


# Investigation of the Fungal Diversity of the Federated States of Micronesia and the Construction of an Updated Fungal Inventory

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## ABSTRACT

The Federated States of Micronesia (FSM) is an island country in the western Pacific and is a known biodiversity hotspot. However, a relatively small number of fungi (236 species) have been reported till July 2021. Since fungi play major ecological roles in ecosystems, we investigated the fungal diversity of FSM from various sources over 2016 and 2017 and constructed a local fungal inventory, which also included the previously reported species. Fruiting bodies were collected from various host trees and fungal strains were isolated from marine and terrestrial environments. A total of 99 species, of which 78 were newly reported in the FSM, were identified at the species level using a combination of molecular and morphological approaches. Many fungal species were specific to the environment, host, or source. Upon construction of the fungal inventory, 314 species were confirmed to reside in the FSM. This inventory will serve as an important basis for monitoring fungal diversity and identifying novel biological resources in FSM.

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## KEYWORDS

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## 1. Introduction

The Federated States of Micronesia (FSM) is an island country in the western Pacific. It consists of four states (from west to east): Yap, Chuuk, Pohnpei, and Kosrae. The climate is warm and humid-tropical rainforest throughout the year, and the landscape of each state varies from low coral atolls to high mountainous terrain. The FSM is a biodiversity hotspot, with 3025 species of animals and 1553 species of plants reported hitherto [1]. However, a relatively small number of fungi (236 species) have been reported [1–19]. The magnitude of fungal species is estimated conservatively to be 1.5 M [20]. Given that the fungus to plant ratio (6:1) is one of the key elements for estimating the number of fungal species, the fungal species reported in the FSM is extremely low.

Fungi play major ecological roles as saprotrophs, symbionts, and pathogens in various environments [21]. Since the FSM comprises various types of habitats, its fungal species is believed to be more diverse than previously reported. Studies on fungal diversity in the FSM were limited to polypores in the forests [7], indoor fungi [16,17,19], and plant pathogens [2,10]. Previous studies on fungal identification in


the FSM were mostly confined to the comparisons of morphological characteristics and seldom on sequence analysis [16,17,19]. Therefore, limited fungal sequence data from the FSM are available.


As part of the projects organized by the Ministry of Ocean and Fisheries in Korea, we investigated fungal diversity from various sources in the FSM in 2016 and 2017. The aim of this study was to survey the fungi in the FSM using combined molecular and morphological approaches and construct a new fungal inventory based on the species identified in this study as well as those from previously reported studies.

