Conference Proceeding

LANGKAWI INTERNATIONAL MULTIDISCIPLINARY ACADEMIC CONFERENCE

Langkawi Seaview Hotel, Pulau Langkawi, MALAYSIA

21 December 2019

Asian Scholar Network (002903215-H)

eISBN: 978-967-16859-4-5

LANGKAWI INTERNATIONAL MULTIDISCIPLINARY ACADEMIC CONFERENCE (LIMAC 2019)

eISBN: 978-967-16859-4-5

Published By:
Asian Scholars Network (002903215-H)
Selangor, Malaysia

TABLE OF CONTENTS

Proceeding: Langkawi International Multidisciplinary Academic Conference (LIMAC 2019)
(eISBN: 978-967-16859-4-5)
Langkawi Seaview Hotel, Pulau Langkawi, MALAYSIA

THE FUNGAL DIVERSITY OF MADAI CAVE, SABAH, MALAYSIA

Ibrahem G. Wasti[1,2*], Jaya Seelan Sathiya Seelan[1]

[1] Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, 88400 Jalan UMS, Kota Kinabalu, Sabah, Malaysia.
[2] Faculty of Natural Science and Sustainability, University College Sabah Foundation, Jalan Sanzac, Sembulan, 88100 Kota Kinabalu. Sabah

*Corresponding Author: ibrahemghaniwasti@ucsf.my.edu

Abstract: Borneo is one of the biodiversity hotspots of the world, but its limestone caves are one of its least studied ecosystems. Studies of the cave mycobiota of Sabah is essentially non-existent. In this study, speleothem (n = 4), cavern water (n =4), and bat guano (n = 4) samples were collected and studied for their fungal diversity and abundance. Opportunistic sampling was utilized and the dilution method was performed during isolation. Fifty-five culturable fungi were isolated of which 32 species from 15 genera, nine orders, and two phyla were observed. Morphological characterization and molecular analysis of the ITS gene region were utilized for identification of the isolates. The average fungal abundance count for speleothem samples were 229.3 CFU/cm2, cavern water was 335.0 CFU/ml, and bat guano was 6266.7 CFU/g. Simpson and Shannon diversity indices indicated that speleothem samples had the highest fungal diversity, followed by bat guano, and cavern water. Speleothem also resulted in the most pure isolates (n=23) and distinct fungal taxa (n=19). Ascomycetes dominated the fungal composition of all sample types, accounting for 53 out of 55 total isolates. The remaining two isolates were both Basidiomycetes. The most abundant genera recovered from cave samples was Penicillium, but Aspergillus spp. had the highest occurrence as they were isolated from all samples except for one. Purpureocillium lilacinum was the species with the highest occurrence, appearing in five separate samples from all three substrate types. This study serves to produce baseline data useful for further research on the mycoflora of Sabah's various ecosystems. We urge that visitors should be more aware of the potential risks and disturbance they cause to microbial communities when entering cave environments.

Keywords: Mycology, Ecology, Speleology

1. Introduction

Caves are unique ecosystems that are underexplored in Borneo with regards to its microorganisms, especially the role of fungi. Distinct from the external environment, caves are dark, relatively cool, humid, and nutrient-limited by nature (Gabriel and Northup, 2013; Zhang et al., 2017; Zhang et al., 2018). The lack of photosynthetic organisms influences the oligotrophic nature of the cave, which in turn influences its mycofloral diversity (Gunde-Cimerman et al., 1998; Hose et al., 2000; Barton and Jurado, 2007; Kuzmina et al., 2012; Ogórek et al., 2013; Gabriel and Northup, 2013). Fungi are some of the most dominant organisms in caves due to the high rate of spore dissemination, colonization capability in various substrates, and tolerance to a wide range of pH values (Nováková, 2009; Bastian et al., 2010; Wang et al., 2010; Ogórek et al., 2013). Over 1150 species of fungi have been recorded from caves throughout the world, in which most are Ascomycota, followed by Basidiomycota and Zygomycota at lesser rates (Vanderwolf et al., 2013). While many if the fungi isolated from caves are ubiquitous and likely stem from the external environment, some suspected obligate troglobitic fungi have been reported, such as

Acaulium caviariforme, *Aspergillus baecitus*, and *Aspergillus thesauricus*. Although, whether there are any true obligate troglobitic fungi is still up for debate. It is estimated that only 3-8% of all fungi have been identified and described and that an overwhelming majority of extant fungi are yet to be discovered (Hawksworth and Lücking, 2017).

