

ENVIRONMENTAL DNA METABARCODING OF FUNGI ASSOCIATED WITH BAT GUANO IN WANG BURMA CAVE (PERLIS, PENINSULAR MALAYSIA)

Sanjeef Kumr Subramaniam^{1,2,3}, Nur Muhammad Taufiq-Makmor⁴, Tan Weng Hui⁴, Mohammed Rizman-Idid^{1,2}, Hasmahzaiti Omar⁴, Abiramy Krishnan⁵, and Siti Aisyah Alias^{1,2,C}

Abstract

Discoveries of unique, undescribed cave-dwelling species exhibiting extreme endemism demonstrate their biodiversity richness and specialization to such exclusive environments. Studies on fungi in caves enable understanding of their diversity, distribution, evolution, and physiology, and also facilitate the discovery of harmful pathogens or beneficial species. We hypothesize that Ascomycota would be the dominant phylum associated with bat guano samples, consistent with previous findings from Asian cave systems. This study is the first to document cave fungi species in Peninsular Malaysia through environmental DNA metabarcoding, specifically to identify the species composition of fungi within bat guano samples from Wang Burma Cave, Perlis. Thirty-two distinct amplicon sequence variants were detected among 380,856 sequence reads, revealing Ascomycota as the dominant fungal phylum (>99.99%). Thirteen species were identified, including five new records for Malaysia: *Aspergillus caninus*, *A. chlamydosporus*, *A. phialosimplex*, *Candida palmioleophila*, and *Mortierella rhinolophicola*. *Penicillium simplicissimum* was the most dominant species (67%) across all sampling sites. This study provides the first molecular record of cave-associated fungi in Peninsular Malaysia and highlights the ecological and conservation significance of subterranean fungal communities as indicators of cave ecosystem health.

INTRODUCTION

Fungi are incredibly diverse and have crucial ecological functions worldwide (Tedersoo et al., 2014; Powell and Rillig, 2018). Fungi can be found in a variety of forms in cave environments; each one adapted to the special circumstances present in these subterranean habitats. A specific collection of environmental factors that affect the growth and development of fungi is present in caves (Farda et al., 2022). These conditions include low light levels, high humidity, stable temperatures, and limited nutrient availability (Ren et al., 2021; Mammola et al., 2022). As a result, cave fungi have evolved fascinating adaptations to thrive in such harsh conditions.

Cave fungi refer to the various species of fungi that are specifically adapted to and found within cave environments. Cave fungi are sometimes also known as troglobitic fungi, and the term “troglobitic” is derived from the Greek words “troglos” (cave) and “bios” (life), indicating their specialized adaptation to cave habitats (Saikia and Ruedi, 2021). Strict definition of troglobitic fungi would refer to a species or population that is bound to a subterranean habitat strictly, in other words, true cave life (Sket, 2008). Real obligate troglobitic fungi imply a cave origin and evolution. However, most of the currently discovered fungi from caves were known from other environments (Vanderwolf et al., 2013; Zhang et al., 2017). Zhang et al. (2017) suggested that cave fungi may have an origin from outside the cave environment. Cave fungi have unique characteristics and adaptations that allow them to thrive in the challenging conditions found within caves. These fungi play important ecological roles within cave ecosystems. They often act as decomposers, breaking down organic matter such as bat guano, decaying plant material, or animal remains and recycling nutrients back into the cave ecosystem (Nieves-Rivera et al., 2009; Karkun et al., 2012; Out et al., 2016). In fact, one of the most excellent substrates for the development and reproduction of cave fungi is bat guano (Ogórek et al., 2016; Dimkić et al., 2021). The diet (frugivorous, insectivorous, or sanguivorous) of the bat may have an impact on the nutrient content of its guano (Emerson and Roark, 2007; Cunha et al., 2020). Studying cave fungi requires specialized methods and techniques due to their unique habitat and adaptations. Once collected, cave fungal specimens are examined and identified by mycologists using morphological, microscopic, and molecular techniques (Alsohaili and Bani-Hasan, 2018). Morphological characteristics, such as spore shape, size, and color, along with the presence or absence of specific structures, are examined under microscopes.