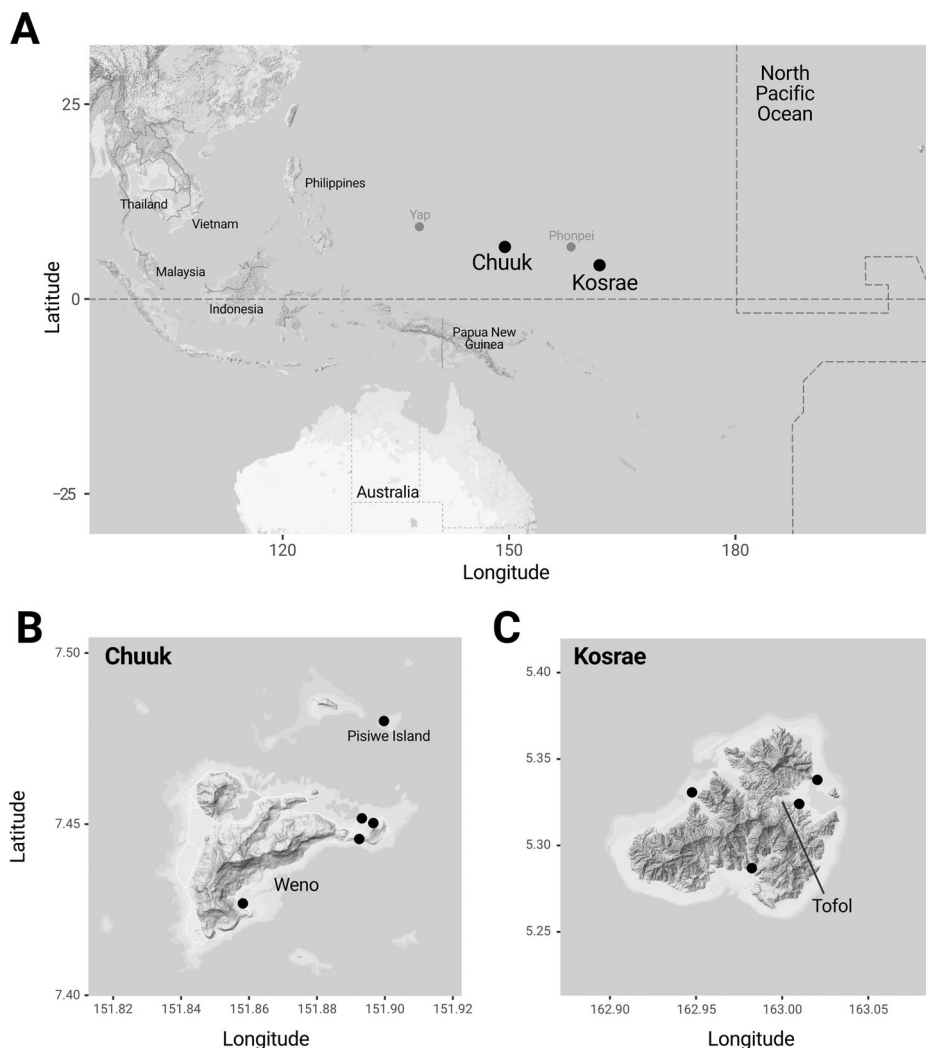
## 2. Materials and methods

### 2.1. Sample collection and isolation

Fungal sampling was performed in the states of Chuuk (January 2016) and Kosrae (January 2017) in the FSM (Figure 1). Fruiting bodies were collected from the forest, while fungal strains were isolated from various sources in both terrestrial (rotten woods) and marine environments (sea sand and seaweeds). Sampling and transport processes were

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 Supplemental data for this article can be accessed [here](#).



**Figure 1.** Location of the sampling sites in Chuuk and Kosrae, the Federated States of Micronesia.

performed with the permission of the FSM Government.

The fruiting bodies were dried completely and transported to the laboratory in Korea. The specimens were initially grouped based on their morphological features [22–27]. Representative specimens (between 1–3) from each group were selected for molecular identification. Specimens were also deposited at the Seoul National University Fungus Collection (SFC) (Table 1).

To isolate the fungal strain, 5 g of sea sand was mixed with ten times its volume of sterile water, and 100  $\mu$ L of the supernatant was plated on Dichloran Rose Bengal Chloramphenicol agar (DRBC; Difco, Becton Dickinson). The rotten wood and seaweed were cut to approximately 5 mm in length and plated on DRBC agar. The plates were transported to the laboratory in Korea in an icebox. All plates were incubated at 25 °C for 5–7 d. Individual fungal strains were transferred to potato dextrose agar (PDA; Difco, Becton Dickinson) plates and stored in 20% glycerol at –80 °C at the SFC (Table 2). Fungal strains were grouped morphologically according to their respective genera.

## 2.2. Molecular identification processes

Genomic DNA was extracted from the fruiting bodies and fungal strains using a modified cetyltrimethylammonium bromide extraction protocol [28]. PCR amplification of the internal transcribed spacer (ITS) of all fruiting bodies and fungal strains was performed using the primers ITS1F and ITS4 [29,30]. Appropriate protein-coding genes were used for the accurate identification of some genera; calmodulin (*CaM*) for *Aspergillus*,  $\beta$ -tubulin (*benA*) for *Penicillium*, and translation elongation factor 1- $\alpha$  (*tef1*) for *Fusarium* and *Trichoderma* were amplified using the primers CF1 and CF4 [31], Bt2a and Bt2b [32], and EF1 and EF2 [33], respectively. All PCRs were performed using AccuPower<sup>®</sup> PCR premix (Bioneer Co., Daejeon, Korea) in a C1000 thermal cycler (Bio-Rad, Richmond, CA). The PCR conditions for all loci were as follows: initial denaturation for 5 min at 95 °C followed by 35 cycles of 40 s at 95 °C, 40 s at 55 °C, and 60 s at 72 °C, with a final extension for 10 min at 72 °C. The PCR products were purified using the Expin<sup>™</sup> PCR Purification Kit (GeneAll Biotechnology, Seoul, Korea). DNA sequencing was conducted using an ABI Prism 3700