Fungi play vital roles in the ecosystems they inhabit, whether as saprophytes, symbionts, parasites, or food sources (Bastian et al., 2010; Araújo and Hughes, 2016). Mycoses are rapidly becoming one of the leading threats to wildlife as numerous epidemics have been reported all over the world, including in tropical regions due to its warm and humid climates (Jurado et al., 2010; Hsu et al., 2012; Fisher et al., 2012). Histoplasmosis is a potentially deadly mammalian disease caused by *Histoplasma capsulatum* and are common in soil enriched with bat guano, and it has been recorded from Malaysian cave environments (Ponnampalam, 1963). *Pseudogymnoascus destructans*, the causal agent of White-nose syndrome (WNS) in bats (Puechmaille et al., 2011), *Batrachochytrium dendrobatidis*, the causal agent of chytridiomycosis in amphibians (Fisher et al., 2009), and *Ophodiomyces ophiodiicola*, the causal agent of Snake fungal disease (Lorch et al., 2016), are all severe, and often fatal, skin related infections widely distributed in the continents they exist in. These pathogenic fungi are able to infect a broad range of species, and they are likely able to persist on abiotic substrates long enough to increase likelihood of successful dispersal to other regions and infect novel host populations (Eskew and Todd, 2013; Lorch et al., 2016). While there are no recorded cases of these deletrious fungi in Sabah, research on microfungi in this region is extremely scant.

Cave fungi have been isolated from many substrates and reservoirs, including sediment, wall, speleothem, guano, water, air, and various fauna (Jurado et al., 2008; Vanderwolf et al., 2013). Endolithic growth of lithobiontic fungi can biologically weather rock surfaces and can help stabilize and preserve rock surface morphology (Hoppert et al., 2004). *Fusarium* spp. are able to biogenically weather concrete and produce calcium organic complexes. Also, it has been shown that entomogenous fungal spores can colonize and grow on any rock surface that has even minute traces of carbon (Wainwright et al., 1993; Barton & Jurado, 2007). Cavern water movement indirectly affect cave mycoflora in various capacities and have been sampled for fungal isolation (Cunningham et al., 1995; Vanderwolf et al., 2013). Non-native fungal species and organic material may be introduced into the cave via water movement in and out of the caves as well as vertical filtration of rainwater from the soil above the cave itself (Dupont et al., 2007; Ikner et al., 2007). Wet surfaces are also less conducive for fungal spore dispersal in caves compared to dry periods due to the differences in surface adhesion (Jones and Harrison, 2004). The moist nature, stable temperatures, and abundance of nutrients in bat guano makes it one of the most dominant fungal substrate in caves, as it can serve as its own micro-ecosystem (Paulson, 1972; Nieves-Rivera et al., 2009).

Subterranean ecosystems are interesting as they can be a source of organisms that have adapted to tolerate relatively unfavorable life conditions, including fungi (Ogórek et al., 2017). There is great potential for fungi, especially undiscovered species, to contribute to industry due to their enzymatic properties (Niehaus et al., 1999). This study is the first of its kind in Madai Cave, and establishing baseline data can work to propel further ecological research and industrial application with regards to fungi studies in Borneo.

2. Materials and Methods

2.1 Site description

Madai Cave, Baturong Madai Forest Reserve, Class VI (Virgin Forest), Kunak, Sabah (4°41'10.01”N, 118°15'4.12"E) was visited on 28-29 November, 2017 (Fig. 1). A small village is located immediately outside of the cave entrance, and there are two main chambers of the cave.

The first one has an entrance at the ground level, which is where the sampling for this study took place. A second chamber is located a short hike up the limestone hill, past some ancient burial sites. The air temperature in the cave on the day of sampling fluctuated around 26 ^{o}C, and rose to around 29 ^{o}C near the cave entrance. The air humidity ranged between 92 to 100%. The cave itself is open to the public during the offseason for swiftlet nest harvesting, often visited by large groups of foreigners and locals alike.

Figure 1: Madai Cave. A. Cave entrance. B. Researcher collecting guano sample. C. Speleothem. D. Guano pile. E. Cave stream deep in the cave. F. Village children playing near the cave entrance. G. Visible graffiti on cave wall. H. Land use for palm oil surrounding Baturong Madai Forest Reserve.

2.2 Sampling and Identification

Opportunistic sampling of speleothem, cavern water, and bat guano was utilized, in which four samples of each substrate were acquired from different parts of the cave. All samples were collected in triplicate. Speleothem were sampled using the swab method (Ikner et al., 2007; Vaughan et al., 2011). Guano samples (10 g) were collected using sterile sample tubes and capped and sealed (Nieves-Rivera et al., 2003). Ten ml of cavern water were also collected and sealed in sterile sample tubes. All samples were chilled in ice (<4 ^{o}C) until transported to the laboratory.

In the laboratory, samples were placed in Petri dishes onto Potato Dextrose Agar (PDA) using serial dilution, which were incubated from 7-14 days at 25 ± 1 ^{o}C. The colonies that appeared on the medium were categorized by Morphological Taxonomic Units (MTU) and counted. Pure isolates were obtained using the three–point method before morphological and genetic analysis. Morphological identification were performed based on universal identification keys described by Raper and Fennell (1965), Klich (2002) and Domsch et al., (1980).

