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Research Article

Species Composition of *Anopheles* Mosquitoes in Danum Valley, Lahad Datu, Sabah

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ABSTRACT

Malaria continues to be a public health concern globally, while in Malaysia, cases remain high among interior communities in Borneo, including in Sabah. We studied the *Anopheles* species in Danum Valley, Lahad Datu, by random sampling of mosquitoes in the virgin forest of the Danum Valley Conservation Area (VF), low-ground regenerated forest (100m above sea level) (LRF) and high-ground regenerated forest (400m above sea level) (HRF). Over 12 trap nights, a total of 839 individuals of *Anopheles* mosquitoes belonging to nine species were collected with mosquito magnet: *Anopheles asiaticus* (94), *An. balabacensis* (12), *An. barbumbrosus* (7), *An. fragilis* (640), *An. interruptus* (38), *An. jamesii* (9), *An. latens* (5), *An. maculatus* (17) and *An. montanus* (17). Among them, are vectors for zoonotic malaria in Malaysia namely *Anopheles balabacensis* (1.43%), *An. maculatus* (2.03%) and *An. latens* (0.60%), albeit relatively low in numbers. HRF had the highest number of *Anopheles* mosquitoes collected (670), followed by LRF (130) and VF (39). Both Simpson's (D) and Shannon-Wiener (H) diversity indices were highest at LRF (D = 2.07; H = 1.2 with highest Species Evenness, E = 0.58), followed by HRF (D = 1.62; H = 0.8; E = 0.39) and VF (D = 1.31; H = 0.47; E = 0.43. Greater numbers of the malaria vectors were found in LRF and HRF, compared to VF suggesting that there may be greater exposure to vectors and vector-associated diseases when entering these regenerated forests. Significant differences ($p < 0.05$) for different forest types were detected for the total number of mosquitoes, total *Anopheles* and *An. fragilis* between different forest types.

Keywords: *Anopheles*; tropical rainforest; malaria vectors; DVFC.

INTRODUCTION

The World Health Organization (WHO) (2015) estimated that about 4.3 million deaths were due to malaria vector mosquitoes between the years 2001 and 2013, making these insects the most dangerous animals in the world. In 2017 alone, approximately 435,000 malaria deaths were recorded worldwide (WHO, 2018).

In Malaysia, 16,500 malaria cases were recorded from 2013 to 2017. Sabah (7150 cases; 43.3%) and Sarawak (5684 cases; 34.4%) were the major contributors of malaria cases (Hussin, 2020). The malaria parasites transmitted between humans in Malaysia are *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, and *Plasmodium ovale.* While the cases of *P. falciparum* and *P. vivax* have decreased, zoonotic malaria originating from monkeys caused by *Plasmodium knowlesi* continues to increase (Cooper et al., 2019). *Plasmodium knowlesi* is the most common malaria causing parasite species in East Malaysia near forest regions, meanwhile more cases of *P. vivax* were recorded in West Malaysia.

Forests in Sabah have long been heavily logged (or exploited) due to economic globalisation and local socio-political dynamics (Gunggut et al., 2014). This has resulted in rise of *P. knowlesi* incidence in humans, and strong linkage between deforestation and *P. knowlesi* cases has been demonstrated (Fornace et al., 2016). The main vectors of *P. knowlesi* belong to the Leucosphyrus group of mosquitoes (Tan et al., 2008; Vythilingam et al., 2008; Hii et al., 1985) with high biting rates for vector *Anopheles* mosquitoes being recorded at farm edges bordering forest and forest areas.

The main agent of forest disturbance in Sabah has been the timber industry and whose logging activities has left relatively small undisturbed lowland dipterocarp forest areas (Marsh & Greer, 1992). Habitats that have been fragmented create transition areas with increased spatial overlap among humans, mosquitoes, and wildlife populations or altered vector ecology can also increase the frequency of disease transmission (Hutchings et al., 2011; Overgaard et al., 2003). The detrimental effects of these environmental alterations at forest edges have been documented for malaria and other vector-borne zoonotic diseases (Lambin et al., 2010; Vanwambeke et al., 2007). Effects of deforestation and land cover changes for agricultural purposes contributed to temperature rise, vector species dynamics and abundance changes with time strongly linked to land use changes (Kweka et al., 2016).

The diversity and species composition of *Anopheles* mosquitoes dwelling in Danum Valley Conservation Area (DVCA) is poorly studied. With monkey malaria cases caused by *P. knowlesi* transmitted by *An. balabacensis* rising in Sabah and becoming a threat to the planned malaria elimination programme in Malaysia (William et al., 2013), more information on the mosquitoes fauna will be needed. Therefore, this study was conducted to determine the composition and diversity of *Anopheles* mosquitoes in the DVCA based on different forest types and hopefully such information will help formulate control strategies.

METHODOLOGY

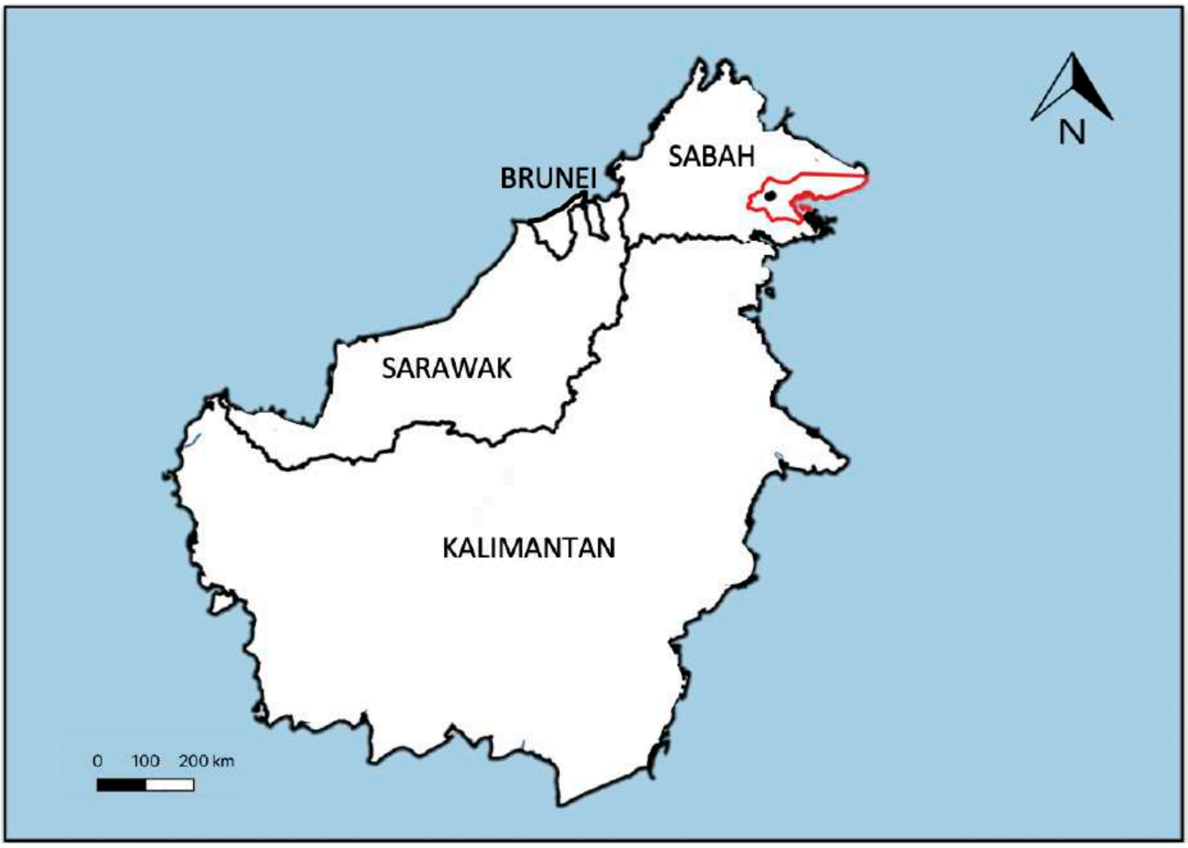

Figure 1: Map of Borneo Island. Danum Valley Conservation Area is demarcated in red and located within Lahad Datu, Sabah.

The Danum Valley Conservation Area (DVCA) (N 04°57.829’ E 117°48.055’) situated in Lahad Datu was selected for this study. The DVCA is located 81.1km from Lahad Datu Airport and it takes approximately 2 hours 30 minutes by four-wheel drive to reach Danum Valley Field Centre (Fig. 1).

The DVCA is a well-known Class 1 Forest Reserve managed by Yayasan Sabah and Royal Society’s Southeast Asia Rainforest Research Programme (SEARRP). The virgin forest area includes the uninhabited 43,800 ha of lowland forest (Marsh & Greer, 1992). The DVCA presents opportunities for ecological research to obtain scientific understanding of ecological processes and evolutionary mechanisms operation within tropical rainforests (Marsh & Greer, 1992) .

Danum Valley comprises dense, undisturbed lowland forests with mostly dipterocarp species, mangroves, montane forest, swamps and transitional forest and re-logged forest at least once (Marsh & Greer, 1992; Reynolds et al., 2011). During rainy periods, animal tracks in the lowland forest easily get flooded; becoming temporary breeding grounds for mosquitoes, long enough for larvae to become adults. Dense tree canopies in the forest minimise sunlight exposure to the ground, which makes the environment moist and humid; perfect for mosquitoes to breed.

The influence of the weather on mast fruiting is reportedly strongest in the eastern part of Borneo (Ashton et al., 1988). During the transition months following the equinoxes (May–June and October–November), the study area experiences highest rainfall, and at the height of the northerly monsoon in December and January (as cited by Clarke & Walsh, 2006). The months of August and September are drier as the south-westerly monsoon reaches its height, and rainfall is also occasionally lower in March and April, which are the months most vulnerable to low rainfall in El Nino Southern Oscillation (ENSO) atmospheric conditions (Walsh & Newbery, 1999).

