of BORNEAN BIODIVERSITY





Edited by Yee Ling Chong Freddy Kuok San Yeo Faisal Ali Anwarali Khan



Glimpses of BORNEAN BIODIVERSITY

Edited by Yee Ling Chong Freddy Kuok San Yeo Faisal Ali Anwarali Khan

Universiti Malaysia Sarawak Kota Samarahan

Contents

FOREWORD	vii
Jane Labadin	
PREFACE	ix
Yee Ling Chong, Freddy Kuok San Yeo and Faisal Ali Anwarali Khan	
1. Introduction	1
Yee Ling Chong, Faisal Ali Anwarali Khan and Freddy Kuok San Yeo	
2. Primate Diversity in Totally Protected Areas of Western Sarawak Norfarbana Mazlan, Yuvarajan Manivannan, Mohammad Zabid Zainal Abidin, Wan Nur Syafinaz Wan Azman and Faisal Ali Anwarali Khan	5
3. Diet Composition of Proboscis Monkeys in Different Habitat Types Kar Hon Ng and Yee Ling Chong	19
4. Bats in Totally Protected Areas of Sarawak: A Review on their Diversity, Distribution and Conservation Status Nursyafiqah Shazali, Praveena Rajasegaran, Isham Azhar, Mohd Ridwan Abd Rahman, Roberta Chaya Tawie Tingga and Faisal Ali Anwarali Khan	33
5. Distribution Records of Terrestrial Small Mammals at Various Habitats in Western Sarawak Sieu Zhien Teo, Jin Kiet Tan, Yang Lee, Yee Ling Ng, Nur Elfieyra Syazana Hamdan, Danielle Levesque, Yee Ling Chong and Andrew Alek Tuen	51
6. Shrews of Malaysian Borneo: Diversity, Distribution and Conservation Status Qbairil Shyamri Rosli, Julius William Dee, Muhd Amsyari Morni, Hasmahzaiti Omar and Faisal Ali Anewarali Khan	69
7. Mangrove Birds of Kuching Wetland National Park and Bako Village in Kuching Karim Nurqamareena, Yee Ling Chong, Isa Sait and Mohamad Fizl Sidq Ramji	79
8. Birds of Satang Island and Talang-Talang Island, Sarawak Hilda Jelembai Neilson Ilan, Mohamad Fizl Sidq Ramji, Badiozaman Sulaiman, Nurul Ashikeen Ab Razak, Frances Hii Dai Sze, Mustafa Abdul Rahman and Nor Salmizar Azmi	97

9. Frog Sound for Species Recognition of Genus <i>Pulchrana</i> and Related Genera <i>Ramlah Zainudin, Marly Matleen Augustine Agob, Hasnizam Abdul Wabid and</i> <i>Muhammad Fadzil Amram</i>	113
10. Notes of Freshwater Fishes from Hillstreams in Bawang, Gading and Santubong Mountains Badiozaman Sulaiman, Nur Nadbirah Izzaty Selamat, Emanuel Benang, Hung Hui Chung and Yee Ling Chong	125
11. Description of Orthetrum sabina and Orthetrum testaceum Based on Morphometric Data and Scanning Electron Microscope Micrographs Hashimatul Fatma Hashim, Yee Ling Chong, Nurulain Samsudin, Ratnawati Hazali, Siti Nurlydia Sazali @ Piksin, Asyirah Azeme, Izzat Muzakkir Mursidi and Nurul Farah Ain Idris	135
12. Community Structure of Termites in Borneo Highland, Padawan, Sarawak Norsyarizan Jamil, Wan Nurainie Wan Ismail and Shiu Hui Ting	143
13. Diversity of Sarawak Rice in Kampung Lebor, Serian – A First Insight Freddy Kuok San Yeo, Meekiong Kalu, Zinnirah Shabdin, Nur Karimah Mohamad, Nor Ain Hussin and Hung Hui Chung	155
14. Diversity of Wild Musa L. (Musaceae) in Sarawak Meekiong Kalu, Freddy Kuok San Yeo, Zinnirab Shabdin and Cheksum @ Supiab Tawan	169
15. Diversity of <i>Mapania</i> Aublet (Mapanioideae; Cyperaceae) in Sarawak Zinnirab Sbabdin, Meekiong Kalu, Freddy Kuok San Yeo and Ivy Geralyn Nangan Melana	185
16. Microfungal Diversity of Balambangan Cave in Taman Tun Mustapha, Sabah (Northern Borneo) Ibrahem G. Wasti and Jaya Seelan Sathiya Seelan	195
GENERAL INDEX	213
INDEX TO SCIENTIFIC NAMES	216
	210

FOREWORD

In conjunction with the Borneo Book Fair 2017 of which Universiti Malaysia Sarawak hosted it for the first time, the idea to celebrate the different viewpoints among interested scholars in their discourse on academic publishing in Borneo was materialised through the organisation of a conference. Thus, providing a platform for the participants and presenters to deliberate on the need to preserve the wealth of Borneo through book publication. With the theme "Contesting and advancing knowledge in Borneo Academic Publishing", majority of the conference papers were distinctly themed Biodiversity. The knowledge and insights that were collected rigorously by the vigorous researchers have resulted in the sharing of the knowledge on the richness of Bornean biodiversity through the publication of this book.

Undeniably, the book editors and their chapters' contributors took up the challenge that was put forward through the conference theme. The aim of publishing this book is obviously to organise the knowledge in such a way that the wisdom and picturesque wealth flow seamlessly within the pages of this book and be transferred and diffused across borders to be enjoyed by every walks of life. This interesting and attractive book had successfully captured glimpses of the wealthy Bornean biodiversity.