DNA from fungi cultured on PDA were extracted using the E.Z.N.A. DNA Fungal Kit (Omega Bio-Tek, USA) using the manufacturer's instructions. The internal transcribed spacer region of fungal rDNA was amplified using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). Polymerase chain reactions were performed in a Bio Rad T100 Thermal Cycler according to Ogórek et al. (2016). The PCR products were electrophoresed in a 1 % agarose gel for 30 mins, which was stained with gel red for visualization. The PCR products were purified using Colum-Pure PCR Clean-Up Kit (Applied Biological Materials, Inc.) according to manufacturer protocols and sequenced by MyTACG (Taiwan).

2.3 Data Analysis

BioEdit Sequence Alignment Editor was used for producing assembled ITS fungal sequences and subsequent analysis. Then, fungi were put through the BLASTn algorithm (http://www.ncbi.nlm.nih.gov/) which compared the obtained sequences with those deposited in the GenBank database.

The obtained data from the serial dilution of fungal colonies cultured in Petri dishes were expressed as colony-forming units (CFU) per centimeter squared for speleothem surfaces, per milliliter for cavern water, and per gram for bat guano samples. This was done using the formula $X = (a \times p)/V$, where "*a*" is the number of colonies, "*V*" is the inoculation aliquot volume, and "*p*" is the dilution factor. The abundance data was then used for diversity and evenness analysis for each substrate using PAST 3.10 software. Linear regression analysis (Ordinary Least Squares) on sample fungal abundance and occurrence data were tested against the distance from cave entrance to analyze any correlation, this was done using R software v 3.4.0.

3.0 Results

Altogether, a total of 55 pure isolates were obtained from four speleothem samples (n=23), four cavern water samples (n=15), and four bat guano samples in Madai Cave (n=17) (Table 1). The fungi were separated into 32 species, 15 genera, nine orders, and two phyla, namely Ascomycota and Basidiomycota. Only two out of 32 species were identified as Basiodiomycota, *Ganoderma australe* and *Pyrrhoderma noxium*. Out of the 55 isolates, 31 were corroborated by BLASTn results, which included 24 out of 32 total species identified (Table 2). All BLASTn results had Identity matches over 96.7%, except for *Talaromyces flavus*, which had and Identity match of 92.2%. The fungus that had the most frequent occurrence was *Purpureocillium lilacinum*, which appeared from five different samples including all three substrates. However, the genus that was isolated most frequently was *Aspergillus*, making up 20 (36.4%) of the 55 total isolates and appeared from all samples collected except for one guano sample. Based on fungal abundance data, *Penicillium* spp. dominated fungal composition accounting for 48.9% of speleothem fungi, 56.3% of cavern water fungi, and 47.1% of bat guano fungi (Fig. 2).

Table 1: Average abundance of culturable fungi of Madai Cave, Sabah (CFU per unit sample).

Sample (Distance from Entrance)	Fungal Taxa	Identification	CFU/*unit*[a]	
			Fungal Taxa	Sample Total
Speleothem				$\bar{x}$ = 229.3
S1 (1 m)	*Aspergillus* sp. 3	Morphology	72.9	554.7
	Aspergillus flavus Link	Morphology	222.7	
	Penicillium paxilli Bainier	Morphology	259.1	
S2 (16 m)	*Aspergillus aculeatus* Iizuka	Morphology	4.4	381.3
	Aspergillus flavus	Morphology	44.9	
	Penicillium citrinum Thom	Morphology	272.4	
	Plectosphaerella cucumerina (Lindf.) W. Gams	DNA[b]	15.1	
	Trichoderma harzianum Rifai	Morphology	44.4	
S3 (55 m)	*Annulohypoxylon nitens* (Ces.) Y.M. Ju, J.D. Rodgers, & H.M. Hsieh	DNA	62.7	175.1
	Aspergillus sp. 1	DNA	1.8	

