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Fungal community composition in arthropogenically-active and non-active northern Borneo caves

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Abstract: Fungal studies are scant within this region of Borneo, and the potential effects of anthropogenic disturbance on fungal communities in Sabahan caves have not been explored. This study involved opportunistic sampling of guano, speleothems, and cavern water from four caves in Sabah, Malaysia, categorised according to the level of anthropogenic activity. The dilution method was used for fungal isolation and abundance calculations. Morphological identification of axenic cultures was conducted, and DNA barcoding of the ITS region of cryptic species corroborated identification. A total of 170 axenic cultures were attained, composed of 69 morphological taxonomic units (MTUs), 33 genera, ten orders, and two divisions – Ascomycota and Basidiomycota. Fifty-seven of the 69 MTUs were identified to the species level. The genera *Aspergillus* and *Penicillium* were particularly prevalent, with a combined total of 86 isolations (50.6%). Notably, twelve species were documented for the first time in cave ecosystems of Sabah. The average fungal abundance across all substrates and fungal taxa occurrence was found to be higher in samples found in active caves. A significant difference in relative abundance was observed between active and non-active cave conditions. Beta regression modelling indicated that anthropogenic activity is a significant factor influencing the composition of cave fungal communities. While anthropogenic activity seems to affect cave mycobiomes, especially by selecting for ubiquitous fungi, it is likely not the sole influencing determinant of their composition.

Keywords: Biodiversity, guano, human activity, limestone, mycology, speleothem, water

INTRODUCTION

Caves are one of the most prominent environments for the proliferation of fungi. These unique underground oligotrophic ecosystems have shown to contain abundant fungal diversity (Vanderwolf *et al.* 2013). In this aspect, bat caves play a crucial role, with fungi predominantly existing as saprophytes, symbionts, and parasites within their respective ecosystems (Bastian *et al.* 2010; Araújo and Hughes 2016). Over 1600 fungal species have been reported from subterranean environments such as caves and mines (Zhang *et al.* 2018, 2020). Factors that affect fungal occurrence in caves include seasonal changes, temperature, humidity, anthropogenic activity, availability of sunlight, and proximity to cave entrances

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(Wang *et al*. 2010; Taylor *et al*. 2014). Caves, much like other subterranean environments, are characterised by their nutrient-poor conditions. They typically present a humid, cool, and dark environment that supports a diverse but particular assemblage of mycota, particularly oligotrophic and psychrotolerant fungi (Vanderwolf *et al.* 2013). Fungi are dominant in caves due to their high rate of spore dissemination and colonisation capability in various types of substrates (Wang *et al.* 2010; Vanderwolf *et al*. 2013). Despite the relatively limited nature of cave ecosystems, organic matter such as guano, dead wood, and animal remains serve as essential nutrient sources for a variety of cave-dwelling fungi (Nieves-Rivera *et al*. 2009; Santamaria and Faille 2007; Vanderwolf *et al.* 2013).

Studies undertaken in bat caves remain limited, especially in tropical and sub-tropical regions (Vanderwolf *et al.* 2013; Cuhna *et al.* 2020). While many cave-dwelling organisms are true troglobites, very few fungi are specialised in the cave ecosystems (Zhang *et al*. 2018). Nonetheless, at least 53 new fungal species have been described following their isolation from the cave environment (Zhang *et al*. 2017, 2020). Many of the fungi isolated from caves are ubiquitous and likely stem from the surrounding external environment. However, there have been reports of fungi suspected to be obligate troglobites, such as *Acaulium caviariforme*, *Aspergillus baecitus*, and *Aspergillus thesauricus*.

Fungal diversity and abundance tend to increase with increasing anthropogenic activities, especially near cave entrances with heavy traffic, and during peak holiday seasons (Wang *et al*. 2010; Shapiro and Pringle 2010; Porca *et al.* 2011; Ogórek *et al.* 2013; Pusz *et al.* 2015). The impact of human activity on cave ecosystems are predominantly detrimental, as they destabilise the biological equilibrium within these ecosystems (Chelius 2009). Aerobiological studies conducted on ambient cave fungi have shown positive correlation between human visitation rates and the concentration of airborne fungi and spores (Wang *et al.* 2010; Porca *et al.* 2011). Additionally, during peak ecotourism seasons, particularly the summer months, there is a notable increase in entomopathogenic fungi (Porca *et al.* 2011). Human visitation into caves introduces sundry foreign substances and potential nutrients for microbes (Jablonsky *et al.*1993). It can also influence changes in cave climate, both of which are known to affect cave microbial community composition (Pflitsch and Piasecki 2003).

Man *et al.* (2015) conducted a comprehensive survey of fungal diversity in Heshang Cave, China, examining sediments, weathered rocks, and bat guanos. Their findings revealed that of the 194 total fungal isolates identified, the highest fungal diversity was found in cave sediments followed by weathered rocks. In a separate study by Nieves-Rivera (2009), fifty species of guanophilic fungi were isolated from three caves in tropical Puerto Rico with *Circinella umbellata* being the most frequently observed. In Lascaux cave, known for its prehistoric paintings, it was suggested that water from the soils above the cave was responsible for introductions of fungal species into the cave (Dupont *et al.* 2007). Among the identified fungi, 23 of them were mitosporic fungi, which produce spores via asexual division. Additionally, Preedanon *et al*. (2023) reported eight novel fungi from Saturn Geopark caves in Thailand.

Reports of mycoflora associated with Bornean caves, a region where tropical diversity is among the top in the world, are limited. Gomantong and Madai caves in Sabah (Borneo), Malaysia are some of the most visited caves by both local and international tourists. However, these caves are situtated within region with extensive amounts of land-use changes due to oil palm cultivation and other development activities surrounding its forest areas (Wasti *et al.* 2020, 2021). Hence, this study aims to compare the fungal community diversity and abundance between speleothem, bat guano, and cavern water samples of anthropogenically -active versus non-active caves.

MATERIALS AND METHODS

Study sites

Northern Borneo, in the state of Sabah, Malaysia, is home to myriad limestone outcrops in which various caves formed. Four accessible limestone caves in Sabah were visited between April 2017 to July 2018 (Figure 1). Three sample types – speleothem, water, and bat guano – were collected for this study. The sampling were conducted during the daytime between 11:00 a.m. to 2:00 p.m. to ensure consistency (Figure 2). All studied caves were located within the sundry forest reserves or virgin jungle reserves within Sabah, except for Balambangan Cave in Balambangan Island, which was gazetted as part of Tun Mustapha Marine Park.

The selection of the four caves in this study were based on the levels of anthropogenic activities within or surrounding the cave sites (Figure 3). The primary factors considered as indicators of human disturbances included the magnitude of tourism and human visitation, land-use patterns in the surrounding areas, and the presence of swiftlet nest harvesting activities (Table 1). The caves classified as active attract thousands of visitors annually, engage in ongoing edible swiftlet nest farming and harvesting, and are surrounded by extensive land-use, predominantly heavy monocrop agriculture of palm oil plantations. These active caves are famous for their swiftlet nest harvesting, with harvesting apparatus visible at multiple points when visiting the cave. The farming and harvesting of the nests of white swiftlet (*Aerodramus fuciphagus*) and black swftlet (*A. maximus*) are common in Sabahan caves (Lundberg and McFarlane 2012).

Bats that roost in these caves include those in the families Molossidae, Pteropodidae, Hippossideridae, Rhinolophidae, Vespetilionidae, and Emballonuridae (Wasti *et al.* 2020; Mahyudin *et al*. 2022). Mounds of bat guano cover almost the entirety of the cave floor, which are inhabited by a plethora of arthropods including cockroaches, centipedes, spiders and beetles. Copious amounts of vandalism on the walls and ceilings of the caves, especially in chambers closest to the entrances are glaringly apparent. From the two active caves, the study collected ten speleothem samples, ten water samples, and eight bat guano samples. The results of the fungal identification from Madai cave were published in a prior research by Wasti *et al.* (2021).

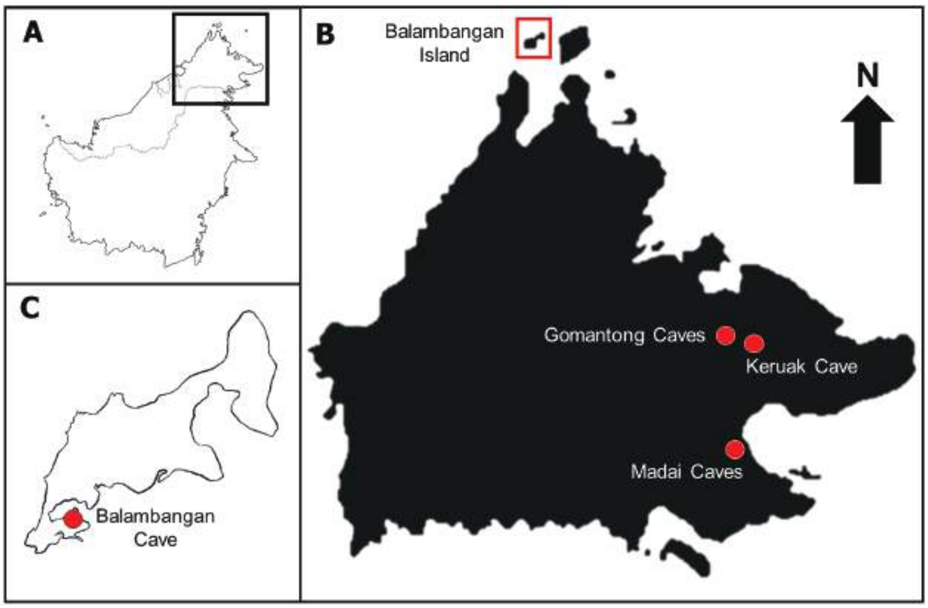

Figure 1. Locations of the four cave sites in Sabah (Malaysia).

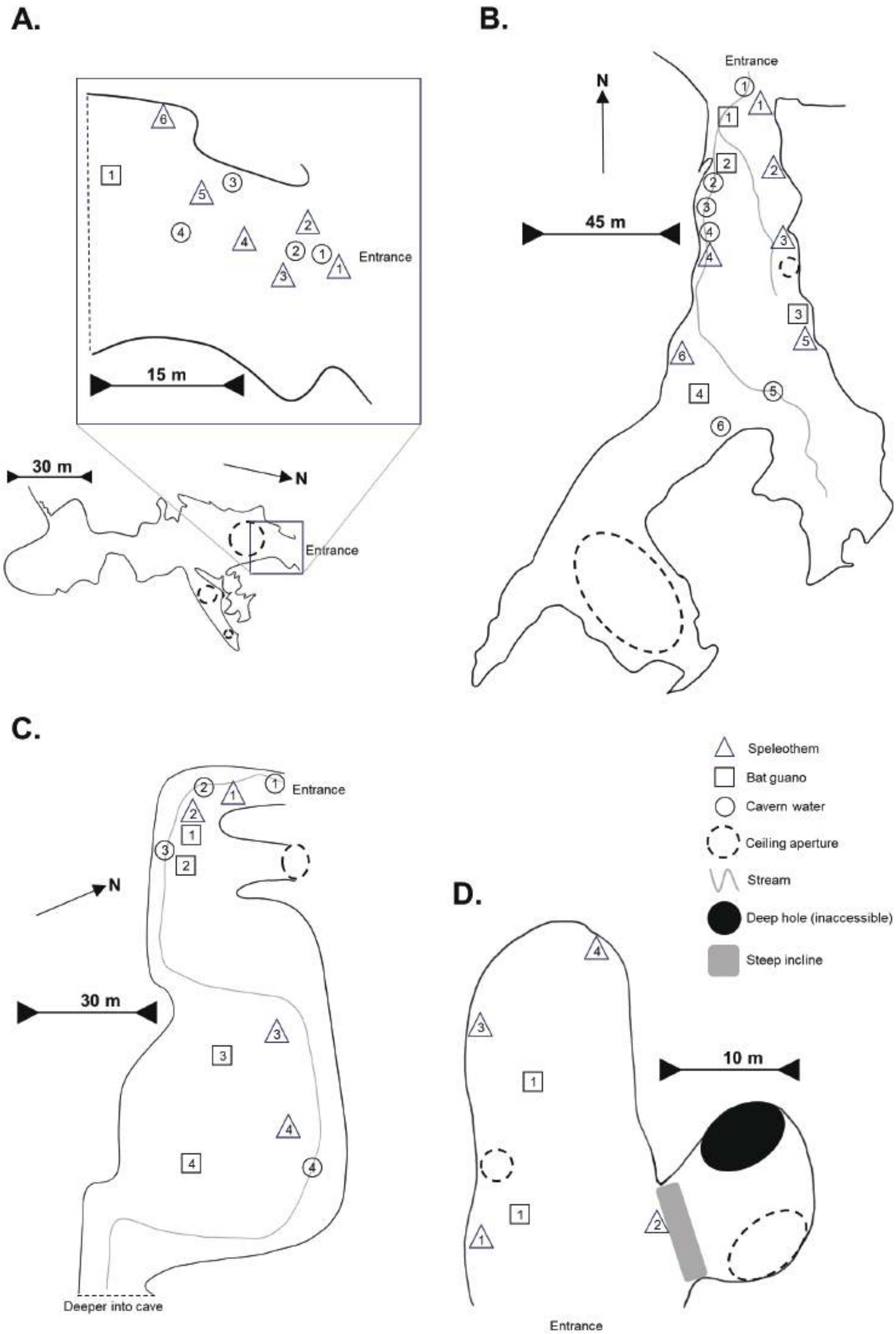

Figure 2. Layout with sampling points of **A.** Balambangan cave; **B.** Gomantong cave (Semud Hitam); **C.** Madai cave (first 100 m); **D.** Keruak cave.