It is hoped that the shores of Borneo will continue to attract learned scholars from every field imaginable to continuously investigate, write and publish books and journals on Borneo.

> Jane Labadin Director, UNIMAS Publisher



PREFACE

his book was conceived in conjunction with the International Conference on Borneo Book Publishing 2017, which was held at The Waterfront Hotel, Kuching from 11 until 13 October 2017. The conference had attracted over a hundred participants from various government and private institutions, including academicians, researchers, school teachers, postgraduate and undergraduate students. This book reflects the strength and scholarships of local academicians on both field and laboratory-based research on Malaysian Borneo biodiversity. It also showcases the authors' major and recent findings on the various topics related to the ecology, taxonomy, and diversity of flora and fauna on this island. One of the biggest challenges for local scientists to perform research on the land of the hornbills (Sarawak) and land below the wind (Sabah) is the difficulty to explore countless inaccessible areas with physico-geographical barriers such as mountainous terrains and river basins. Besides, the lack of financial support and skilled manpower to deploy field excursion, inadequate state-of-the-art field equipment, and laboratory facilities are among other major limitations faced by the local researchers. However, these do not hinder the passion and determination of researchers to continue to explore the biodiversity of this island.

This book captures the unique and diverse flora and fauna in Malaysian Borneo. In producing this book, we are grateful to the authors for their contributions and the reviewers for their critical comments. The research findings in this book were supported by numerous grants and acknowledged separately in the respective chapters. We hope that this work will contribute to better awareness of our wealth in biodiversity, together we safeguard our biodiversity and ecosystems in the island of Borneo.

> Yee Ling Chong Freddy Kuok San Yeo Faisal Ali Anwarali Khan



INTRODUCTION

Yee Ling Chong, Faisal Ali Anwarali Khan and Freddy Kuok San Yeo

orneo has always attracted many natural historians including Alfred Russel Wallace with its unique and rich diversity. Borneo, located between the archipelagos of the Philippines and Indonesia, is the third largest island in the world and also known as one of the mega-biodiversity hotspots. The high complexity of tropical rainforests support many niches that can accommodate the rich diversity of flora, fauna, and microorganisms. Many of these are rare and protected species, while some of these are Bornean endemics. There are more than 277 species of land and marine mammals, including 63 endemic species; about 673 bird species, including 59 endemic species; at least 400 described freshwater fishes with 149 of these endemic in Borneo; and over 200 species of herpetofauna, which include amphibians and reptilians. Borneo has at least 15,000 flowering plant species spread across mangrove, peat swamp and freshwater swamp forests, lowland dipterocarp forests, hill dipterocarp forests, and ironwood forests. Among which, more than 3,000 species of trees and more than 1,700 species of orchids are recorded. Approximately half of the plant species are endemic to Borneo. Our tropical rainforests are also rich storehouses of biota – plant and animal species, having potential properties for curing human illness or as dietary supplements to improve human health. However, many new species are yet to be discovered as most of the recorded species concentrated in easily accessible areas due to the nature of the geographical barriers on this island.

Although Borneo is well known for its magnificent tropical rainforests, regrettably Borneo also has the highest deforestation rate around the world. This has resulted in degraded forest patches due to forest conversion for agricultural uses, urbanisation, or illegal timber harvesting. The unprecedented anthropogenic activities, together with climate changes, are witnessing some of the mammalian fauna at the brink of extinction in Borneo. These include Sumatran Rhino, Sarawak Langur, Kutai Langur and Abbot's Gibbon, whereas some others are rapidly declining in population size such as Proboscis Monkey, Orangutan, all gibbons, all langurs, Banteng, Tufted Ground Squirrel, Banded Civet and Pangolin as a result of habitat degradation. A similar pattern is also observed in other major groups including fishes, birds, frogs, and snakes that adapt to specific habitats. The anthropogenic activities also affected the plant diversity, resulting in various plant species that are now 'totally protected' (e.g. Rafflesia and Ensurai) or 'protected' (e.g. Pitcher plants, Orchids and Engkabang).

Although Borneo harbors a vast wealth of flora and fauna, as well as other natural resources, not much of this information is published in book deplorably. In total, there are less than 100 book titles on Bornean biodiversity published where most of the studies are published in scientific journals which are not easily accessible by the public. More importantly, this knowledge is yet to reach the public that plays a big role in safeguarding the diversity as well as managing them accordingly. Therefore, it is critical that documents such as coffee table books or other light reading books are made available to the public to increase their awareness to the environment as well as to appreciate the uniqueness of Bornean diversity.

In lieu of this importance, this book is published to provide glimpses of the biodiversity wealth in Malaysian Borneo. The next 15 chapters in this book discuss various topics related to the discovery of fauna as well as macro- and microflora in Malaysian Borneo using both field-sampling and laboratory-based approaches. Chapter 2 offers the first compilation of the distribution and habitat preferences of primate species in National Parks of Western Sarawak, including those currently found in captivity in Matang Wildlife Centre. The diet composition and the foraging activities of Proboscis Monkey, one of the keystone and endemic primate species in Borneo, in both mangrove and beach forests are presented in Chapter 3.

