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CHAPTER 2

DNA barcoding, taxonomy, and IUCN updates on Malaysian fungal biodiversity

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Abstract: The island of Borneo, renowned for its unparalleled biodiversity, is home to a vast array of mushroom species that have remained understudied, despite their ecological, economic, and medicinal significance. Fungi play crucial roles in understanding the health of forest ecosystems, from decomposing organic matter to forming symbiotic relationships with other lifeforms, while mushroom species essentially provide support for food, medicine, and bioactive compounds. However, the process to identify and catalogue Borneo's unique fungi at present is often plagued with challenges, from inadequate support towards research and development, human resources capital for mycological expertise, and compounded by the present threat of deforestation and habitat loss. To uncover the potential of Borneo's fungal diversity, a collaborative and multidisciplinary approach is presently vital, especially involving taxonomic studies for identification for species, exploring greater nuance for its ecological research, while understanding their symbiotic roles, and the optimisation towards innovative biotechnological exploration. Borneo has the potential to develop a thriving mushroom industry, contributing significantly to the wealth and prosperity of its people, benefited by its many diverse local traditional communities, as this will be a unique proposition for future generations. The journey to fully understand and harness the potential of Borneo's mushrooms is remaina journey far from complete, but with concerted effort, holds immense promise for science, society, and the environment.

INTRODUCTION

Fungi form an extremely diverse group of organisms. They occur everywhere—not only in forests, but also in urban environments, beaches, mangroves, and even inside other living organisms. Without fungi, ecosystems would function differently, and yet fungi do not receive as much research attention and funding compared to flora and fauna.

People associate fungi mainly with mushrooms—the fruiting bodies that can be seen to the naked human eye. However, most fungi occur within soil, substrates, plants, animals, and other organisms. They are able to interact with plants and animals and form highly diverse ecological networks. Other than mushrooms, fungi exist as moulds, yeasts, and many microscopic forms, only observed via specialised equipment. It is these microscopic fungi that are the most visually striking, where they may stand individually or in clusters, some with unique colouration, forms, and shapes. And yet they often go unnoticed by the public, owing to their often diminutive stature.

Many fungi are beneficial to humans, while some can be harmful. They function as important natural recyclers, contributing greatly to nutrient cycling and decomposition. Decomposition is closely linked to carbon processes, yet fungal contributions to carbon dynamics remain an area that requires more research studies. Fungi also form partnerships with plants and animals that can be beneficial, neutral, or even harmful.

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A common example of this interaction is the relationship between nitrogen-fixing microbes, fungi, and plant roots. In this relationship, atmospheric nitrogen is too stable to be utilised by plants, instead nitrogen-fixing microbes convert atmospheric nitrogen, N_2 into ammonia, NH_3, which can readily be absorbed by the plant. Fungi, on the other hand, can form minute tendrils that spread through the soil, transporting nutrients, such as phosphorus, and moisture to the plant. In return, the plant provides sugars, lipids, and amino acids, complimenting the nutritional needs of the microbes and fungi (Liu-Xu *et al.* 2024).

On the other hand, the planet's warming atmosphere and its effects in shifting weather patterns, especially due to increased rainfall may have negative implications for fungi species that are deleterious. The warmer climate enables the proliferation of various fungi species into uncharted territory, and along with it, fungal diseases that may impact previously safe species. For example, amphibians in the warming Palaearctic are facing increasing vulnerability to the fungal pathogen *Batrachochytrium salamandrivorans* (Luedtke *et al.* 2023). Closer to home, Malaysian vegetable farmers may be adversely impacted by fungal pathogens such as *Pythium* spp., *Phytophthora* spp., and *Fusarium* spp., which thrives in waterlogged soils, arising from frequent rainfall. Besides the immediate livelihood concerns, fungal pathogens in a warming climate pose risks to a nation's food sovereignty and biodiversity health as well (Abd Murad and Mohd Zainudin 2017).

Global estimates of fungal species diversity have increased substantially over time. Earlier estimates suggested approximately 1.5 million fungal species (Hawksworth 2001), while recent estimates have proposed several million species worldwide (Hawksworth and Lücking 2017). Only a relatively small proportion of this has been formally described, amounting to approximately 205,000 species (Royal Botanic Gardens, Kew 2026).