Anopheles mosquitoes were collected with Mosquito Magnet® Independence MM3200 traps (MMTs) using the mosquito attractants, Lurex3 and Octenol. The cartridge of the Lurex3 contained lactic acid, a chemical compound found in human skin odour (Steib et al., 2001), whereas Octenol mimics human breath (Chaiphongpachara et al., 2018). The MMTs were powered by four AA batteries and the combustion process was facilitated by a 30kg propane gas cylinder. During the combustion of propane, MMTs produce carbon dioxide. Supplemented with a cartridge of Lurex3 and Octenol for each trap, MMTs give off heat, humidity, lactic acid provided by the cartridge of Lurex3, human breath from Octenol and carbon dioxide gas simulating the human presence (Sant'Ana et al., 2014).

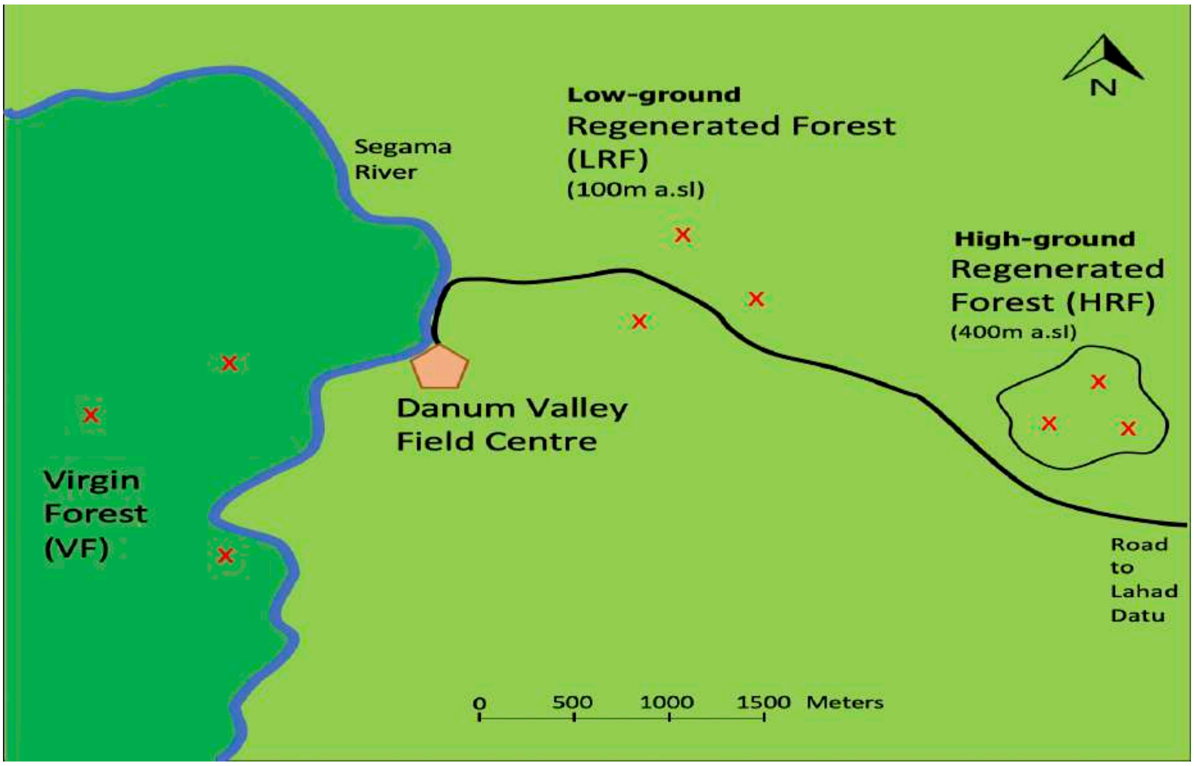

Figure 2: The symbols, X, indicate the trap sites within different forest types of Danum Valley: Virgin Forest (DVCA) = VF, Low-ground Regenerated Forest =LRF, High-ground Regenerated Forest = HRF (400m a.s.l.).

The MMTs were set at random sites within the virgin forest (VF), low-ground regenerated forest (LRF) and 400m above sea level high-ground regenerated forest (HRF) (Fig. 2); approximately 1 km apart from each trap. MMTs were set up at 8.00 am on Day 1 and remained switched on until 8.00 am of Day 2. The MMT nets containing mosquitoes were collected every 24 hours and new fresh nets were placed daily for a total of 12 trap nights at each site. In the sampling months of May and January, three MMT were used, but in October only one

MMT was used due to mechanical issues. Mosquito sampling was carried out in the first week of May 2019, October 2019 and January 2020. A total of six trap nights for the month of May 2019 sampling, and three trap nights for October 2019 and January 2020 were carried out at each site.

MMT nets with *Anopheles* mosquitoes were taken to the Danum Valley Field Centre (DVFC) Scientific Laboratory for further processing. The mosquitoes were killed in a –30°C freezer, mounted individually and placed into 1.5 µl microcentrifuge tubes and stored in the –30°C freezer temporarily prior to being transferred out from Danum Valley. They were later carefully packaged and transported to the Faculty of Medicine and Health Sciences Entomology Laboratory in Universiti Malaysia Sabah for identification process.

Anopheles mosquitoes were identified to the species level using the identification keys of Rattanarithikul et al., (2006) and Sallum et al. (2005). Species richness, along with species diversity, were assessed through the application of Simpson's Reciprocal Diversity Index (D), Shannon-Wiener Diversity Index (H), and Evenness (E). Statistical analysis was done using R programming language for statistical analysis (version 3.5.2). A generalized linear mixed-effect model (GLMM, with R glmmTMB package) was used to analyse mosquito abundance (*An. fragilis*, the most abundant species and total *Anopheles*) as a function of the dependent variables (VF, LRF and HRF), using day and site as random factors, and Poisson distribution as the underlying distribution. The numbers for the other species were too low and too zero inflated for meaningful analysis. In all analyses, different forest types were considered as a fixed effect. Month of sampling was fit alternatively as a fixed (to predict monthly values) or random effect (to test for differences between forest types while controlling for seasonal variation). Poisson distribution was used in the analysis of mosquito abundance.

Models testing associations between response variables (mosquito abundance) explanatory variables (forest types and month of sampling) were assessed through comparison on the basis of having higher log-likelihood and lower Akaike information criterion (AIC) values. Tukey post hoc contrasts were used to differentiate the nature of statistical differences between forest types.

RESULTS

A total of 839 female *Anopheles* mosquitoes belonging to nine different *Anopheles* species were collected from all sites (Fig. 3). The dominant species was *Anopheles fragilis* (76.28%), followed by *An. asiaticus* (11.20%), *An. interruptus* (4.53%), *An. maculatus* and *An. montanus* 2.03% respectively, *An. balabacensis* (1.43%), *An. jamesii* (1.07%), *An. barbumbrosus* (0.83%) and *An. latens* (0.60%). The total *Anopheles* recorded in HRF constituted 78.9% of all mosquitoes caught, while in VF it was only 4.65%. Mosquito traps were set up in the various sampling sites using similar amounts of the same baits. Thus, the catch should reflect mosquito abundance. It is unlikely that under sampling has occurred on one site and not another.

Three species of vectors, namely *Anopheles balabacensis, An. latens* and *An. maculatus* were recorded at LRF and HRF (Fig. 1). These vectors were collected in small numbers at LRF: *Anopheles balabacensis* (4), *An. latens* (4), and *An. maculatus* (13). The numbers of the same three species from HRF were 6, 1 and 4, respectively, while at VF, only two individuals of *An. balabacensis* were collected.

Higher numbers of *Anopheles* mosquito at HRF were caught in all three sampling dates in May, October 2019 and January 2020. The number of *Anopheles* mosquitoes trapped at VF was the lowest with 39 individuals. Furthermore, *An. balabacensis* was caught in all three sites whereas *An. latens* and *An. maculatus* were only caught in LRF and HRF (Fig. 3).

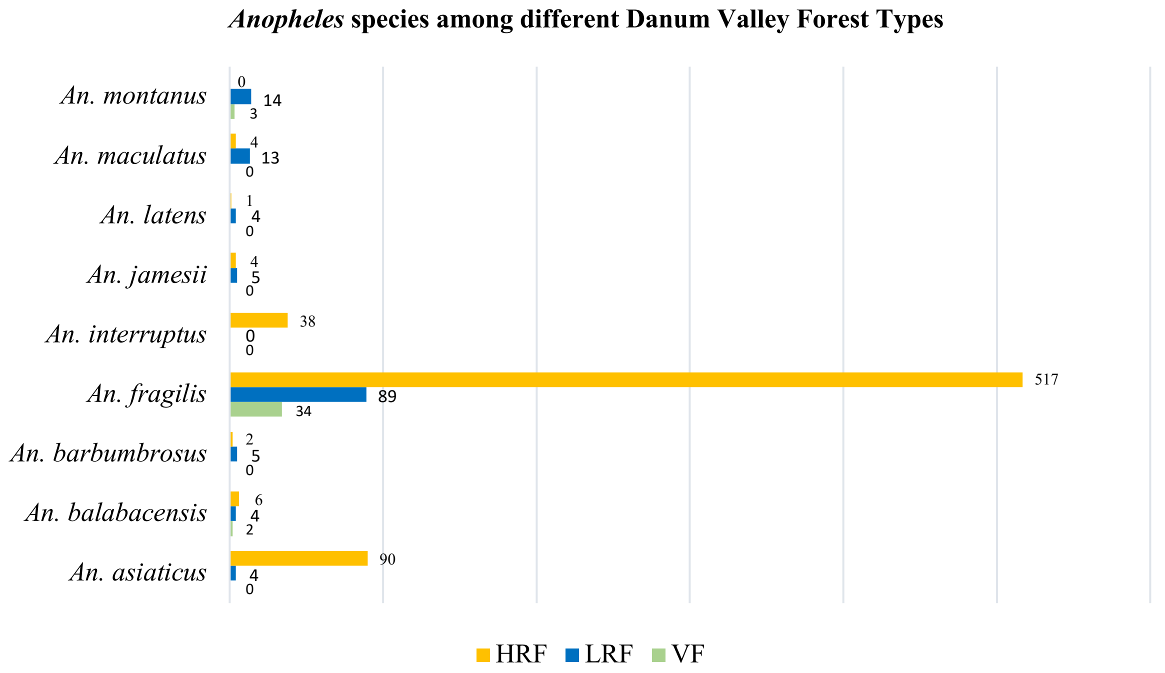

Figure 3: The number of individuals for each *Anopheles* sp. collected from different forest types. VF = virgin forest; LRF = low-ground regenerated forest; HRF = high-ground regenerated forest.