	Aspergillus europaeus Hubka, A. Nováková, Samson, Houbraken, Frisvad, M. Kolařík	Morphology	4.4	
	Aspergillu niger Tiegh.	DNA	0.9	
	Ganoderma australe (Fr.) Pat.	DNA	49.8	
	Penicillium bilaiae Chalab.	DNA	44.4	
	Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson	DNA	4.9	
	Talaromyces flavus (Klöcker) Stolk & Samson	DNA	0.4	
	Trichoderma asperellum Samuels, Lieckf. & Nirenberg	Morphology	1.3	
	Xylaria feejeensis (Berk.) Fr.	DNA	4.4	
S4 (75 m)	*Aspergillus* sp. 2	Morphology	13.3	86.2
	Aspergillus nomius Kurtzman, B.W. Horn & Hesselt.	DNA	49.3	
	Penicillium citrinum	Morphology	9.3	
	Purpureocillium lilacinum	DNA	13.3	
	Trichoderma harzianum	Morphology	0.9	
Cavern Water				$\bar{x}$ = 335.0
W1 (3 m)	*Aspergillus japonicus* Saito	Morphology	25.6	744.4
	Penicillium sp. 1	Morphology	716.7	
	Purpureocillium lilacinum	DNA	2.2	
W2 (17 m)	*Aspergillus aculeatus*	Morphology	3.3	488.9
	Aspergillus flavus	Morphology	17.8	
	Aspergillus sydowii (Bainier & Sartory) Thom & Church	DNA	123.3	
	Purpureocillium lilacinum	DNA	176.7	
	Pyrrhoderma noxium (Corner) L.W. Zhou & Y.C. Dai	DNA	167.8	
W3 (30 m)	*Aspergillus aculeatus*	Morphology	36.7	81.1
	Aspergillus nomius	DNA	13.3	
	Penicillium paxilli	Morphology	30.0	
	Trichoderma harzianum	Morphology	1.1	
W4 (85 m)	*Aspergillus japonicus*	DNA	14.4	25.6
	Penicillium citrinum	DNA	7.8	
	Phaeosphaeriopsis sp.	DNA	3.3	
Bat Guano				$\bar{x}$ = 6266.7
G1 (26 m)	*Paecilomyces variotii* Bainier	Morphology	33.3	8,955.6
	Penicillium paxilli	Morphology	8,922.2	
G2 (30 m)	*Annuhypoxylon nitens*	DNA	111.1	13,622.2
	Aspergillus aculeatus	DNA	1,122.2	
	Cladosporium cladosporioides (Fresen.) G.A. de	DNA	111.1	

	Vries			
	Curvularia lunata (Wakker) Boedijn	DNA	1,111.1	
	Penicillium simplicissimum (Oudem.) Thom	DNA	2,877.8	
	Pochonia chlamydosporia (Goddard) Zare & W. Gams	DNA	1,111.1	
	Talaromyces minioluteus (Dierckx) Samson, Yilmaz, Frisvad & Seifert	DNA	5,944.4	
	Trichoderma paraviridescens Jaklitsch, Samuels & Voglmayr	DNA	1,233.3	
G3 (53 m)	*Aspergillus nomius*	DNA	1,111.1	1,111.1
G4 (75 m)	*Aspergillus* sp. 1	DNA	1,122.2	1,377.8
	Aspergillus niger	Morphology	22.2	
	Paecilomyces variotii	DNA	200.0	
	Penicillium sp. 2	Morphology	11.1	
	Purpureocillium lilacinum	Morphology	11.1	
	Trichoderma asperellum	DNA	11.1	

[a] CFU/cm^2 for speleothem samples, CFU/ml for cavern water samples, and CFU/g for bat guano samples.

[b] All molecular characterization is corroborated by morphological characterization.

Table 2: Culturable cave fungi of Madai Cave, Sabah BLASTn (GenBank) analysis.

Fungal Taxa	Source Sample	Temple	Query Cover (%)	Identities (%)	*E* value
Annulohypoxylon nitens	G2	KU684021	92%	99.3%	0.0
Annulohypoxylon nitens	S3	FN252415	98%	99.0%	0.0
Aspergillus sp. 1	G4	MK638758	100%	96.9%	0.0
Aspergillus sp. 1	S3	MH517369	100%	96.9%	0.0
Aspergillus aculeatus	G2	MK280716	100%	100.0%	0.0
Aspergillus japonicus	W4	KF800630	100%	100.0%	0.0
Aspergillus niger	S3	MK203789	100%	100.0%	0.0
Aspergillus nomius	G3	MH279416	99%	99.7%	0.0
Aspergillus nomius	S4	MH279388	99%	99.8%	0.0
Aspergillus nomius	W3	MH279387	100%	100.0%	0.0
Aspergillus sydowii	W2	KX674612	100%	100.0%	0.0
Cladosporium cladosporioides	G2	EF405864	99%	100.0%	0.0
Curvularia lunata	G2	JN116704	93%	100.0%	0.0
Ganoderma australe	S3	LC084692	94%	99.4%	0.0
Paecilomyces variotii	G4	FJ345354	99%	100.0%	0.0
Penicillium bilaiae	S3	LN901118	100%	96.7%	0.0
Penicillium citrinum	W4	GU566273	99%	99.5%	0.0
Penicillium simplicissimum	G2	HQ607866	99%	99.8%	0.0