HISTORY OF MYCOLOGY RESEARCH IN SABAH

The earliest fungal documentations and collections in Borneo were made in Sarawak by Odoardo Beccari during his first expedition to this region, between 1865 and 1868 (Cesati 1879). In Sabah (formerly North Borneo), early systematic mycological work began later, primarily undertaken in the 1950s. However, the earliest collections were generally deposited in overseas herbaria rather than in local collections. Between the 1950s and 1960s, Professor E.J.H. Corner, who travelled extensively around the Mount Kinabalu region, had collected fungal specimens (Corner 1964). Some of these collections are now kept in Singapore, Edinburgh, and in the Kew Gardens. Historically, knowledge of Borneo's macrofungi was mainly recorded from Corner's studies. Later regional documentation was featured in David Pegler's *The Large Fungi of Borneo* (Pegler 1997).

Most of the earlier specimens were identified using traditional morphological taxonomy. Consequently, many organisms were classified as varieties rather than distinct species. Today, mycologists aim to revisit and revise these historical collections. Unfortunately, some specimens are missing or their locations are uncertain. Nevertheless, researchers can return to clarify the scientific descriptions and to determine what species were documented in the past, which may represent new species, and which are new records for particular regions.

In recent years, extensive survey and documentation of macrofungi were carried out during expeditions to forests and other conservation areas in Sabah, including the Taman Botanikal Sepilok Forest Reserve, Kadamaian Forest, Imbak Canyon Conservation Area, Danum Valley Conservation Area, Tawau Hills Park, Mount Kinabalu, and limestone caves in Sabah (Paul *et al.* 2019; Subari *et al.* 2020; Mohammad Hafiz Syukri *et al.* 2020; Dukit *et al.* 2024; Ibrahem *et al.* 2024, Lim and Jaya Seelan 2024), all these have significantly

expanded our understanding of Bornean fungal diversity. At present, several guidebooks have documented the macrofungal diversity of Sabah (Pegler 1997; Piakong *et al.* 2018; Jaya Seelan and Yap 2026).

DNA BARCODING AND FUNGAL RESEARCH IN SABAH

Significant knowledge gap exists for tropical fungi within the region, with many taxa awaiting molecular DNA barcoding. Recent molecular studies are increasingly relying on ribosomal DNA (rDNA) sequencing, specifically targeting the Internal Transcribed Spacer (ITS) region, which serves as the DNA barcode for fungi, and this has helped resolve species complexes. As examples, Jaya Seelan *et al.* (2015), Lim and Jaya Seelan (2024), as well as Dukit and Jaya Seelan (2024) had revised and updated the species information for some of the Bornean macrofungi, particularly Polyporaceae, Panaceae, and *Cantharellus cuticulatus* using molecular approaches.



Figure 1. *Cantharellus cuticulatus*. Scale bar = 20mm. Photo by Aderina Dukit.

The Institute for Tropical Biology and Conservation (ITBC) at Universiti Sabah Malaysia (UMS) employs an integrative taxonomic approach that combines traditional taxonomy and morphology with specimen collections. Morphological descriptions, observations, notes, and distinguishing characteristics are carefully documented and integrated with molecular identification. DNA-based approaches enable the investigation on phylogenetic and evolutionary relationships among the fungal species.

More recently, ITBC have significantly worked on developing new approaches involving chemotaxonomy, which uses chemical characteristics as additional taxonomic markers. For instance, one mushroom species may contain particular bioactive compounds that are absent from another species. Combining these morphology, molecular information, phylogeny, and chemistry gives us a much stronger basis for identifying and differentiating lineages.

One of the case studies in the long-term fungi monitoring and research in Sabah involves *Chlorogaster dipterocarpi*, a new gasteroid fungus species collected in a dipterocarp forest in Sabah (Læssøe and Jalink 2004). This species has been tracked by ITBC for almost 12 years. Characterised by its distinctive durian-like appearance and striking green colouration, the fungus is a particularly notable organism of Sabah's forest biodiversity. Based on these observations, the species appears to be endemic to Sabah. Its populations have been monitored from several locations, particularly in south-western Sabah, including key conservation areas such as Danum Valley and Tawau. This long-term monitoring provides valuable information on the species distribution, contributing to a better understanding of its ecology and informing future conservation assessments to these areas.