Research has revealed diverse fungal communities, with species belonging to common genera like *Aspergillus*, *Penicillium*, and *Cladosporium* (Docampo et al., 2011; Cunha et al., 2020). These examples represent a glimpse into the research conducted on cave fungi in Asia. Research on cave fungi in Asia has yielded valuable insights into the

¹Institute of Ocean and Earth Sciences, Advanced Studies Complex, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

²National Antarctic Research Centre, Advanced Studies Complex, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

³Institute for Advanced Studies, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

⁴Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

⁵MAHSA University Malaysia, Bandar Saujana Putra, 42610 Jenjarom, Selangor, Malaysia

^CCorresponding author: saa@um.edu.my.

biodiversity, ecology, and adaptations of these unique cave-dwelling fungi. Asian countries with extensive cave systems, such as China, Thailand, Cambodia, Vietnam, and Malaysia, have been significant focal points for such studies (Durringer et al., 2012). In China, studies have documented several new species and provided insights into their taxonomy, distribution, and ecological roles. For instance, research in Guangxi and Guizhou provinces revealed diverse fungal communities in caves, including species from genera like *Aspergillus*, *Penicillium*, and *Trichoderma* (Man et al., 2015; Huang et al., 2021; Zhang et al., 2021). In the northern region of Thailand, investigation of cave fungal diversity and physiology reported the occurrence of the genera *Fusarium*, *Aspergillus*, and *Penicillium*, among others (Cyske et al., 2021; Suphaphimol et al., 2022). While, in Cambodia and Vietnam, investigations have been conducted in caves across different regions, including the famous Bayon temple sandstone of Angkor Thom and Phong Nha-Ke Bang National Park, respectively (Lan et al., 2010; Trinh et al., 2018). Researchers have discovered new cave fungal species and examined their phylogenetic relationships. Studies have also explored the potential biotechnological applications of cave fungi, such as their production of enzymes with industrial relevance (Tavares et al., 2018; Paula et al., 2019; Fernández-Remacha et al., 2022).

Cave fungi in Malaysia have been the subject of scientific research, particularly in the cave-rich regions of Sarawak and Sabah on the island of Borneo (Hunt et al., 2007; Wasti et al., 2020). Although the richness of fungal species in caves is well established, this study uniquely documents fungi associated with bat guano within Wang Burma Cave, contributing novel records for Peninsular Malaysia. Existing published research on cave fungi in Malaysia has only employed the extraction of DNA and alignment of nucleotide sequences from pure isolates. Environmental DNA (eDNA) metabarcoding is used to determine the evolutionary relationships and species boundaries of the collected fungi. Cave fungi are often challenging to cultivate in the laboratory due to their specialized adaptations and nutrient requirements. However, researchers may attempt eDNA metabarcoding approaches to study the fungal diversity by sequencing of amplicons (Hamad et al., 2017). Therefore, the present study will be the first to employ eDNA metabarcoding for the identification of fungi and the analysis of fungal diversity in a cave in Peninsular Malaysia. The chosen cave for this study is known as Wang Burma Cave, located in the pristine limestone karst landscape of Perlis State Park in northern Peninsular Malaysia. Although the cave is a prominent natural heritage site under UNESCO with conservation efforts on charismatic cave species, such as bats and rare invertebrates, the cave's microbial biodiversity—particularly fungi—remains largely overlooked. Thus, studying the fungal communities associated with bat guano from this cave through eDNA metabarcoding will contribute toward Malaysia's cave fungal species diversity checklist.

MATERIALS AND METHODS

Study Site and Location of the Cave

The study was conducted at Wang Burma 1 Cave, which is a totally dark and dry cave (Anuwar et al., 2020). The Wang Burma 1 Cave is a part of the permanently reserved forest of Perlis State Park, Perlis, Peninsular Malaysia (6°41'51"N 100°11'53"E), and is accessible to guided visitors. Three bat guano sample collection sites (designated as WBA, WBB and WBC) were established within the middle chamber, approximately 100 m from the entrance, with WBA and WBB located 5 m apart and WBC located near the sidewall. A schematic cave map indicating sampling sites is included in Figure 1. Each of the collection sites was fixed at least 5 m apart from one another.

