. (2022)
	Dai 17507	China	MH390433	MH390399			Wu et al. (2022)
Fulvifomes rimosus	M 2392655	Australia	MH628255	MH628017			Hattori et al. (2014)
Fulvifomes robiniae	JV1307/6	USA		MH390377			Wu et al. (2022)
	CBS 211.36	USA	AY558646	AF411825			Wagner and Ryvarden (2002)
Fulvifomes siamensis	XG 2	Thailand	JX104709	JX104756			Sakayaroj et al. (2012)
	TUFC 13803	Japan	LC315791				Hattori et al. (2022)
	Dai 18309	Vietnam	MH390434	MH390389			Wu et al. (2022)
Fulvifomes squamosus	USM 258349	Peru/Piura	MF479269	MF479264			Salvador-Montoya et al. (2018)
Fulvifomes subindicus	Dai 17743	China	MH390435	MH390393			Wu et al. (2022)
	Cui 13908	China	MH390436	MH390394			Wu et al. (2022)
Fulvifomes submerrillii	Dai 17911	China	MH390405	MH390371			Wu et al. (2022)
Fulvifomes thailandicus	LWZ 20140731–1	Thailand	KR905672	KR905665			Zhou (2015)
Fulvifomes tubogeneratus	GXU2468	China	MT580805	MT580800			Zheng et al. (2021)
Fulvifomes xylocarpicola	MU 8	Thailand	JX104676	JX104723			Sakayaroj et al. (2012)
Fulvifomes yoroui	OAB 0097	Benin	MN017126	NG088091			Olou et al. (2019)

margin, 7−8 pores per mm, but irregularly elongated to sinuous at the centre; dissepiments entire, thick. *Tubes* brown (6E7), stratified, woody hard, up to 3.5 cm thick in total, each annual layer up to 7 mm thick without layer of contextual tissue in between. *Context* darker than tubes, woody hard, up to 1.5 cm thick.

Hyphal system dimitic; generative hyphae hyaline to pale yellowish (3A3), thin- to slightly thick-walled, frequently septate, occasionally branched, present mostly in subhymenium and trama, rare in context, 1.8–3.3 µm wide; skeletal hyphae yellow (3B8) to dark brown (7F7), thick-walled, aseptate, unbranched, dominant in both trama and context, 2–3 µm wide in trama, 1.8–3.2 µm wide in context, IKI−, CB−.

Basidia rarely observed in the type material, barrel-shaped to subutriform, 4-sterigmate, simple septate, 11.8 × 4.8 µm. *Basidiospores* subglobose to broadly ellipsoid, yellowish orange (4A8) to brownish-yellow (5C8), thick-walled, smooth, IKI−, CB−, (3.7–)4.1–4.8(–5) × (2.9–)3.2–3.9(–4.1) µm, L = 4.44 µm, W = 3.57 µm, Q = 1.25 (n = 60/2). *Cystidioles* absent. *Setae* absent.

Additional specimen examined: SFC20160126-34; Federated States of Micronesia, Chuuk States, Weno Island; on a rotten trunk of *Xylocarpus granatum*, 26 January 2016. GenBank accession numbers: OR168712 (ITS) and OR168722 (nLSU).

Notes: *Fulvifomes labyrinthus* is phylogenetically closely related and morphologically similar to *Fulvifomes xylocarpicola* T. Hatt., Sakayaroj & E.B.G. Jones, they share the same host, *Xylocarpus granatum* (Hattori et al. 2014). However, *F. labyrinthus* can be distinguished from *F. xylocarpicola* by a reddish-brown pileus and partly irregular pores.

Fulvifomes rhizophorus H. Suh, Y. Cho, D. Kim & Y.W. Lim, sp. nov. (Fig. 5)

MycoBank Number: MB849264

Diagnosis: *Fulvifomes rhizophorus* grows on the trunk of the mangrove tree, *Rhizophora apiculata*, and is characterised by an ungulate basidiome.

Holotype: Federated States of Micronesia, Kosrae Lelu, 18 January 2017. SFC20170118-26 (dried specimen). GenBank accession numbers: OR168715 (ITS) and OR168725 (nLSU).

Etymology: The epithet 'rhizophorus' referring to the genus of its host tree.