Another significant find in this region, is the Spongiforma squarepantsii, which was first discovered in Lambir Hills National Park in Sarawak. It gained popularity because of its resemblance to a cartoon character, SpongeBob SquarePants. The species belongs to a bolete-related group and forms an ectomycorrhizal relationship with forest trees, particularly dipterocarps. These fungi have a symbiotic or mutualistic association with their host trees. The Bornean species has distinctive characteristics and differ from similar-looking species reported from Peninsular Malaysia and Thailand. This highlights an important taxonomic significant difference: whereby the organisms that look similar may actually represent or belong to a different species.

The common cup fungi is another example which is commonly encountered in the forests. Despite their apparent abundance, these fungi harbour a surprising vast diversity. Studies have revealed cryptic species and new records. *Cookeina sinensis* is reported as a new record from Malaysia, and was collected for the first time from Sabah (Subari *et al.* 2022). Asian specimens display considerable variation in colour and morphology, while specimens from subtropical and tropical regions, such as Mexico, Suriname, and Brazil may represent other forms or species. Only a handful of species had ever been recorded in Malaysia. Morphological and phenotypic variation makes it difficult to determine whether specimens belong to the same species or a different species. Only through molecular evidence enables us to separate some of these groups into distinct lineages, and to be recommended formally as new species.

A newly described macrofungus species, *Calocybe kinabaluensis*, was collected from the Kinabalu UNESCO Global Geopark in Sabah. This species is distinguished by its deep blue to indigo-blue cap, and whitish to pale cream gills. This extraordinary species has placed the region's significance for its fungal biodiversity.

Medicinal and edible fungi

A well-known example of medicinal fungi is the tiger milk mushroom. Many people became familiar with this mushroom through its commercial use in supplements, particularly for respiratory health. It is also regarded as an important medicinal mushroom in Malaysia. In Peninsular Malaysia, some populations may be threatened because habitats where the mushroom was historically recorded have experienced deforestation and development. In Borneo, populations may still occur in relatively intact habitats, although information on their distribution has historically been limited.

Research by ITBC examined the species present in Borneo and compared them with those in Peninsular Malaysia. *Lignosus hainanensis* was also identified as an additional record related to a species previously reported from China (Chong *et al*. 2024). The phenology of these mushrooms was observed in order to better understand when they occur. Their underground structures are also important taxonomic characteristics that are difficult to

appreciate unless researchers observe the fungi directly in the field. In addition to field-based research, the laboratory has also successfully cultivated certain species using agricultural cultivation approaches. One objective is to increase the size and biomass of the cultivated fungi. Larger fruiting bodies can potentially increase their economic value.



Figure 2. *Lignosus hainanensis* collected in Sabah. Scale bar = 1 cm.

An example of an edible mushroom used by local communities in Sabah is *Pleurotus tuber-regium*. There has been taxonomic uncertainty because similar fungi occur in Africa and Asia. The African form possesses a tuber-like structure, whereas the Asian form appears to differ morphologically. This suggests that what was once regarded as a single species may actually represent a different species. Some of these mushrooms have also shown potential bioactive compounds, including possible anti-nematode properties.

Another interesting group of fungi is the *Amauroderma* spp., which have been traditionally associated with treatments for epilepsy. Orang Asli communities in Peninsular Malaysia have traditionally used these for medicinal and traditional purposes. Specifically, it is used to prevent fits, with the traditional preparation requiring the mushroom's hollow stipe (stem) to be worn around the neck (Chang and Lee 2004). To investigate these mushrooms scientifically, research is being conducted by ITBC to identify whether specimens identified as *Amauroderma* represent a single species or several species. Preliminary findings indicate substantial variation, with different forms potentially representing distinct species. Several may still require formal description.

Hericium coralloides, also known as coral tooth mushroom, was recorded for the first time in Sabah. This fungus produces bioactive compounds (e.g. hericonones, polysaccharides, erinacines) with antioxidant, immunomodulatory, cytotoxic, and neuroprotective properties, including potential therapeutic applications for age-related immune decline and Alzheimer's disease.



Figure 3. Coral-like, branched fruiting body of *Hericium coralloides*.