The sampling sites had bat inhabitants roosting on the cave ceiling (Fig. 2), with an estimated bat count ranging between 120 and 150 individuals in the chamber. Bat guano substrates were also observed on the ground. The guano samples appeared moist to semi-dry, light brown, and heterogeneous in texture, containing fine particulates of digested insect remains. Although no bat identification survey was performed during sampling, observational data suggest the colony consisted predominantly of *Hipposideros* spp. and *Rhinolophus* spp., which are commonly recorded in Peninsular Malaysian caves (Nordin et al., 2021; Hazrizal Fuad et al., 2024). Both bat genera consist of insectivores, which may be attributed to the characteristics of the bat guano sampled across the three sampling sites.

There were not many distinct characteristics between the bat guano substrates of WBA and WBB, while WBC had some dead insects and litter (small organic debris and detritus). Surface temperature of bat guano measured using a Testo 925 digital thermocouple thermometer ($\pm 0.5^\circ\text{C}$ accuracy) revealed similar temperatures for all three collection sites (25°C). This temperature is within the range of values reported by other studies of caves in Malaysia (Baharim et al., 2018; Rajasegaran et al., 2018; Wasti et al., 2021).

Collection of Bat Guano Samples

Triplicates of 10 g of bat guano samples were collected randomly from each site at a depth of 5–10 cm from the surface. All sampling in this study adhered to safety protocols. The collected guano samples were then transferred into a 100 mL Falcon tube. The nine guano samples were kept cold in ice to slow down the metabolism of microscopic fungi (micro-fungi), referring to filamentous and yeast-like fungi in the samples, and transported to Universiti Malaya. Guano samples

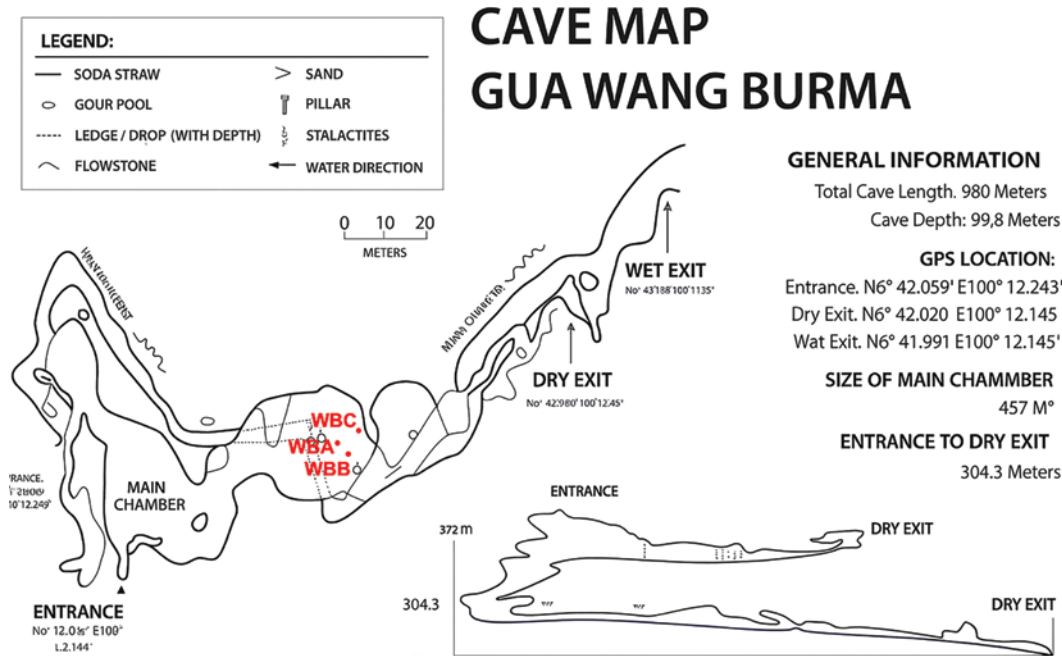


Figure 1. Schematic map of Wang Burma Cave taken on site. Locations for collection points (WBA, WBB, and WBC) are indicated with dots on the map.