**Table 1.** List of species identified from the fruiting bodies collected from Chuuk and Kosrae, the Federated States of Micronesia.

Phylum	Order	Species	Number of specimens		Substrate*	
			Chuuk	Kosrae		
Ascomycota	Pezizales	<i>Cookeina sinensis</i>		2	Br, Ma	
		<i>Annulohyphoxylon urceolatum</i>	1		Ma	
	Xylariales	<i>Daldinia eschscholtzii</i>	1	2	Ar, Ho, Pr	
		<i>Xylaria allantoidea</i>		1	Rh	
Basidiomycota	Agaricales	<i>Xylaria arbuscula</i>	1	1	Br, Ma	
		<i>Xylaria haemorrhoidalis</i>	1	1	Co, Ma	
		<i>Coprinellus disseminatus</i>	1	2	Ar, Br	
		<i>Coprinopsis cinerea</i>	1		Co	
		<i>Coprinopsis strossmayeri</i>		1	S	
		<i>Favolaschia manipularis</i>		2	Br, Ho	
		<i>Galerina sulciceps</i>		2	Co, Un	
		<i>Gymnopilus crociphyllus</i>		1	Co	
		<i>Gymnopilus lepidotus</i>	1		Co	
		<i>Gymnopus dysodes</i>	1		Ma	
		<i>Gymnopus indoctus</i>	1		Br	
		<i>Marasmius palmivorus</i>	3	1	Ar, Co	
		<i>Pleurotus djamor</i>	2		Br, Rh	
		<i>Pluteus septocystidiatus</i>	1		Co	
		<i>Psathyrella luteopallida</i>	2		Br, S	
		<i>Schizophyllum commune</i>	4	2	Ba, Co, Ma, Rh	
		<i>Volvariella volvacea</i>		2	Br, Mu	
		Auriculariales	<i>Auricularia polytricha</i>	5	4	Ar, Br, Co, Ho, Ma, Pr, Rh, Un
			Dacrymycetales	<i>Dacryopinax spathularia</i>	1	
		Geastrales	<i>Geastrum mirabile</i>		1	Br
		Hymenochaetales	<i>Fuscoporia senex</i>	1		Un, Ar
			<i>Hyphodontia tropica</i>	2		Ar, Rh
		Phelliales	<i>Phellinus noxius</i>	1	2	Br, Ma, Pr
			<i>Phallus atrovolutus</i>	1		S
		Polyporales	<i>Earliella scabrosa</i>	2	1	Br, Ma
			<i>Favulus grammacephalus</i>	3	3	Br, Eu, Ma, Un
			<i>Fomitopsis ostreiformis</i>	4	3	Co, Ma, Mo, Rh
			<i>Funalia aspera</i>	2	3	Br, Ma, Rh, Un
			<i>Ganoderma gibbosum</i>		3	Ar, Br, Ho
			<i>Ganoderma orbiforme</i>		2	Ac, Br
			<i>Hexagonia tenuis</i>	1	2	Co, Ma, Rh
			<i>Leiotrametes menziesii</i>	3	8	Ac, Ar, Co, Ho, Ma, Mo, Rh, Un
			<i>Lentinus squarrosulus</i>	2	1	Ar, Ma
<i>Lentinus tuber-regium</i>	1			S		
<i>Nigroporus vinosus</i>			1	Un		
<i>Phlebiopsis flavidoalba</i>			1	Ho		
<i>Polyporus arcularius</i>	2			Ar, Ma		
<i>Pycnoporus sanguineus</i>			2	Ma, Mo		
<i>Rigidoporus microporus</i>			2	Co, Ho		
<i>Rigidoporus vinctus</i>			1	Ho		
<i>Tinctoporellus epimiltinus</i>	2		2	Ac, Ar, Br, Ma		
<i>Trametes polyzona</i>	2		Ma			