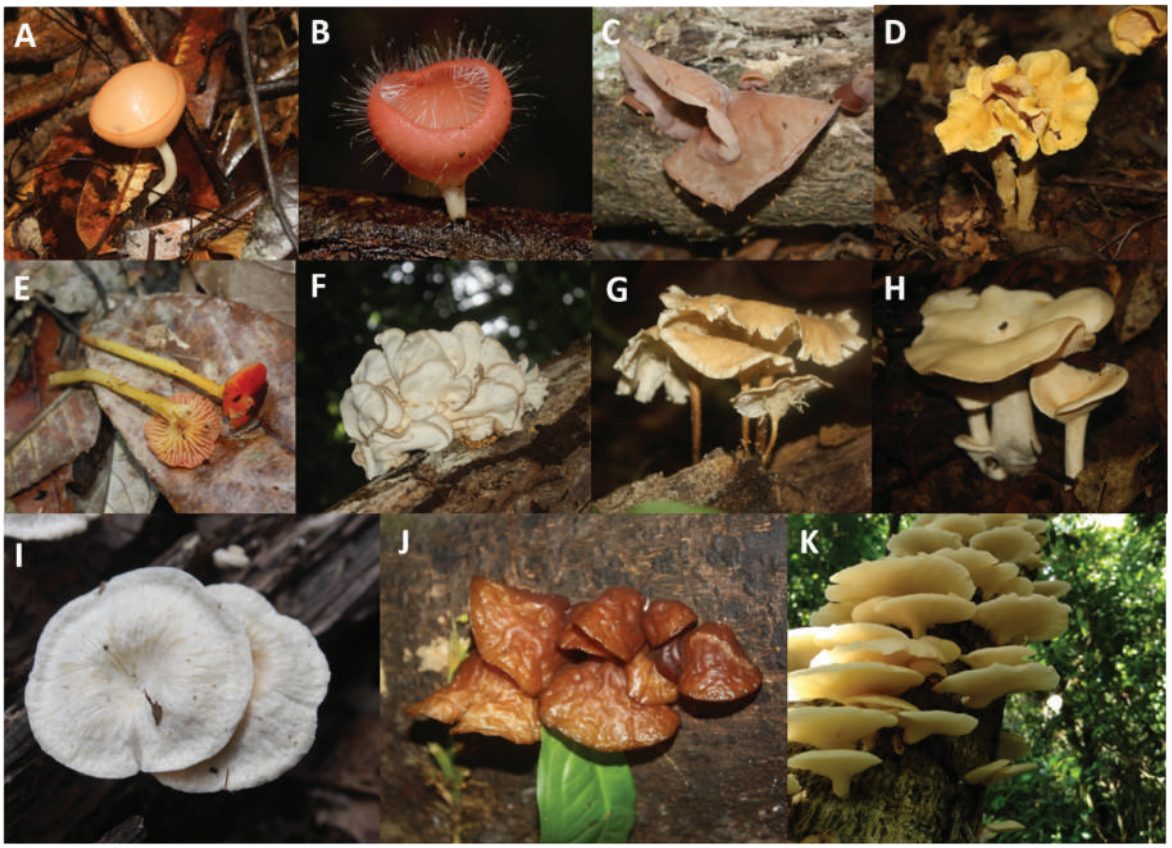

Figure 4. Wild edible mushrooms species found in Imbak Canyon Conservation Area, Sabah. **A.** *Cookeina sulcipes*; **B.** *Cookeina tricholoma*; **C.** *Auricularia polytricha*; **D.** *Chantarellus* sp.; **E.** *Hygrocybe miniata*; **F.** *Pleurotus* sp.; **G.** *Pleurotus tuberregium*; **H.** *Hydnum* sp.; **I.** *Lentinus squarrosulus*; **J.** *Auricularia delicata*; and **K.** *Favolus* sp.

"Zombie fungi"

Entomopathogenic fungi, commonly referred to as "zombie fungi", are parasitic fungi associated with insects and other arthropods. These fungi have attracted broader public attention through portrayals in popular media such as the American television serial, *The Last of Us*. In nature, they are extraordinary organisms that exhibit remarkable biological adaptation that allows them to infect, kill, and in some cases, manipulate their hosts. At the same time, they may contain compounds or ecological properties that could be useful to humans. Many entomopathogenic fungi are highly host-specific. Different ant species, for example, may be infected by different fungal species. Other fungi may specialise to target beetles, cicadas, spiders, or other hosts. These fungi potentially contain compounds with antifungal, antibacterial, and biocontrol properties.