Description: *Basidiome* perennial, pileate, sessile, broadly attached, solitary, woody hard and without odour

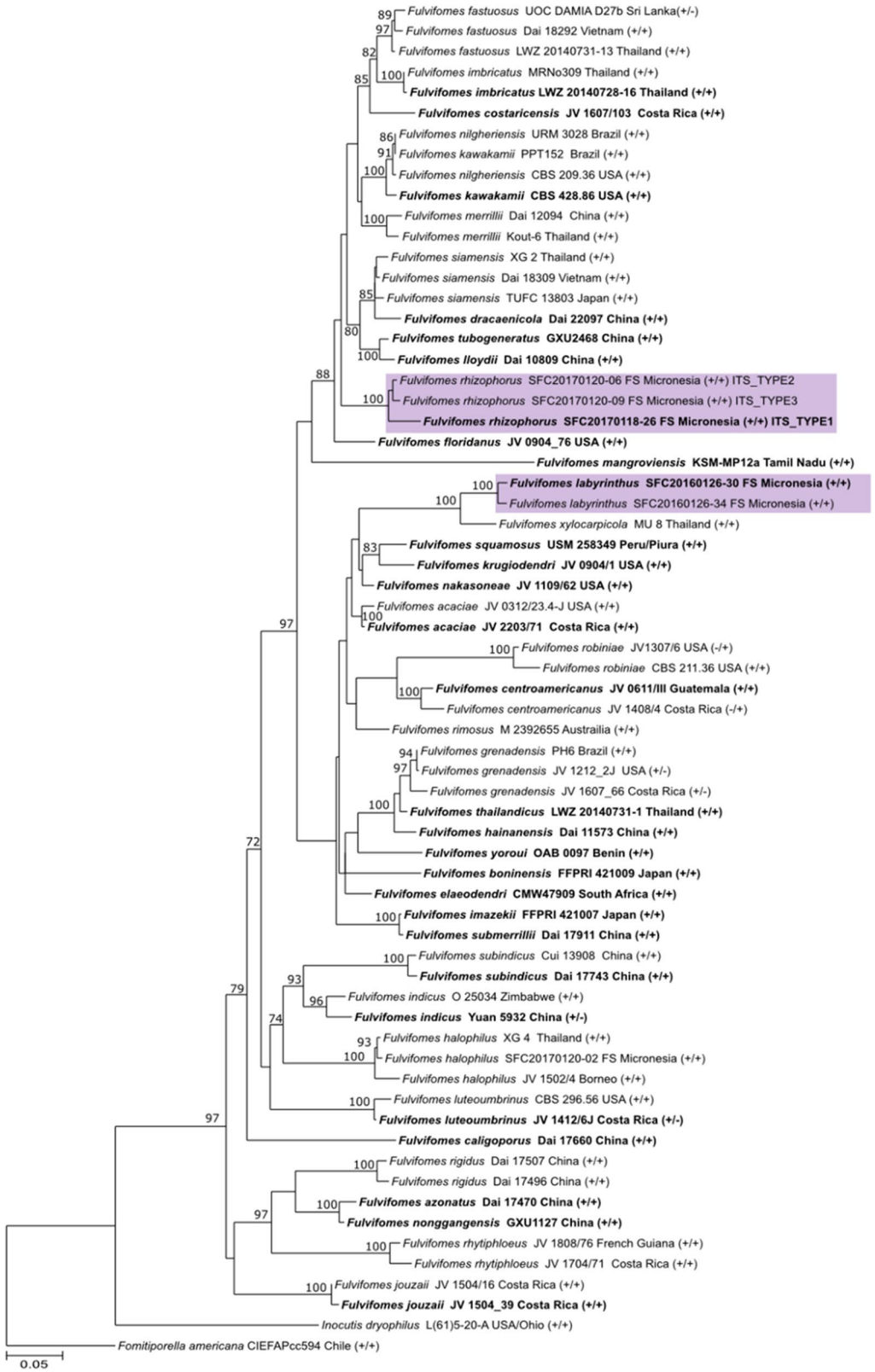

Fig. 2 Maximum likelihood tree of *Fulvifomes* constructed based on ITS and nLSU regions. The symbols (+/+) indicate the availability of ITS/nLSU sequences or the absence (−/−). Taxa in bold correspond to the type specimens. Purple boxes indicate new species from the FS Micronesia