Chapter 4 reviews the diversity, distribution, and conservation status of 68 species of bats in 15 totally protected areas of Sarawak. The composition of bat species found in 10 types of vegetation is discussed in Chapter 4. A checklist and the species distribution of terrestrial small mammals at four different habitats (i.e. forests, oil palm plantations, villages, and urban areas) in Kuching, Sarawak is discussed in Chapter 5. In Chapter 6, the authors described the diversity, distribution, and provided a taxonomic review of elusive shrews in Sarawak and Sabah.

Notes on mangrove birds in Kuching Wetland National Park and Bako Village, Kuching are provided in Chapter 7. The authors recorded 42 bird species at both sites and sighted Asian Black Hornbill, a 'totally protected' species under Sarawak Wild Life Protection Ordinance, 1998. Chapter 8 reports 22 bird species on the offshore islands (namely Satang Island and Talang-Talang Island) of Western Sarawak based on mist-netting and boat-cruise circumnavigation.

In Chapter 9, the call characteristics of Bornean frog sounds from the genus *Pulchrana* are described. Dichotomous keys, which were constructed based on the advertisement calls, for species recognition of *Pulchrana* frogs were further explained by the authors. The distribution of 11 species of freshwater fishes from the hill-streams of three mountains located in Western Sarawak is elucidated in Chapter 10. Chapter 11 portrays the morphological variations of two dragonfly species from the genus *Orthetrum* found in Sarawak based on morphometric data and nano-surface structures using a scanning electron microscope. The community structure of termites in Borneo Highland, Padawan is highlighted in Chapter 12. In this chapter, the authors reported 29 termite species and classified them based on different feeding groups.

A catalogue of Sarawak rice diversity based on data collected from Lebor Village, Serian is presented in Chapter 13. The authors presented 19 lowland rice landraces which have variations in grain size, shape, and colour. The diversities and distributions of Wild *Musa* L. (*pisang*), and *Mapania* (*Pandan Tikus* or *Rumput Serapat*) recorded in Sarawak are highlighted in Chapter 14 and 15, respectively. Lastly, the mycobiota of Balambangan Cave in Northern Borneo is described in Chapter 16. This chapter described the microfungal diversity at different lighting zones and categorised them by the types of substrates within the caves.

In conclusion, this book archives the authors' diverse knowledge on the flora and fauna in Malaysian Borneo, ranging from taxonomy, diversity, distribution, habitat preferences, and their conservation status where some of these were new geographic records. It is optimistic that these informative data are valuable, not only to inform the public on the wealth of biodiversity in Malaysian Borneo, but also as part of the conservation efforts by informing this baseline information to important stakeholders, including related government agencies and the nongovernment organisations. Only with the involvement of all parties that would be possible to conserve our natural resources in Borneo.



Bornean Orangutan (Pongo pygmaeus) in captivity at Matang Wildlife Centre.

MICROFUNGAL DIVERSITY OF BALAMBANGAN CAVE IN TAMAN TUN MUSTAPHA, SABAH (NORTHERN BORNEO)

Ibrahem G. Wasti and *Jaya Seelan Sathiya Seelan *Corresponding author: seelan80@ums.edu.my

SUMMARY

espite Borneo being one of the top biodiversity hotspots, the mycobiota of caves within Borneo has scarcely been studied. The effects of cave mycobiota has on the flora and fauna of its ecosystem must be evaluated to maintain the integrity of the cave ecosystem. Balambangan cave is in Balambangan Island, which is about 40 km from the northern tip of Kudat, Sabah. It is a relatively inactive cave with regards to anthropogenic activity. The aims of this study were to isolate and characterise the microfungi in speleothem surfaces, cavern water, dead arthropods, and bat guano. Distances from the entrance of the sampling sites and lighting zones within the cave were recorded in order to evaluate whether they affect fungal diversity and abundance. Seven speleothem surfaces, four cavern water pools, and two guano piles were sampled. No dead arthropods were collected in this study. A total of 64 pure fungal isolates were obtained from the samples. They were identified to the genus level based on morphological identification. The genera identified were Aspergillus (31.4%), Penicillium (31.4%), Fusarium (17.1%), Trichoderma (5.7%), Verticillium (5.7%), Blastomyces (2.9%), and Paecilomyces (2.9%). The sample size was too small for statistical analysis to be significant, but this data will be compiled with data from other sites for future analysis.

Cool, humid, dark cave environment comprise of diverse array of microscopic fungi. This is mainly attributed to the wide variety of substrates, such as cave sediment, guano, speleothem, cadavers, and other organic materials, that provide nutrients for saprophytic fungi. Microfungi are not only consumers but a major food source for other cave-dwelling organisms [1-7]. Due to the various fauna that either reside in caves or visit caves regularly, particularly bats and arthropods, it is not surprising that caves provide a reservoir for parasitic or pathogenic microfungal species as well [8-10].

Not much is known about the microscopic fungal biodiversity and abundance of caves in Borneo despite it being one of the last known major biodiversity hotspots in the world. However, there has been a significant increase in travel and eco-tourism in this region as well as increased economic ventures that take advantage of cave ecology and its surrounding forest areas. First, translocation and introduction of a novel pathogen into a habitat with the naïve host can be potentially fatal to some of the local cave fauna, as may be the case with the White-Nose Syndrome epidemic affecting in America [6,11-16]. Furthermore, increased anthropogenic influences within and around cave environments have been shown to affect the endemic microfungi [1,4,17-23]. As the rate of mycoses continues to rise globally, disturbances to the microflora in these caves raise concerns of conservation as well as health safety due to potential negative repercussions to the overall cave ecology [24]. Changing the ecology of a biome may lead to limiting environmental changes that can serve as the driving force of altering interactions between pathogen and host species, increasing the former's pathogenicity or weakening the latter's immune system. Even worse, limiting conditions of a changing environment may not only be favourable to an already existing pathogen but lead to the emergence of new diseases by the production of conditions that were previously rare [11,25,26].