\*Ac: *Acacia* sp.; Ar: *Artocarpus altilis*; Ba: *Bambusa* sp.; Bl: Broad-leaved tree; Co: *Cocos nucifera*; Eu: *Eucalyptus deglupta*; Ho: *Horsfieldia* sp.; Ma: *Mangifera indica*; Mo: *Morinda citrifolia*; Mu: *Musa* sp.; Pr: *Premna* sp.; Rh: *Rhizophora* sp.; S: Soil; Un: Unknown species.

Genetic Analyzer (Life Technologies, Gaithersburg, MD, USA) at MacroGen (Seoul, Korea).

The sequences were assembled, proofread, and edited using MEGA5 [34]. The revised sequences were uploaded to GenBank and the accession numbers are listed in Supplementary Table 1. Genus-by-genus phylogenetic analyses were performed for molecular identification. Multiple generic sequence alignments were performed using MAFFT v7 [35]. Neighbor-joining trees were constructed with MEGA5 using the Kimura 2-parameter model [36] with 1000 bootstrap replicates.

### 2.3. Species inventory

A list of previously reported fungal species in the FSM was compiled using the occurrence data from the Global Biodiversity Information Facility [1] (<https://www.gbif.org/occurrence/download/>

0204233-200613084148143), New Zealand Fungal Herbarium [2], and other publications (Table S1). The species newly reported in this study were also included in this fungal inventory.

## 3. Results

### 3.1. Species identification

A total of 118 fruiting bodies were collected from Chuuk ( $n = 56$ ) and Kosrae ( $n = 62$ ). Based on the morphological features and phylogenetic analysis of ITS sequences, 46 different species (two phyla, nine orders, 22 families, and 38 genera) were identified (Table 1). Among the 118 fruiting bodies, 107 (90.7%) belonged to Basidiomycota and 11 (9.3%) belonged to Ascomycota (Figure 2(A)). The most dominant order was Polyporales (50.0%,  $n = 59$ ),

**Table 2.** List of species identified from the fungal strains isolated from different sources in Chuuk and Kosrae, the Federated States of Micronesia.