Phaeosphaeriopsis sp.	W4	KF800300	99%	99.5%	0.0
Plectosphaerella cucumerina	S2	EU326201	97%	99.5%	0.0
Pochonia chlamydosporia	G2	EU266591	97%	99.8%	0.0
Purpureocillium lilacinum	S3	KY951911	100%	99.7%	0.0
Purpureocillium lilacinum	S4	MH860675	99%	99.8%	0.0
Purpureocillium lilacinum	W1	MH860675	100%	99.7%	0.0
Purpureocillium lilacinum	W2	MH860675	99%	98.6%	0.0
Pyrrhoderma noxium	W2	KU194338	99%	98.5%	0.0
Talaromyces flavus	S3	MH857785	99%	92.2%	0.0
Talaromyces minioluteus	G2	MH857785	100%	99.5%	0.0
Trichoderma asperellum	G4	KY623504	99%	100.0%	0.0
Trichoderma paraviridescens	G2	MF782827	99%	99.8%	0.0
Xylaria feejeensis	S3	KY951907	99%	99.7%	0.0

In speleothem samples, the average fungal abundance was 229.3 CFU cm^{2-1}. The single isolate which had the highest abundance count from speleothem samples was *Penicillium citrinum* (S1) with 272.4 CFU cm^{2-1}. The average fungal abundance of cavern water samples were 335.0 CFU ml^{-1}, and *Penicillium* sp. 1 (W1) had the highest abundance for a single isolate with 716.7 CFU ml^{-1}. In bat guano samples, the average fungal abundance was 6,266.7 CFU g^{-1}, and the single isolate which had the highest abundance count was *Penicillium paxilli* (G1) with 8,922.2 CFU g^{-1}. Utilizing the fungal abundance data, diversity and linear regression analyses were performed for each substrate. Shannon and Simpson alpha diversity indices showed that speleothem samples had the most diverse fungal communities, followed by bat guano, and then cavern water (Table 3). The Linear Regression analysis showed negative statistically significant relation ($r^2 = 0.0703$, $p < 0.05$) between fungal abundance in speleothem and distance from cave entrance (Table 4; Fig. 3). However, no other statistically significant result was observed between the remaining fungal abundance of the occurrence data and their relationship with distance from cave entrance.

Table 3: Diversity index scores based on abundance counts of all taxa isolated.

Cave	**Sample**	**Simpson** (1-D)	**Shannon** (*H*)	**Evenness** (e^H/S)
Madai	Bat guano	0.7914	1.906	0.4205
	Speleothem	0.8378	2.152	0.4526
	Cavern water	0.6694	1.532	0.3855

Table 4: Linear regression analysis (OLS method) correlating fungal occurrence and abundance with distance from cave entrance.

Substrate	**Distance (m)**	**Fungal occurrence**			**Fungal abundance**		
		r^2	**y=**	**p value**	**r^2**	**y=**	**p value**
Speleothem	6.3 – 28.7	0.7364	0.075x + 2.9949	0.1419	0.9703	-6.0631x + 522.15	0.0150*
Cavern water	3.0 – 85.0	0.1305	-0.0096x +	0.6388	0.6654	-7.7769x	0.1843

			4.0751			+ 597.47	
Bat guano	26.0 – 75.0	0.0033	0.0084x + 3.8632	0.9422	0.7059	-225.96x + 16661	0.1598

***** *p*-value for significance less than 0.05.

4. Discussion and Conclusion

Studies in limestones cave in Sabah have been limited to studies on its fauna, geology, or anthropogenic value. This is the first study on fungi in Madai Cave, as microbiology is often overlooked when studies are conducted in the cave ecosystem. Microorganisms are important in most ecosystems they are a part of mainly due to their role as decomposers, which makes them responsible for carbon cycling (Nielsen et al., 2011). In caves, microbes also act as food sources for invertebrates and act as parasites to cave insects (Vanderwolf et al., 2013).

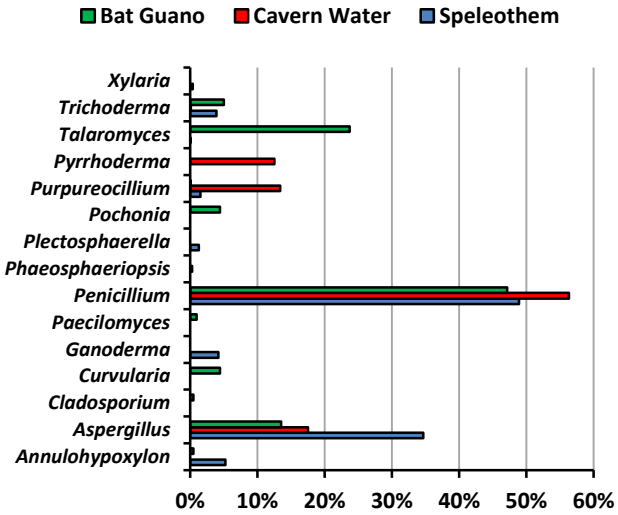


Figure 2: The percentage fungi by genus isolated from Madai Cave contributing to the total abundance count in the different substrates.