Baseline data of microfungi in Bornean caves are virtually non-existent. This study, conducted in a limestone cavern in Balambangan Island of North Borneo, is the first of its kind in this region. Undoubtedly the first study of many, the goal is to identify microfungal species on various substrates within limestone caves in Borneo and collect data as to the fungal biodiversity and abundance stemming from different distances from the cave entrance. Data from this study should be built upon in the near future and lead to even more informative studies regarding microfungi and limestone cave ecology in Borneo.

SAMPLING SITE

Balambangan Island is located about 40 km north from the town of Kudat, Sabah, a coastal town in Northern Borneo. Caves in Balambangan can be found

in various shapes and sizes due to prolonged chemical weathering and erosion, mainly caused by rainfall, dissolving the limestone karst formation [27]. The study site is the largest known limestone caves in the Bongaya sedimentary rock formation of Balambangan Island, reaching about 300 m in length and can get up to 80 m in width in certain areas [27]. Although the cave is deep and has a few narrow corridors, it is relatively well lit due to several apertures in the ceiling. In fact, in the widest, most spacious area of the cave, starting around 30 m from the entrance, there is a very large opening that lights up this area of the cave. The opening is a sinkhole when viewed from the forest above, thus canopy cover can be seen from inside the cave looking up. Directly below this opening, piles of large limestone fragments are scattered across the cave floor, suggesting that the ceiling had caved in some time in the past. 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. A number of holes in the ceiling of the cave contain roosting bats, where guano piles or guano-enriched soil can be located directly below them.



Photo taken within the cave under the large hole on Balambangan Island.



Map layout of the Balambangan cave adapted from Mohamed & Ali (2007). (a) The area indicated in the red box is the sampling area. (b) The large ceiling hole starting about 30 m from the entrance. (c) Cave entrance seen from inside the cave. (d) The north wall of the cave about 20 m from the entrance. (e) Large stalagmites inside the cave around the area under the large ceiling hole.

SAMPLING PROCEDURES

A 30 m transect line was laid out starting from the cave entrance into the cave. Light zones within the cave were categorised into three distinct zones: (1) lighted zones (0.0 m - 9.57 m); (2) twilight zones (9.57 m - 15.11 m, 27.0 m - 30.0 m); and (3) dark zones (15.11 m - 27.0 m). The lighted zones are defined as areas of the cave that are exposed to direct sunlight. The twilight zones are areas not exposed to direct sunlight but are visible without the help of a flashlight. The dark zones are areas not exposed to neither direct nor indirect sunlight. Zones were defined at high noon. Any speleothems, cavern water, dead arthropods, or guano that fell within the length of the transect line, from the east wall to the west wall, were sampled for fungi. The only exception to this rule was the second out of two guano piles sampled in this study, which was measured to be 28.5 meters into the cave from the entrance and considered to be in a twilight zone due to exposure to indirect light from the largest aperture of the cave. Speleothems were sampled using the swab method [17,28]. For each speleothem, an area of 25 cm² was swabbed using a sterile cotton swab wetted with sterile distilled water. The area of 25 cm² was determined by measuring a 5 cm by 5 cm square on the surface of a speleothem prior to swabbing. The cotton tip of the swab was then cut directly into a sterile air-tight twist-cap tube containing 4 mL of sterile distilled water and capped immediately. This method was done in triplicate for each speleothem sample. A different area on each speleothem was swabbed for every replicate on the same site. There were seven speleothems (n=7) sampled in this study, ranging from 0.8 m - 19.66 m from the cave entrance. Four different water pools (n=4) were sampled within the study area, ranging from 1.8 m - 20.0 mfrom the cave entrance. The water was collected using small sterile glass pipettes in which 3 mL would be secured into sterile air-tight twist-cap tubes. This was done in triplicate for each cavern water pool samples. Bat guano was collected in triplicate from two different piles (n=2), 20.0 m and 28.5 m from the main entrance respectively, into large-sized sterile air-tight twist-cap tubes. About 25 mL of guano was collected for each guano sample using a scoop that was cleaned and sterilized after every use. Both surface and submerged guano were collected. No dead arthropods were found and collected in this study. All samples were chilled in a cooler and transported to the laboratory within three days, where they were stored in cold temperature (<4°C) until further identification.

The samples were cultured directly onto potato dextrose agar with streptomycin and incubated for 3-7 days at room temperature in the dark. Any colony from a specific sampling site is considered unique as long as there is no redundancy in morphological characteristics for the same site. All unique isolates were sub-cultured until pure isolates were obtained. Pure isolates were evaluated by their colonial morphology and micromorphology under a compound microscope. Dyes utilised for micromorphological evaluation included lactophenol blue, congo red, and acid fuschin. The morphology of the colonies, hyphae, conidiophores, and conidia were identified according to the universal diagnostic keys described in Domsch *et al.* [29], Klich [30], and Watanabe [31].



Map of the sampling area along the 30 m transect line. (a) The transect line is placed starting from the entrance of the cave. The different light zones are represented in the figure using different colours. (b) One of the stalagmites sampled from the dark zone of the cave. (c) The guano pile 28.5 m from the entrance, nearby but not directly under the large ceiling hole. (d) Cavern water reservoir sampled from the dark zone of the cave.