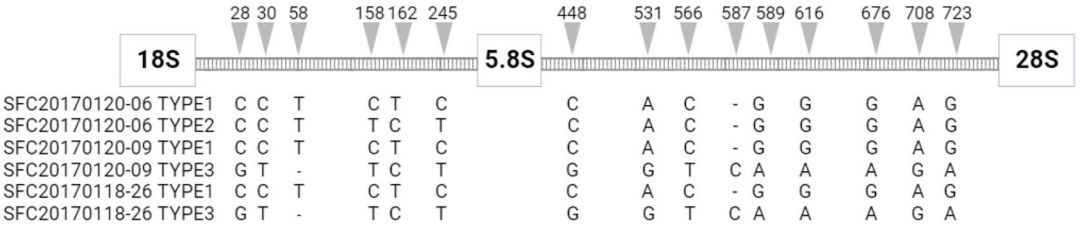

Fig. 3 ITS sequence types of *Fulvifomes rhizophorus*. Three ITS types were identified in *Fulvifomes rhizophorus* and two different types of ITS were present in each specimen. In the ITS1 region, there were five substitutions and one indel. In the ITS2 region, there were eight substitutions and one indel

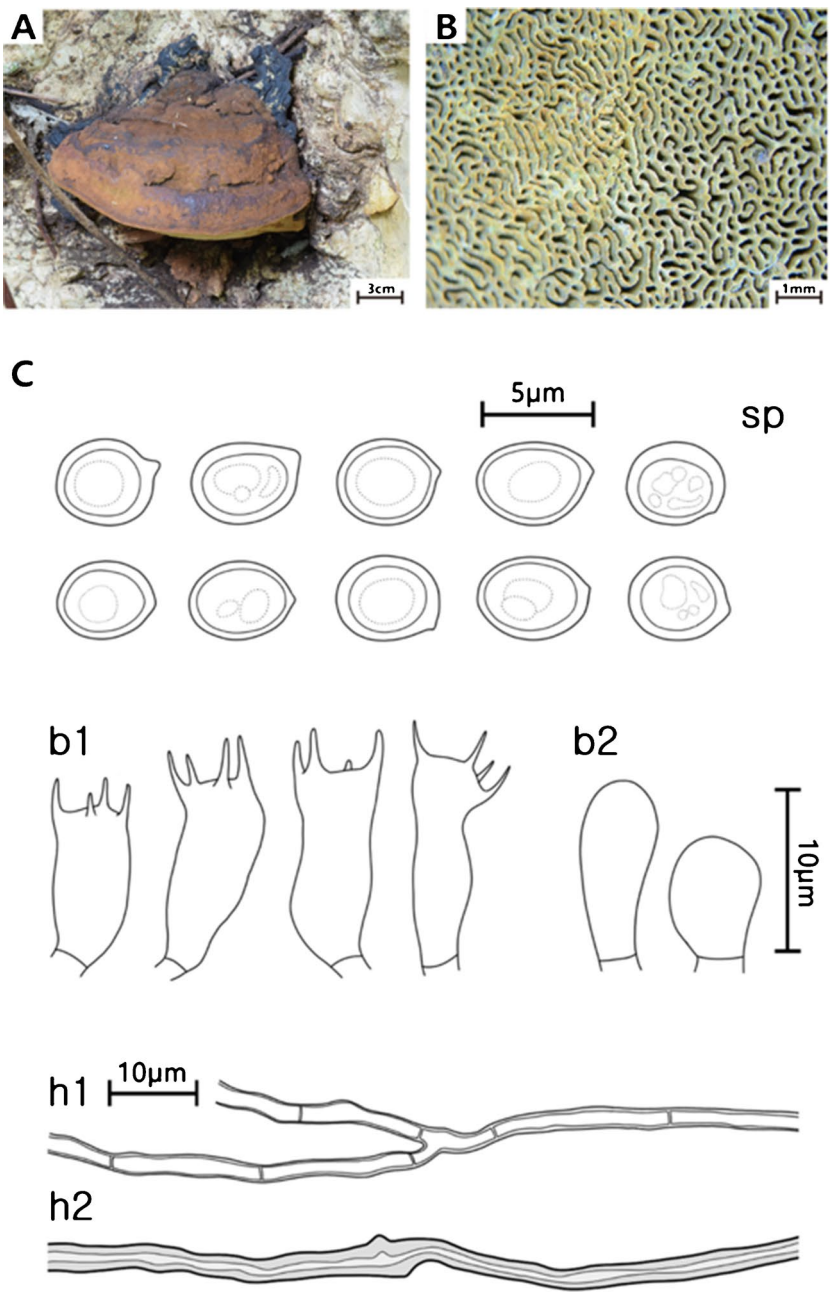

Fig. 4 Morphological characters of *Fulvifomes labyrinthus* (SFC20160126-30, holotype). **A** Basidiome. **B** Pore