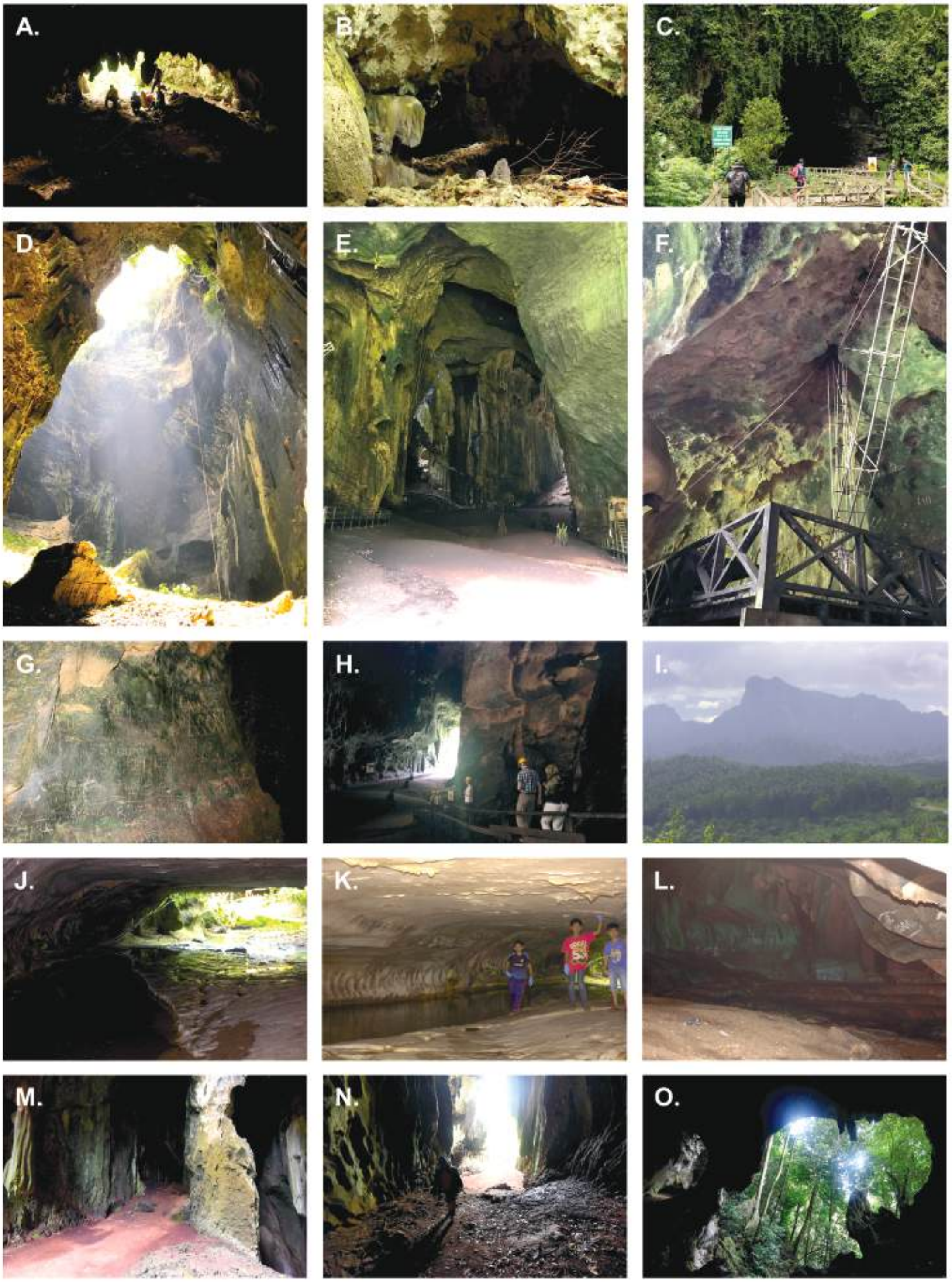

Figure 3. A. Balambangan cave entrance; **B.** Balambangan cave under ceiling aperture; **C.** Gomantong cave entrance; **D.** Gomantong cave chamber opposite entrance; **E.** Gomantong cave view of main chamber from entrance; **F.** Gomantong cave swiftlet nest harvesting equipment; **G.** Vandalism on Gomantong cave walls; **H.** Tourists walking withing Gomantongcave wooden path; **I.** Palm oil plantation surrounding karst formation near Madai cave area; **J.** Entrance to Madai cave; **K.** Village children loitering and playing within Madai cave; **L.** Vandalism on Madai cave walls; **M.** Keruak cave main chamber seen from entrance; **N.** Keruak cave main chamber seen from end of chamber; **O.** Canopy cover seen from wall aperture from within Keruak cave.

Table 1. Four chosen cave sites separated based on anthropogenic activity.

Cave	Forest Reserve	Type	Location	Tourism & Visitation	Land Use Activity (i.e., Monocrop Agriculture)	Swiftlet Nest Harvesting
Gomantong Cave System	Gomantong Forest Reserve (Virgin Forest)	Active	Kinabatangan, Sabah (5°31'30"N 118°04'15"E)	High	Yes	Yes
Madai Cave	Baturong Madai Forest Reserve (Virgin Forest)	Active	Kampung Madai, Kunak, Sabah (4°41'10.01"N 118°15'4.12"E)	High	Yes	Yes
Balambangan Cave	Taman Tun Mustapha	Non-Active	Balambangan Island, Sabah (7°13.313"N 116°53.070"E)	Low	No	No
Keruak (Cave)	Keruak VJR	Non-Active	Kinabatangan, Sabah (5°31'06.4"N 118°17'27.8"E)	Low	Yes	No

Figure 4. Photos of the various substrates from all four caves. **A-C.** Speleothem; **D-F.** Bat guano; **G-I.** Cavern water.

Sampling

Opportunistic sampling of three types of substrates were collected, namely speleothems, bat guano, and cavern water. The distances from the cave entrance for every sample collected were recorded. Speleothem surfaces were sampled using the swab method (Ikner *et al.* 2007; Vaughan *et al.* 2011), and an area of 25 cm^2 was swabbed per sample. The swabs were immersed in sterile distilled water in sterilised canonical centrifuge tubes, capped, and sealed with parafilm. Guano samples were collected using sterile scoops and stored in sterile canonical centrifuge tubes (Nieves-Rivera 2003). Ten grams of guano was collected per replicate. Cavern water samples were collected at 10 ml per replicate and sealed in sterile canonical centrifuge tubes.

The samples were systematically labelled according to substrate type, accompanied by a numerical designation corresponding to the increasing distance from the cave entrance (e.g., speleothem sample 1 = S1; speleothem samples 2 = S2). All samples were collected in triplicate from each sampling site and were transported to the mycology laboratory in the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah under chilled conditions (<4 °C). The samples were subsequently stored in a chiller at <4 °C until the isolation process.

Fungal identification, DNA extraction, sequencing and analysis

Samples were subjected to serial dilution with sterile distilled water in a ten-fold manner, extending up to a dilution factor of 10^{-4}. Aliquots of 1 ml from the 1×10^{-2}, 1×10^{-3} and 1×10^{-4} dilutions were evenly distributed onto culture plates in triplicate. The samples were placed in Petri dishes via serial dilution onto Potato Dextrose Agar (PDA) infused with 40 mg/L streptomycin sulfate, which were incubated for 7 – 28 days at 25 ± 1 °C in the dark (Wasti *et al.* 2021). Dilutions and plating were performed in triplicate.

The colonies that developed in the media were categorised by Morphological Taxonomic Units (MTUs) and quantified for fungal abundance. Pure isolates were obtained using the three-point inoculation method on streptomycin sulfate-infused PDA prior to conducting morphological and genetic analysis. All pure isolates underwent morphological identification based on morphological characteristics (Domsch *et al.* 2007; Klich 2002). Subsequently, selective molecular identification was used to confirm or identify cryptic fungal taxa that could not be identified through morphological examination.

DNA for molecular identification was extracted from pure cultures aged between 7 – 14 days old, using the E.Z.N.A. DNA Fungal Kit (Omega Bio-Tek, USA) following manufacturer's instructions. The internal transcribed spacer (ITS) region of fungal rDNA was amplified using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.* 1990). Polymerase chain reaction (PCR) amplifications were performed in a Bio-Rad T100 Thermal Cycler according to Ogórek *et al.* (2016). The amplification products were electrophoresed in a 1% agarose gel for 30 mins, which was stained with gel red for visualisation. The PCR products were then purified using Column-Pure PCR Clean-Up Kit (Applied Biological Materials, Inc.) according to manufacturer protocols, and DNA sequencing was performed by MyTACG Bioscience SDN. BHD. (Kuala Lumpur, Malaysia). The newly generated ITS sequences were assembled using BioEdit Sequence Alignment Editor version 7.2.5. These sequences were all deposited to the NCBI GenBank database using the BLASTn algorithm to obtain top hit sequences (http://www.ncbi.nlm.nih.gov).

Fungal species occurrence is defined as the number of times the same species (or MTU) was isolated as pure culture (maximum of once per sample). Fungal abundance data was obtained during the serial dilution process and were calculated and expressed as colony-forming units (CFU). The calculation was performed with the formula $X = (a \times p)/V$

for each MTU for every dilution plate— “a” being the number of colonies, “p” the dilution factor, and “V” the inoculation aliquot volume. The abundance for each MTU per sample is the average of nine dilution plates, and the total fungal abundance for each sample is the sum of the abundance of the MTUs isolated from that sample.

Statistical analyses

All analyses were undertaken in R software, Version 2023.03.0+386 (R Core Team 2023). Relative abundance was calculated as the proportion of CFU per isolate relative to the total CFU from its corresponding active or non-active site. Relative abundance was selected as it allows comparison of microbial populations across different substrates and reconciles the differences in fungal abundance units among the substrates. Kruskall-Wallis analysis was performed to determine differences in the relative abundances across different anthropogenic conditions, substrates, and cave localities. To further investigate specific differences, pairwise Wilcoxon rank-sum tests were carried out. These tests compared the relative abundances of fungal communities between active and non-active sites, between caves, and across various substrates. To address the increased risk of Type I errors from multiple pairwise comparisons, a Bonferroni correction was applied. Given that the dependent variable, relative abundance, takes values between 0 and 1, beta regression was used to estimate the effects of distance from the cave entrance and site anthropogenic activity (active or non-active) on the fungal community. The Akaike information criterion corrected for small samples (AICc) (Burnham and Anderson 2002) was used to determine the best model to explain changes in cave fungal community.

RESULTS

Fungal composition

A total of 45 samples from all four caves were collected across all substrates. From these samples, 170 isolates were identified and classified into 69 distinct molecular taxonomic units (MTU). Of these, 57 were identified to the species level and the remaining 12 to the genus level. The identified fungi comprised of 33 genera, spanning ten orders and two divisions, namely Ascomycota and Basidiomycota (Table 2), which were confirmed by morphological characters and ITS region molecular analysis. DNA extraction (ITS) was carried out on 80 isolates to validate morphological identification and assist in cryptic taxa identification. BLASTn sequence analysis confirmed 49 taxa to the species level and two taxa to the genus level (Table 3).