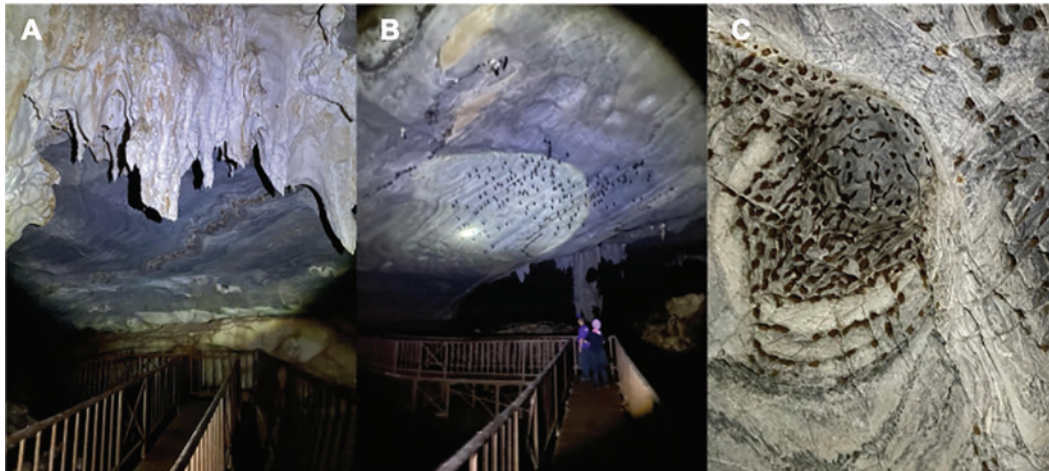


Figure 2. (a) Interior of Wang Burma Cave I; (b) bats roosting on the cave ceiling; (c) bats roosting on the cave ceiling 100 m from the entrance of the cave.

(5'-GGAAGTAAAAGTCGTAACAAGG-3') (Gardes and Bruns, 1993) and the reverse primer ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (White et al., 1990). Each 25 μ L PCR comprised of 12.5 μ L of 2 $\times$ REDiant 2 $\times$ PCR Master Mix (1st BASE), 0.5 μ L of each primer (10 μ M), 1–2 μ L of template DNA (~10 ng), and nuclease-free water to volume. Thermocycling conditions were as follows: initial denaturation at 95 °C for 3 min; followed by 30 cycles of denaturation at 98 °C for 20 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; with a final extension at 72 °C for 5 min. Successful amplification and visualization of PCR products were confirmed using 1% TAE agarose gel electrophoresis.

Library Construction and Amplicon Sequencing

The first library construction was performed through amplification of the fungal ITS1 regions with KOD-Multi & Epi-® (Toyobo) using locus-specific sequence primers with forward (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and reverse (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3') overhang adapters. For the second part of library construction, dual indices were attached to the amplicon PCR with the use of the Illumina Nextera XT Index

were kept frozen at 0 °C for long-term storage until needed for molecular genetics work.

DNA Extraction and Gel Electrophoresis

Total genomic DNA (gDNA) from the bat guano samples was extracted using NucleoSpin® Soil Kit according to the manufacturer's conditions (Vautier et al., 2020) with modifications. This involved a double extraction procedure of the same samples to maximize the yield of extracted gDNA from bat guano. Then, 1% TAE (Tris-acetate-EDTA) agarose gel electrophoresis was run and visualized to check the quality of the eluted gDNA. The concentration of gDNA was determined using NanoDrop and fulfilled the criterion of ≥ 20 ng/ μ L as good quality DNA. The gDNA samples were then stored at -20 °C until needed for amplicon sequencing.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) of the fungal internal transcribed spacer 1 (ITS1) region was performed using the gDNA templates, including the forward primer ITS1F

Kit v2 in accordance with the manufacturer's protocols. The Agilent Bioanalyzer 2100 System, with the Agilent DNA 1000 Kit, was used to measure the quality of libraries, whereas the Helixyte Green™ Quantifying Reagent was used to perform fluorometric quantification. Next, the libraries were normalized and pooled as prescribed by Illumina and were sequenced using the MiSeq platform (2 × 300 bp) with 300 bp paired-end (PE) according to the standard protocol. Library preparation and quality control were performed by Apical Scientific following the authors' initial extraction and PCR amplification in the Universiti Malaya laboratory. Sequencing was carried out by Apical Scientific Sdn. Bhd., generating raw paired-end reads in FASTQ format.