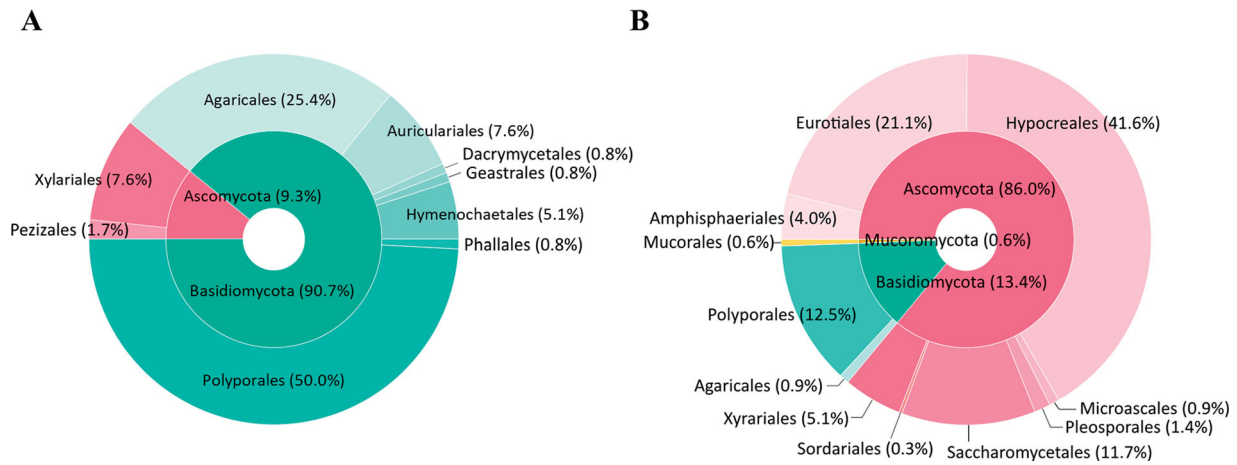
Phylum	Order	Species	Number of strains		Number of strains (source)			
			Chuuk	Kosrae	Seaweed	Sea sand	Wood	
Ascomycota	Amphisphaeriales	<i>Pestalotiopsis humus</i>		2	2			
		<i>Pestalotiopsis knightiae</i>	3			2	1	
		<i>Pseudopestalotiopsis indica</i>	4		1	3		
		<i>Robillarda sessilis</i>	5			5		
		<i>Aspergillus aculeatinus</i>	6				6	
	Eurotiales	<i>Aspergillus brunneoviolaceus</i>	6		2	3	1	
		<i>Aspergillus costaricensis</i>	2		2			
		<i>Aspergillus flavus</i>	2		2			
		<i>Aspergillus neoniger</i>	9	1	6	3	1	
		<i>Aspergillus pseudonomius</i>	1		1			
		<i>Aspergillus sydowii</i>	1		1			
		<i>Aspergillus tubingensis</i>		1		1		
		<i>Aspergillus welwitschiae</i>		1	1			
		<i>Aspergillus westerdijkiae</i>	1			1		
		<i>Paecilomyces formosus</i>	2				2	
		<i>Penicillium antarcticum</i>	1				1	
		<i>Penicillium brocae</i>		1	1			
		<i>Penicillium chermesinum</i>		2	2			
		<i>Penicillium citrinum</i>	27	5	4	20	8	
		<i>Penicillium ramusculum</i>		1	1			
		<i>Penicillium rubens</i>	1		1			
		<i>Penicillium singaporense</i>	1				1	
		<i>Penicillium velutinum</i>	2		1		1	
		Hypocreales	<i>Beauveria felina</i>		1	1		
			<i>Fusarium equiseti</i>	1				1
	<i>Fusarium incarnatum</i>		1				1	
	<i>Fusarium longipes</i>		1				1	
	<i>Fusarium mangiferae</i>		1				1	
	<i>Fusarium pseudensiforme</i>		1		1			
	<i>Fusarium solani</i>		6				6	
	<i>Gliomastix polychroma</i>			1		1		
	<i>Myrothecium roridum</i>		1			1		
	<i>Purpureocillium lilacinum</i>			1		1		
	<i>Trichoderma afroharzianum</i>			1	1			
	<i>Trichoderma brevicompactum</i>			1		1		
	<i>Trichoderma guizhouense</i>			1	2			
	<i>Trichoderma lentiforme</i>		10				10	
	<i>Trichoderma reesei</i>		115		6	7	102	
	<i>Trichoderma rifai</i>			1	1			
	<i>Trichoderma turrialbense</i>		1				1	
	Microascales	<i>Ceratocystis ethacetica</i>	1		1			
		<i>Ceratocystis paradoxa</i>	1		1			
		<i>Graphium basitruncatum</i>	1			1		
	Pleosporales	<i>Deniquelata barringtoniae</i>	1				1	
		<i>Epicoccum draconis</i>	1				1	
		<i>Leptosphaerulina chartarum</i>	1				1	
		<i>Paraphaeosphaeria arecacearum</i>		2	2			
	Saccharomycetales	<i>Galactomyces candidum</i>	40	1	39	1	1	
		<i>Chaetomium globosum</i>	1		1			
	Sordariales	<i>Annulohyphoxylon stygium</i>		1	1			
	Xylariales	<i>Daldinia eschscholtzii</i>	17			1	16	
		<i>Coprinellus disseminatus</i>	1			1		
		<i>Coprinellus radians</i>	2				2	
		<i>Fomitopsis ostreiformis</i>	37				37	
		<i>Lentinus squarrosulus</i>	3				3	
	Basidiomycota	Agaricales	<i>Trametes polyzona</i>	4			4	
			<i>Gongronella butleri</i>		1		1	
		Polyporales	<i>Poitrasia circinans</i>	1				1