surface. **C** Drawings of microscopic features: 'sp' basidiospores, 'b1' basidia, 'b2' basidioles, 'h1' generative hypha and 'h2' skeletal hypha in trama

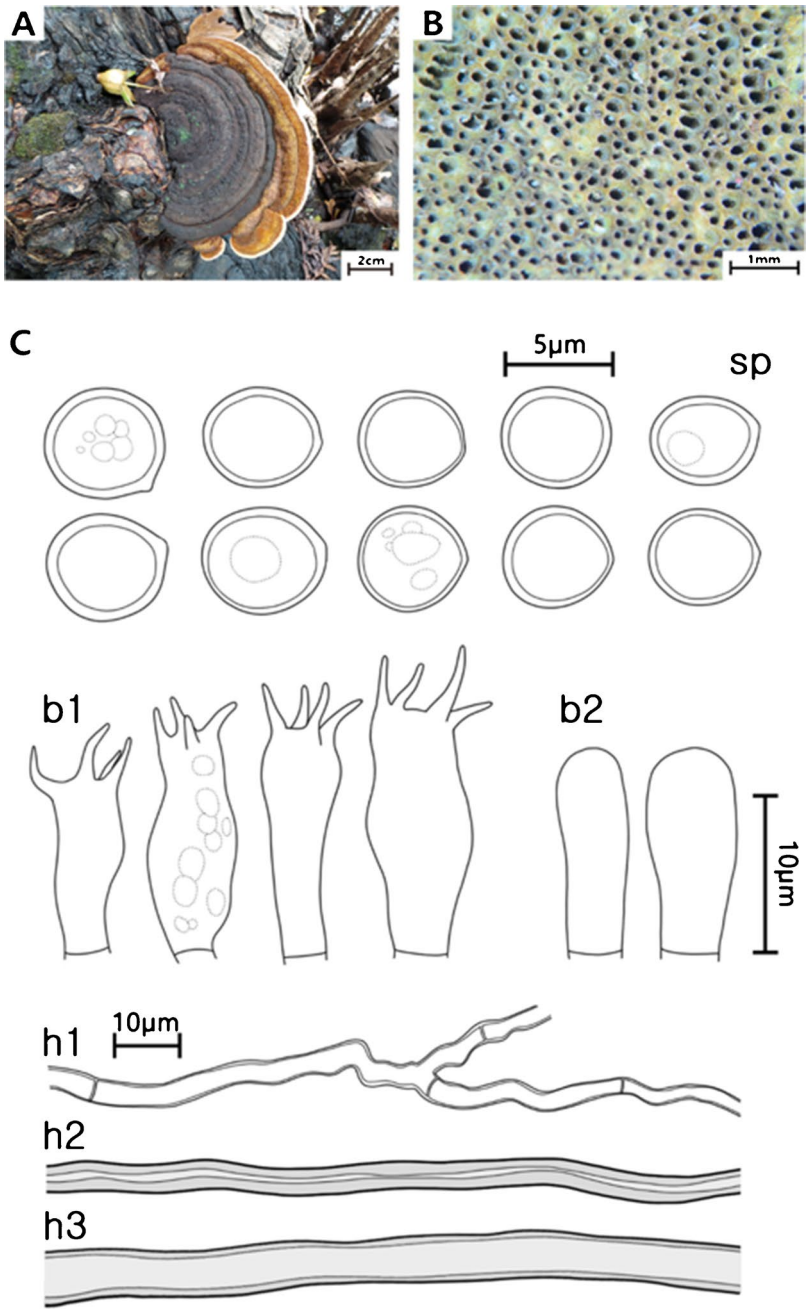

Fig. 5 Morphological characters of *Fulvifomes rhizophorus* (SFC20170118-26, holotype). **A** Basidiome. **B** Pore surface. **C** Drawings of microscopic features: 'sp' basidiospores, 'b1' basidia, 'b2' basidioles, 'h1' generative hypha, 'h2' skeletal hypha in trama, and 'h3' skeletal hypha in context