Assignment of Amplicon Sequence Variants

Raw reads obtained were demultiplexed and processed using the DADA2 plugin in QIIME 2 (version 2024.10.1). Quality filtering, denoising, paired-end read merging, and chimera removal were conducted using default parameters. The resulting high-quality sequencing data were assigned to unique amplicon sequence variants (ASVs), which represent exact biological sequences present in the samples, and were trimmed to the region amplified by the primers used in this study. Taxonomic assignment of ASVs was achieved through BLAST search of the UNITE Database (<https://unite.ut.ee/>). All raw reads and ASV datasets were deposited in GenBank Biosample (Submission SUB15333087) with accession number SAMN48597270.

Taxonomic Abundance and Community Composition

Taxonomic abundance and community composition were analyzed based on the ASV table generated following taxonomic assignment. Relative abundance at various taxonomic levels (phylum, class, order, family, and genus) was calculated to determine the composition of fungal communities across samples. The fungi identified were visualized using a chord diagram and compared with fungal diversity reported by other studies in Malaysian caves to provide a comprehensive checklist of Malaysian cave fungi.

RESULT AND DISCUSSION

Taxonomic Abundance and Diversity

Sequencing of the ITS1 region yielded a total of 491,691 raw reads, with 380,856 reads remaining after merging, dereplication, and chimera removal. In total, 32 ASVs representing two phyla, five known classes, four known orders, five known families, five known genera, and six known species were reported (Table 1).

Phylum Ascomycota was found to be the most dominant phylum (consisting of >99.99% of the 380,856 sequence reads), similar to findings by Wasti et al. (2020, 2021).

The dominance of Ascomycota aligns with previous reports from Malaysian and Southeast Asian caves, possibly due to their adaptive capacity to nutrient-poor and oligotrophic guano environments (Cunha et al., 2020; Wasti et al., 2020). Another phylum that was identified was Mortierellomycota, which consisted of only a mere 21 sequences.

Of the 32 ASVs, 14 were identified successfully to the species level, representing six species: *Aspergillus caninus*, *A. chlamydosporus*, *A. phialosimplex*, *Candida palmioleophila*, *Mortierella rhinolophicola*, and *Penicillium simplicissimum* (Fig. 3). Seven ASVs were identified only to the genus level, and these included the genera *Aspergillus*, *Candida*, *Mortierella*, *Penicillium*, and *Sagenomella*. Some ASVs were identified only to higher taxonomic levels (e.g., family, order, class, or phylum), which may be attributed to (1) low quality of the sequence reads or (2) limitations of barcode references in repository databases. Regardless, all of these ASVs were confidently classified as the phylum Ascomycota. In addition, three species, such as *A. chlamydosporus*, *C. palmioleophila*, and *P. simplicissimum*, were recorded in samples from all three sites. The three most abundant ASVs identified were ASV4 (33.6%) and ASV5 (33.8%), which are two strains of *P. simplicissimum*, and ASV16 (15.3%), which represents a strain of *C. palmioleophila*.

Eurotiomycetes and Eurotiales (73%) were the most abundant class and order, respectively, followed by class Saccharomycetes and order Saccharomycetales (21%), and class Leotiomycetes and order Helotiales (6%) (Fig. 4). The most dominant family for all three sites was Aspergillaceae (72%), while the other family under Eurotiales found in the samples, *Trichocomaceae* (<1%), had significantly lower abundance compared to its sister family. The relative abundance of Mortierellaceae was similar to the order Mortierellales, as Mortierellaceae is a monotypic family. Myxotrichaceae was similar in abundance to the order Helotiales, thus implying that there were no other fungi of other families under this order that were present in the samples. This was also the case for the enigmatic/uncertain family under Saccharomycetales labeled as "Incertae familiae."

Under Aspergillaceae, two genera were identified, which were *Aspergillus* (<1%) and *Penicillium* (99%). *Candida* (21%) was the only genus under *incertae familiae* (Saccharomycetales) found in the samples; hence, the relative abundance was similar to its family. *Mortierella* and *Sagenomella* were also the only identified genera of their respective families; hence, their relative abundance values were also similar to those of their families.

Table 1. Relative abundance of ASVs identified based on fungal ITS1 sequences in bat guano from three sites (WBA, WBB, and WBC) in Wang Burma Cave.