Among the isolates, 96.5% (164 isolates) were Ascomycota, and 3.5% (6 isolates) were Basidiomycota. Of the Ascomycota, the most prominent genera were *Aspergillus* (25.3%), *Penicillium* (25.3%), *Trichoderma* (13.5%), and *Fusarium* (5.9%) (Figure 5). The genera *Aspergillus* and *Penicillium* comprised 16 and 12 different species, respectively, which were greater compared to the other genera. The species with the highest occurrence was *Penicillium citrinum*, having been cultured from 14 different samples (i.e., guano, speleothem, and water). This was also the only species of fungi isolated from all four cave sites. The other two species cultured from ten or more samples were *Penicillium paxilli* (12 samples) and *Trichoderma harzianum* (10 samples). Six isolates were identified as Basidiomycetes, each identified as a different species and were isolated only once.

Speleothem yielded 82 isolates (48.2%) comprised of 44 species from 21 genera, and seven orders. Cavern water yielded 53 isolates (31.2%) comprised of 34 species from 21 genera and eight orders. Bat guano yielded 35 isolates (20.6%) comprised of 28 species from 14 genera and six orders. The average number of species isolated per sample was 4.10 ± 2.57 for speleothem, 3.79 ± 1.58 for cavern water, and 3.18 ± 2.18 for bat guano (Supplement 2). *Penicillium citrinum* had the highest occurrence from speleothem samples (n = 9), *Penicillium paxilli* had the highest occurrence from cavern water samples (n = 5), and *Trichoderma harzianum* had the highest occurrence for bat guano (n = 3). The highest number of fungal taxa recovered from any one substrate type was ten taxa, which occurred in speleothem samples. Madai cave had the highest average fungal abundance count for speleothem samples (299.3 CFU/cm^2), cavern water samples (335.0 CFU/ml), and bat guano samples (6266.7 CFU/g) (Supplement 3). *Aspergillus* represented the highest proportion of fungal isolates in speleothem and bat guano (Figure 6A). *Penicillium* fungi represented the highest proportion of isolates in cavern water distribution, and were the second highest proportion for speleothem and bat guano as well.

Of the 170 isolates, 56 isolates were from non-active sites (i.e., Balambangan Cave and Keruak Cave) and 114 isolates were from active sites (i.e., Gomantong Cave and Madai Cave). The non-active cave isolates comprised 19 different genera, while active cave isolates comprised 21 different genera. From the active cave sites, the average fungal occurrence per sample was 4.07 ± 2.11 taxa (Table 4). Forty-eight species were identified from the active cave isolates, and 33 of those were unique to the active caves. *Penicillium paxilli* had the highest occurrence (n = 12), amounting to 10.53% of all active cave isolates. For the non-active sites, the average fungal occurrence was 3.29 ± 2.31 taxa per sample. Thirty-five species were identified from non-active cave isolates, in which 20 of those were unique to the non-active caves. *Penicillium citrinum* was most frequently isolated from both the non-active sites (n = 5). Active caves showed greater relative abundance of Eurotiales and Hymernochaetales fungi compared to non-active caves (Figure 6B). However, active caves has lower relative abundance of other Orders, especially Pleosporales, Polyporales, and Xylariales fungi.

Table 2. Fungal taxa cultured from caves in Sabah, Malaysia. The number of occurrences for each fungal taxa in each cave are shown. Data from Wasti *et al.* (2021) was used for Madai cave isolates.

	Cave			
Fungi	**Balambangan Cave**	**Gomantong Cave**	**Madai Cave**	**Keruak Cave**
Ascomycota				
Capnodiales				
Cladosporium cladosporioides	0	1 D[1]	1 D	0
Eurotiales				
Aspergillus sp. 1	0	2 M[2]	1 M	0
Aspergillus sp. 2	0	0	1 M	0
Aspergillus sp. 3	0	1 M	0	0
Aspergillus sp. 4	0	1 M	0	1 M
A. aculeatus	1 M	1 M	1 D, 3 M	0
A. bertholletiae	1 M	1 M	2 D	0
A. chevalieri	0	1 D	0	0
A. europaeus	0	1 M	1 M	0
A. flavus	1 M	0	3 M	0
A. japonicus	1 D, 2 M	0	1 D, 1 M	0
A. luchuensis	1 M	1 M	0	0
A. niger	1 M	2 M	1 D, 1 M	0
A. nomius	0	0	3 D	0
A. parasiticus	1 M	0	0	0
A. sydowii	0	0	1 D	0
A. versicolor	0	0	0	2 D
Paecilomyces sp. 1	1 M	0	0	0
Pa. variotii	0	0	1 D, 1 M	0
Penicillium sp. 1	3 M	0	0	0
Penicillium sp. 2	0	0	1 M	0
Penicillium sp. 3	0	0	1 M	0
P. bilaiae	0	0	1 D	0
P. brevistipitatum	0	0	0	1 M

P. chrysogenum	1 M	0	0	0
P. citrinum	3 M	3 D, 3 M	1 D, 2 M	2 D
P. coprophilum	0	0	0	1 D
P. ochrochloron	0	1 M	0	0
P. oxalicum	0	2 D	0	2 D
P. paxilli	0	5 D, 4 M	3 M	0
P. simplicissimum	1 M	1 M	1 D	0
Talaromyces sp. 1	0	0	1 D	0
T. flavus	0	1 M	1 D	0
Hypocreales				
Clonostachys rosea	1 D, 2 M	0	0	0
Fusarium sp. 1	2 M	0	0	0
Fusarium sp. 2	2 M	0	0	0
F. proliferatum	0	5 D, 1 M	0	0
Lecanicillium fusisporum	1 D	0	0	0
Metarhizium carneum	1 M	0	0	0
Pochonia chlamydosporia	0	0	1 D	0
Purpureocillium lilacinum	1 M	1 D	4 D, 1 M	0
Tolypocladium album	0	1 D	0	0
Trichoderma asperellum	0	2 D, 1 M	1 D, 1 M	1 D
T. citrinum	0	1 D	0	0
T. harzianum	2 M	1 D, 4 M	3 M	0
T. koningii	0	2 M	0	0
T. paraviridescens	2 M	1 D	1 M	0
Incertae sedis				
Plectosphaerella cucumerina	0	0	1 D	0
Verticillium sp.	2 M	0	0	0
Onygenales				
Blastomyces sp.	1 M	0	0	0
Pleosporales				
Curvularia lunata	0	0	1 D	0
C. verruculosa	0	1 D	0	0
Microsphaeropsis arundinis	0	0	0	1 D
Phaeosphaeriopsis sp.	0	1 D	1 D	0
Pseudopithomyces maydicus	1 D	0	0	0
Trichosphaeriales				
Nigrospora oryzae	1 D	0	0	0
Xylariales				
Annulohypoxylon nitens	0	0	2 D	0
A. stygium	0	1 D	0	0
Daldinia eschscholtzii	0	1 D	0	0
Hypoxylon anthochroum	0	0	0	1 D
Nodulisporium sp.	3 M	1 D	0	1 D
Pestalotiopsis sp.	0	1 M	0	0
Xylaria feejeensis	0	0	1 D	0
Basidiomycota				
Hymenochaetales				
Pyrrhoderma noxium	0	0	1 D	0
Polyporales				
Irpex laceratus	0	1 D	0	0
Fomitopsis meliae	0	0	0	1 D
Ganoderma australe	0	0	1 D	0
Lentinus sajor-caju	0	0	0	1 D
Phanerochaete ericina	1 D	0	0	0

[1] = identified by DNA BLASTn (GenBank) and phylogenetic analysis and corroborated by morphological characterisation.
[2] = identified by morphological characterisation only.

Table 3. ITS gene region BLASTn results of Sabahan cave fungi.

Fungal Taxa	Source Sample	NCBI Identification	Query Cover (%)	Identities (%)	*E* value	NCBI Accession
Annulohypoxylon nitens	C3G2	KU684021	92%	99.30%	0.0	MN783056
Annulohypoxylon nitens	C3S3	FN252415	98%	99.00%	0.0	MN783036
Annulohypoxylon stygium	C2G4	MF770832	99%	99.90%	0.0	MN783042
Aspergillus aculeatus	C3G2	MK280716	100%	100.00%	0.0	MN783053
Aspergillus bertholletiae	C3G4	MK638758	100%	96.90%	0.0	MN783044
Aspergillus bertholletiae	C3S3	MH517369	100%	96.90%	0.0	MN783034
Aspergillus chevalieri	C2G1	MH752443	100%	100.00%	0.0	MN783093
Aspergillus japonicus	C1S3	KC128815	100%	100.00%	0.0	MN783088
Aspergillus japonicus	C3W4	KF800630	100%	100.00%	0.0	MN783040
Aspergillus niger	C3S3	MK203789	100%	100.00%	0.0	MN783035
Aspergillus nomius	C3G3	MH279416	99%	99.70%	0.0	MN783061
Aspergillus nomius	C3S4	MH279388	99%	99.80%	0.0	MN783075
Aspergillus nomius	C3W3	MH279387	100%	100.00%	0.0	MN783057
Aspergillus sydowii	C3W2	KX674612	100%	100.00%	0.0	MN783070
Aspergillus versicolor	C4G2	KX894661	100%	100.00%	0.0	MN783085
Aspergillus versicolor	C4S3	MF476080	100%	100.00%	0.0	MN783097
Cladosporium cladosporioides	C2W6	KU366279	100%	99.66%	0.0	MN783064
Cladosporium cladosporioides	C3G2	EF405864	99%	100.00%	0.0	MN783059
Clonostachys rosea	C1S5	MH869057	99%	98.85%	0.0	MN783019
Curvularia lunata	C3G2	JN116704	93%	100.00%	0.0	MN783060
Curvularia verruculosa	C2S2	JN116709	96%	100.00%	0.0	MN783027
Daldinia eschscholtzii	C2W1	KU304335	100%	99.32%	0.0	MN783086
Fomitopsis meliae	C4S2	KC585351	97%	98.24%	0.0	MN783083
Fusarium proliferatum	C2S1	HQ176445	99%	99.83%	0.0	MN783052
Fusarium proliferatum	C2S2	MH055399	100%	100.00%	0.0	MN783028
Fusarium proliferatum	C2S4	MH055399	100%	100.00%	0.0	MN783051
Fusarium proliferatum	C2S5	MH055399	100%	100.00%	0.0	MN783066
Fusarium proliferatum	C2W1	MH055399	100%	99.83%	0.0	MN783089
Ganoderma australe	C3S3	LC084692	94%	99.40%	0.0	MN783025
Hypoxylon anthochroum	C4G2	MH712243	91%	94.44%	0.0	MN783082
Irpex laceratus	C2G3	KF850375	100%	100%	0.0	MN783065
Lecanicillium fusisporum	C1S3	MF770736	93%	99.83%	0.0	MN783092
Lentinus sajor-caju	C4G2	KP283493	99%	98.99%	0.0	MN783084
Microsphaeropsis arundinis	C4S1	MK990140	98%	100.00%	0.0	MN783081
Nigrospora oryzae	C1W4	HQ607925	99%	100.00%	0.0	MN783090
Nodulisporium sp.	C2W5	KR016438	96%	99.46%	0.0	MN783033
Nodulisporium sp.	C4G2	KF494820	94%	99.16%	0.0	MN783080
Paecilomyces variotii	C3G4	FJ345354	99%	100.00%	0.0	MN783021