or taste. *Pileus* dimidiate, applanate, projecting 6.5 cm up, 11.5 cm wide and 3 cm thick at the base. *Pileal surface* yellowish (5E8) to reddish-brown (8F8), furrowed, concentrically sulcate with deep grooves, radially cracked. *Pore surface* golden-brown (5D7) to linoleum-brown (5E7); sterile margin distinct, up to 4 mm wide. *Pores* primarily round in shape, featuring closed slits, typically not connected to adjacent pores, 6−7 pores per mm; dissepiments entire, thick. *Tubes* light brown (6D8) to dark brown (6F8), stratified, woody hard, up to 1.4 cm thick in total, each annual layer up to 1 cm thick without layer of contextual tissue in between. Context darker than tubes, woody hard, duplex, the lower layer dark blond (5D4), up to 8 mm thick, the upper layer dark yellow (4C8), up to 9 mm thick.

Hyphal system dimitic; generative hyphae hyaline to pale yellowish (3A3), thin- to slightly thick-walled, frequently septate, occasionally branched, present mostly in subhymenium and trama, rare in context, 2.5–3 μm wide in trama, 3.3–5.2 wide in context; skeletal hyphae aseptae, unbranched, dominant in both trama and context, brown (6E8), in trama thick-walled with narrow lumen, 3.7–7.5 μm wide, dark brown (7F7), thick-walled with wide lumen, 6.2–8 μm in context, IKI−, CB−.

Basidia rarely observed in the type material, clavate to subutriform, 4-sterigmate, simple septate, 10.8 × 4.7 µm. *Basidiospores* subglobose to broadly ellipsoid, yellowish-orange (4A8) to brownish-yellow (5C8), thick-walled, smooth, IKI−, CB−, (5–)5.3–6(–6.8) × (4.3–)4.4–5.2(–6) µm, L = 5.6 µm, W = 5 µm, Q = 1.14 (n = 81/3). *Cystidioles* absent. *Setae* absent.

Additional specimens examined: SFC20170120-06; Federated States of Micronesia, Kosrae Tafunsak, on a trunk of *R. apiculata*, 20 January 2017. GenBank accession numbers: ITS OR168713 and nLSU OR168723. SFC20170120-09; Federated States of Micronesia, Kosrae Tafunsak, on a trunk of *R. apiculata* tree on 20 January 2017. GenBank accession numbers: OR168714 (ITS) and OR168724 (nLSU).

Notes: *Fulvifomes rhizophorus* is found on trunks of *Rhizophora apiculata*, which is submerged in the shallow waters of the mangrove forest. Amongst the *Fulvifomes* species in the FS Micronesia, *Fulvifomes rhizophorus* can be distinguished by larger basidiospores compared to those of *Fulvifomes labyrinthus* (4.1–4.8 × 3.2–3.9 µm) and *Fulvifomes halophilus* (4–5.5 × 3.5–5 µm) (Hattori et al. 2022).

Discussion

During the exploration of FS Micronesia in 2016 and 2017, six fruiting bodies of *Fulvifomes* were found. These were identified as three species, including two newly proposed species, based on morphological examination and phylogenetic analyses. Two specimens are described here as *Fulvifomes labyrinthus* sp. nov. and three are described as *Fulvifomes rhizophorus* sp. nov. The remaining specimen was identified as *Fulvifomes halophilus*, which is being reported for the first time in the FS Micronesia. *Fulvifomes rhizophorus* has three different types of ITS sequences, whereas other *Fulvifomes* species in FS Micronesia have only one ITS type. Various ITS types within a single species have been observed across *Basidiomycota* (Anderson and Kohn 2007; Lim et al. 2008). With various environmental changes occurring in mangrove forests, this phenomenon may indicate the initiation of sympatric speciation, where divergence occurs under non-disruptive selection (Giraud et al. 2008; Druzhinina et al. 2010). Therefore, our results suggest that *Fulvifomes rhizophorus* was introduced early in the FS Micronesia with some geographical and host restrictions, resulting in a diverse population (Burnett 1983; Griffith and Hedger 1994).