Anopheles species richness, *S* at HRF and LRF are eight and three species found in VF (Table 1). Both Simpson's and Shannon Wiener Diversity Indices were highest at LRF (D = 2.29, H = 1.28). HRF (D = 1.58, H = 0.76) and lowest at virgin forest (D = 1.31, H = 0.47) (Table 1). The three *Anopheles* species found at VF are *An. balabacensis, An. fragilis* and *An. montanus* (Fig. 3).

Table 1: *Anopheles* species richness and diversity indices by forest types. VF = virgin forest; LRF = low-ground regenerated forest; HRF = high-ground regenerated forest.

Species richness and diversity Index	VF	LRF	HRF
Species richness, *S*	3	8	8
Simpson Reciprocal Index, *D*	1.31	2.29	1.58
Shannon-Weiner Index, *H*	0.47	1.28	0.76
Evenness, *E*	0.43	0.62	0.36

Significant differences in the total number of *Anopheles* mosquitoes and the most abundant species, *An. fragilis* were detected for different forest types (Table 2). HRF had the highest mean catch per trap per night (46.5±10.24), but LRF had the lowest mean catch per trap per night (16.2±3.64) although it had significantly higher predicted mean catch per trap per night than VF (22.6± 5.03) for total *Anopheles* mosquitoes. Similarly, significant differences were observed in mosquito catch across the months ($P < 0.1$). January 2020 had a significantly higher number of total mosquitoes caught per trap per night (50.6±11.20) than May 2019 (30.4±6.65) and October 2019 (11.0±2.56).

The dominant *Anopheles* species is *An. fragilis* (78.9% of all *Anopheles*) (Figure 3) and the lowest predicted mean catch per trap per night for *An. fragilis* at VF was (1.35±0.42). A formal comparison of this dominant species in all three types of forest revealed this species has a significant preference in HRF with highest predicted mean per trap per night (20.55±5.34) followed by LRF (3.54±0.98) and VF (1.35±0.42).

Table 2: Fitting GLMM models for total *Anopheles* and *An. fragilis* trapped at Danum Valley. The predicted means and 95% standard error were calculated from the model.

	Forest type	Predicted mean catch/trap/night	p-value for difference of 2 means	AIC
Total *Anopheles*	High-ground regenerated forest	26.96±6.75 [a]	a-b <0.0001	424.81
	Low-ground regenerated forest	5.62±1.47 [b]	a-c <0.0001	
	Virgin forest	1.59±0.47[c]	b-c <0.0001	
Anopheles fragilis	High-ground regenerated forest	20.55±5.34 [a]	a-b <0.0001	397.92
	Low-ground regenerated forest	3.54±0.98 [b]	a-c <0.0001	
	Virgin forest	1.35±0.42 [c]	b-c =0.001	

DISCUSSION

Anopheles mosquitoes in Danum Valley

This study presents initial findings regarding the species composition and diversity of *Anopheles* species across different forest types within Danum Valley. To our knowledge, this is the first study conducted in Danum Valley concerning the diversity of *Anopheles* mosquitoes. Our analysis showed that the highest diversity of *Anopheles* mosquitoes was found in HRF whereas VF has the lowest diversity of *Anopheles* mosquitoes. Only three species of *Anopheles* mosquitoes were recorded from VF. These suggest that human disruptive activities in regenerated forests gives rise to more mosquito species than that of an undisturbed forest. The Intermediate Disturbance Hypothesis (IDH) provides an explanation for the role of disturbance coexistence of climax and colonist species (Loaiza et al., 2017). Assuming that, in undisturbed VF, disturbance-intolerant species (climax species) tend to monopolise resources (e.g., space and food sources), driving less competitive species to local extinction and reducing overall species diversity.

In our study *An. fragilis* is the predominant species found in all forest types and the highest number caught was in HRF despite being a more disturbed area. According to Reid (1965), *An. fragilis* (Aitkeni group) is known to be distributed on lowland in Malaya, Indonesia, Borneo and the Philippines. The Aitkeni group has not been found to be involved in disease transmission yet, but more information is needed on their biology and ecology. Forest land alteration at HRF environment seems to be more favourable to *An. fragilis* than LRF and VF. There is limited knowledge on why *An. fragilis* is found in abundance in HRF. However, the current knowledge by Byrne et al. (2021) shows that land use change is creating more suitable habitats for *Anopheles* vector larvae and this may be contributing to the abundance of *An. fragilis* in the regenerated forest of Danum Valley. The forest floor of the virgin forest tends to be shaded and covered with thick leaf litter and acidic water environments whereas logged lands tend to have sunlit neutral water environments (Brant, 2011). Logged lands are also prone to form temporary breeding puddles. Habitat requirements vary across different mosquito species and this gives a succession of vector species through different forest types (Patz et al., 2000; Norris, 2004). Landscape changes strongly provide significant affect to mictoclimate of a habitat, such as temperature, runoff, evapotranspiration (Patz & Olson, 2006). Hence, these factors are key in determining the abundance, survivorship and diversity of *Anopheles* mosquitoes (Patz & Olson, 2006).

In May and January, higher numbers of mosquitoes were collected because these months coincide with Sabah's two main rainy seasons (December to February and May to July). The increase in mosquito number could be due to more temporary breeding grounds for mosquitoes such as road puddles, rain pools, animal prints in the forest, potholes and tyre prints in human settlements, resulting from the rain (Hamza & Rayah, 2016; Hawkes et al., 2019). The number of trap nights for the first sampling trip at the DVCA was longer compared to the other two sampling trips in October and January. Three MMT were used in the first and third sampling trips which may be one of the reasons why the number of *Anopheles* mosquitoes was the highest in the month of May.

In October, the number of *Anopheles* mosquitoes collected was the lowest; a possible reason may be due to less rain as compared with May and January. The fruiting season in Danum Valley roughly corresponds to the period from the end of the rainy to the dry season, and the period at the beginning of the rainy season (Tomoko et al., 2010). The abundance and richness of mosquitoes highly depends on biotic factors (oviposition, presence of hosts for food and plants as shelter) and to abiotic factors (relative humidity, seasonality and temperature) (Abella-Medrano et al., 2020). *Plasmodium knowlesi* has been shown to be very infectious, with rhesus monkeys (*Macaca mulatta*) developing blood stage infections if one or more *P. knowlesi*-infected female *Anopheles* mosquitoes successfully feed on them (Murphy et al., 2014). Our temporal variation data to show the correlation between rainforest types and the effect on mosquito abundance is still lacking. There are other limitations such as difficulties in identifying microscopically incomplete or damaged mosquitoes resulting from over handling and transportation of fragile mosquitoes from DVCA to the UMS faculty laboratory, and loss of characters in specimens stored over an extended period of time. There was also limited equipment for the molecular screening of parasites in the mosquitoes.

Relationship between *Anopheles* mosquitoes and forest types

GLMM model (Table 2) fitting showed that in general, the highest number of mosquitoes caught were from the high-ground regenerated forest. The INFAPRO Tower was built around HRF for the purpose of tourism and meteorological study station for scientists. The construction of a gravel road up to the tower and the clearing of trees to build the tower had changed the environment of the ecosystem. Other alterations of the forested environment include logging of the rain forest with notably abandoned logging tracks, gullies, heavily disturbed or compacted areas causing detrimental effects on the terrain components and leading to soil erosion (Clarke & Walsh, 2006). Landscape features largely control the ability of female vectors to spread from their breeding sites to hosts, increases host-vector contacts (Raffy & Tran, 2005). Land cover alterations, such as deforestation and agricultural expansion had been associated with altered dynamics and geographical distribution of malaria and other vector borne diseases (Lambin et al., 2010) caused by *P. knowlesi* in Sabah.

Forest habitats and the linkage of emerging diseases

Some of the *Anopheles* species caught are confirmed vectors of zoonotic malaria. In Sabah, *An. balabacensis* (Wong et al., 2015*;* Ang et al., 2020) and *An. latens* (Vythilingam et al., 2006) are *P. knowlesi* malaria vectors belonging to the Leucosphyrus group*. Anopheles maculatus* is a vector of human malaria in Peninsular Malaysia (Vythilingam et al*.*, 1995). The majority of these *Anopheles* vectors are found in LRF and HRF in the DVCA where there was a degree of man-initiated disturbances to the forest. People will have a greater risk of exposure to zoonotic diseases when living at the forest fringe and working or entering the forest, reason being their proximity with the malaria parasite reservoir monkeys and the *Anopheles* vectors (Singh & Daneshvar, 2013; Anstey & Grigg, 2019). Our findings are consistent with recent findings where *An. balabacensis* were found to increase in abundance in disturbed forests (Brant et al., 2016; Chua et al., 2019; Wong et al., 2015).

Plasmodium knowlesi is responsible for approximately 70% of the reported malaria cases affecting humans in Malaysian Borneo (William et al., 2013). This could be due to the vector *An. balabacensis* which transmits the malaria parasite, being frequently found in disturbed forest habitats of regenerated forests as a result of human activities involving land-use changes. Such alterations create a pathway for both macaque hosts and highly adaptable vectors to migrate closer to human settlements, increasing interactions between man and mosquitoes. At night, when female mosquitoes generally feed, most of the hosts like humans and animals are immobile (Lambin et al., 2010). Logging and deforestation create a strong bridge, in which it increases interactions between humans, mosquito vectors and the macaque hosts of *P. knowlesi.* Evidence suggests that agricultural expansion and forest fragmentation affect *P. knowlesi* exposure, which supports linkages between land use change and *P. knowlesi* transmission (Fornace et al., 2019).

Factors that influence the diversity, abundance and range of host and *Anopheles* vector species are socio ecological processes such as man-made land use change, globalization of agricultural settings, population growth, and climate change (Davidson et al., 2019). The effect of land cover changes according to Fornace et al. (2019) are associated with the increased risks of *Plasmodium knowlesi* exposure and disease transmission. Alterations of landscapes have massive potential to threaten any future malaria eradication efforts (Fornace et al., 2021). Our findings indicate that land use alteration creates more favourable habitats for Anopheles vector larvae, thus contributing to more diverse Anopheles mosquitoes in the regenerated forest of Danum Valley. Byrne et al. (2021) working elsewhere in Sabah had similar finding."