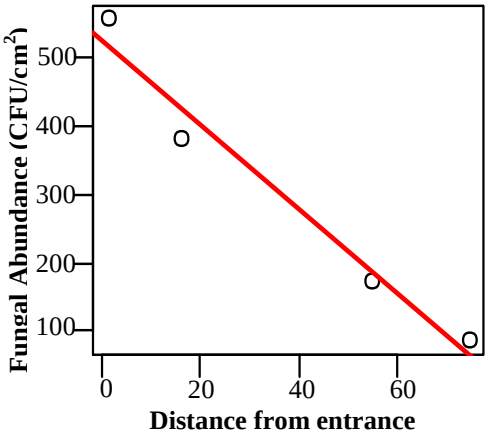


Figure 3: Scatterplot and regression line of the statistically significant outcome of linear regression analysis (OLS) for speleothem fungal abundance versus distance.

The phylum Ascomycota usually dominates the fungal composition of cave ecosystems, followed by Basidiomycota, Zygomycota, and others (Vanderwolf et al., 2013). Our results fell in line with previous fungal studies from cave ecosystems, as all but two pure isolates cultured were Ascomycota, especially with fungi in the genera *Penicillium* and *Aspergillus* being some of the most abundant fungi throughout all substrates. According to previous reviews on cave fungi, *Annulohypoxylon nitens*, *Aspergillus aculeatus*, *A. europaeus*, *A. nomius*, *Ganoderma australe*, *Penicillium bilaiae*, *Pyrrhoderma noxium*, *Talaromyces minioluteus*, *Trichoderma asperellum*, *T. paraviridescens*, and *Xylaria feejeensis* were discovered from cave samples worldwide for the first time in this study (Vanderwolf et al., 2013; Nováková et al., 2018). Many fungi, especially microfungi, can only be identified for certain to the genus level when morphological analysis or only a single gene marker is used due to overlap in similarities of phenotypes and genotypes (Schoch et al., 2012). Thus, in this study the utilization of a combination of traditional morphological characterization and modern molecular analysis proved extremely useful for identification to the species level.

Madai Cave is infested with various invertebrates and act as roosting sites for fauna such as bats and swiftlets. Animals are known to harbour fungi and act as likely disseminators of fungal spores within caves, either as hosts, vectors, or cadavers (Vanderwolf et al., 2013; Nováková et al., 2018. Evidence shows that human traffic into caves causes contamination of indigenous fungal species by non-indigenous microorganisms and are also responsible for introducing nutrients into the cave (Shapiro and Pringle, 2010; Porca et al., 2011; Griffin et al., 2014). Increased human visitation is also correlated to lower levels of fungal diversity. It is hard to determine exactly how anthropogenic activity influences Madai Cave's mycobiota, although evidence suggests that the fungal composition of this cave is likely to differ from less visited caves in Sabah.

This study on Madai Cave's mycobiome serves to develop baseline data to propel future research. Hitherto, due to the lack of effort and research on microfungi in Borneo, we are unaware of any deleterious fungal diseases towards the fauna and humans in Sabah's limestone caves, but opportunistic fungal pathogens do exist in Malaysia, including some of the *Aspergillus* spp. isolated in this study (Velayuthan and Denning, 2016). Borneo, especially Sabah, has a strong ecotourism industry, and with growing environmental limitations due to global climate change, tourists and researchers should be aware to the potential risks cave fungi and emerging infectious fungal diseases can pose to humans and local fauna. Ongoing studies on fungi from various caves in Sabah is currently in progress. We urge more mycological studies and surveys to be conducted not only to better understand their tremendous ecological impacts, but their enormous biological and industrial potential.

References

Araújo, J. P. M., & Hughes, D. P. (2016). Diversity of Entomopathogenic Fungi. Which Groups Conquered the Insect Body? *Advances in Genetics*, *94*, 1–39. https://doi.org/10.1016/bs.adgen.2016.01.001

Barton, H. A., & Jurado, V. (2007). What's Up Down There? Microbial Diversity in Caves Microorganisms in caves survive under nutrient-poor conditions and are metabolically versatile and unexpectedly diverse. *Microbe*, *2*(3), 132–138.

Bastian, F., Jurado, V., Nováková, A., Alabouvette, C., & Saiz-Jimenez, C. (2010). The microbiology of Lascaux Cave. *Microbiology*, 156(3), 644–652.

Cunningham, K. I., Northup, D. E., Pollastro, R. M., Wright, W. G., & LaRock, E. J. (1995). Bacteria, fungi and biokarst in Lechuguilla Cave, Carlsbad Caverns National Park, New Mexico. *Environmental Geology*, *25*(1), 2–8. https://doi.org/10.1007/BF01061824

Domsch, K. H., Gams, W., & Anderson, T. H. (1980). *Compendium of soil fungi. Volume 1*. Academic Press (London) Ltd..

Dupont, J., Jacquet, C., Dennetière, B., Lacoste, S., Bousta, F., Orial, G., & Roquebert, M. F. (2007). Invasion of the French Paleolithic painted cave of Lascaux by members of the Fusarium solani species complex. *Mycologia*,*99*(4), 526-533.