followed by Agaricales (25.4%,  $n = 30$ ), Auriculariales (7.6%,  $n = 9$ ), and Xylariales (7.6%,  $n = 9$ ) (Figure 2(A)). The dominant species were *Leiotrametes menziesii* ( $n = 11$ ), followed by *Auricularia polytricha* ( $n = 9$ ), *Fomitopsis ostreiformis* ( $n = 7$ ), *Favolus grammocephalus* ( $n = 6$ ), and *Schizophyllum commune* ( $n = 6$ ) (Table 1, Figure 2(A), Figure 3(A–H)).

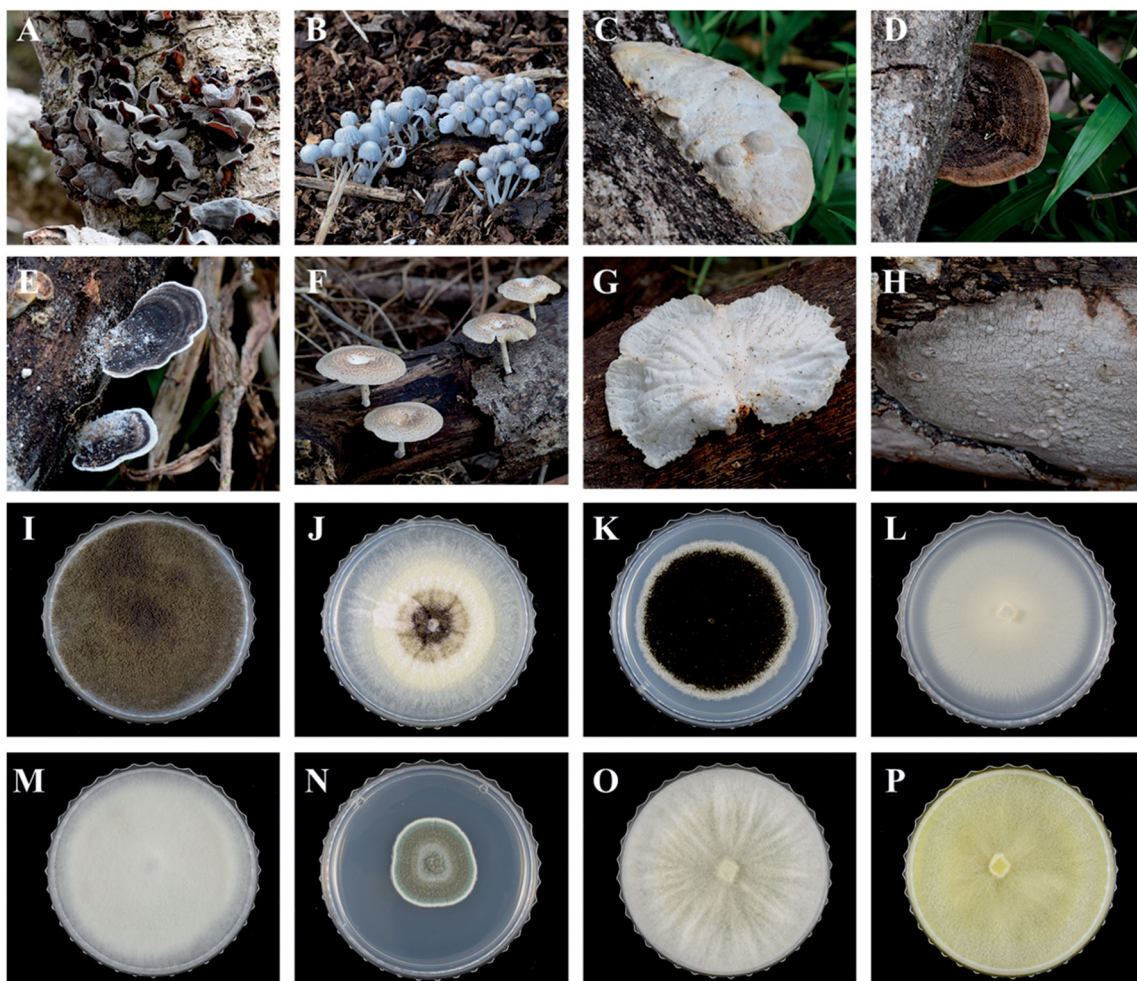
A total of 351 fungal strains were isolated from Chuuk ( $n = 325$ ) and Kosrae ( $n = 26$ ), of which 58