CONCLUSIONS

This study focused on mosquito diversity within different forest types of Danum Valley and their medical importance. We believe this study has provided significant baseline data on vector mosquitoes within the pristine forest of Sabah that can be used for future research directions. The findings will be useful to plan a strategic vector control programme for villagers staying near forest fringes. More research is needed to gather data on the effects of wind, rainfall and feeding time of mosquitoes for effective fogging treatments for *Anopheles* in Sabah. Molecular work for pathogen screening will reveal more about the *Plasmodium* parasite species and uncover newly emergent malaria parasites carried by the *Anopheles* mosquitoes found in the pristine rainforest of Borneo

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Research Article

Woody Plants of Ulu Muda Forest Reserve Kedah, Malaysia

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ABSTRACT

The woody plants species inventory and diversity of primary, old secondary and secondary forest patches of Ulu Muda Forest Reserve (UMFR), Kedah, Peninsular Malaysia were assessed in this study. This was done to understand the current status of the forest towards ensuring proper conservation plans. Plants with diameter at breast height of not less than 10 were identified and enumerated within 10m radius (both left and right) of a 1500m transect laid at each site. Two hundred and thirty-five (235) plant species belonging to 56 families and 144 genera were enumerated in all the sites. The percentage composition of lianas (14.53%) was found to be very minimal to tree species (76.49%) which denotes the health status of the forest. *Polyalthia jenkensii*, *Spondias cytherea* and *Intsia palembanica* were the most common plants in the three sites. The Shannon diversity index of all the sites was observed to be greater than 2, which shows the high diverse nature of the forest despite some past disturbances. The old secondary forest patch was reported to have recuperated over the years due to its highest diversity indices.

Keyword: Conservation; Dipterocarp; Euphorbiaceae; Forest; Kedah.

INTRODUCTION

Tropical forests globally are known to be highly valuable due to the ecosystem services they provide such as controlling soil erosion and serving as habitats to both plants and animals (Anbarashan & Parthasarathy, 2013). These tropical forests are threatened severely by anthropogenic influences and require specific interventions towards the management and maintenance of their overall carbon productivity, biodiversity and sustainability (Kumar et al., 2006). To ensure the management and sustainability of forest ecosystems, it is important to understand their tree species composition and structures (Kacholi, 2014). The knowledge of the forests' structure, tree species richness, and their characteristics is a useful tool towards an effective long-term conservation of the forests (Ifo et al., 2015). The main anthropogenic threat faced by tropical forests globally is the issue of logging and this has gotten the attention of most ecologists in studying how these forests have reacted to the threat (Saiful & Latiff, 2014). This makes it more expedient for tropical forests to be protected to conserve biodiversity and mitigate climate change (Berry et al., 2010). Consequently, most tropical countries have now adopted a more sustainable way of scrutinizing logging activities in their forests through closed supervision (Sadeghi et al., 2014).

Similarly, Malaysian forests are known to be highly diverse in plant species and are economically useful to the country. About 44.7% of the total land mass in Peninsular Malaysia is composed of forests (Ghollasimood, 2011). Based on the elevation and soil types ranging from coastal areas to the hills, about 16 different types of forests have been classified across the world (Whitmore & Sayer, 1992). The Ulu Muda Forest Reserve (UMFR) can be described as a dipterocarp forest (Mardan et al., 2013). Both primary and secondary forests have to be protected due to their importance in maintaining balance in the environment (Berry et al., 2010).

Over the years, there has been a consistent decrease in the number of plant species at the UMFR due to logging activities (Saiful et al., 2008). This is why there is need for continuous assessment of the plant species diversity in the forest so as to guide effective conservation planning and management. This study aims to produce an inventory of the richness and diversity of plant species (mainly angiosperms) found at the Ulu Muda Forest Reserve (UMFR), Kedah, Malaysia. This is to give an updated record of the plant species diversity of this forest which was lacking in previous studies (Mardan et al., 2013).

METHODOLOGY

Study area

Ulu Muda Forest Reserve (UMFR) which has an average size of about 160,000 ha is located in Kedah, Northwest of Peninsular Malaysia. The city where this forest is located has an average annual rainfall of about 2000mm with the peak in October. This forest comprises both hill and lowland dipterocarp forest vegetations. It also houses other vegetation types such as limestone and riparian vegetations. Due to the proximity of the forest to Southern Thailand, a few Thailand flora are found in this forest (Suksuwan, 2008). Apart from these, the forest serves a lot of economical uses such as water supply, recreation and logging to the natives of Kedah and neighbouring states (Mariana et al., 2008). Consequently, it has remained threatened by these anthropogenic activities. The most prevalent threats on the UMFR are the legal and illegal unstainable logging activities.

Sampling technique and data analysis

The forest was divided into three (3) study sites namely secondary forest (site 1), old secondary forest (site 2), and primary forest (site 3). The geographic coordinates of the sites are presented in Table 1.

Sampling was done using a 1,500m line transect laid at each site. Plants particularly with diameter at breast height (dbh) of not less than 10cm, found along the transect and within the radius of 10m to the right and left of the transects were identified. Plant identification was done on-site and only those with uncertain identities were collected and identified at the herbarium of Universiti Sains Malaysia, Penang.

Table 1: Location of the study sites.

Site	Latitude (N)	Longitude (E)	Altitude	Forest type
1	6° 6'47.03"	100°57'52.59"	130 m	Secondary Forest
2	6° 5'48.92"	100°58'27.19"	185 m	Old Secondary Forest
3	6° 6'21.12"	100°58'16.39"	210 m	Primary Forest

The diversity indices such as Simpson index, Shannon index, species evenness and Margalef index were estimated using the PAST 3.0 software. Rarefaction analysis was done to determine the species richness using the Biodiversity Pro software. The similarity in plant species between the studied forests was assessed using a Sorenson similarity index as calculated using the equation below:

$$Sc = (2W/a)\ x\ 100\%\ (Sorenson, 1948)$$

Sc represents the similarity coefficient. W symbolized the number of plant species found common to all the sites. Also, a is the sum of the total number of plants species recorded at each of the sites.

RESULTS AND DISCUSSION

A total of 235 plant species comprising the following plant types; 15 emergent, 25 main canopy, 128 understory, 12 treelets, 18 shrubs, 34 lianas, 2 slender climbers and 1 large strangler were identified at all the sites in this forest (Fig. 1). These 235 plant species belong to 56 families and 144 genera (Appendix 1).

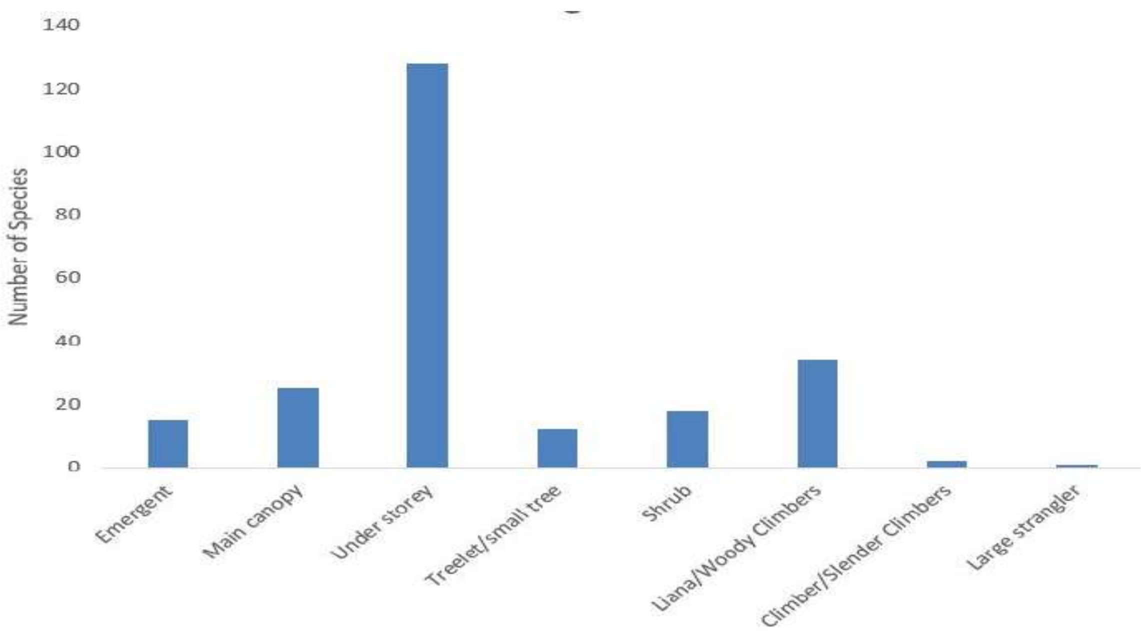

Figure 1: The plant types and the corresponding number of species.

Sites 1, 2 and 3 have 46, 199, and 135 plant species respectively. The rarefaction curve shows that site 2 has the highest plant species richness, followed by site 3, while site 1 has the lowest (Fig. 2).