Eskew, E. A., & Todd, B. D. (2013). Parallels in amphibian and bat declines from pathogenic fungi. *Emerging Infectious Diseases*, *19*(3), 379–385. https://doi.org/10.3201/eid1903.120707

Fisher, M. C., Garner, T. W. J., & Walker, S. F. (2009). Global Emergence of Batrachochytrium dendrobatidis and Amphibian Chytridiomycosis in Space, Time, and Host . *Annual Review of Microbiology*, *63*(1), 291–310. https://doi.org/10.1146/annurev.micro.091208.073435

Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., & Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature*, *484*(7393), 186.

Gabriel, C. R., & Northup, D. E. (2013). Microbial ecology: caves as an extreme habitat. In *Cave*

microbiomes: a novel resource for drug discovery (pp. 85-108). Springer, New York, NY.
Griffin, D. W., Gray, M. A., Lyles, M. B., & Northup, D. E. (2014). The transport of nonindigenous microorganisms into caves by human visitation: a case study at Carlsbad Caverns National Park. *Geomicrobiology Journal*, *31*(3), 175-185.
Hawksworth, D. L., & Lücking, R. (2017). Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology spectrum*, *5*(4).
Hoppert, M., Flies, C., Pohl, W., Günzl, B., & Schneider, J. (2004). Colonization strategies of lithobiontic microorganisms on carbonate rocks. *Environmental Geology*, *46*(3-4), 421-428.
Hsu, L. Y., Wijaya, L., Ng, E. S. T., & Gotuzzo, E. (2012). Tropical fungal infections. *Infectious disease clinics of North America*, *26*(2), 497-512.
Ikner, L. A., Toomey, R. S., Nolan, G., Neilson, J. W., Pryor, B. M., & Maier, R. M. (2007). Culturable microbial diversity and the impact of tourism in Kartchner Caverns, Arizona. *Microbial ecology*, *53*(1), 30-42.
Jones, A. M., & Harrison, R. M. (2004). The effects of meteorological factors on atmospheric bioaerosol concentrations–a review. *Science of the Total Environment*, *326*(1), 151-180.
Jurado, V., Sanchez-Moral, S., & Saiz-Jimenez, C. (2008). Entomogenous fungi and the conservation of the cultural heritage: A review. *International Biodeterioration and Biodegradation*, *62*(4), 325–330. https://doi.org/10.1016/j.ibiod.2008.05.002
Jurado, V., Laiz, L., Rodriguez-Nava, V., Boiron, P., Hermosin, B., Sanchez-Moral, S., & Saiz-Jimenez, C. (2010). Pathogenic and opportunistic microorganisms in caves. *International Journal of Speleology*, *39*(1), 15–24. doi:10.5038/1827-806x.39.1.2
Klich, M. A. (2002). Biogeography of Aspergillus species in soil and litter. Mycologia, 94(1), 21-27.
Kuzmina, L. Y., Galimzianova, N. F., Abdullin, S. R., & Ryabova, A. S. (2012). Microbiota of the Kinderlinskaya cave (South Urals, Russia). *Microbiology*, *81*(2), 251–258. https://doi.org/10.1134/S0026261712010109
Lorch, J. M., Knowles, S., Lankton, J. S., Michell, K., Edwards, J. L., Kapfer, J. M., … Blehert, D. S. (2016). Snake fungal disease: An emerging threat to wild snakes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1709). https://doi.org/10.1098/rstb.2015.0457
Niehaus, F., Bertoldo, C., Kähler, M., & Antranikian, G. (1999). Extremophiles as a source of novel enzymes for industrial application. *Applied microbiology and biotechnology*, *51*(6), 711-729.
Nielsen, U. N., Ayres, E., Wall, D. H., & Bardgett, R. D. (2011). Soil biodiversity and carbon cycling: A review and synthesis of studies examining diversity-function relationships. *European Journal of Soil Science*, *62*(1), 105–116. https://doi.org/10.1111/j.1365-2389.2010.01314.x
Nieves-Rivera, Á. M. (2003). Mycological survey of Río Camuy Caves Park, Puerto Rico. *Journal of Cave and Karst Studies*, *65*(1), 23-28.
Nieves-Rivera, Á. M., Santos-Flores, C. J., Dugan, F. M., & Miller, T. E. (2009). Guanophilic fungi in three caves of southwestern Puerto Rico.
Nováková, A. (2009). Microscopic fungi isolated from the Domica Cave system (Slovak Karst National Park, Slovakia). A review. *International Journal of Speleology*, *38*(1), 71–82. https://doi.org/10.5038/1827-806X.38.1.8
Nováková, A., Kubátová, A., Sklenář, F., & Hubka, V. (2018). Microscopic fungi on cadavers and skeletons from cave and mine environments. *Czech Mycology*, *70*(2), 101–121.
Ogórek, R., Lejman, A., & Matkowski, K. (2013). Fungi isolated from Niedźwiedzia Cave in Kletno (Lower Silesia, Poland). *International Journal of Speleology*, *42*(2), 161–166. https://doi.org/10.5038/1827-806X.42.2.9

Ogórek, R., Pusz, W., Zagożdżon, P. P., Kozak, B., & Bujak, H. (2017). Abundance and Diversity of Psychrotolerant Cultivable Mycobiota in Winter of a Former Aluminous Shale Mine. *Geomicrobiology Journal*, *34*(10), 823–833. https://doi.org/10.1080/01490451.2017.1280860

Paulson, T. L. (1972). Bat guano ecosystems. *Bulletin of the National Speleological Society*, *34*(2), 55-59.