species (three phyla, 11 orders, 21 families, and 28 genera) were identified based on genus-by-genus phylogenetic analyses of ITS, *act*, *benA*, *CaM*, and *tef1* (Table 2). Five out of the 58 species were also obtained as fruiting bodies. Of the 351 strains, 302 (86.0%) belonged to Ascomycota, 47 (13.4%) belonged to Basidiomycota, and two (0.6%) belonged to Mucoromycota (Figure 2(B)). Hypocreales (41.6%,  $n = 146$ ), Eurotiales (21.1%,  $n = 74$ ), and Polyporales (12.5%,  $n = 44$ ) were the dominant orders (Figure





**Figure 2.** Composition of fruiting bodies (A) and fungal strains (B) from Chuuk and Kosrae, the Federated States of Micronesia.



**Figure 3.** Major species of fruiting bodies (A–H) and fungal strains (I–P) from Chuuk and Kosrae, the Federated States of Micronesia. (A) *Auricularia polytricha*, (B) *Coprinellus disseminates*, (C) *Fomitopsis ostreiformis*, (D) *Funalia aspera*, (E) *Leiotrametes menziesii*, (F) *Lentinus squarrosulus*, (G) *Marasmius palmivorus*, (H) *Tinctoporellus epimiltinus*, (I) *Aspergillus aculeatinus*, (J) *Aspergillus brunneoviolaceus*, (K) *Aspergillus neoniger*, (L) *Galactomyces candidum*, (M) *Gongronella butleri*, (N) *Penicillium citrinum*, (O) *Trichoderma guizhouense*, and (P) *Trichoderma reesei*.

2(B)). The dominant species were *Trichoderma reesei* ( $n = 115$ ), followed by *Galactomyces candidum* ( $n = 41$ ), *F. ostreiformis* ( $n = 37$ ), and *Penicillium citrinum* ( $n = 32$ ) (Table 2, Figure 2(B), Figure 3(I–P)).

### 3.2. Source comparison

Fruiting bodies were collected from various host trees: *Mangifera indica* (19 fungal species from 21 strains), *Cocos nucifera* (13 spp. from 19 strains),

*Artocarpus altilis* (12 spp. from 14 strains), *Rhizophora* sp. (9 spp. from 14 strains), and others (31 spp. from 50 strains) (Table 1). *L. menziesii* and *A. polytricha* were commonly found across various host trees, whereas many other species were found on only one or two different host trees. Fruiting bodies of four species (*Coprinopsis strossmayeri*, *Lentinus tuberregium*, *Phallus atrovolvatus*, and *Psathyrella luteopalida*) were found in the forest soil.

Fungal strains were isolated from both marine (39 spp. from 139 strains) and terrestrial environments (27 spp. from 212 strains) (Table 2). *G. candidum* was commonly isolated from marine environments, while *T. reesei* was more abundant in the terrestrial environment. Eight species, including *P. citrinum* and *T. reesei*, were isolated from both environments; however, most species were isolated from either marine (31 spp.) or terrestrial (19 spp.) environments. Various species were found in the seaweed (27 spp. from 85 strains), sea sand (18 spp. from 54 strains), and rotten wood (27 spp. from 212 strains) (Table 2). *Aspergillus brunneoviolaceus*, *A. neoniger*, *G. candidum*, *P. citrinum*, and *T. reesei* were the species shared across all sources, while many were unique to each source: seaweed (20 spp.), sea sand (10 spp.), and rotten wood (19 spp.). *G. candidum*, *P. citrinum*, and *T. reesei* were the dominant species residing in seaweed, sea sand, and rotten wood, respectively (Table 2).