Penicillium bilaiae	C3S3	LN901118	100%	96.70%	0.0	MN783041
Penicillium citrinum	C2S4	MH858380	99%	100.00%	0.0	MN783029
Penicillium citrinum	C2S6	KT758118	87%	89.04%	0.0	MN783067
Penicillium citrinum	C2W1	MH858380	99%	100.00%	0.0	MN783050
Penicillium citrinum	C3W4	GU566273	99%	99.50%	0.0	MN783043
Penicillium citrinum	C4S1	MH858380	99%	99.83%	0.0	MN783076
Penicillium citrinum	C4S2	MG733673	100%	100.00%	0.0	MN783094
Penicillium coprophilum	C4S1	MK450685	99%	99.84%	0.0	MN783071
Penicillium ochrochloron	C2G4	MK720828	99%	100.00%	0.0	MN783072
Penicillium oxalicum	C2W1	KF152942	100%	100.00%	0.0	MN783096
Penicillium oxalicum	C2W2	KF152942	99%	99.84%	0.0	MN783063
Penicillium oxalicum	C4G1	KF367495	99%	99.84%	0.0	MN783074
Penicillium oxalicum	C4S2	KX674635	99%	99.68%	0.0	MN783079
Penicillium paxilli	C2G4	JN617709	100%	100.00%	0.0	MN783046
Penicillium paxilli	C2S2	JN617709	99%	99.36%	0.0	MN783039
Penicillium paxilli	C2S4	JN617709	100%	100.00%	0.0	MN783030
Penicillium paxilli	C2W4	JN617709	99%	99.67%	0.0	MN783031
Penicillium paxilli	C2W6	JN617709	99%	99.84	0.0	MN783068
Penicillium simplicissimum	C3G2	HQ607866	99%	99.80%	0.0	MN783054
Phaeosphaeriopsis sp.	C2W1	JQ936185	97%	99.48%	0.0	MN783018
Phaeosphaeriopsis sp.	C3W4	KF800300	99%	99.50%	0.0	MN783087
Phanerochaete ericina	C1S6	KP135146	98%	94.25%	0.0	MN783095
Plectosphaerella cucumerina	C3S2	EU326201	97%	99.50%	0.0	MN783077
Pochonia chlamydosporia	C3G2	EU266591	97%	99.80%	0.0	MN783069
Pseudopithomyces maydicus	C1W1	MF919624	97%	100.00%	0.0	MN783091
Purpureocillium lilacinum	C2S1	MH860675	100%	100.00%	0.0	MN783037
Purpureocillium lilacinum	C3S3	KY951911	100%	99.70%	0.0	MN783024
Purpureocillium lilacinum	C3S4	MH860675	99%	99.80%	0.0	MN783073
Purpureocillium lilacinum	C3W1	MH860675	100%	99.70%	0.0	MN783020
Purpureocillium lilacinum	C3W2	MH860675	99%	98.60%	0.0	MN783058
Pyrrhoderma noxium	C3W2	KU194338	99%	98.50%	0.0	MN783062
Talaromyces flavus	C3G2	MH857785	100%	99.50%	0.0	MN783055
Talaromyces sp.	C3S3	MH857785	99%	92.20%	0.0	MN783022
Tolypocladium album	C2W4	AB457005	95%	98.08%	0.0	MN783032
Trichoderma asperellum	C2S5	KR856222	99%	100.00%	0.0	MN783048
Trichoderma asperellum	C2W4	MG171157	99%	100.00%	0.0	MN783045
Trichoderma asperellum	C3G4	KY623504	99%	100.00%	0.0	MN783026
Trichoderma asperellum	C4G1	MK253266	100%	99.84%	0.0	MN783078
Trichoderma citrinum	C2W4	DQ000630	96%	97.53%	0.0	MN783038
Trichoderma harzianum	C2G2	MF782821	99%	99.84%	0.0	MN783047
Trichoderma paraviridescens	C3G2	MF782827	99%	99.80%	0.0	MN783049
Xylaria feejeensis	C3S3	KY951907	99%	99.70%	0.0	MN783023

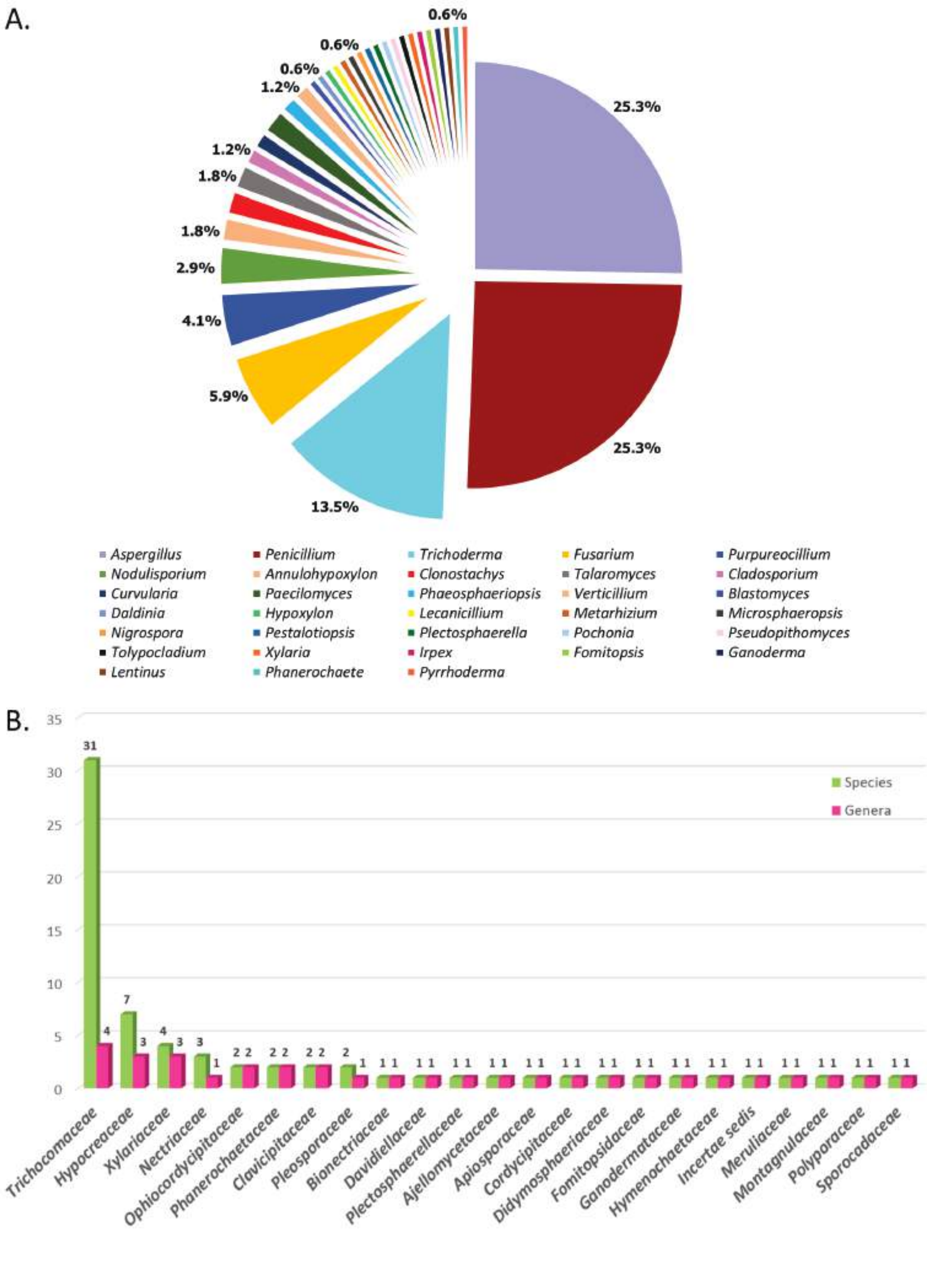

Figure 5. A. Proportion of fungal isolates by genus (n = 170) by genera;
B. Most diverse fungal families observed in this study.

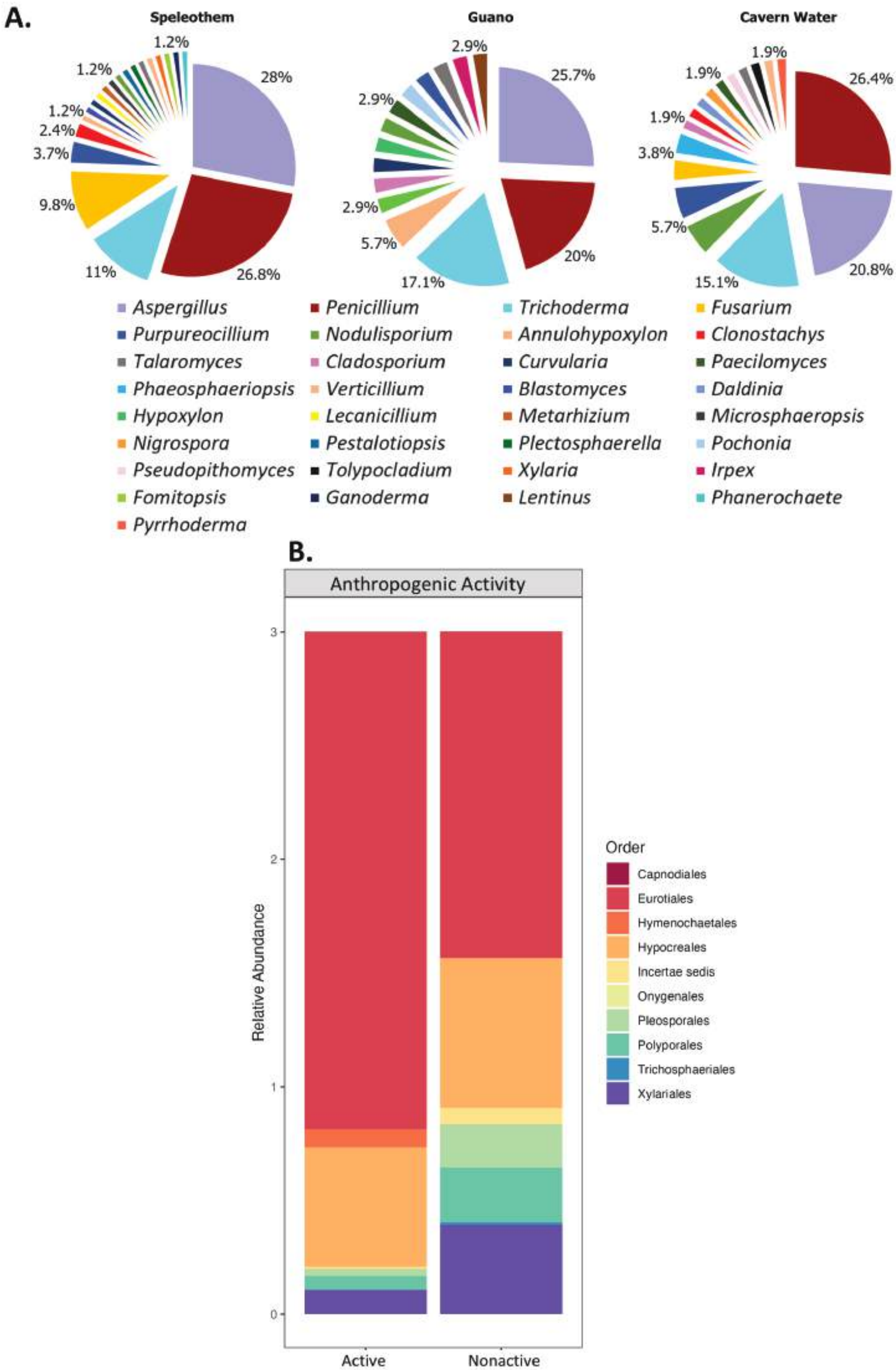

Figure 6. A. Distribution of fungal isolates in from various substrates by genus; **B.** Relative abundance of fungal Orders in active and nonactive caves.

Table 4. Average fungal occurrence and fungal abundance of different substrates between active and non-active caves.

Substrates	Average occurrence / Abundance	
	Active	Non-active
Fungal occurrence	4.07 ± 2.11 taxa sample^{-1}	3.29 ± 2.31 taxa sample^{-1}
Speleothem fungal abundance	190.6 ± 179.5 CFU / cm^2	72.3 ± 60.0 CFU / cm^2
Cavern water fungal abundance	212.9 ± 240.1 CFU ml^{-1}	50.3 ± 40.4 CFU ml^{-1}
Bat guano fungal abundance	5627.8 ± 4935.4 CFU g^{-1}	3318.5 ± 2950.1 CFU g^{-1}

Variations in relative abundances across conditions, substrates, and caves

There were differences in relative abundance observed across the active and non-active sites (Kruskal-Wallis $\chi^2 = 11.37$, $p = 0.0007$), as well as between locality (Kruskal-Wallis $\chi^2 = 15.17$, $p = 0.0017$). Significant variations were also found across cavern water (Kruskal -Wallis $\chi^2 = 11.95$, $p = 0.00056$) and guano (Kruskal-Wallis $\chi^2 = 4.73$, $p = 0.030$) of active versus non-active caves. However, there were no significant differences detected within the speleothem samples.