Ponnampalam, J. (1963). Isolation of Histoplasma capsulatum from the soil of a cave in central Malaya. *The American journal of tropical medicine and hygiene*, *12*(5), 775-776.

Porca, E., Jurado, V., Martin-Sanchez, P. M., Hermosin, B., Bastian, F., Alabouvette, C., & Saiz-Jimenez, C. (2011). Aerobiology: An ecological indicator for early detection and control of fungal outbreaks in caves. *Ecological Indicators*, *11*(6), 1594–1598. https://doi.org/10.1016/j.ecolind.2011.04.003

Puechmaille, S. J., Frick, W. F., Kunz, T. H., Racey, P. A., Voigt, C. C., Wibbelt, G., & Teeling, E. C. (2011). White-nose syndrome: Is this emerging disease a threat to European bats? *Trends in Ecology & Evolution*, *26*(11), 570-576. doi:10.1016/j.tree.2011.06.013

Raper, K. B., & Fennel, D. I. (1965). Aspergillus terreus group. *The genus Aspergillus. The Williams & Wilkins Co., Baltimore, Md*.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., ... & Fungal Barcoding Consortium. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, *109*(16), 6241-6246.

Shapiro, J., & Pringle, A. (2010). Anthropogenic influences on the diversity of fungi isolated from caves in Kentucky and Tennessee. *The American Midland Naturalist*, *163*(1), 76-87.

Vanderwolf, K. J., Malloch, D., Mcalpine, D. F., & Forbes, G. J. (2013). A world review of fungi, yeasts, and slime molds in caves. *International Journal of Speleology*, *42*(1), 77–96. https://doi.org/10.5038/1827-806X.42.1.9

Vaughan, M. J., Maier, R. M., & Pryor, B. M. (2011). Fungal communities on speleothem surfaces in Kartchner Caverns, Arizona, USA. *International Journal of Speleology*, *40*(1), 8.

Velayuthan, R. D., & Denning, D. W. (2016). Estimation of serious fungal disease burden in Malaysia.*Fungal disease epidemiology & clinical trials*.

Wainwright, M., Ali, T. A., & Barakah, F. (1993). A review of the role of oligotrophic micro-organisms in biodeterioration. *International Biodeterioration & Biodegradation*, *31*(1), 1-13.

Wang, W., Ma, X., Ma, Y., Mao, L., Wu, F., Ma, X., & Feng, H. (2010). Seasonal dynamics of airborne fungi in different caves of the Mogao Grottoes, Dunhuang, China. *International Biodeterioration & Biodegradation*, 64(6), 461-466.

Zhang, Z. F., Liu, F., Zhou, X., Liu, X. Z., Liu, S. J., & Cai, L. (2017). Culturable mycobiota from Karst caves in China, with descriptions of 20 new species. *Persoonia - Molecular Phylogeny and Evolution of Fungi*, *39*(1), 1–31. https://doi.org/10.3767/persoonia.2017.39.01

Zhang, Z., Zhao, P., & Cai, L. (2018). Origin of Cave Fungi, *9*(June), 1–10. https://doi.org/10.3389/fmicb.2018.01407

LANGKAWI INTERNATIONAL MULTIDISCIPLINARY ACADEMIC CONFERENCE

(LIMAC 2019)

ORGANIZING COMMITTEE

Chairman

Ts. Dr. Zahari Abu Bakar

Treasurer

Hanisah Taib

Technical Committee

Rohaida Hanum Mohd Hassan

Muhammad Aminuddin Zahari

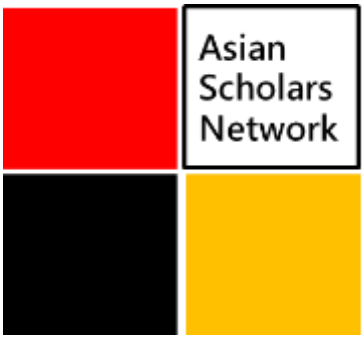
Technical Reviewer

Dr. Safaie Mangir

Dr. Lee Khai Loon

Dr. Mohd Yazid Md Taib

Published by:
Asian Scholars Network
(002903215-H)



eISBN 978-967-16859-4-5