### 3.3. Species inventory update

By analyzing previous records of fungi in the FSM, dating from 1913, we made a list of 236 species from Yap, Chuuk, Pohnpei, and Kosrae (Table S1). Only 58 of these had been identified using sequence data. The listed fungal species were detected in various sources, including diseased plants (101 spp.), rotting wood (66 spp.), house dust (44 spp.), lichens (14 spp.), and forest soil (3 spp.). Some species (8 spp.) from the marine environment were identified using metagenomics. Most of the listed species belonged to Ascomycota (144 spp., 61.0%) and Basidiomycota (90 spp., 38.1%), while three and one species belonged to Mucoromycota and Glomeromycota, respectively (Table S1).

We added 78 new species to the FSM fungal inventory, resulting in a total of 314 fungal species as follows: Ascomycota ( $n = 193$ , 61.5%), Basidiomycota ( $n = 117$ , 37.3%), Mucoromycota ( $n = 3$ , 1.0%), and Glomeromycota ( $n = 1$ , 0.3%).

## 4. Discussion

We conducted a survey of fungal diversity at Chuuk and Kosrae states in the FSM. Based on molecular

and morphological identification, we identified a total of 99 fungal species from various environments. Although 236 fungal species have been previously reported in the FSM, sequence information is available for only a few species [4,16,17]. Morphological features are not enough to identify most fungal species at the species level because of their similar morphological characteristics and variations depending on the environment [17,37–40]. Therefore, we verified the identification of the 99 species collected in this study by performing a thorough sequence analysis, in addition to morphological identification. The morphology-based identifications are currently being verified by sequence-based identification. Sequence analysis plays an important role in distinguishing morphologically similar species and reducing the rate of misidentification. Hence, it is necessary to develop a standardized method for each taxon, based on sequencing, and construct a reliable global database [41]. The sequence information generated in this study will be useful to researchers for the accurate identification of various fungi and for conducting further studies on the fungal diversity of the FSM.

Environmental filtering by hosts, habitats, and sources is an important factor influencing fungal communities [42–44]. In this study, although the sample size was small, many fruiting bodies showed host preference. A previous study has also reported that some fruiting bodies (polypore fungi) in the FSM show host and habitat specificity [7]. The fungal diversity in our study also varied according to the habitat and source. Only eight of the 58 isolated fungal species were commonly found in both marine and terrestrial habitats, while most of the species were unique to their sources: sea sand, seaweed, and wood. We also found that many species isolated from the marine environment were previously not recorded in the FSM. The discovery of many unrecorded species in our study may be due to the inclusion of new environments in the survey. Recent studies have shown that various fungal species inhabit marine environments [45–47]. *Annulohyphoxylon stygium*, an unrecorded species, was isolated only from seaweeds in this study. It produces various secondary metabolites and enzymes [48,49] and provides nutrients to *Tremella fuciformis* for the growth and development of fruiting bodies [50]. Recently, *A. stygium* isolated from seaweed has been shown to produce novel molecules that can be used as UV filters [51]. Therefore, investigating various environments may lead to the discovery of industrially and medicinally useful fungal species.

Though the FSM is a small country, it is known as a biodiversity hotspot with high plant and animal

diversity. Despite this, there has been relatively little research on its fungal diversity. Although we surveyed only a limited number of sites and sources, we confirmed the presence of 99 fungal species, including at least 78 previously unrecorded species in the FSM, based on morphological features and sequence information. In addition, we constructed a new fungal inventory of the FSM, along with the results of previous studies. Further extensive research across both marine and terrestrial environments should be conducted to discover new fungal species. This study will be an important basis for the discovery of new bioresources and the monitoring of fungi based on ecological changes caused by urban development in the FSM.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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