Aspergillus fungi represented the highest proportion of fungal isolates in both active caves, while *Penicillium* dominated in the non-active caves (Figure 7A). From these isolates, only seven genera (representing 15 species) were shared between both active and non-active caves (Figure 7B). The variations in relative abundances between conditions, substrates, and locality were assessed using Wilcoxon Rank-Sum Test. The analysis revealed that fungal communities in non-active caves had significantly higher relative abundances than those in active caves ($p = 0.0008$) (Figure 8A). Additionally, the fungal communities in Balambangan showed significantly higher relative abundance than in Gomantong ($p = 0.0024$), but no significant differences were found between other cave sites (Figure 8B). No significant differences in fungal community relative abundances were observed between the different substrate types (Figure 8C).

Effects of anthropogenic activities on cave fungal communities

Three beta regression models were employed, with relative abundance as the response variable, and anthropogenic activity and distance from cave entrance as the predictor variables. The best and most plausible model following Akaike's information criterion corrected for small samples (AICc) indicated that anthropogenic activity is a significant factor that influences the relative abundance of fungal communities (Table 5).

Table 5. Regression relationships between relative abundance and various predictor variables analysed using beta regression modelling.

Response Variable	Parameters (Predictor Variables)	Estimate	Standard Error	z-value	Pr(>|z|)	AICc (Δ)
A. Relative abundance	(Intercept)	–3.505	0.176	–19.961	< 2e^{-16} ***	–667.3
	Anthropogenic activity	0.572	0.172	3.332	**0.000862*****	(2.1)
	Distance from entrance	–0.0004	0.003	–0.138	0.891	
B. Relative abundance	(Intercept)	–3.5212	0.1358	–25.930	< 2e^{-16} ***	–669.4
	Anthropogenic activity	0.582	0.1580	3.685	**0.000229*****	(0.00)
C. Relative abundance	(Intercept)	–3.178	0.142	–22.450	< 2e^{-16} ***	–658.8
	Distance from entrance	–0.003	0.002	–1.418	0.156	(10.6)

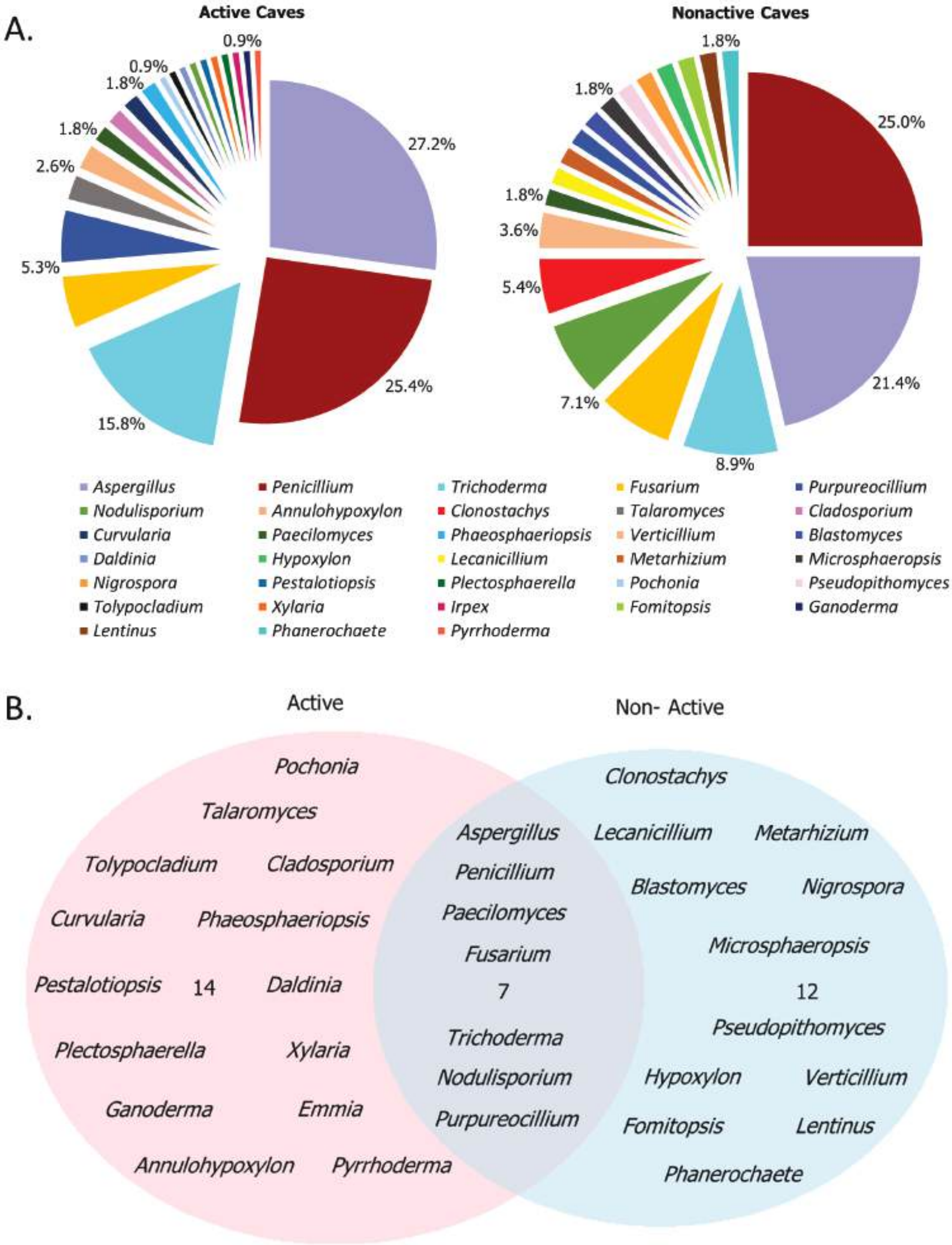

Figure 7. A. Distribution of fungal isolates in active caves versus non-active caves;
B. Venn diagram of fungal genera obtained from active versus non-active caves in this study.

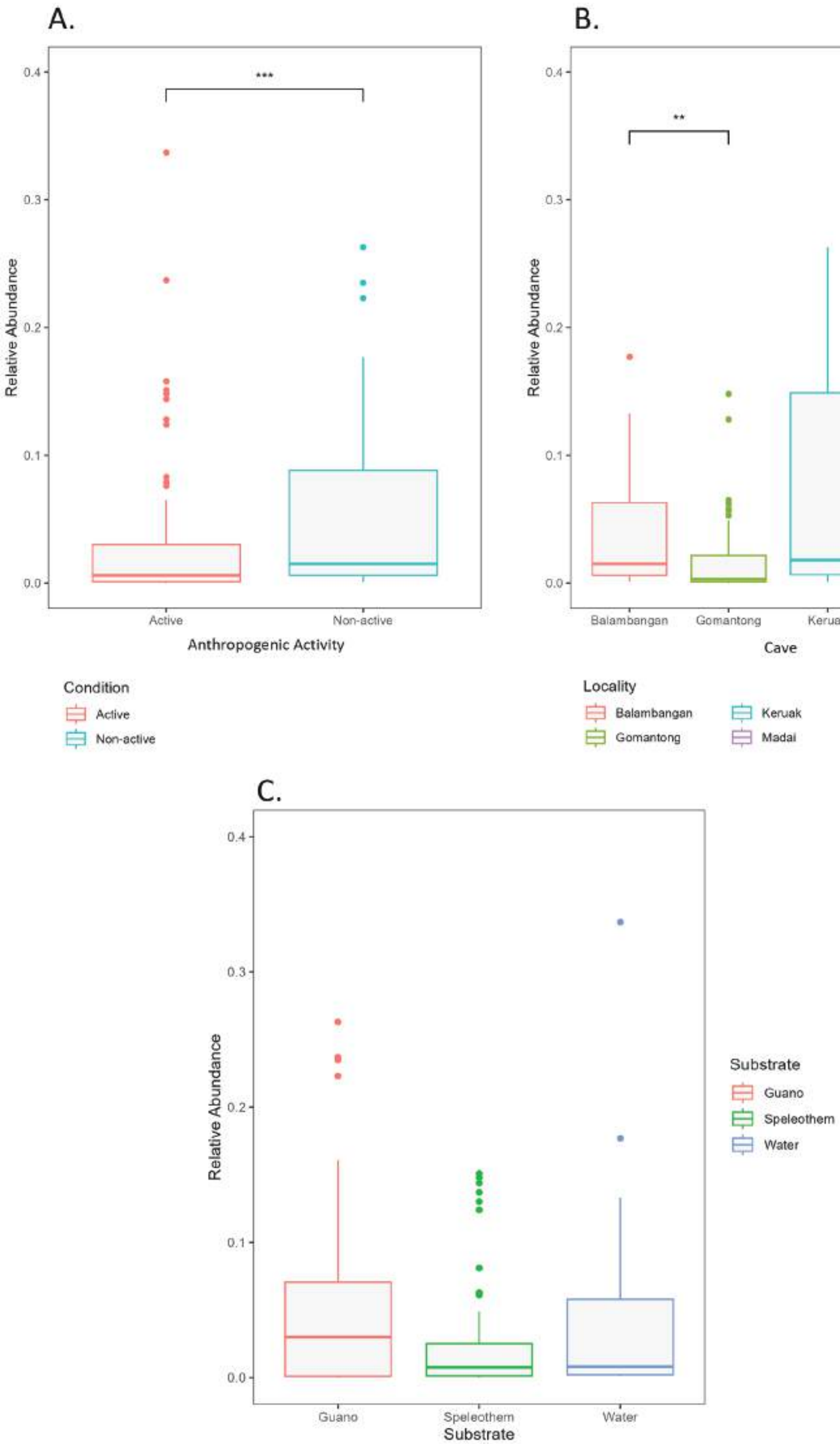

Figure 8. Box plots of the pairwise comparative analyses of fungal abundance by **A.** Anthropogenic activity; **B.** Cave locality; **C.** Substrate type.

DISCUSSION

The diversity of fungal community composition in caves remains largely unexplored, and their potential applications are still unknown (Poli *et al.* 2024). This study investigated the community composition of culturable fungi in various substrates of four Bornean karst caves, focusing on the potential influence of anthropogenic activity. The current research described 69 different taxa from 33 genera, 10 orders, originating from two divisions: Ascomycota and Basidiomycota. *Aspergillus* (25.3%), *Penicillium* (25.3%), *Trichoderma* (13.5%), and *Fusarium* (5.9%) were the most dominant genera recorded. These findings are congruent with previous reports of fungal diversity from other regions, where fungi from these genera are some of the most dominant fungi isolated from cave environments (Vanderwolf *et al.* 2013; Nováková *et al.* 2017; Zhang *et al.* 2020).

The isolation of *Aspergillus* spp. and *Penicillium* spp. dominated all environmental substrates, collectively accounting for the majority of fungal isolates. The results suggested that these fungi area ubiquitously distributed within and among cave systems. *Aspergillus aculeatus*, *A. flavus*, *A. niger*, and *P. simplicissimum* were isolated from three out of four cave sites studied, while *P. citrinum* was isolated from 14 samples across all four cave sites. In a study by Grishkan *et al*. (2004), most fungal species recovered from caves were found at only one sampling point. The appearance of fungal isolates in multiple cave sites suggest their ubiquitous nature in cave environments.

This study also identified six Basidiomycota isolates, with only two originating from guano samples. Basidiomycota fungi are less frequently reported in caves compared to external environments, likely because they are typically associated with large and nutrient-rich substrates, such as wood, leaf litter, and tree roots (Subari *et al.* 2022), which are generally not abundant in subterranean environments. Notably, four of the 33 fungal genera identified in this study – *Blastomyces*, *Nodulisporium*, *Phaeosphaeriopsis*, and *Pyrrhoderma* – are reported for the first time in cave environments in this study (Vanderwolf *et al.* 2013; Zhang *et al.* 2017; Nováková *et al.* 2018; Zhang *et al.* 2020). Additionally, 12 fungal species were isolated from cave environment for the first time, namely *Aspergillus luchuensis*, *A. bertholletiae*, *Curvularia verruculosa*, *Daldinia eschscholtzii*, *Fomitopsis meliae*, *Hypoxylon anthochroum*, *Irpex laceratus*, *Lentinus sajor-caju*, *Metarhizium carneum*, *Penicillium brevistipitatum*, *Phanerochaete ericina*, and *Trichoderma citrinum*. This list excludes species reported by Wasti *et al.* (2021).

From the different substrates collected across all four caves, 44 fungal species were isolated from speleothem samples, 34 species from cavern water, and 28 species from bat guano. Vanderwolf *et al.* (2013) reported that cave walls and bat guano make up a much larger proportion of fungal taxa than cavern water. Most of the speleothem samples in this study were wet, indicating that they were biologically active (Dodge-Wan and Min 2013). Bat guano creates a moist microclimate rich in nutrients, which is an oligotrophic ecosystem (Paulson 1972; Nieves-Rivera *et al.* 2009), making it ideal for fungal growth. In this context, bats are most likely one of the largest sources of organic carbon for cave fungi.