ASV	Identified Taxa	Sequence Reads (n)			Relative Abundance	
		WBA	WBB	WBC	N	%
ASV5	<i>Penicillium simplicissimum</i>	26,413	49,105	53,226	128,744	33.80
ASV4	<i>Penicillium simplicissimum</i>	66,980	54,553	6,606	128,139	33.64
ASV16	<i>Candida palmioleophila</i>	19,228	1,284	37,897	58,409	15.34
ASV9	<i>Myxotrichaceae</i> ^a	3,846	17,606	1,951	23,403	6.14
ASV19	<i>Candida palmioleophila</i>	0	642	15,690	16,332	4.29
ASV6	<i>Penicillium</i> sp. 3 ^b	0	9,569	6,050	15,619	4.10
ASV23	<i>Sagenomella</i> ^b	3,051	0	123	3,174	0.83
ASV27	<i>Aspergillus caninus</i>	1,444	0	0	1,444	0.38
ASV14	<i>Candida palmioleophila</i>	673	60	496	1,229	0.32
ASV7	<i>Lecanoromycetes</i> ^c	0	0	1,077	1,077	0.28
ASV25	<i>Aspergillus</i> sp. 1 ^b	1,045	0	0	1,045	0.27
ASV26	<i>Aspergillus chlamydosporus</i>	429	261	43	733	0.19
ASV21	<i>Ascomycota</i> ^d	0	315	7	322	0.085
ASV22	<i>Ascomycota</i> ^d	57	172	0	229	0.06
ASV18	<i>Candida palmioleophila</i>	0	26	180	206	0.05
ASV28	<i>Aspergillus phialosimplex</i>	200	0	0	200	0.05
ASV8	<i>Lecanoromycetes</i> ^c	0	0	123	123	0.03
ASV24	<i>Eurotiales</i> ^e	0	121	0	121	0.03
ASV29	<i>Penicillium</i> sp. 4 ^b	41	28	0	69	0.02
ASV11	<i>Lecanoromycetes</i> ^c	42	0	0	42	0.01
ASV30	<i>Penicillium</i> sp. 5 ^c	0	0	33	33	<0.01
ASV32	<i>Penicillium</i> sp. 6 ^c	28	0	0	28	<0.01
ASV1	<i>Penicillium simplicissimum</i>	0	25	0	25	<0.01
ASV31	<i>Penicillium simplicissimum</i>	0	0	23	23	<0.01
ASV2	<i>Penicillium</i> sp. 1 ^b	0	19	0	19	<0.01
ASV12	<i>Mortierella rhinolophicola</i>	0	0	16	16	<0.01
ASV15	<i>Candida palmioleophila</i>	0	0	16	16	<0.01
ASV10	<i>Myxotrichaceae</i> ^a	0	10	0	10	<0.01
ASV17	<i>Candida palmioleophila</i>	0	0	10	10	<0.01
ASV3	<i>Penicillium</i> sp. 2 ^b	6	0	0	6	<0.01
ASV13	<i>Mortierella rhinolophicola</i>	0	5	0	5	<0.01
ASV20	<i>Candida palmioleophila</i>	0	0	5	5	<0.01
		123,483	133,801	123,572	380,856	100

^aASV9 and ASV10 can only be identified up to the family level.^bASV2, ASV3, ASV6, ASV25, ASV29, ASV30, and ASV32 can only be identified up to the genus level.^cASV7, ASV8, and ASV11 can only be identified up to the class level.^dASV21 and ASV22 can only be identified up to the phylum level.^eASV24 can only be identified up to the order level.

Insights into the Identified Fungi Associated with Bat Guano

The fungal community profiles across the three sampling sites (WBA, WBB, and WBC) revealed clear dominance patterns, as well as site-specific fluctuations in several medically relevant taxa. The most abundant species overall was *Penicillium simplicissimum*, which consistently represented the largest proportion of ASVs across all samples (up to 67%). This strong and uniform dominance suggests that *P. simplicissimum* is a core and persistent component of the cave mycobiome. Although non-pathogenic, some strains of this species are known to function as plant growth-promoting fungi and have been explored for use as biofertilizers (Shimizu et al., 2013), indicating ecological versatility rather than pathogenic relevance within this cave environment.