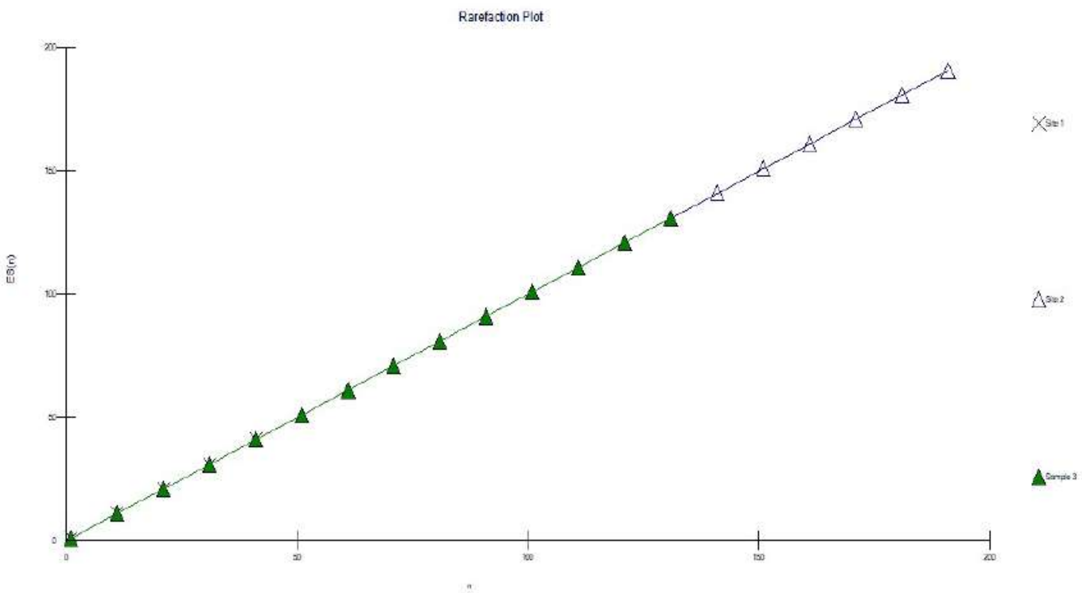

Figure 2: Rarefaction plot showing the species richness of the sample sites.

In this analysis, the estimated species richness for each site was very close to the observed species richness. The lianas have 14.53% composition as compared with the 76.49% of trees (emergent, main canopy, understory and treelets) in these forests. This low liana composition indicates that the forests are less disturbed and more stable in terms of tree richness and diversity (Villagra et al., 2013). Abundance of lianas in tropical forests has been reported to be positively correlated with high disturbance and also interfere with tree diversity (Addo-Fordjour & Rahmad, 2015; García León et al., 2018).

Euphorbiaceae, Annonaceae, and Meliaceae are the most common families having 22, 18, and 14 number of species respectively. These same plant families have been reported to be dominant in many other tropical forests (Françoso et al., 2016; Rahmad et al., 2018). A previous study at the hill dipterocarp forest of UMFR also confirmed that Euphorbiaceae was the most important family in the forest (Sadeghi et al., 2014). However, this could have been due to the removal of some Dipterocarpaceae members during past logging (Saiful et al., 2008). In site 1, *Polyalthia jenkensii*, *Spondias cytherea* and *Pterocymbium javanicum* were the most common species. In site 2, *Lagerstromia speciosa*, *Polyalthia jenkensii*, and *Spondias cytherea* were the most common species. However, in site 3, *Intsia palembanica*, *Spondias cytherea,* and *Bombax valetonii* were the most common species. This then made *Polyalthia jenkensii*, *Spondias cytherea*, and *Intsia palembanica* to overall be highly distributed species in all the three sites.

Site 2 which is an old secondary forest, was observed to have the highest Simpson (0.995), Shannon (5.288) and Margalef (37.25) indices, followed by site 3 which is a primary forest. Site 1 was the one with the least of all the diversity indices estimated. This is very understandable due to the recent level of disturbance (logging) faced by site 1, being a secondary forest. None of the studied forests had a Shannon index that less than two. This shows that they are all highly diverse and stable in plant species (Barbour et al., 1999). The Sorenson similarity index coefficient between the three study sites is 8.97%. This is very low and it indicates that there is very little similarity between the study sites in terms of plant species. The three forests are different in level of disturbance. This could explain the reason for the low similarity index between them.

Table 2: The diversity indices of plants species at the Ulu Muda Forest, Kedah.

	Site 1	**Site 2**	**Site 3**
Number of species	46	198	135
Simpson index	0.978	0.995^{b}	0.993^{b}
Shannon index	3.829^{a}	5.288^{d}	4.905^{b}
Species evenness	1^{a}	1^{a}	1^{a}
Margalef index	11.75^{c}	37.25^{d}	27.32^{e}

The inventory and diversity of plants reported in this study could be a useful tool for the conservation of these forests in future (Jayakumar et al., 2011). Generally, tropical forests to which UMFR belongs, are described as very rich in species diversity and are providers of important ecosystem services including sources of timber and non-timber forest products (NTFPs), carbon stock and sequestration etc. (Bradon, 2014). As done in this study, it is important to periodically ascertain the status of forests which are known to provide lots of ecosystem services that are very essential to the survival of life on earth (Cardinale et al., 2012; Steur et al., 2020). This will aid in implementing appropriate conservation measures by policy makers or conservationists in the forests whose ecosystem services have been identified to be threatened (IPBES, 2019). There is a direct positive relationship between forest biodiversity richness and ecosystem services (Harrison et al., 2014). This means that conservation of forest biodiversity will also aid in the preservation of its ecosystem services (Steur et al., 2020). Specifically, higher plant diversity of a forest is expected to lead to a higher ecosystem service since the plants are the primary producers in such forest (Quijas et al., 2010).

CONCLUSIONS

This study has revealed that the old secondary forest (site 2) has undergone fast recuperation over the years and has become richer and more diverse in plant species as compared with the primary forest (site 3) at the UMFR. This shows that the efforts made by the government in conserving this forest reserve is yielding positive results and it is commended. The current level of supervised logging activities should be maintained or further minimized so as to keep the forest more stable in the provision of ecosystem services and diverse in plant species.

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Appendix 1: List of plants identified at Ulu Muda Forest, Kedah

S/N	Family	Species	Local name	Plant type	Site 1	Site 2	Site 3
1	Actinidiaceae	*Saurauia pentapetala*	Sauraya	T	0	1	0
2	Alangiaceae	*Alangium griffithii*	Mentulang	U	0	1	0
3	Alangiaceae	*Alangium ridleyi*	Mentulang	U	0	1	0
4	Anacardiaceae	*Bouea macrophylla*	Kundang daun besar	U	0	1	0
5	Anacardiaceae	*Bouea oppositifolia*	Kundang rumenia	U	1	0	1
6	Anacardiaceae	*Dracontomelon dao*	Sengkuang	U	1	0	1
7	Anacardiaceae	*Mangifera macrocarpa*	Macang hutan	U	0	0	1
8	Anacardiaceae	*Mangifera sp.1*	Macang hutan	U	0	0	1
9	Anacardiaceae	*Spondias cytherea*	Umrah/Amra	E	1	1	1
10	Annonaceae	*Cananga odorata*	Kenanga hutan	U	1	0	0
11	Annonaceae	*Desmos chinensis*	Akar mempisang	L	0	1	1
12	Annonaceae	*Desmos cochinchinensis*	Akar mempisang	L	0	1	1
13	Annonaceae	*Desmos dumosa*	Akar mempisang	L	0	1	0
14	Annonaceae	*Fissistigma manubriatum*	Larak	L	1	1	1
15	Annonaceae	*Goniothalamus tenuifolius*	Seteru putih	S	0	1	1
16	Annonaceae	*Goniothalamus tortilipetalus*	Seteru hitam	S	0	1	1
17	Annonaceae	*Mitrella kentii*	Akar mempisang	L	1	1	1
18	Annonaceae	*Polyalthia clavigera*	Mempisang	U	0	1	0
19	Annonaceae	*Polyalthia jenkensii*	Mempisang	U	1	1	1
20	Annonaceae	*Polyalthia lateriflora*	Mempisang	U	0	0	1
21	Annonaceae	*Polyalthia rumphii*	Mempisang	U	0	1	1
22	Annonaceae	*Polyalthia sclerophylla*	Mempisang	U	0	0	1
23	Annonaceae	*Popowia pisocarpa*	Mempisang	U	0	1	1
24	Annonaceae	*Pyramidanthe prismatica*	Akar mempisang	L	0	1	0
25	Annonaceae	*Trivalvaria macrophylla*	Mempisang	U	0	1	1
26	Annonaceae	*Uvaria pauci-ovulata*	Akar mempisang	L	0	1	0
27	Annonaceae	*Xylopia magna*	Banit kijang	U	0	1	0
28	Apocynaceae	*Tabernaemontana corymbosa*	Jelutong badak	U	0	1	0
29	Apocynaceae	*Tabernaemontana pauciflora*	Jelutong kecil	U	0	1	0
30	Araliaceae	*Arthrophyllum diversifolium*	Susun pelepah	U	0	1	0

S/N	Family	Species	Local name	Plant type	Site 1	Site 2	Site 3
31	Araliaceae	*Macropanax maingayi*	None	C	0	1	0
32	Araliaceae	*Schefflera heterophylla*	None	U	0	1	0
33	Araliaceae	*Trevesia burckii*	Jari hantu	S	0	1	1
34	Bignoniaceae	*Diplanchea bancana*	Cenderu	C	1	0	0
35	Bombacaceae	*Bombax valetonii*	Kekabu hutan	E	0	1	1
36	Bombacaceae	*Durio singaporensis*	Durian hutan	C	0	1	0
37	Burseraceae	*Santiria laevigata*	Kedondong kerantai licin	C	0	1	1
38	Burseraceae	*Santiria tomentosa*	Kedondong kerantai bulu	C	1	0	0
39	Burseraceae	*Trioma malaccensis*	Kedondong kijal	C	0	1	1
40	Cecropiacaeae	*Poikilospermum suaveolens*	Akar tumpang	L	0	1	0
41	Chloranthaceae	*Chloranthus erectus*	None	S	1	1	0
42	Combretaceae	*Terminalia citrina*	Jelawai belang rimau	C	0	0	1
43	Compositae	*Vernonia arborea*	None	U	0	1	0
44	Connaraceae	*Rourea emarginata*	None	L	0	1	0
45	Connaraceae	*Rourea memosoides*	None	L	0	1	0
46	Connaraceae	*Rourea minor*	None	L	0	1	0
47	Connaraceae	*Rourea rugosa*	None	L	0	1	1
48	Dilleniaceae	*Dillenia reticulata*	Simpoh gajah	C	0	1	0
49	Dilleniaceae	*Tetracera akara*	Akar mempelas	L	0	1	0
50	Dilleniaceae	*Tetracera macrophylla*	Akar mempelas	L	0	1	0
51	Dilleniaceae	*Tetracera maingayi*	Akar mempelas	L	0	1	0
52	Dipterocarpaceae	*Anisoptera costata*	Mersawa kesat	E	0	1	0
53	Dipterocarpaceae	*Dipterocarpus costulatus*	Keruing kipas	E	0	1	1
54	Dipterocarpaceae	*Dipterocarpus fagineus*	Keruing bukit	E	0	1	0
55	Dipterocarpaceae	*Dipterocarpus hasseltii*	Keruing ropol	E	0	1	0
56	Dipterocarpaceae	*Hopea mengarawan*	Merawan penak	E	0	1	0
57	Dipterocarpaceae	*Shorea guiso*	Balau membatu	E	0	1	1
58	Dipterocarpaceae	*Shorea leprosula*	Meranti tembaga	E	0	1	1
59	Dipterocarpaceae	*Shorea parvifolia*	Meranti sarang punai	E	0	0	1
60	Dipterocarpaceae	*Vatica pauciflora*	Resak laru	E	1	0	0
61	Ebenaceae	*Diospyros andamanica*	Kayu arang	U	0	1	1