Tropical regions harbour high entomopathogenic fungi diversity and abundance. However, there are limited studies on entomopathogenic fungi in Borneo. In Sarawak, Lavery *et al.* (2021) investigated the spatial dynamics of ants infected by *Ophiocordyceps unilateralis* in Gunung Mulu National Park, Sarawak, Borneo, and found that the height at which infected ants are located is driven by microclimatic conditions that optimise fungal growth and spore release. Moreover, recent inventories in Sabah's conservation areas, including Mount Kinabalu, Danum Valley, and Maliau Basin have established the baseline for entomopathogenic fungi diversity in Borneo.

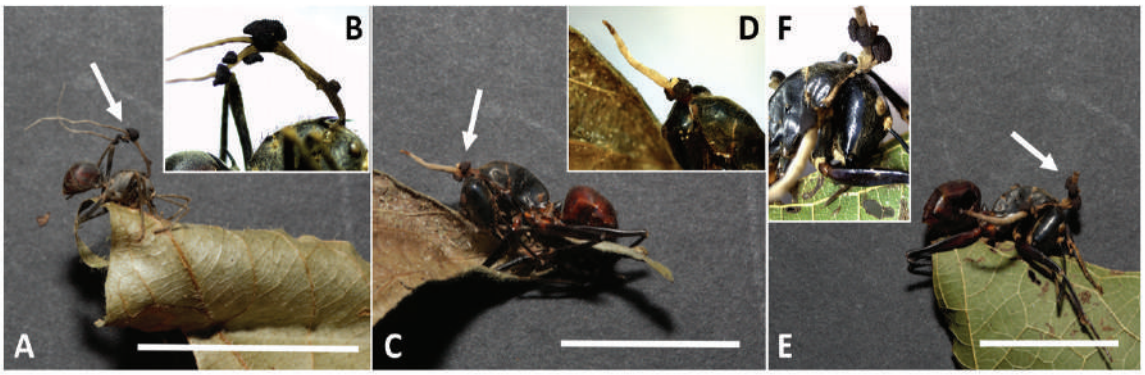

Figure 5. *Ophiocordyceps unilateralis* complex. **A.** Stroma arising anteriorly from the pronotum of ant, firmly attached on the edge of leaf; **B.** The fruiting body of fungus with a few perithecia on the stroma (white arrow); **C.** Single stroma arising anteriorly from the pronotum of ant attached on the edge of leaf; **D.** Single perithecia close to the dorsal pronotum of ant; **E.** Two stroma grew from the dorsal pronotum of the ant attached on the edge of the leaf; and **F.** Perithecia on stroma. Scale bars = 1 cm.

Recent studies in Sabah revealed several notable additions to Malaysia's fungal diversity. Shahbaz *et al*. (2026a) documented five entomopathogenic fungi species for Borneo, including *Cordyceps jakajanicola*, *C. tenuipes*, *Blackwellomyces calendulinus*, *Purpureocillium lilacinum*, and *Simplicillium formicae* in oil palm plantation of Sabah. Four new species of entomopathogenic fungi for Malaysia are Leptobacillium geminatum (Shahbaz *et al.* 2026b), *Akanthomyces infaproensis* (Shahbaz *et al.* 2026c), *Metarhizium tawauensis* (Shahbaz *et al.* 2026d), *Pleurocordyceps cornusynnemata* (Shahbaz *et al.* 2026e), were discovered in Sabah. In addition to these new species, six new records for Malaysia were reported, such as, *Simplicillium formicae* (Shahbaz *et al.* 2026a), *Leptobacillium leptobactrum*. *Leptobacillium symbiotic*, and *L. coffeanum* isolated from spiders, *Simplicillium puwenense*, isolated from a wasp (Shahbaz *et al.* 2026b) and *Pleurocordyceps aurantiaca*, isolated from Diptera (Shahbaz *et al*. 2026e) collected from Sabah. Of these, *Simplicillium formicae* (Shahbaz *et al.* 2026a), *S. puwenense* (Shahbaz *et al*. 2026b) and *Pleurocordyceps cornusynnemata* (Shahbaz *et al.* 2026e) are the hyperparasitic fungi infecting other entomopathogenic fungi.