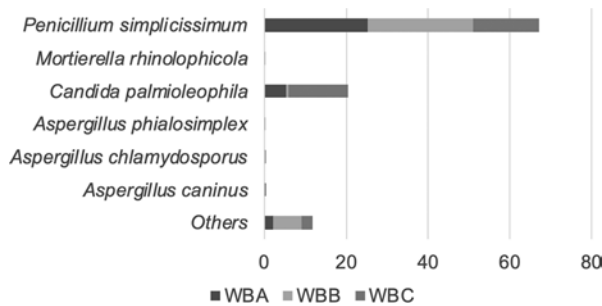


Figure 3. Relative abundance (%) of fungal species identified across three sampling sites (WBA, WBB, and WBC) based on ASV proportions.

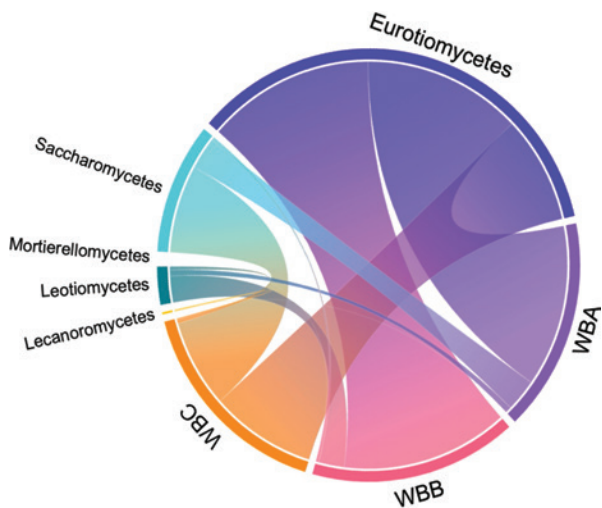


Figure 4. Chord diagram showing major identified fungal classes associated with bat guano identified from three sites (WBB, WBC, and WBA) in Wang Burma Cave.

tus has similarly never been documented. Instead, prior cave studies in Malaysia have reported other pathogenic *Aspergillus* species such as *A. flavus*, *A. niger*, and *A. nomius* (Marr et al., 2002; Caira et al., 2012; Wasti and Seelan, 2019; Wasti et al., 2020). The low abundance of *Aspergillus* taxa in the current dataset therefore suggests limited representation of this genus within Gua Wang Burma compared to other Malaysian cave systems.

Overall, the combination of highly dominant non-pathogenic taxa (e.g., *P. simplicissimum*), heterogeneous but medically relevant yeasts (e.g., *C. palmioleophila*), and rare occurrence of specialized or low-pathogenicity species (e.g., *Mortierella* spp. and *Aspergillus* spp.) reflects a fungal community possibly shaped by both microhabitat variation and the unique ecological constraints of the cave environment.

Diversity of Cave Fungi in Malaysia

To provide a more comprehensive analysis of available baseline data on cave fungi associated with bat guano in Malaysia, we have compiled a checklist of 34 cave fungal species reported in the present study and two previous studies conducted in Sabah caves (Table 2; Wasti et al., 2020, 2021). The present study contributes five new records, including *Aspergillus caninus*, *A. chlamydosporus*, *A. phialosimplex*, *Candida palmioleophila*, and *Mortierella rhinolphicola*. The addition of these five previously unreported bat guano-associated fungi from Peninsular Malaysia suggests that Malaysian cave mycobiomes may harbor regionally distinct assemblages shaped by local environmental gradients, guano chemistry, and bat species composition.

CONCLUSION

This study highlighted the cave fungi associated with bat guano in Wang Burma Cave, Perlis. eDNA metabarcoding analysis revealed the diverse fungal community, some of which have potential applications in biotechnology and conservation efforts. Understanding these microbial interactions will contribute to better conservation management of cave ecosystems and their associated biodiversity.

In contrast, *Candida palmioleophila* exhibited marked variability in relative abundance among sites, approximately 16% in WBA, 2% in WBB, and 44% in WBC, making it the second most prominent taxon in the dataset and the only species demonstrating strong spatial heterogeneity. *C. palmioleophila* is an ascomycetous yeast with documented opportunistic pathogenicity, particularly in immunocompromised patients, where it is associated with candidemia and severe complications including dermal infections, respiratory failure, and fatal systemic infections (Jensen and Arendrup, 2011; Pierantoni et al., 2020). Its uneven distribution across the cave sites may therefore indicate microhabitat-driven niche suitability rather than widespread establishment.