S/N	Family	Species	Local name	Plant type	Site 1	Site 2	Site 3
62	Ebenaceae	*Diospyros buxifolia*	Meribut	U	0	1	0
63	Ebenaceae	*Diospyros pendula*	Kayu arang	U	0	1	0
64	Ebenaceae	*Diospyros singaporensis*	Kayu arang	U	0	1	1
65	Ebenaceae	*Diospyros sumatrana*	Kayu arang	U	0	1	1
66	Elaeocarpaceae	*Elaeocarpus stipularis*	Mendung	U	0	1	0
67	Euphorbiaceae	*Alchornea rhodophylla*	Julong jantan	T	0	1	0
68	Euphorbiaceae	*Aporosa arborea*	Sebasah	U	1	1	1
69	Euphorbiaceae	*Aporosa aurea*	Sebasah	U	0	1	1
70	Euphorbiaceae	*Aporosa confusus*	Sebasah	U	0	1	0
71	Euphorbiaceae	*Baccaurea brevipes*	Rambai hutan	U	1	1	1
72	Euphorbiaceae	*Baccaurea kunstleri*	Tampoi	U	0	0	1
73	Euphorbiaceae	*Baccaurea parviflora*	Setambun tahi	U	0	1	1
74	Euphorbiaceae	*Baccaurea racemosa*	Setambun jantan	U	0	0	1
75	Euphorbiaceae	*Croton erythrostachys*	Hujan panas	U	1	1	1
76	Euphorbiaceae	*Elateriospermum tapos*	Perah	C	0	1	1
77	Euphorbiaceae	*Endospermum diadenum*	Sesenduk	C	1	1	0
78	Euphorbiaceae	*Erismanthus obliquus*	None	T	0	1	1
79	Euphorbiaceae	*Glochidion glomeratum*	Ubah	U	1	0	0
80	Euphorbiaceae	*Glochidion sericeum*	Ubah	U	0	1	0
81	Euphorbiaceae	*Macaranga diepenhorstii*	Mahang	U	1	1	1
82	Euphorbiaceae	*Macaranga gigantea*	Mahang gajah	U	1	1	0
83	Euphorbiaceae	*Macaranga hosei*	Mahang	U	0	1	0
84	Euphorbiaceae	*Macaranga hypoleuca*	Mahang tumu putih	U	0	1	0
85	Euphorbiaceae	*Macaranga lowii*	Mahang hutan	U	0	1	0
86	Euphorbiaceae	*Mallotus griffithianus*	Balik angin	U	0	1	0
87	Euphorbiaceae	*Sapium baccatum*	Ludai	U	1	1	0
88	Euphorbiaceae	*Sauropus androgynus*	Pucuk manis hutan	S	0	1	1
89	Fagaceae	*Castanopsis curtisii*	Berangan	C	0	1	0
90	Fagaceae	*Castanopsis lucida*	Berangan	C	0	1	1
91	Fagaceae	*Lithocarpus rassa*	Mempening	C	1	1	1
92	Flacourtiaceae	*Casearia sp.2*	None	T	0	1	1

S/N	Family	Species	Local name	Plant type	Site 1	Site 2	Site 3
93	Flacourtiaceae	*Casearia tuberculata*	None	T	0	1	1
94	Flacourtiaceae	*Flacourtia rukam*	Rukam hutan	T	0	0	1
95	Flacourtiaceae	*Hydnocarpus castanea*	Setumpol	U	0	1	1
96	Flacourtiaceae	*Hydnocarpus woodii*	Setumpol	U	0	1	1
97	Flacourtiaceae	*Ryparosa fasciculata*	Duku hutan	U	0	1	1
98	Flacourtiaceae	*Ryparosa kunstleri*	Duku hutan	U	0	1	1
99	Flacourtiaceae	*Scolopia spinosa*	Telusuk ayam	U	0	1	1
100	Gnetaceae	*Gnetum macrostachyum*	Akar meninjau	T	0	1	0
101	Guttiferae	*Garcinia nervosa*	Kandis beruang	U	0	1	1
102	Guttiferae	*Garcinia parvifolia*	Kandis	U	0	1	1
103	Icacinaceae	*Stemonurus malaccensis*	Sampul keris	U	0	1	1
104	Irvingiaceae	*Irvingia malayana*	Pauh kijang	E	0	0	1
105	Lauraceae	*Actinodaphne sesquipedalis var.sesquipedalis*	Medang payung	U	1	0	0
106	Lauraceae	*Cryptocarya infectoria*	Medang kunyit	U	0	1	1
107	Lauraceae	*Dehasia incrassata*	Medang	U	0	1	1
108	Lauraceae	*Litsea elliptica*	Medang	U	0	1	0
109	Lauraceae	*Litsea grandis*	Medang	U	0	0	1
110	Vitaceae	*Leea indica*	Memali	S	1	1	0
111	Leguminosae	*Albizia splendens*	Batai hutan	C	0	1	0
112	Leguminosae	*Bauhinia bidentata*	Tapak kuda	L	0	1	1
113	Leguminosae	*Caesalpinia andamanica*	Gorek	L	0	1	1
114	Leguminosae	*Caesalpinia sumatrana*	Gorek	L	0	1	1
115	Leguminosae	*Callyera atropurpurea*	Tulang daing	C	0	1	1
116	Leguminosae	*Crudia curtisii*	Merbau kera	U	1	0	0
117	Leguminosae	*Derris malaccensis*	None	L	1	0	0
118	Leguminosae	*Dialium platysepalum*	Keranji	C	0	0	1
119	Leguminosae	*Intsia palembanica*	Merbau	E	0	1	1
120	Leguminosae	*Parkia timoriana*	Petai kerayong	C	0	1	0
121	Leguminosae	*Saraca declinata*	Gapis	U	0	1	1
122	Leguminosae	*Sindora coriacea*	Sepetir licin	C	0	0	1

S/N	Family	Species	Local name	Plant type	Site 1	Site 2	Site 3
123	Leguminosae	*Spatolobus ferrugineus*	None	L	1	1	1
124	Loganiaceae	*Strychnos flavescens*	Tarik gajah	L	0	1	1
125	Loganiaceae	*Strychnos ignatii*	Tarik gajah	L	0	1	1
126	Lytraceae	*Lagerstromia speciosa*	Bungor	E	1	1	1
127	Magnoliaceae	*Magnolia elegans*	Cempaka hutan	U	0	1	0
128	Magnoliaceae	*Magnolia lilifera var.lilifera*	Cempaka hutan	U	0	1	0
129	Malvaceae	*Hibiscus macrophyllus*	Tutor	C	0	1	1
130	Melastomataceae	*Clidermia hirta*	Kelentit nyamuk	S	1	1	0
131	Melastomataceae	*Oxyspora bullata*	Senduduk gajah	S	0	1	0
132	Melastomataceae	*Oxyspora hirta*	Senduduk gajah	S	0	1	0
133	Melastomataceae	*Phyllagatis hirta*	Tapak sulaiman	S	0	1	1
134	Melastomataceae	*Phyllagatis magnifica*	Tapak sulaiman	S	0	1	1
135	Melastomataceae	*Pternandra coerulescens*	Sial menahun	U	0	1	0
136	Melastomataceae	*Pternandra echinata*	Sial menahun	U	0	1	0
137	Meliaceae	*Aglaia argentea*	Bekak	U	0	1	1
138	Meliaceae	*Aglaia crassinervia*	Bekak	U	1	0	0
139	Meliaceae	*Aglaia elliptica*	Bekak	U	0	1	0
140	Meliaceae	*Aglaia grandis*	Bekak	U	0	1	1
141	Meliaceae	*Aglaia lawii ssp.oligocarpa*	Bekak	U	1	0	0
142	Meliaceae	*Aglaia leucophylla*	Bekak	U	0	1	0
143	Meliaceae	*Aglaia macrocarpa*	Bekak	U	0	1	1
144	Meliaceae	*Aglaia pachyphylla*	Bekak	U	0	0	1
145	Meliaceae	*Aglaia palembanica*	Bekak	U	0	1	1
146	Meliaceae	*Aglaia tenuicaulis*	Bekak	U	0	1	1
147	Meliaceae	*Aphanamixis polystachya*	Kulim burung	U	1	0	1
148	Meliaceae	*Aphanamixis sumatrana*	Kulim burung	U	1	1	0
149	Meliaceae	*Chisocheton ceramicus*	Pasak lingga	U	0	1	0
150	Meliaceae	*Lansium domesticum*	Langsat hutan	U	1	1	1
151	Memecylaceae	*Memecylon dichotomum*	Nipis kulit	U	0	1	1
152	Memecylaceae	*Memecylon megacarpum*	Nipis kulit	U	0	1	1
153	Memecylaceae	*Memecylon oleifolium*	Nipis kulit	U	0	0	1