MICROFUNGAL DIVERSITY

Sixty-six pure isolates were successfully obtained from the collected samples, where 49 were successfully identified at least up to the genus level. Speleothem had the highest number of unique isolates (42) comprising of seven genera, cavern water has 20 unique isolates comprising four genera, and guano has two unique isolates from one genus. When categorised by light zones, the lighted zones had the highest number of unique isolates (28), the dark zones had 20 unique isolates, followed by the twilight zones with 16. Speleothem site number seven, which was in a dark zone, had the highest number of unique isolates of unique isolates with 15. Of the eight genera identified from this cave, three were unique to speleothem, one was unique to water, three were found on water and speleothem, and one was found on both speleothem and guano. Sterile mycelia were isolated from all three substrates. In order of abundance, eight genera of identified isolates were *Aspergillus* (31.4%), *Penicillium* (31.4%), *Fusarium* (17.1%), *Trichoderma* (5.7%), *Verticillium* (5.7%), *Blastomyces* (2.9%), and *Paecilomyces* (2.9%) – percentages exclude sterile mycelia.

This study is the first survey of microfungi in a Bornean cave in Sabah. The known distributions of the identified fungi in Borneo is only Balambangan cave since this is the first study of its kind in this region. This cave is isolated on an island and has relatively minimal amounts of human visitation. No swiftlet-nest farming or land use change can be accounted for anywhere near the vicinity of this cave, which for this reason has been calcified as a cave of low anthropogenic activity. This present study gave preliminary results from several different taxa sampled from three different substrates, in which both macromorphological and micromorphological identification techniques were used. Forty-nine of the pure isolates were identified to at least the genus level. *Aspergillus* and *Penicillium* isolates accounted for 62.8% of all identified taxa, followed by *Fusarium* (17.1%) and *Trichoderma* (5.7%). This falls in line with the most frequently observed taxa that occur in caves [6,23,32].

The report of *Aspergillus* coming from a cave with bat hibernacula in Borneo is not surprising as *Aspergillus* spp. have been isolated from bats in Sarawak, Malaysia, including a pathogenic variant [33,34]. *Aspergillus* species are ubiquitously isolated from caves in continents across the globe and have been sampled isolated from speleothem, soil sediment, bats, bat guano, and arthropods [10,33,35-38]. *Penicillium* spp. are also ubiquitous in cave environments have been isolated from sediment, air, bat guano, mammalian dung, earthworm casts, and isopod diplopod feces from the Domica Cave system alone [2]. *Penicillium* was the most abundant genus of the culturable fungi from Heshang Cave, China regardless of substrates (i.e. soil sediment, weathered rocks, and bat guano) [38].

All, except two, of the taxa reported from this study, have been reported to be isolated from cave sites prior to 2013: *Paecilomyces carneus* and *Blastomyces* sp. [6]. The presence of *Blastomyces* sp. is of interest due to the pathogenicity of *Blastomyces dermititidis* [39]. It is a zoonotic fungal species and does not only affect humans. The fungus has been isolated from the lung and liver of bats, specifically *Rhinopoma hardwickei* [40,41]. The entomopathogenic fungus, *Isaria fumosorosea*, isolated in this study was not directly from an insect or insect cadaver. This may have been possible if conidia produced from a recently infected arthropod somehow adhered to the water. This could pose a threat to arthropods that come in contact with any water within the cave acting as reservoirs for *I. fumosorosea*'s known detrimental effects on *Diaphorina citri*, vectors of citrus greening disease, make them suitable candidates for biological control agents for organic growers and farmers in developing countries where chemical insecticides are relatively expensive [42-46].

The light zones of the sampling sites within the cave were recorded and attributed to the isolated fungi. In this study, the lighted zones of the cave had the highest number of unique fungal taxa per substrate (5.6), followed by the twilight zones (5.3), and the dark zones with the least (4). Hsu & Agoramoorthy [36] had similar results, with the cave entrance (light zones) having the highest average relative abundance of thermophilous fungi and twilight zones having the least.

Looking at the various types of substrate sampled, speleothem consists the highest number of unique isolates per individual substrate (6), followed by cavern water (5), and bat guano (1). This corresponds well with the culturable fungi in Heshang Cave, China (temperate), where the α -diversity analysis, the fungal diversity was highest in soil sediment, followed by weathered rocks, and least in bat guano [38]. However, this is in contrast with the results from the Domica Caves system (temperate), where bat guano had the highest number of microfungal taxa found out of various substrates which included soil sediment, air, mammalian dung, isopod diplopod feces, vermiculations, and cadavers [2].

Molecular analysis was not conducted on the samples and cultures from this trip, but general sequencing using the fungal-specific ITS primer will be conducted in the future. The fungal diversity from the guano samples may be lower than the actual amount, mainly due to the method of culturing fungi from these particular set of samples. For the future, fungal abundance will be considered for analysis and the serial dilution method will be utilized for all substrates' samples.

Even from the first site of a larger study, the general microfungal diversity of an inactive limestone cave in Sabah is realized. Fungi play a crucial link in the trophic chain as they recycle nutrients within every ecosystem they are a part of. To discern the specific ways these fungi, play a role, whether as saprotrophs, parasites, or pathogens and their corresponding biochemical pathways, baseline data needs to be obtained. While many of the taxa identified in this study fall in line with other types of fungi sampled from caves prior, the data acquired from this study will prove valuable for the future. Since this is only one cave, comparisons between anthropogenically active versus inactive limestone caves in Borneo could not be conducted. Furthermore, potentially two strains of pathogenic fungi were isolated from Balambangan Cave. Further studies should be conducted in this site to estimate risk, for humans and other fauna, as visitations to the area will likely increase with the continuous rise of ecotourism in Malaysia.