The detection of *Mortierella rhinolphicola*, although occurring at negligible abundances across all sites, represents a noteworthy finding. This species was first described from bat carcasses in a cave in Yunnan, China (Karunarathna et al., 2020) and may include cave-adapted or host-associated strains. Members of *Mortierella* are generally non-pathogenic, and no evidence exists thus far to suggest pathogenic potential in *M. rhinolphicola*. Its minimal presence in our samples is consistent with both its rarity in global mycobiome reports and the possibility of a specialized ecological niche within cave ecosystems.

Among the *Aspergillus* species detected (including *A. caninus*, *A. phialosimplex*, and *A. chlamydosporus*), all occurred only at trace proportions in the ASV dataset. *Aspergillus caninus* is of particular interest due to its ability to cause severe infections in canines (Kano et al., 2019; Yang et al., 2020). While the other detected *Aspergillus* species have not been associated with significant human pathogenicity, the genus as a whole contains several major agents of invasive fungal infections in immunocompromised individuals (Beck-Sagué and Jarvis, 1993). Notably, *A. fumigatus*, the primary etiological agent of aspergillosis (a lung disease), was absent from all cave samples, aligning with previous studies in Malaysian caves, where *A. fumigatus* has similarly never been documented.

Table 2. Occurrence of fungi associated with bat guano in caves of Malaysia.

Species	Wang Burma Cave (Present Study)	Madai Cave, Sabah (Wasti et al., 2021)	Gomantong Cave, Sabah (Wasti et al., 2020)
<i>Annulohyphoxylon nitens</i>		a	
<i>Aspergillus aculeatus</i>		a	
<i>Aspergillus caninus</i>	a,b		
<i>Aspergillus chlamydosporus</i>	a,b		
<i>Aspergillus flavus</i>			a
<i>Aspergillus niger</i>		a	
<i>Aspergillus nomius</i>		a	
<i>Aspergillus ochraceus</i>			a
<i>Aspergillus phialosimplex</i>	a,b		
<i>Aspergillus restrictus</i>			a
<i>Aspergillus</i> sp. 1	a	a	
<i>Aspergillus fumigatus</i>			
<i>Blastobotrys malaysiensis</i>			
<i>Candida palmioleophila</i>	a,b		
<i>Cladosporium cladosporioides</i>		a	
<i>Curvularia lunata</i>		a	
<i>Mortierella rhinolophicola</i>	a,b		
<i>Paecilomyces variotii</i>		a	
<i>Penicillium citrinum</i>			a
<i>Penicillium paxilli</i>		a	a
<i>Penicillium simplicissimum</i>	a	a	
<i>Penicillium</i> sp. 1	a	a	
<i>Penicillium</i> sp. 2	a	a	
<i>Penicillium</i> sp. 3	a		
<i>Penicillium</i> sp. 4	a		
<i>Penicillium</i> sp. 5	a		
<i>Penicillium</i> sp. 6	a		
<i>Pestalotiopsis</i> sp.			a
<i>Pochonia chlamydosporia</i>		a	
<i>Purpureocillium lilacinum</i>		a	
<i>Talaromyces minioluteus</i>		a	
<i>Trichoderma asperellum</i>		a	
<i>Trichoderma paraviridescens</i>		a	
<i>Xylaria feejeensis</i>			a
Total	13	17	7

^aFungi from bat guano.^bNew records of identified species.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Higher Education (MOHE) for its funding program—Higher Institution Centre of Excellence (HiCoE) (Grant No. IOES-2014G) and the Wang Burma Rangers and the Perlis Forestry Department for their assistance in the field.

AUTHOR CONTRIBUTION STATEMENT

SAA and MR-I conceived the idea for the project. NMT-M and TWH collected bat guano samples and carried out DNA extraction and metabarcoding analysis. SKS and NMT-M analyzed and discussed the metabarcoding results. AK assisted with fungal isolation and identification. SAA and MR-I supervised the findings of this work and verified the

analytical methods and results from the metabarcoding analysis. HO consulted on bat guano as a substrate for cave fungi. All authors discussed the results and contributed to the final manuscript.

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