Fulvifomes species have relatively simple basidiome features, which make them difficult to distinguish amongst species. Species differentiation based on microscopic features is challenging because setae and cystidioles are absent. Furthermore, basidia are difficult to find, where some studies have noted that basidia are not observed (Zhou 2014; Olou et al. 2019; Salvador-Montoya et al. 2022). Similarly, basidia of these three species have been extensively searched. We observed basidia from the pore layer closer to the context of our perennial specimens by thin-sectioning in the vertical direction. Basidia may not be the key morphological character in distinguishing *Fulvifomes* species, however, providing the overall morphological profile of a species when designating a new species may ensure greater confidence in species delimitation.

According to Gilbert et al. (2008), four species were found in the FS Micronesia, namely *Aurificaria luteoumbrina*, *Phellinus fastuosus*, *P. mangrovicus* and *P. merrillii*, which have been transferred to *Fulvifomes* (Bondarceva et al. 1992; Hattori et al. 2014; Zhou 2015; Wu et al. 2022). As phylogenetic analyses had not been thoroughly conducted at the time, these species were classified and identified based only on morphological characteristics. Besides *Fulvifomes* species, other polypores were also transferred to other genera later on (Zmitrovich and Malysheva 2013). It is essential to re-identify the FS Micronesian specimens collected in the past using DNA sequencing and morphological characterisation to fully understand the *Fulvifomes* and other fungal distribution in this area.

Geographical and ecological features help to distinguish *Fulvifomes* from other genera in *Hymenochaetaceae*. Amongst the species that have validated sequences, three species were found in the temperate region: *Fulvifomes robiniae* from USA in the early 1900s (Murrill 1914), *F. rimosus* of which the lectotype was found in Tasmania, and *F. imazekii* from Kochi, Japan (Hattori et al. 2022). Other *Fulvifomes* species have been detected mainly in tropical and subtropical regions worldwide (Fig. 1). *Fulvifomes halophilus*, an unrecorded species in the FS Micronesia, also followed the distribution pattern of the majority of *Fulvifomes*; this species was also found in Thailand and Borneo, in the tropical and subtropical regions of Southeast Asia (Sakayaroj et al. 2012; Wu et al. 2022).

Limited protein-coding gene sequences have been found for *Fulvifomes* in open databases, such as for *RPB2* and *TEF1*, which are commonly analysed in phylogenetic studies of *Hymenochaetaceae*. In this study, we provide sequences of protein-coding genes (*RPB2* and *TEF1*) for the three *Fulvifomes* species, hoping to increase the resolution of phylogenetic analyses in future studies. Until now, a total of 40 *Fulvifomes* species have been discovered around the world. Two new species (*Fulvifomes labyrinthus* and *Fulvifomes rhizophorus*) and one previously unrecorded species (*Fulvifomes halophilus*) were identified in this study. Including species previously reported in the FS Micronesia, a total of seven *Fulvifomes* species are detected in the

country. Considering the unique environments of the Pacific Islands in tropical and subtropical areas (Mueller-Dombois et al. 1998; Rehman et al. 2013), a more intensive study in these regions may reveal more *Fulvifomes* species.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11557-024-01946-4.

Acknowledgements We are grateful to Chuuk and Kosrae State Government, the Federated States of Micronesia, for allowing this research. This study results from a collaborative project between Micronesia and the Korean government. Therefore, there is no violation of the ABS protocol, and the results are being shared with Micronesia.

Author contribution All authors contributed to the study conception and design. Material preparation and data collection were performed by HS and YC. Phylogenetic analysis was performed by CS and drawings were done by DK. The first draft of the manuscript was written by HS and YC, and YWL commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open Access funding enabled and organized by Seoul National University. This research was supported by the grant of the Ministry of Oceans and Fisheries (PM59731).

Data availability GenBank accession ITS: OR168710–5, nLSU: OR168720–5, *TEF1*: OR215495–8, *RPB2*: OR196705–7.

Declarations

Competing interests The authors declare no competing interests.

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