Number of unique isolates obtained per sampling site versus the distance from the main cave entrance.



Venn diagram of the identified genera categorised by the substrates they were isolated from.



The number of unique isolates their proportion relative to the total number of unique isolates identified is represented by the pie chart above. *Aspergillus* and *Penicillium* both share the mode with 31.4%, as both genera had 11 unique isolates identified.



Pure cultures (Left) pictured with their microscopic photos showing distinguishing microstructures, which include various conidiophores, conidia, and hyphae (Right). Row 1 (Left to Right): *Paecilomyces carneus*, and *Aspergillus japonicus*. Row 2 (Left to Right): *Aspergillus niger*, and *Verticillium* sp.. Row 3 (Left to Right): *Blastomyces* sp., and *Penicillium* sp.. Row 4 (Left to Right): *Penicillium* sp., and sterile mycelia.



Pure cultures (Left) pictured with their microscopic photos showing distinguishing microstructures, which include various conidiophores, conidia, and hyphae (Right). Row 1 (Left to Right): *Trichoderma* sp., and *Fusarium* sp.. Row 2 (Left to Right): *Aspergillus parasiticus*, and *Aspergillus flavus*. Row 3 (Left to Right): *Penicillium* sp., and *Fusarium solanii*. Row 4 (Left to Right): *Aspergillus ochraceous*, and *Aspergillus nidulans*.

ACKNOWLEDGEMENTS

Authors thank Mat Sain Mohd Buang, Kalipin Sampin, Yabainus Juhailin, and all other members of Sabah Parks who were involved in the Expedition of Tun Mustapha Park in April of 2017. Authors also thank the Institute of Tropical Biology and Conservation (ITBC), University Malaysia Sabah (UMS) for the support and contributions. Many thanks to UMS Great (GUG0143-1/2017) and Fundamental Research Grant Scheme (FRG046-2017) provided by Universiti Malaysia Sabah (UMS) and the Ministry of Higher Education (MOHE), respectively.

REFERENCES

- Santamaria, S. & Faille, A. (2007). Rhachomyces (Ascomycota, Laboulbeniales) parasites on cave-inhabiting Carabid beetles from the Pyrenees. *Nova Hedwigia*, 85(1-2): 159-186.
- [2] 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.
- [3] Bastian, F., Jurado, V., Nováková, A., Alabouvette, C. & Saiz-Jimenez, C. (2010). The microbiology of Lascaux cave. *Microbiology*, 156(3): 644-652.
- [4] 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.
- [5] Ogórek, R., Lejman, A., Pusz, W., Miłuch, A. & Miodyńska, P. (2012). Characteristics and taxonomy of *Cladosporium* fungi. *Mikologia lekarska*, 19(2): 80-85.
- [6] Vanderwolf, K., Malloch, D., Mcalpine, D. & Forbes, G. (2013). A world review of fungi, yeasts, and slime molds in caves. *International Journal of Speleology*, 42(1): 77-96.
- [7] Walochnik, J. & Mulec, J. (2015). Free-living amoebae in carbonate precipitating microhabitats of karst caves and a new vahlkampfid amoeba, *Allovahlkampfia spelaea* gen. nov., sp. nov. *Acta Protozoologica*, 48(1): 25-33.
- [8] Puechmaille, S.J., Verdeyroux, P., Fuller, H., Gouilh, M.A., Bekaert, M. & Teeling, E.C. (2010). White-Nose Syndrome Fungus (*Geomyces destructans*) in Bat, France. *Emerging Infectious Diseases*, 16(2): 290-293.
- [9] Hoyt, J.R., Langwig, K.E., Sun, K., Lu, G., Parise, K.L., Jiang, T. & Kilpatrick, A.M. (2016Host persistence or extinction from emerging infectious disease: insights from white-nose syndrome in endemic and invading regions. In *Proceedings of the Royal Society B* 283(1826): 20152861.
- [10] Vanderwolf, K.J., Malloch, D. & McAlpine, D.F. (2016). Ectomycota associated with arthropods from bat Hibernacula in Eastern Canada, with particular reference to *Pseudogymnoascus destructans. Insects*, 7(2): 16.
- [11] Rachowicz, L.J., Hero, J., Alford, R.A., Taylor, J.W., Morgan, J.A., Vredenburg, V.T. & Briggs, C.J. (2005). The novel and endemic pathogen hypotheses: Competing explanations for the origin of emerging infectious diseases of wildlife. *Conservation Biology*, 19(5): 1441-1448.
- [12] Cryan, P.M., Meteyer, C., Boyles, J.G. & Blehert, D.S. (2010). Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. *BMC Biology*, 8(1): 135.
- [13] 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.
- [14] U.S. Fish & Wildlife Service. (2012, January 17). North American bat death toll exceeds 5.5 million from white-nose syndrome [Press release]. https://www.whitenosesyndrome.org/sites/ default/files/files/wns_mortality_2012_nr_final_0.pdf. Downloaded on 25 May 2016.