Bioluminescent fungi

Bioluminescent fungi are well studied in Peninsular Malaysia, where approximately 20 species can be found (Chew *et al*. 2014; Thi and Siti Nuratikah 2025). However, no data are available for Borneo. Through the funding under the 12th Malaysia Plan, we were able to identify how many different groups of bioluminescent fungi are present in Borneo. Collaborations involving government agencies and the Sabah Forestry Department have enabled fungal surveys, expeditions, specimen collection, identification services, and investigations into potential bioprospecting value of mushrooms. Malaysia still has major gaps in fungal biodiversity information. There are many species proposed for assessment, but only a portion have been formally accepted and published through the IUCN process.

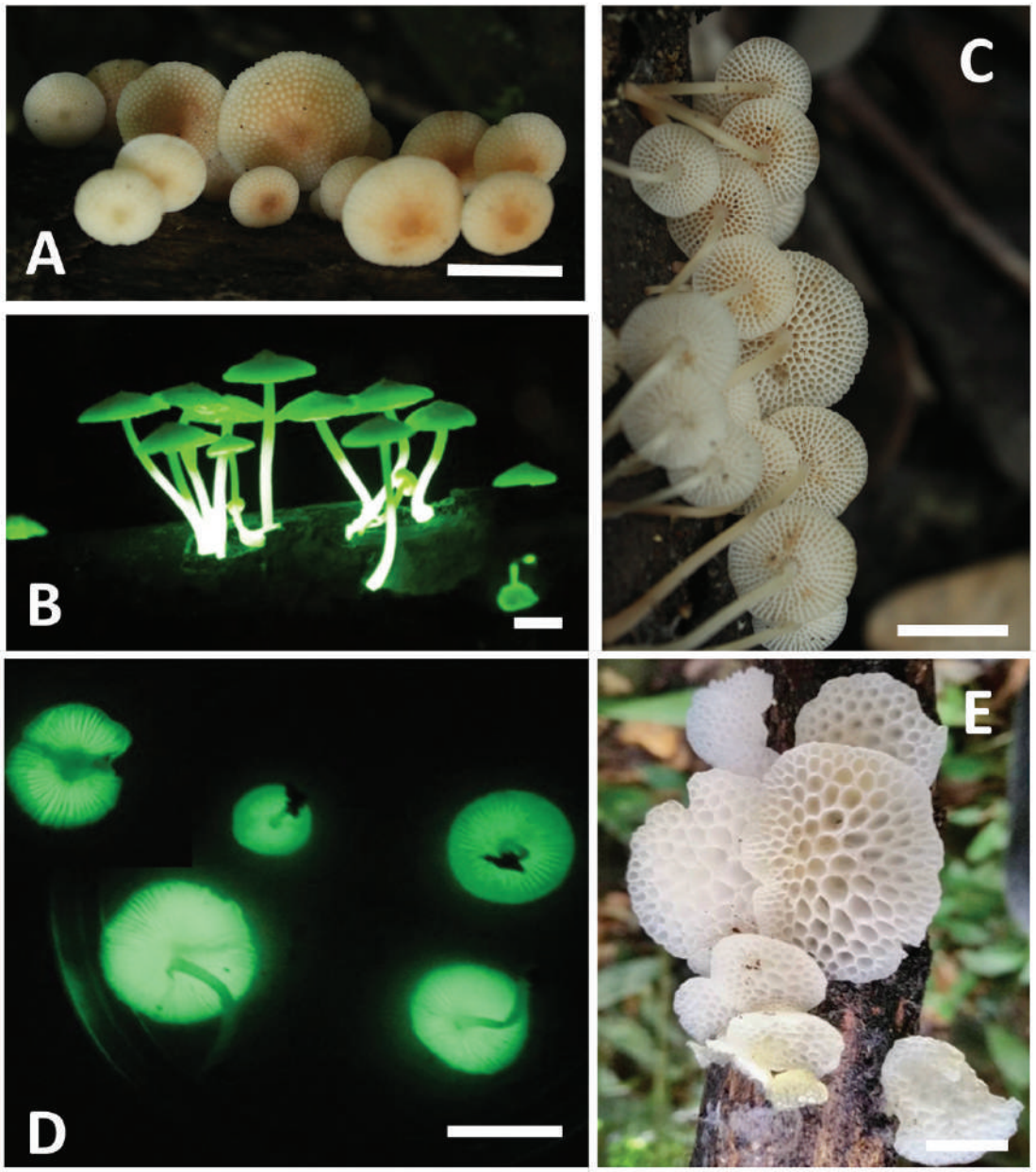

Figure 5. Bioluminescence mushrooms collected from Kadamaian Forest. **A-C**. *Filoboletus manipularis*, a convex, slightly umbonate pileus with hymenopores; **D.** *Mycena chlorophos*, pileus convex with striate margin and closed lamellae; and **E.** *Favolaschia cyatheae*. Scale bars = 1 cm.

Mycophagy

Fungi occurrence in the forest ecosystem contributes to the diets of other wildlife. For instance, the Asian brown giant tortoise (*Manouria emys*), was observed feeding on *Russula virescens* in Danum Valley Conservation Area (Jaya Seelan *et al.* 2025). Malaysian primates, such as the long-tailed macaque (*Macaca fascucularis*), Bornean Orangutan (*Pongo pygmaeus morio*), and the red langur (*Presbytis rubicunda*) includes fungi, albeit in extremely minute quantities, in their diets (Kanamori *et al.* 2010; Ruslin *et al*. 2018; Cheyne *et al.* 2019). Besides vertebrates, Malaysian insects, including beetles and grasshoppers are known to consume a variety of fungi species (Monk and Samuels 1990; Meghma Bera 2018). Mycophagy is a symbiotic relationship between the fungi and the fauna, where the fungi is able to disperse its spores through the feces of the fauna that consumes them and propagate the fungi to new territory, while the fauna is able to access nutrients that are made bioavailable through the decomposition of detritus.

FUNGI CONSERVATION IN MALAYSIA

Fungi conservation still requires continuous systemic biodiversity surveys, which not only covers monitoring of wild populations, but also broadening the existing fungi culture deposits. This will enormously build a comprehensive DNA barcode reference library (ITS and secondary markers). This improved baseline understanding of fungi in Malaysia, compliments the forest landscapes and ecological importance of the habitat in the region, factoring a zonation classification for national biodiversity and conservation plan and its policies.