A total of 124 fungal isolates (68.9%) were extracted from active caves, compared to 56 (31.1%) from non-active caves. There were higher MTUs and unique species identified from active caves than non-active caves. Also, the average fungal occurrence and fungal abundance for all substrates were higher for active caves sites. Madai cave, an active cave, consistently ranked the highest for average fungal abundance across all substrate types. It should be noted that sampling opportunities in non-active caves were reduced due to their relatively smaller sizes. For instance, Keruak cave lacked cavern water to sample from, and Balambangan cave had only one bat guano pile, which yielded a single fungal isolate.

The presence of humans and various fauna influences fungal assemblages in caves (Griffin *et al*. 2014; Bastian *et al.* 2009). Extensive studies have been conducted on the interaction of cave fungi and bats, with bats known to harbor and disperse a wide variety of fungi, and thus shaping the cave mycobiome (Seelan and Anwarali 2009; Holz *et al.* 2018). Given the large number of visitors to Gomantong and Madai caves annually, it is likely that human activity inadvertently introduces a range of foreign substances and potential nutrients for fungi, unintentionally selectively promoting the abundance of certain taxa and disrupting the microbial equilibrium (Griffin *et al.* 2014; Porca *et al*. 2011).

Statistical analyses and beta regression modelling showed anthropogenic activity to be a significant factor affecting fungal composition in caves. Increased human traffic into caves can cause contamination to indigenous fungal communities by introducing non-native microorganisms and nutrients into the cave environment (Chelius *et al.* 2009; Porca *et al.* 2011; Griffin *et al*. 2014). The introduction of these nutrients and increased food source may promote a more metabolically diverse range of microbes, resulting in high-impact zones becoming more organically rich than other non-disturbed areas of the cave. Human visitation indirectly alters cave conditions, such as air temperature and airflow currents (Pflitsch and Piasecki 2003), both of which are known to affect cave microbial community composition. Anthropogenic disturbance may also account for the increased relative abundance of Eurotiales fungi in the active cave sites, especially generalist cosmopolitan fungi such as *Aspergillus* and *Penicillium*. These fungi, which are better adapted to fluctuating environment, may start to outcompete other fungal taxa that are more fastidious and slower growing.

Pairwise comparison highlighted a significant difference in fungal composition, particularly between Balambangan and Gomantong cave. Balambangan cave is located on the relatively small Balambangan island 44 km north of Kudat, a town on the northern coast of Borneo. As the only island cave in this study, Balambangan cave contrasts sharply with the other three caves, which are located on the Borneo mainland. The mainland caves may share more ecological similarities with each other despite their differences in extent of human disturbance. Gomantong cave is the most visited cave in Sabah, Borneo, attracting tens of thousands of visitors from all over the world annually (Wasti *et al*. 2020). The ecological and geographical differences between the caves likely to account for differences in their fungal composition, in addition to the influence of anthropogenic activity.

The Wilcoxon analyses and beta regression model in this study indicated that neither substrate type nor distance from entrance were significant factors in affecting fungal relative abundance. Arthropods and bats are active dispersers of fungi and fungal spores (Šustr *et al.* 2005; Wasti *et al*. 2020). Compound that with potential environmental fungal spore dispersers such as water flow (Ikner *et al.* 2007) and airflow (Pusz *et al.* 2015; Ogórek *et al.* 2013), these factors may facilitate movement of fungal spores throught the cave once they are already introduced, allowing their spread across various substrates.

Ultimately, anthropogenic activity appeared to be a determinant factor of fungal community composition in caves. However, each individual cave is unique, and previous data suggested that fungal communities tend to differ between caves due to a multitude of environmental, physiochemical, and ecological factors (Vanderwolf *et al*. 2013; Nováková *et al*. 2018; Pfendler *et al*. 2018; Zhang *et al.* 2019).

In this study, the medium of choice for isolation of culturable fungi was PDA, as it is one of the most common types of media selected for culturing cave fungi (Vanderwolf *et al*. 2013). The use of non-selective agar allowed for the maximising of diversity during dilution and isolation process. Other common agar types have been used to isolate cave fungi, including Malt agar and Sabouraud agar, and the type of agar media used likely affects the variety of fungi observed in the cultures (Visagie *et al.* 2019, Out *et al*. 2016). In recent years, non-culture methods of identifying cave fungi are becoming more common, particularly genetic approaches. Modern molecular methods allow for direct genetic sampling of the environment, including metagenomic analysis (Zepeda Mendoza *et al*. 2016; Lindner *et al.* 2011; Zhang *et al.* 2019). More studies on cave fungi in tropical regions should be conducted using both culture-dependent and non-culture methods in the future to enhance the understanding of the cave fungi communities.

CONCLUSION

The fungal communities of Sabahan caves appeared to be influenced by both anthropogenic activity and substrate type. Most of the fungal isolates were Ascomycota, specifically *Aspergillus* and *Penicillium* fungi. Basidiomycota fungi made up a small percentage of the isolates, especially those belong to Order Polyporales. Statistical analyses and beta regression modeling showed that anthropogenic activity was a significant factor affecting relative fungal abundance in thes studied limestone caves. Future cave microbial studies should integrate metagenomics and culture-independent methods to complement culture-based methods. Furthermore, mycobiome comparisons between the entrance and deep zones in caves with various morphologies and anthropogenic rates should be further investigated. The findings of this study provide essential baseline data on the mycobiome of tropical caves and help to increase the understanding of cave ecologies, especially at sites absent of white nest swiftlets.

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Supplement 1. Site descriptions of the four caves in North Borneo (Sabah).

The Gomantong cave system is located in Gomantong Forest Reserve, Kinabatangan, Sabah, which is a 3,297-hectare Class VI Virgin Jungle Reserve (VJR). The cave system is part of the largest limestone outcrop in the area, the Gomantong Hill (Lundberg and McFarlane 2012). The cave is composed of two main sections: Semud Hitam (Black Cave) and Semud Putih (White Cave). The area surrounding the forest reserve is almost exclusively utilised for monocrop agriculture of oil palm plantation. The cave system is famous for its swiftlet nest farming and harvesting (Ismail 1999; Hobbs 2004; Lundberg and McFarlane 2012), which are conducted by municipal employees. Birds nest harvesting apparatus are visible throughout the cave. At dusk, thousands of bats can be seen leaving the cave as birds fly into the cave. The heaps of guano that cover the cave floor are inhabited by a plethora of arthropods, predominantly cockroaches, beetles and centipedes. The guano exudes a pungent smell of ammonia that can be perceived dozens of yards before entering the cave itself. Ceiling apertures allow for water to enter the cave from above, and streams of water flow through the cave and beyond the entrance. Due to the high ceiling, the only speleothem available for sampling were located near or on the walls of the cave. According to the Sabah Wildlife department, Gomantong caves average between 13,000 to 15,000 visitors annually, mostly comprised of foreign tourists. Semud Hitam's boardwalk is located near the walls of the cave, resulting in some sections of the cave walls being marked or vandalised by visitors. Semud Hitam span about 100 m from the mouth of the cave to the end of the board-walk. Six speleothem samples, six water samples, and four bat guano samples collected from this cave.

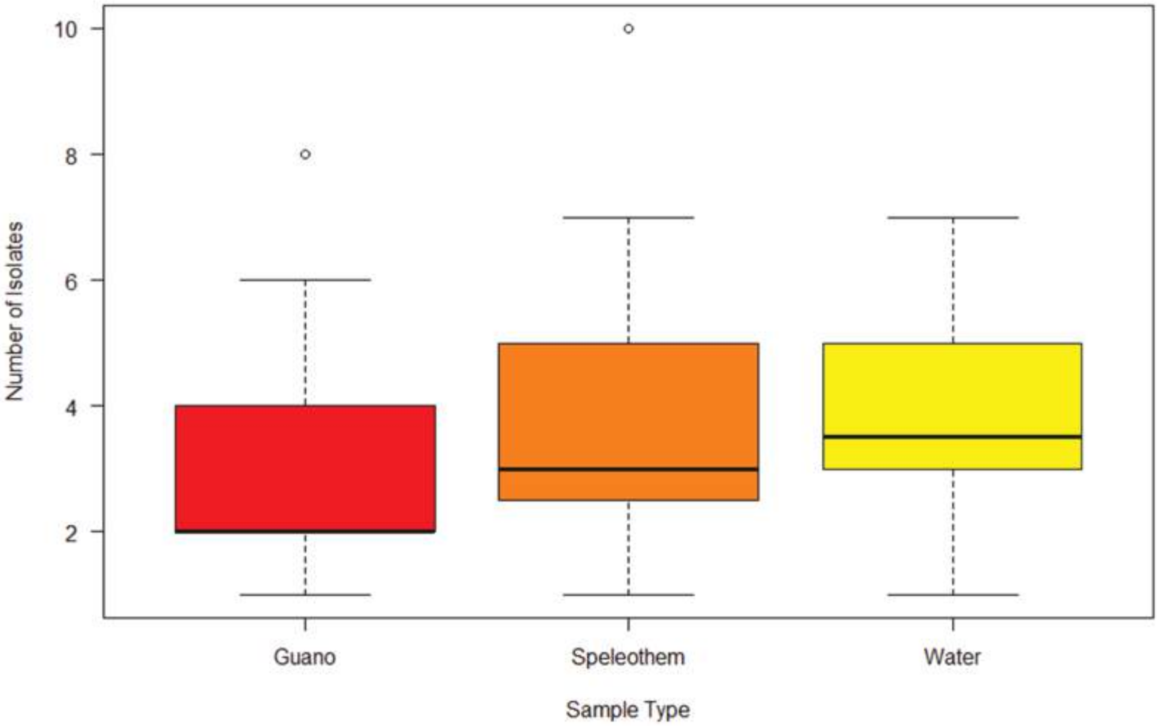
The Madai cave system is located in Baturong Madai Forest Reserve, Kunak, Sabah, which is a 3,436 hectare Class VI VJR. According to Sabah Wildlife Department, the cave is part of the inheritance of the Idahan people. Similar to Gomantong Forest Reserve, the area surrounding the Baturong Madai Forest Reserve is dominated by monocrop production of palm oil. There is a village located directly in front of the cave entrance, where families of swiftlet nest harvesters reside. In contrast to Gomantong caves, swiftlet nest harvesting in Madai cave is performed by the community itself. A percentage of royalty payments are made to the government and Sabah Wildlife Department, which help decide the best suitable dates to harvest the nests. Madai cave is also known to host large groups of tourists during the off seasons of swiftlet nest harvesting. Bat colonies were found throughout the cave ceiling, leading to sections of the cave floor covered with piles of guano. Visitors are allowed to enter the cave during the off season of swiftlet nest harvesting. The cave is full of arthropods such as cockroaches, centipedes, beetles, and spiders. However, there were marks of vandalism on the walls of many sections near the entrance due to the cave getting its fair share of visitors and the local children from the community. A large stream of water runs through the cave. Most sections within the cave had high ceilings, but some areas had low ceilings that allowed for the sampling of speleothem. The cave does not have a boardwalk or path. Four speleothem samples, four water samples, and four bat guano samples were collected from this cave.

Balambangan cave is located on Balambangan Island, which is situated about 40 km norths of the town of Kudat, Sabah, a coastal town in northern Borneo. It is under the jurisdiction of Sabah Parks as it has been gazetted as a part of Tun Mustapha Marine Park. This study site is the largest known limestone cave in the Bongaya sedimentary rock formation of the island, reaching up to 300 m in depth and up to 8 m in width in certain areas (Ali *et al.* 2007). The cave is relatively well lit due to a number of apertures in the ceiling, especially a large aperture around 30 m inside the cave from the cave entrance. Canopy cover can be seen from inside the cave when looking up at the aperture. Forest detritus such as rotting wood and leaves are scattered underneath areas of these ceiling openings, as well as areas of wet soil and water puddles that exist after periods of heavy rain. Many stalagmites and stalactites are spread across the cave floor and ceiling, respectively. Not many arthropods were seen within the cave except the occasional spider, and no swiftlet nests were present at the time of the visit. Several holes in the ceiling of the cave contain roosting bats, where guano piles or guano-enriched soil can be located directly below them. Seven speleothem samples, four water samples, and one bat guano sample were acquired from Balambangan cave.