- [15] Eskew, E.A. & Todd, B.D. (2013). Parallels in Amphibian and bat declines from pathogenic fungi. *Emerging Infectious Diseases*, 19(3): 379–385.
- [16] Reynolds, H.T. & Barton, H.A. (2014). Comparison of the white-nose syndrome agent *Pseudogymnoascus destructans* to cave-dwelling relatives suggests reduced saprotrophic enzyme activity. *PLoS ONE*, 9(1): e86437.
- [17] 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.
- [18] Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature*, 451(7181): 990-993.
- [19] Chelius, M.K., Beresford, G., Horton, H., Quirk, M., Selby, G., Simpson, R.T. & Moore, J.C. (2009). Impacts of alterations of organic inputs on the bacterial community within the sediments of Wind Cave, South Dakota, USA. *International Journal of Speleology*, 38(1): 1-10.
- [20] 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): 76–86.
- [21] Porca, E., Jurado, V., Martin-Sanchez, P.M., Hermosín, B., Bastian, F., Alabouvette, C. & Sáiz-Jiménez, C. (2011). Aerobiology: an ecological indicator for early detection and control of fungal outbreaks in caves. *Ecological Indicators*, 11(6): 1594-1598.
- [22] Tulsiani, S.M., Cobbold, R.N., Graham, G.C., Dohnt, M.F., Burns, M.A., Leung, L.P. & Craig, S.B. (2011). The role of fruit bats in the transmission of pathogenic leptospires in Australia. *Annals of Tropical Medicine & Parasitology*, 105(1): 71-84.
- [23] 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.
- [24] 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): 186-194.
- [25] Carey, C., Cohen, N. & Rollins-Smith, L. (1999). Amphibian declines: An immunological perspective. Developmental & Comparative Immunology, 23(6): 459-472.
- [26] Hochberg, M.E., Gomulkiewicz, R., Holt, R.D., & Thompson, J.N. (2000). Weak sinks could cradle mutualistic symbioses-strong sources should harbour parasitic symbioses. *Journal of Evolutionary Biology*, 13(2): 213-222.
- [27] Mohamed, K.R. & Ali, C.A. (2007). Gua dalam batu kapur Balambangan, Pulau Balambangan, Sabah (Caves of Balambangan limstone, Balambangan Island, Sabah). *Geological Society of Malaysia*, 53: 119-123.
- [28] 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): 65-77.
- [29] Domsch, K.H., Gams, W. & Anderson, T.H. (1980). Compendium of soil fungi. Volume 1. London: Academic Press (London) Ltd.
- [30] Klich, M.A. (2002). Biogeography of Aspergillus species in soil and litter. Mycologia, 94(1): 21-27.
- [31] Watanabe, T. (2002). Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species. Second Edition. Washington, DC: CRC Press.
- [32] Docampo, S., Trigo, M.M., Recio, M., Melgar, M., García-Sánchez, J. & Cabezudo, B. (2011). Fungal spore content of the atmosphere of the Cave of Nerja (southern Spain): Diversity and origin. *Science of the Total Environment*, 409(4): 835-843.
- [33] Seelan, S.S.J., Anwarali, F.A., Sepiah, M. & Abdullah, M.T. (2008). Bats (chiropteran) reported with *Aspergillus* species from Kubah National Park, Sarawak, Malaysia. *Journal of Tropical Biology and Conservation*, 4(1): 81-97.
- [34] Seelan, S.S.J. & Anwarali, F. (2009). Case report of a new pathogenic variant of Aspergillus fumigatus isolated from Hipposideros cervinus (Chiroptera: Hipposideridae) in Sarawak, Malaysia. Journal of Threatened Taxa, 1(3): 190-191.
- [35] 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.

- [36] Hsu, M.J. & Agoramoorthy, G. (2001). Occurrence and diversity of thermophilous soil microfungi in forest and cave ecosystems of Taiwan. *Fungal Diversity*, 7: 27-33.
- [37] Pusz, W., Ogórek, R., Knapik, R., Kozak, B. & Bujak, H. (2015). The occurrence of fungi in the recently discovered Jarkowicka cave in the Karkonosze Mts. (Poland). *Geomicrobiology Journal*, 32(1): 59-67.
- [38] Man, B., Wang, H., Xiang, X., Wang, R., Yun, Y. & Gong, L. (2015). Phylogenetic diversity of culturable fungi in the Heshang Cave, central China. *Frontiers in Microbiology*, 6(1158).
- [39] Wisconsin Department of Health Services, Division of Public Health. (2006). Wisconsin Department of Health Services. https://www.dhs.wisconsin.gov/publications/p4/p42030.pdf. Downloaded on 30 September 2017.
- [40] Khan, Z.U., Randhawa, H.S. & Lulla, M. (1982). Isolation of Blastomyces dermatitidis from the lungs of a bat, Rhinopoma hardwickei hardwickei Gray, in Delhi. *Sabouraudia*, 20(2): 137-144.
- [41] Randhawa, H.S., Chaturvedi, V.P., Kini, S. & Khan, Z.U. (1985). Blastomyces dermatitidis in bats: First report of its isolation from the liver of Rhinopoma hardwickei hardwickei Gray. Sabouraudia: Journal of Medical and Veterinary Mycology, 23(1): 69-76.
- [42] Garnier, M., Jagoueix-Eveillard, S., Cronje, P.R., Le Roux, H.F. & Bove, J.M. (2000). Genomic characterization of a liberibacter present in an ornamental rutaceous tree, Calodendrum capense, in the Western Cape Province of South Africa. Proposal of Candidatus Liberibacter africanus subsp. capensis'. *International Journal of Systematic and Evolutionary Microbiology*, 50(6): 2119-2125.
- [43] Subandiyah, S., Nikoh, N., Sato, H., Wagiman, F., Tsuyumu, S. & Fukatsu, T. (2000). Isolation and characterization of two entomopathogenic fungi attacking *Diaphorina citri* (Homoptera, Psylloidea) in Indonesia. *Mycoscience*, 41(5): 509-513.
- [44] Halbert, S.E. & Manjunath, K.L. (2004). Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. *Florida Entomologist*, 87(3): 330-353.
- [45] Meyer, J.M., Hoy, M.A. & Boucias, D.G. (2008). Isolation and characterization of an *Isaria fumosorosea* isolate infecting the Asian citrus psyllid in Florida. *Journal of Invertebrate Pathology*, 99(1): 96-102.
- [46] Hunter, W.B., Avery, P.B., Pick, D. & Powell, C.A. (2011). Broad spectrum potential of *Isaria fumosorosea* against insect pests of citrus. *Florida Entomologist*, 94(4): 1051-1054.