At the same time, citizen science documentation of fungi can be promoted through platforms such as Malaysia Biodiversity Information System (MyBIS); especially for environmental education programmes in improving public awareness on the ecological importance of fungi. There is an urgent need to encourage inter-agency collaboration on species-specific conservation action plans for classifying rare, endemic, or threatened fungi.

Presently, the spores are in collections, and cultivated as germplasm or tissue cultures, which can be a repository for future studies and research use. However, this cryo-preservation comes with a high cost in maintaining it for a long run. An alternative method for this preservation is in urgent need to find an alternative, and as a result of the collection that has gathered approximately 2,400 cultures from edible and medicinal mushrooms, 800 cultures from limestone cave fungi, and about 1,000 cultures of entomopathogenic fungi. These samples are comprehensively studied at present, enabling future fungi researchers to explore how these mushrooms benefit human society at large.

Staff of Yayasan Sabah, Sabah Parks, Sabah Forestry, including rangers and guides have received training and knowledge on fungi. They, in turn, share their observation with the researchers, contributing to the existing knowledge bank on Bornean fungi. Borneensis Collection Centre is the primary repository for preserved fungi specimens in Sabah, serving as a major hub for fungi taxonomy and DNA studies.

Public engagement initiative via educational modules, outreach especially for children, on the importance and awareness of mushrooms has been promising. This is further incorporated into indigenous folklore and art projects, where children are able to interact closely with what they learnt about fungi, and transform that into simple and easy story narrative associating with fungi. Mushrooms Culinary of Sabah, is another project where students collect edible mushrooms, this activity was popular since during the COVID period. Fungi of Borneo Mushroom Art Exhibition was another major highlight using citizen science and drawing the public to contribute a collage of mushroom photographs. All these culminated into a book, in collaboration with Shangri-La Sabah.

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