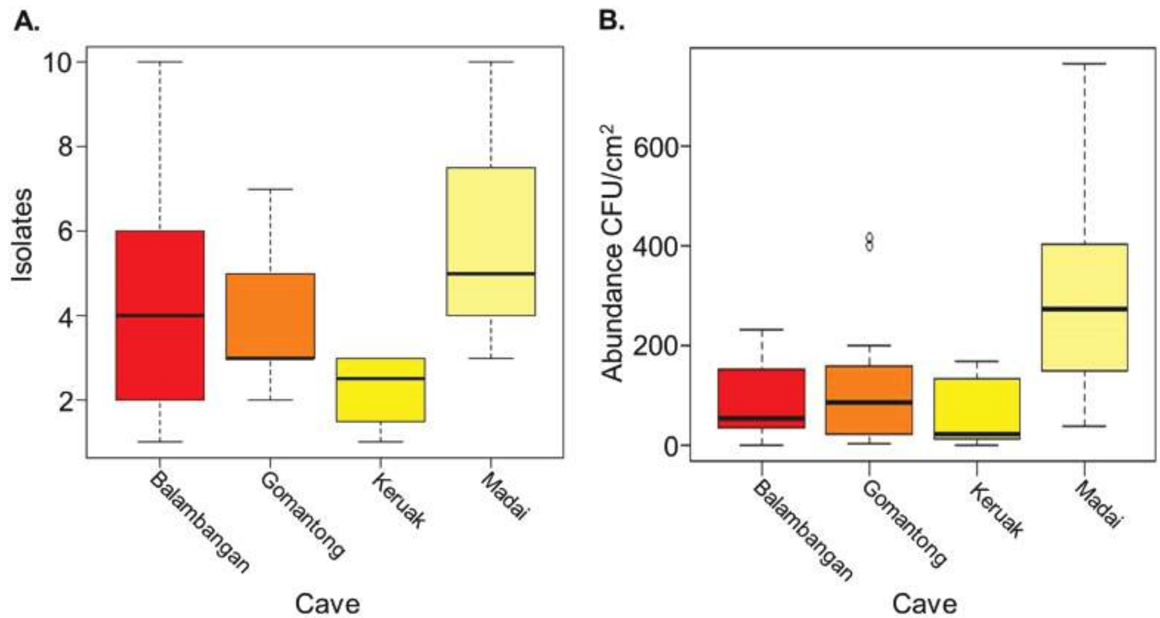
Keruak Cave is located inside a limestone outcrop nearby the banks of the Kinabatangan River, Sabah, in the Keruak Class VI VJR. It is the smallest of the four caves in this study, spanning only 30 m in length. The cave is only accessible by a trail about 1.5 km northwest from the closest village, Sukau. The land use surrounding the jungle reserve are either by the Sukau village community or monocrop production of palm oil. Nonetheless, tourist activity in this area is significantly lower than the popular Gomantong and Madai caves. No swiftlet nest harvesting occurs in Keruak Cave. Unlike the other caves described in this study, none of the sections in this cave are completely dark as the mouth of the cave is very tall and the cave is relatively short. Only two bat guano piles were found in the cave, and there are no pools of water or streams available to be sampled. There is a section of the cave that is inaccessible without spelunking gears and guidance from trained professionals. Four speleothem samples and two bat guano samples were acquired from Keruak cave. No water samples were collected from this cave due to the lack of water pools or streams.

Supplement 2. Box plots of fungal isolate and abundance data.

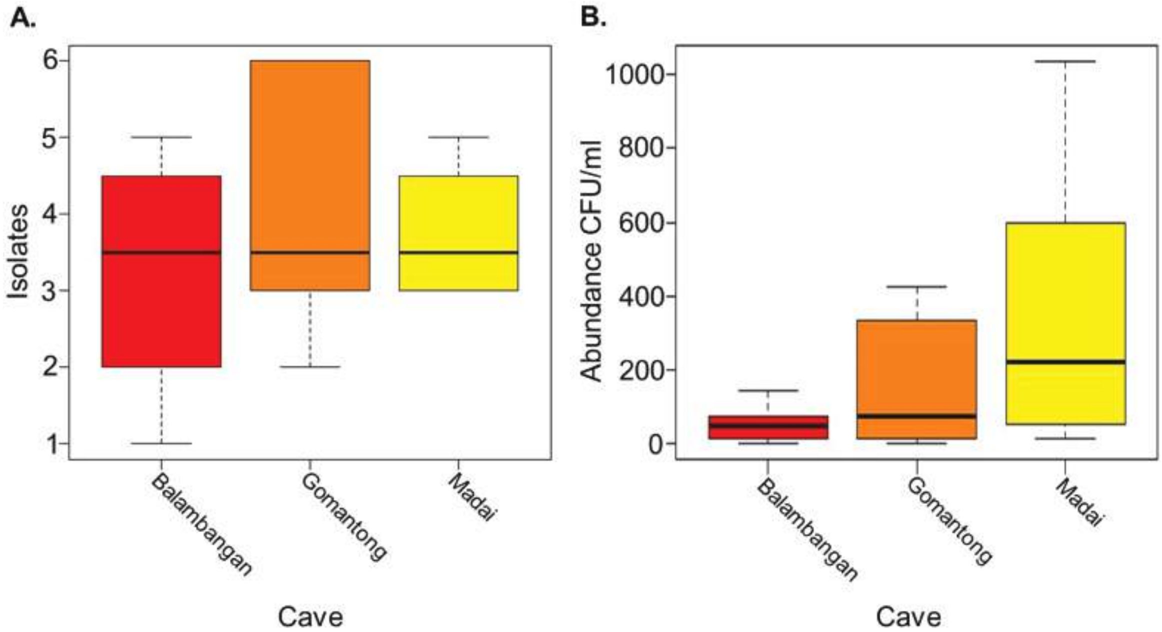
S2.1. Box plot of fungal occurrence per sample from different substrates in Sabahan caves.



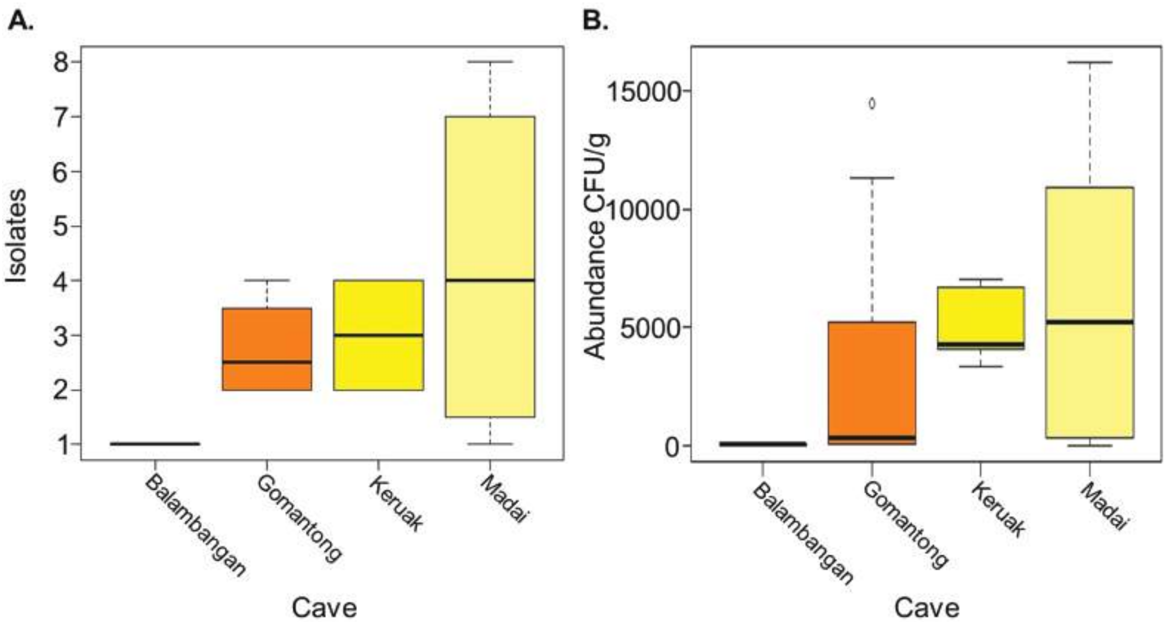
S2.2. Boxplot of speleothem samples comparison of all triplicates. **A.** Number of pure isolates; **B.** Fungal abundance.



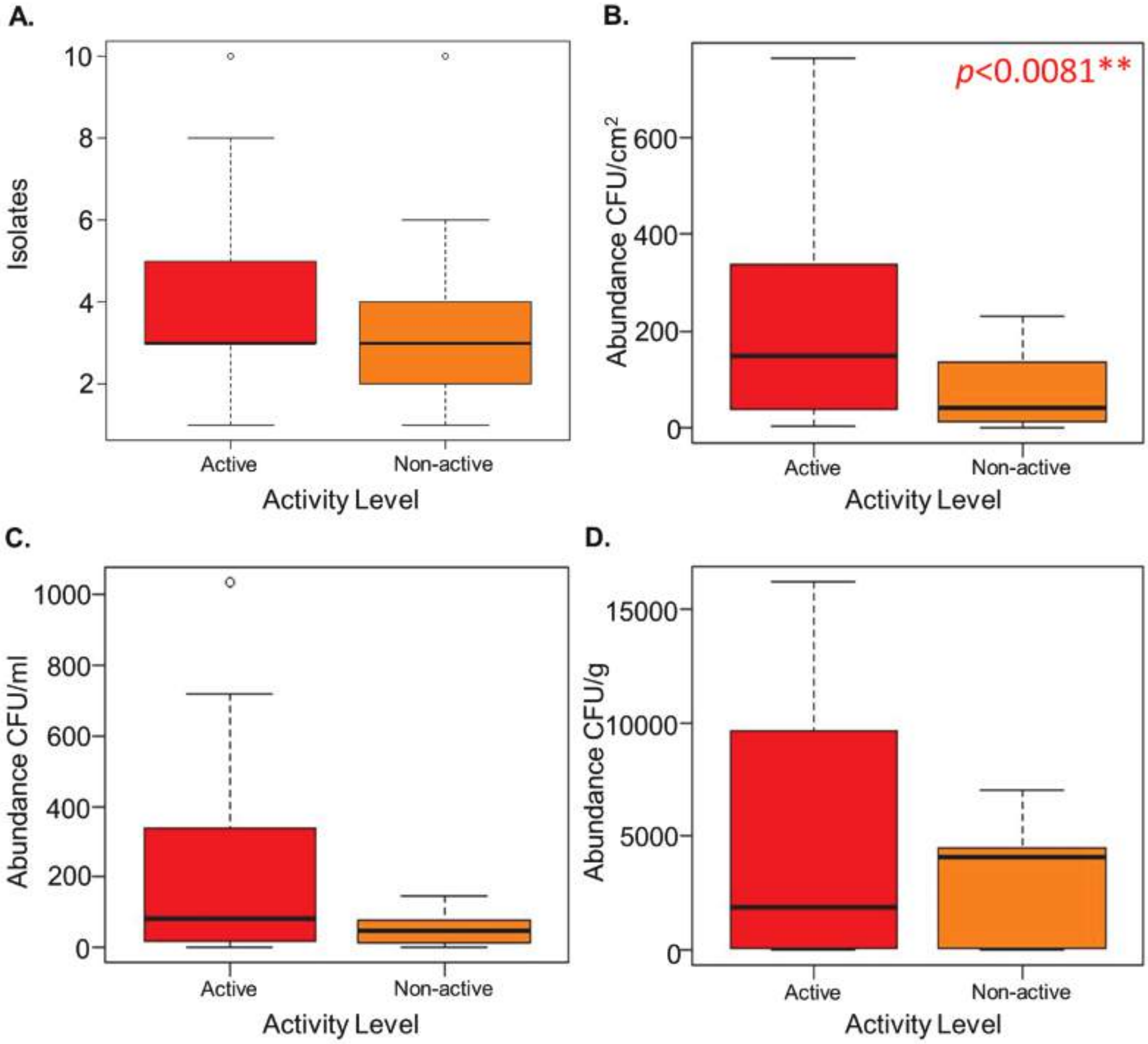
S2.3. Boxplot comparison of cavern water samples of all triplicates. **A.** Number of pure isolates; **B.** Fungal abundance



S2.4. Boxplot comparison of bat guano samples by cave. **A.** Number of pure isolates; **B.** Fungal abundance.