Identified fungal isolates from different substrates and light zones.

Substrate	Distance from entrance (m)	Light Zone	Genus/Species/Isolate
		Lighted	Penicillium sp. isolate S1.2
	0.80		Penicillium sp. isolate S1.4
			Verticillium sp. isolate S1.3
			Sterile mycelia isolate S1.6
			Sterile mycelia isolate S1.7
			Verticillium sp. isolate S2.1
	4.01		Sterile mycelia isolate S2.2
			Aspergillus japonicus
			Aspergillus nidulans
	4.80		Blastomyces sp. isolate \$3.2
			Fusarium sp. isolate S3.4
			Paecilomyces carneus
			Sterile mycelia isolate S3.6
			Penicillium sp. isolate S4.3
			Penicillium sp. isolate S4.6
	11.92		Penicillium sp. isolate \$4.7
		Twilight	Penicillium sp. isolate \$4.9
Speleothem			Sterile mycelia isolate S4.1
			Sterile mycelia isolate S4.2
			Sterile mycelia isolate S4.4
			Sterile mycelia isolate S4.5
	17.7	Dark	Aspergillus parasiticus
			Penicillium sp. isolate S5.1
	17.92		Aspergillus ochraceus
			Sterile mycelia isolate S6.1
	19.66		Aspergillus flavus
			Aspergillus nidulans
			Aspergillus niger
			Aspergillus ochraceus
			Aspergillus parasiticus
			Fusarium sp. isolate S7.1
			Fusarium sp. isolate S7.4
			Trichoderma sp. isolate S7.7
			Sterile mycelia isolate S7.3
			Sterile mycelia isolate S7.3

Water	1.80	Lighted	Fusarium sp. W1.6
			Isaria fumosoroseus
			Penicillium sp. isolate W1.4
			Sterile mycelia isolate W1.5
	4.11		Fusarium sp. W2.1
			Fusarium sp. W2.7
			Penicillium sp. W2.3
			Sterile mycelia isolate W2.5
	14.42 Tw	Twilight	Aspergillus japonicus
			Aspergillus niger
			Penicillium sp. W3.1
			Penicillium sp. W3.2
Bat Guano	20.0	Dark	Sterile mycelia isolate G1.1
	28.5		Trichoderma sp. G2.1

Checklist of identified fungal taxa from Balambangan cave and their known current distribution in cave within Malaysian Borneo.

Taxa Name	Distribution
Aspergillus flavus (Link, 1809)	Balambangan, Sabah
Aspergillus japonicus (Saito, 1906)	Balambangan, Sabah
Aspergillus nidulans (G. Winter, 1884)	Balambangan, Sabah
Aspergillus niger (Tieghem, 1867)	Balambangan, Sabah
Aspergillus ochraceus (Wilhelm, 1877)	Balambangan, Sabah
Aspergillus parasiticus (Speare, 1912)	Balambangan, Sabah
Blastomyces (Gilchrist & Stokes, 1898)	Balambangan, Sabah
Fusarium (Link, 1809)	Balambangan, Sabah
Isaria fumosoroseus (Wize, 1904)	Balambangan, Sabah
Paecilomyces carneus (Duché & Heim, 1957)	Balambangan, Sabah
Penicillium (Link, 1809)	Balambangan, Sabah
Trichoderma (Link, 1809)	Balambangan, Sabah
Verticillium (Nees, 1816)	Balambangan, Sabah

BORNEAN

This book provides an updated information on the selected ecological and biodiversity wealth in the fascinating land of Malaysian Borneo. The high complexity of tropical rainforest offers various niches that can house the mega diversity of flora, fauna, and microorganisms in this land. Individual chapters showcase recent findings on the various topics related to the ecology, taxonomy, and diversity of the selected flora and fauna on this island. This book will be a useful baseline data for public, biologists, related government agencies and other important stakeholders, in managing and protecting the resources of this magnificent tropical rainforest.







Freddy Kuok San Yeo received his PhD from Wageningen University. He currently serves as a senior lecturer at the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. His research focuses on Sarawak rice breeding. He is conducting multi-disciplinary research on pests and diseases of rice, phytochemicals for pest and disease control, and diversity of Sarawak rice.



Faisal Ali Anwarali Khan graduated with his bachelor's in Biotechnology Resource from Universiti Malaysia Sarawak. He later completed both his MSc and PhD at Texas Tech University in the field of Zoology. He is a lecturer at the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. His research interest focuses on the systematics and molecular evolution of Southeast Asian mammals; particularly bats. He hopes to be able to describe the patterns that shape the mammalian diversity in Southeast Asia.



ISBN 978-967-2008-77-4

008774

789672

65.40