S/N	Family	Species	Local name	Plant type	Site 1	Site 2	Site 3
154	Memecylaceae	*Memecylon pubescens*	Nipis kulit	U	0	1	1
155	Menispermaceae	*Coscinium fenestratum*	Mengkunyit	U	1	1	1
156	Moraceae	*Artocarpus elasticus*	Terap nasi	L	0	1	1
157	Moraceae	*Artocarpus fulvicortex*	Keledang bulu	C	0	0	1
158	Moraceae	*Ficus kerkhovenii*	Ara	LS	0	1	1
159	Moraceae	*Ficus lepicarpa*	Ara	U	0	1	0
160	Moraceae	*Ficus scortechinii*	Ara	S	0	1	0
161	Myristicaceae	*Horsfieldia polyspherula var.sumatrana*	Penarahan	U	0	1	1
162	Myristicaceae	*Horsfieldia sucosa*	Penarahan	U	0	1	1
163	Myristicaceae	*Knema curtisii*	Penarahan	U	1	1	0
164	Myristicaceae	*Knema furfuracea*	Penarahan	U	0	1	1
165	Myristicaceae	*Knema hookeriana*	Penarahan	U	0	1	1
166	Myristicaceae	*Knema lamellaria*	Penarahan	U	1	0	1
167	Myristicaceae	*Knema scortechinii*	Penarahan	U	1	1	1
168	Myristicaceae	*Myristica malaccensis*	Penarahan arang	U	1	0	0
169	Myrsinaceae	*Ardisia chlorantha*	Mata pelanduk	T	0	1	1
170	Myrsinaceae	*Ardisia fulva*	Mata pelanduk	S	0	1	0
171	Myrsinaceae	*Ardisia korthalsiana*	Mata pelanduk	T	0	1	1
172	Myrsinaceae	*Ardisia pachysandra*	Mata pelanduk	T	0	1	1
173	Myrtaceae	*Syzygium cinereum*	Kelat	U	0	1	1
174	Myrtaceae	*Syzygium griffithii*	Kelat	U	0	1	1
175	Myrtaceae	*Syzygium polyalthum*	Kelat	U	1	1	0
176	Myrtaceae	*Syzygium scortechinii*	Kelat	U	0	1	1
177	Myrtaceae	*Syzygium sp.6*	Kelat	U	0	1	1
178	Olacaceae	*Ochanostachys amentacea*	Petaling	U	0	1	0
179	Olacaceae	*Strombosia javanica*	Dedali	U	0	1	0
180	Ophiliaceae	*Champereia manillana*	Cemperai	T	0	1	1
181	Pandaceae	*Galearia fulva*	Ekor tupai	T	0	1	1
182	Polygalaceae	*Xantophyllum ellipticum*	Minyak berok	U	1	0	0
183	Polygalaceae	*Xantophyllum eurynchum*	Minyak berok	U	0	1	1

S/N	Family	Species	Local name	Plant type	Site 1	Site 2	Site 3
184	Rhamnaceae	*Ziziphus affinis*	Bidara hutan	L	0	1	1
185	Rhamnaceae	*Ziziphus calophylla*	Bidara hutan	L	0	1	1
186	Rhamnaceae	*Ziziphus kunstleri*	Bidara hutan	L	0	1	1
187	Rhamnaceae	*Ziziphus oenoplia*	Bidara hutan	L	0	1	1
188	Rhamnaceae	*Ziziphus pernettyoides*	Bidara hutan	L	0	1	1
189	Rubiaceae	*Aidia densiflora*	Menterbang	U	0	1	0
190	Rubiaceae	*Canthium horridum*	Akar kait kecil	L	0	1	1
191	Rubiaceae	*Chasalia culviflora*	Puding hutan	S	0	1	0
192	Rubiaceae	*Coptosapelta tomentosa*	None	L	0	1	1
193	Rubiaceae	*Diplospora malaccensis*	Gading-gading	U	0	1	1
194	Rubiaceae	*Fagerlindia fasciculata*	None	L	0	1	1
195	Rubiaceae	*Greenea corymbosa*	None	U	0	1	0
196	Rubiaceae	*Lasianthus griffithii*	None	S	0	1	1
197	Rubiaceae	*Lasianthus inaequalis*	None	S	0	1	1
198	Rubiaceae	*Lasianthus oblongus*	None	S	0	1	1
199	Rubiaceae	*Lasianthus sp.6*	None	S	0	1	0
200	Rubiaceae	*Nauclea offcinalis*	Mengkal	U	0	1	0
201	Rubiaceae	*Uncaria attenuata*	Akar kait-kait	L	0	1	1
202	Sapindaceae	*Lepisanthes senegalensis*	Rambutan hutan	U	0	1	0
203	Sapindaceae	*Nephelium maingayi*	Redan	U	0	0	1
204	Sapindaceae	*Paranephelium macrophyllum*	Rambutan hutan	U	1	1	1
205	Sapindaceae	*Pometia pinnata*	Kasai daun besar	U	1	1	1
206	Sapotaceae	*Madhuca kunstleri*	Nyatoh	U	0	0	1
207	Sapotaceae	*Palaquium maingayi*	Nyatoh tembaga	U	0	1	1
208	Sapotaceae	*Payena maingayi*	Nyatoh durian	U	0	1	1
209	Smilacaceae	*Smilax lanceifolia*	Ubi jaga	CL	1	1	0
210	Smilacaceae	*Smilax setosa*	Ubi jaga	CL	0	1	0
211	Sterculiaceae	*Pterocymbium javanicum*	Menglembu	C	1	1	1
212	Sterculiaceae	*Pterospermum javanicum*	Bayur	C	0	1	1
213	Sterculiaceae	*Pterygota alata*	Kasah	C	1	1	0

S/N	Family	Species	Local name	Plant type	Site 1	Site 2	Site 3
214	Sterculiaceae	*Scaphium linearicarpum*	Kembang semangkuk bulat	U	0	1	0
215	Sterculiaceae	*Sterculia macrophylla*	Kelumpang	U	0	1	0
216	Symplocaceae	*Symplocos ophirensis*	Jirak	U	0	1	1
217	Tetramelaceae	*Tetrameles nudiflora*	Mengkundur	E	0	1	1
218	Theaceae	*Gordonia multinervis*	Samak pulut	U	0	1	0
219	Tiliaceae	*Grewia laevigata*	Akar cenderai	L	0	1	1
220	Tiliaceae	*Microcos hirsuta*	Cenderai	U	0	1	1
221	Tiliaceae	*Microcos latifolia*	Cenderai	U	0	1	1
222	Tiliaceae	*Microcos latistipulata*	Cenderai	U	0	1	0
223	Tiliaceae	*Microcos laurifolia*	Cenderai	U	0	1	1
224	Tiliaceae	*Microcos tomentosa*	Cenderai	U	1	0	0
225	Tiliaceae	*Schoutenia furfuracea*	Bayur	U	1	1	0
226	Ulmaceae	*Celtis philipinensis*	Kaum hampas tebu	C	0	1	0
227	Ulmaceae	*Celtis rigescens*	Kaum hampas tebu	C	0	1	0
228	Ulmaceae	*Gironniera nervosa*	Hampas tebu	U	0	1	1
229	Verbenaceae	*Callicarpa maingayi*	Tampang besi	U	0	1	1
230	Verbenaceae	*Sphenodesma pentandra var.pentandra*	Akar leban	L	0	1	1
231	Verbenaceae	*Teijsmanniodendron pteropodum*	Entapuloh	U	0	1	0
232	Verbenaceae	*Vitex gamosepala*	Leban	U	0	1	0
233	Verbenaceae	*Vitex pinnata*	Leban	U	0	1	0
234	Verbenaceae	*Vitex vestita*	Leban	U	0	1	0

Key: 1 - present; 0 - absent; E - emergent; C – main canopy; U – understory; T – treelet / small tree; S -shrub; L – liana; CL – slender climbers; LS – large strangler.

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Research Article

Checklist of Parasitic Plants in Marilog District, Southern Philippines

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ABSTRACT

Parasitic plants are interesting flowering plants that are present around the world, except in the coldest regions. These species are categorized either as hemiparasites or holoparasites depending on their mode of nutrition to their hosts. Botanical fieldworks through repeated transect walks and opportunistic samplings were carried out in the five forest patches of the three Barangays in Marilog District, Davao City, Philippines on February 2018 to September 2019. Data revealed that there were six species of parasitic plants in the area belonging to five genera in four families. These species include *Balanophora papuana* Schltr. (Balanophoraceae), *Amyema curranii* (Merr.) Danser, *Amyema seriata* (Merr.) Barlow and *Decaisnina ovatifolia* (Merr.) Barlow (Loranthaceae), *Mitrastemon yamamotoi* Makino (Mitrastemonaceae), and *Christisonia schortechinii* Prain (Orobanchaceae). *Amyema seriata* was the most frequently encountered species, whereas *A. curranii* and *M. yamamotoi* (collected only in Site 2 – Mount Malambo) and *C. scortechinii* (collected only in Site 5 – Sitio Matigsalug) were less frequently recorded. Host plants of these species are provided in this paper and the habitat and ecology for each species are discussed. It is imperative that Mount Malambo, the only place where *M. yamamotoi* was documented, should be protected and conserved since this species is rare in the wild. The rapid habitat destruction and conversion of forest lands into agricultural and residential properties in areas near these mountains may lead to depletion of these species. Thus, conservation management strategies of these parasitic plants should be relayed to different policy makers and local people in the area.

Keywords: Parasitic Plants; Hemiparasites; Holoparasites; Biodiversity Conservation; Habitat Destruction.

INTRODUCTION

The world is full of fascinating floras extending from various carnivorous plants to different parasitic plants. Parasitic plants exist almost around the globe and are classified as hemiparasites (photosynthetic) or holoparasites (non-photosynthetic), and are also classified based on their host dependency. Some economically important parasitic plants, such as *Striga* Lour. and *Cuscuta* L. are completely dependent on their host plants for survival and are termed as obligatory parasites. On the other hand, other parasitic plants, such as *Triphysaria versicolor*

Fisch. & C.A.Mey. and *Phtheirospermum japonicum* (Thunb.) Kanitz of the family Orobanchaceae, can complete their life cycle in the absence of their host plants, but are able to parasitize under certain conditions (Kolka & Melnyk, 2018). Such parasites are classified as facultative parasites (Heide-Jørgensen, 2008; Spallek et al., 2013).

Parasitic plants are known to have an invasive organ called haustorium, penetrating the host plant tissue for water, nutrients, and sugar source (Rubiales & Heide-Jorgensen, 2011). Basically, all haustoria are just modified roots (Nickrent, 2002). Parasitic plants occur all over the world in all types of plant communities, except for the aquatic ecosystem. About 4,500 species of parasitic plants are known to science around the world (Rubiales & Heide-Jorgensen, 2011). Hence, this study aimed to increase our understanding of parasitic plants in the Philippines by collecting data on the distribution of these plants in Mindanao Island.