S2.5. Fungal occurrence and abundance comparisons between active and non-active caves. **A.** Fungal occurrence in all samples; **B.** Fungal abundance in speleothem samples; **C.** Fungal abundance in cavern water samples; **D.** Fungal abundance in bat guano samples.



Supplement 3. Fungal identification and abundance data from all samples from the four Sabahan caves.

S3.1. Abundance of culturable fungi from speleothem samples.

Sample (Distance from entrance)	Fungal Taxa	Identification	CFU / cm^3	
			Fungal Taxa	Sample Total
Balambangan				**Mean = 81.2**
C1S1 (0.8 m)	*Clonostachys rosea*	Morphology	4.4	113.3
	Penicillium sp. 1	Morphology	0.9	
	Penicillium citrinum	Morphology	58.2	
	Penicillium simplicissimum	Morphology	45.3	
	Verticillium sp.	Morphology	4.4	
C1S2 (4.0 m)	*Nodulisporium* sp.	Morphology	23.1	33.3
	Trichoderma paraviridescens	Morphology	10.2	
C1S3 (4.8 m)	*Aspergillus aculeatus*	Morphology	58.2	81.8
	Aspergillus japonicus	DNA	12.4	
	Blastomyces sp.	Morphology	0.9	
	Fusarium sp. 1	Morphology	0.4	
	Lecanicillium fusisporium	DNA	1.3	
	Metarhizium carneum	Morphology	8.4	
C1S4 (11.9 m)	*Penicillium* sp. 1	Morphology	9.8	58.7
	Penicillium citrinum	Morphology	44	
	Trichoderma paraviriscens	Morphology	4.9	
C1S5 (17.7 m)	*Clonostachys rosea*	DNA	6.7	6.7
C1S6 (19.7 m)	*Aspergillus acidus*	Morphology	0.4	193.3
	Aspergillus bertholletius	Morphology	89.8	
	Aspergillus flavus	Morphology	44.9	
	Aspergillus japonicus	Morphology	0.9	
	Aspergillus niger	Morphology	5.3	
	Aspergillus parasiticus	Morphology	14.7	
	Fusarium sp. 1	Morphology	10.7	
	Fusarium sp. 2	Morphology	4.9	
	Phanerochaete ericina	DNA	11.1	
	Trichoderma harzianum	Morphology	10.7	
Gomantong				**Mean = 118.1**
C2S1 (2.0 m)	*Aspergillus niger*	Morphology	4.9	159.1
	Fusarium proliferatum	DNA	5.8	
	Penicillium citrinum	Morphology	103.6	
	Pertalotiopsis sp.	Morphology	0.4	
	Purpureocillium lilacinum	DNA	44.4	
C2S2 (19.8 m)	*Curvularia verruculosa*	DNA	0.9	17.8
	Fusarium proliferatum	DNA	15.1	
	Penicillium paxilli	DNA	1.8	
C2S3 (44.3 m)	*Fusarium proliferatum*	Morphology	88.9	142.2
	Trichoderma harzianum	Morphology	53.3	
C2S4 (63.9 m)	*Penicillium citrinum*	DNA	1.8	15.6
	Penicillium paxilli	DNA	0.4	
	Fusarium proliferatum	DNA	13.3	
C2S5 (86.7 m)	*Aspergillus* sp. 1	Morphology	7.1	35.1
	Aspergillus acidus	Morphology	17.8	
	Aspergillus niger	Morphology	0.4	
	Fusarium proliferatum	DNA	3.1	
	Penicillium paxilli	Morphology	0.9	
	Penicillium simplicissimum	Morphology	0.9	
	Trichoderma asperellum	DNA	4.9	
C2S6 (102.0 m)	*Penicillium citrinum*	DNA	27.6	339.1
	Penicillium paxilli	Morphology	266.7	
	Trichoderma harzianum	Morphology	44.9	

Madai				**Mean = 299.3**
C3S1 (1.0 m)	*Aspergillus* sp. 2	Morphology	72.9	554.7
	Aspergillus flavus	Morphology	222.7	
	Penicillium paxilli	Morphology	259.1	
C3S2 (16.0 m)	*Aspergillus aculeatus*	Morphology	4.4	381.3
	Aspergillus flavus	Morphology	44.9	
	Penicillium citrinum	Morphology	272.4	
	Plectosphaerella cucumerina	DNAb	15.1	
	Trichoderma harzianum	Morphology	44.4	
C3S3 (55.0 m)	*Annulohypoxylon nitens*	DNA	62.7	175.1
	Aspergillus bertholletius	DNA	1.8	
	Aspergillus europaeus	Morphology	4.4	
	Aspergillus niger	DNA	0.9	
	Ganoderma australe	DNA	49.8	
	Penicillium bilaiae	DNA	44.4	
	Purpureocillium lilacinum	DNA	4.9	
	Talaromyces sp.	DNA	0.4	
	Trichoderma asperellum	Morphology	1.3	
	Xylaria feejeensis	DNA	4.4	
C3S4 (75.0 m)	*Aspergillus* sp. 1	Morphology	13.3	86.2
	Aspergillus nomius	DNA	49.3	
	Penicillium citrinum	Morphology	9.3	
	Purpureocillium lilacinum	DNA	13.3	
	Trichoderma harzianum	Morphology	0.9	
Keruak				**Mean = 58.9**
C4S1 (6.3 m)	*Microsphaeropsis arundinis*	DNA	93.8	113.3
	Penicillium coprophilum	DNA	10.2	
	Penicillium citrinum	DNA	9.3	
C4S2 (6.7 m)	*Fomitopsis meliae*	DNA	0.4	100.0
	Penicillium citrinum	DNA	99.1	
	Penicillium oxalicum	DNA	0.4	
C4S3 (22.6 m)	*Aspergillus versicolor*	DNA	13.3	13.8
	Penicillium brevistipitatum	DNA	0.4	
C4S4 (28.7 m)	*Aspergillus* sp. 4	Morphology	8.4	8.4

S3.2. Abundance of culturable fungi from cavern water samples.

Sample (Distance from entrance)	Fungal Taxa	Identification	CFU / cm^3	
			Fungal Taxa	Sample Total
Balambangan				**Mean = 50.3**
C1W1 (1.8 m)	*Fusarium* sp. 2	Morphology	22.2	96.7
	Nodulisporium sp.	Morphology	1.1	
	Paecilomyces sp.	Morphology	25.6	
	Pseudopithomyces maydicus	DNA	12.2	
	Purpureocillium lilacinum	Morphology	35.6	
C1W2 (4.1 m)	*Clonostachys rosea*	Morphology	24.4	65.6
	Nodulisporium sp.	Morphology	1.1	
	Penicillium citrinum	Morphology	26.7	
	Verticillium sp.	Morphology	13.3	
C1W3 (14.4 m)	*Aspergillus japonicus*	Morphology	11.1	36.7
	Penicillium sp. 1	Morphology	1.1	
	Penicillium chrysogenum	Morphology	24.4	
C1W4 (20.0 m)	*Nigrospora oryzae*	DNA	2.2	2.2
Gomantong				**Mean = 131.5**
C2W1 (0.0 m)	*Aspergillus* sp. 3	Morphology	2.2	25.6
	Daldinia eschscholtzii	DNA	2.2	
	Fusarium proliferatum	DNA	2.2	
	Penicillium citrinum	DNA	13.3	
	Penicillium oxalicum	DNA	1.1	

	Phaeosphaeriopsis sp.	DNA	1.1	
	Trichoderma koningii	Morphology	3.3	
C2W2 (34.4 m)	*Penicillium citrinum*	Morphology	33.3	35.6
	Penicillium oxalicum	DNA	1.1	
	Trichoderma asperellum	Morphology	1.1	
C2W3 (38.0 m)	*Aspergillus* sp. 4	Morphology	113.3	254.4
	Aspergillus bertholletius	Morphology	3.3	
	Penicillium paxilli	Morphology	13.3	
	Trichoderma koningii	Morphology	124.4	
C2W4 (42.1 m)	*Penicillium paxilli*	DNA	23.3	296.7
	Talaromyces flavus	Morphology	124.4	
	Tolypocladium album	DNA	122.2	
	Trichoderma asperellum	DNA	13.3	
	Trichoderma citrinum	DNA	2.2	
	Trichoderma paraviridescens	Morphology	11.1	
C2W5 (110.1 m)	*Nodulisporium* sp.	DNA	1.1	113.3
	Penicillium paxilli	Morphology	112.2	
C2W6 (120.0 m)	*Cladosporium cladosporioirdes*	DNA	2.2	63.3
	Penicillium paxilli	DNA	23.3	
	Trichoderma harzianum	Morphology	37.8	
Madai				**Mean = 299.3**
C3W1 (3.0 m)	*Aspergillus japonicus*	Morphology	25.6	744.4
	Penicillium sp. 2	Morphology	716.7	
	Purpureocillium lilacinum	DNA	2.2	
C3W2 (17.0 m)	*Aspergillus aculeatus*	Morphology	3.3	488.9
	Aspergillus flavus	Morphology	17.8	
	Aspergillus sydowii	DNA	123.3	
	Purpureocillium lilacinum	DNA	176.7	
	Pyrrhoderma noxium	DNA	167.8	
C3W3 (30.0 m)	*Aspergillus aculeatus*	Morphology	36.7	81.1
	Aspergillus nomius	DNA	13.3	
	Penicillium paxilli	Morphology	30.0	
	Trichoderma harzianum	Morphology	1.1	
C3W4 (85.0 m)	*Aspergillus japonicus*	DNA	14.4	25.6
	Penicillium citrinum	DNA	7.8	
	Phaeosphaeriopsis sp.	DNA	3.3	

S3.3. Abundance of culturable fungi from bat guano samples.

Sample (Distance from entrance)	Fungal Taxa	Identification	CFU / cm^3	
			Fungal Taxa	Sample Total
Balambangan				**Mean = 22.2**
C1G1 (20.0 m)	*Trichoderma harzianum*	Morphology	22.2	22.2
Gomantong				**Mean = 3,141.7**
C2G1 (12.2 m)	*Aspergillus* sp. 1	Morphology	177.8	200.0
	Aspergillus chevalieri	DNA	22.2	
C2G2 (21.5 m)	*Aspergillus europaeus*	Morphology	11.1	133.3
	Penicillium citrinum	Morphology	11.1	
	Trichoderma harzianum	DNA	111.1	
C2G3 (75.2 m)	*Aspergillus aculeatus*	Morphology	11.1	1,233.3
	Irpex laceratus	DNA	1,222.2	11,000.0
C2G4 (110.0 m)	*Annulohypoxylon stygium*	DNA	2,444.4	
	Penicillium ochrochloron	DNA	4,822.2	
	Penicillium paxilli	DNA	2,333.3	
	Trichoderma harzianum	Morphology	1,400.0	
Madai				**Mean = 6,266.7**
C3G1 (26.0 m)	*Paecilomyces variotii*	Morphology	33.3	8,955.6
	Penicillium paxilli	Morphology	8,922.2	

C3G2 (30.0 m)	*Annuhypoxylon nitens*	DNA	111.1	13,622.2
	Aspergillus aculeatus	DNA	1,122.2	
	Cladosporium cladosporioides	DNA	111.1	
	Curvularia lunata	DNA	1,111.1	
	Penicillium simplicissimum	DNA	2,877.8	
	Pochonia chlamydosporia	DNA	1,111.1	
	Talaromyces minioluteus	DNA	5,944.4	
	Trichoderma paraviridescens	DNA	1,233.3	
C3G3 (53.0 m)	*Aspergillus nomius*	DNA	1,111.1	1,111.1
C3G4 (75.0 m)	*Aspergillus bertholletius*	DNA	1,122.2	1,377.8
	Aspergillus niger	Morphology	22.2	
	Paecilomyces variotii	DNA	200.0	
	Penicillium sp. 3	Morphology	11.1	
	Purpureocillium lilacinum	Morphology	11.1	
	Trichoderma asperellum	DNA	11.1	
Keruak				**Mean =1,769.5**
C4G1 (7.8 m)	*Penicillium oxalicum*	DNA	2,622.2	2,111.1
	Trichoderma asperellum	DNA	1,600.0	
C4G2 (19.3 m)	*Aspergillus versicolor*	DNA	11.1	1,427.8
	Hypoxylon anthochroum	DNA	2,344.4	
	Lentinus sajor-caju	DNA	2,222.2	
	Nodulisporium sp.	DNA	1,133.3	