Marilog Forest Reserve in Davao City has a total land area of 63,000 ha and is dominated by the Manobo-Matigsalug tribal communities. This forest reserve hosts diverse unbotanized flora and in 2018, two plant species were reported and hold new records in the Philippines (see Amoroso et al., 2018a; Acma et al., 2019). Studies on tree species diversity and stand structure (Coritico et al., 2022) and diversity of ferns and lycophytes (Rufila et al., 2022) were also recently conducted in the forest patches of Marilog District. Further, this area is home to enigmatic and vivid-looking parasitic plants which were recently collected during repeated botanical fieldwork. Thus, this paper reports the parasitic plants in Marilog District, which bears significance as they act as keystone resources and structures in forest ecosystems (Watson, 2001).

MATERIALS AND METHODS

Entry protocol

Prior to conducting the study, necessary permits were obtained from the respective agencies, such as Barangay resolutions from Barangay Baganihan, Barangay Datu Salumay, and Marahan Proper; Prior Informed Consent (PIC); and Memorandum of Agreement (MOA) among Central Mindanao University, the Matigsalug-Manobo Tribal People Council of Elders Davao, Inc. (MAMATRIPCEDI), and Local Government Units (LGU's) of Marilog District, Davao. A Wildlife Gratuitous Permit (WGP) was obtained from the Department of Environment and Natural Resources (DENR) - Region XI and was used as the collecting permit. Collection of specimens was done between March and November 2019.

Site description

This study was conducted at Barangay Baganihan and Barangay Datu Salumay, which are situated in the northern part and Marahan Proper in the southern part of Marilog District, Davao City (Fig. 1). Sites 1–4 are tropical lower montane rainforests, while Site 5 is a mixed to agro forest ecosystem with elevations ranging from 1,000–1,345 m asl. Among the sites, site 2 had the highest elevation ranging from 1,197–1,345 m asl.

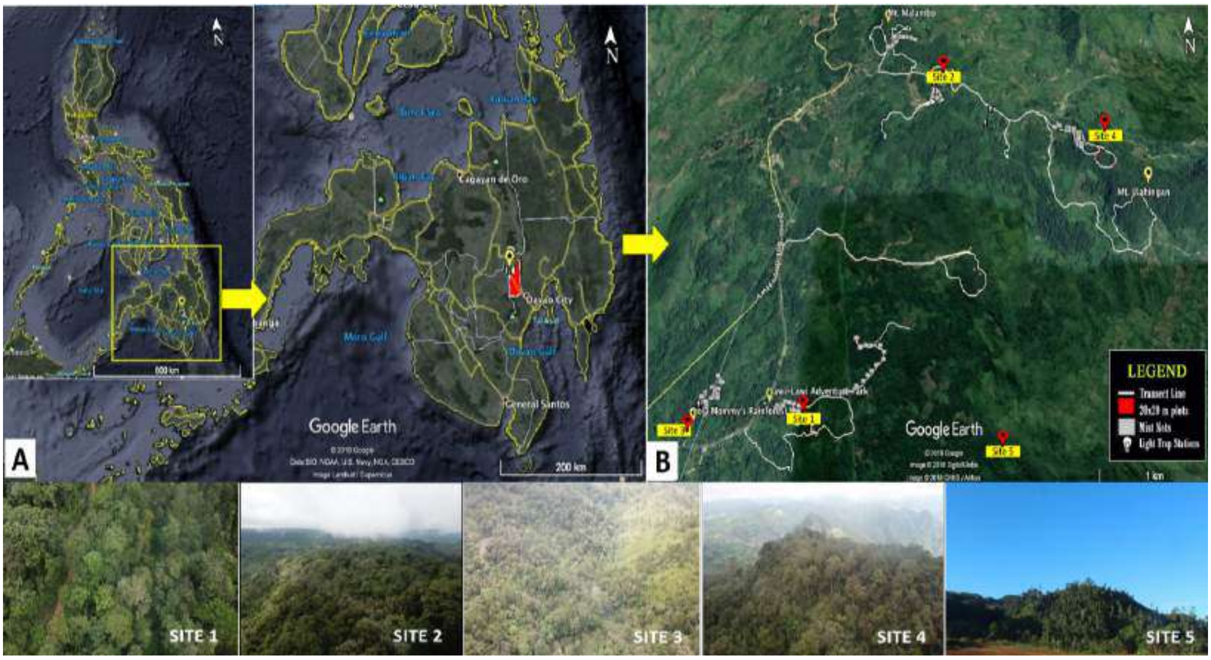

Figure 1: Study site. **A –** Map of the Mindanao Island (inset: Philippine map); **B –** Map of Marilog District showing the forest trails (white lines) and five sampling sites (yellow boxes) (©2018 Google, image ©2018 CNES/Airbus).

Botanical fieldwork

Field expeditions were carried out at Sitio New Calinan, Barangay Baganihan (Site 1), Mount Malambo, Barangay Datu Salumay (Site 2), Lola Mommy's Rainforest, Barangay Baganihan (Site 3), Mount Ulahingan, Barangay Datu Salumay (Site 4), and Sitio Matigsalug, Marahan Proper (Site 5) in Marilog District, Davao City, Philippines. Repeated transect walks and opportunistic samplings were carried to survey and collect parasitic plants in the area. A 2 km transect line was employed using a rope and was established in 100 m from the main trail. The 2 km transect line provides proper estimate of plant species present in a particular study area following the study of Amoroso et al. (2018b), Coritico et al. (2022), and Rufila et al. (2022). Parasitic plants encountered along the transect were recorded and documented. On the other hand, opportunistic sampling was done whenever parasitic plants were observed away from the established transect (Amoroso et al., 2018b).

Species were listed using a field notebook and pencil. Habitat of each species and detailed floral structures were photographed to clearly distinguish the diagnostic characteristics of each species. Furthermore, habitat characterization including the vegetation types and host plants were noted.

Identification of the specimens

The digital database from Co's Digital Flora of the Philippines (Pelser et al., 2011) and related published articles (Mendez et al., 2018; Amoroso et al., 2018a; Pelser et al., 2011) were used for the identification and classification of the collected species.

Assessment of conservation status and endemism

The conservation status of parasitic plants in Marilog District was assessed following the status listed by Fernando et al. (2022) and the IUCN (2023). The endemism of each species was listed following Pelser et al. (2011).

RESULTS AND DISCUSSION

Species composition and occurrence

A total of six species distributed in five genera and four families of parasitic plants were collected in Marilog Forest Reserve, Southern Philippines. Of these, three parasitic plants are categorized as hemiparasitic, namely, *Amyema curranii* (Merr.) Danser, *A. seriata* (Merr.) Barlow, and *Decaisnina ovatifolia* (Merr.) Barlow. Meanwhile, the other three species are categorized as holoparasitic and these are *Balanophora papuana* Schltr., *Mitrastemon yamamotoi* Makino and *Christisonia schortechinii* Prain (Table 1). The data gathered is much lower as compared to 4,500 known parasitic plants around the world (Rubiales & Heide-Jorgensen, 2011); in China with 678 species (Zhang et al., 2018). However, considering these species that occur in a small forest reserve, it is already noteworthy because the area houses diverse parasitic plants distributed in different families.

Table 1: Parasitic plants recorded in Marilog District, Davao City.

Mode of Parasitism	Family	Species
Hemiparasitic	Loranthaceae	*Amyema curranii* (Merr.) Danser
		A. seriata (Merr.) Barlow
		Decaisnina ovatifolia (Merr.) Barlow
Holoparasitic	Balanophoraceae	*Balanophora papuana* Schltr.
	Mitrastemonaceae	*Mitrastemon yamamotoi* Makino
	Orobanchaceae	*Christisonia schortechinii* Prain

The occurrence of parasitic plants differs in each site (Table 2). The most recorded species was *A. seriata* which is present in sites 1, 4, and 5, whereas the least noted species was *A. curranii* and *M. yamamotoi* (collected only in Site 2 – Mount Malambo) and *C. scortechinii* (collected only in Site 5 – Sitio Matigsalug). The diverse parasitic plants in the area could be due to the fact that there were nine species of host plants recorded, in which these species were found to occur in the sampling sites. Furthermore, according to Nickrent (2002), some factors, such as ratio of parasites to hosts, number of individual parasites in a host, and length of time required for the life cycle of parasitic plants could affect the occurrence and number of species.

Table 2: Species occurrence of parasitic plants in five forest patches of Marilog District, Davao City.

Species	Site				
	1	2	3	4	5
Amyema curranii (Merr.) Danser	X	/	X	X	X
Amyema seriata (Merr.) Barlow	/	X	X	/	/
Balanophora papuana Schltr.	X	/	X	/	X
Christisonia schortechinii Prain	X	X	X	X	/
Decaisnina ovatifolia (Merr.) Barlow	X	/	X	/	X
Mitrastemon yamamotoi Makino	X	/	X	X	X

Remarks: / means present; X means absent.

Hemiparasitic plants

(i) Amyema curranii (Fig. 2)

This species has a creeping stem with multiple haustoria. Leaves oblanceolate to lanceolate, umbel inflorescence with 8–15 pink flowers having a yellowish corolla apex. Fruits green when young and red when ripe. *A. curranii* parasitizes on *Melastoma malabatrichum* L. and *Lithocarpus* spp. According to Pelser et al. (2011), this species occurs in Luzon in Benguet province and is endemic to the Philippines. Recently, Tubongbanua et al. (2023) documented the presence of this species in Marilog District, Davao City.

Figure 2: *Amyema curranii.* **A –** Habit; **B –** Inflorescence; **C –** Close up view of the inflorescence.

(ii) Amyema seriata (Fig. 3)

This species has a solitary haustorial attachment to its host. Leaves obovate to oblanceolate, umbel inflorescence with 5–10 red flowers with dark red corolla apex. *Amyema seriata* parasitizes on *M. malabatrichum* and *Ficus* spp. This species was recorded only in Mindanao in Zamboanga del Norte and is endemic to the Philippines (Pesler et al. 2011). This species was also recorded by Tubongbanua et al. (2023) in Marilog District, Davao City.

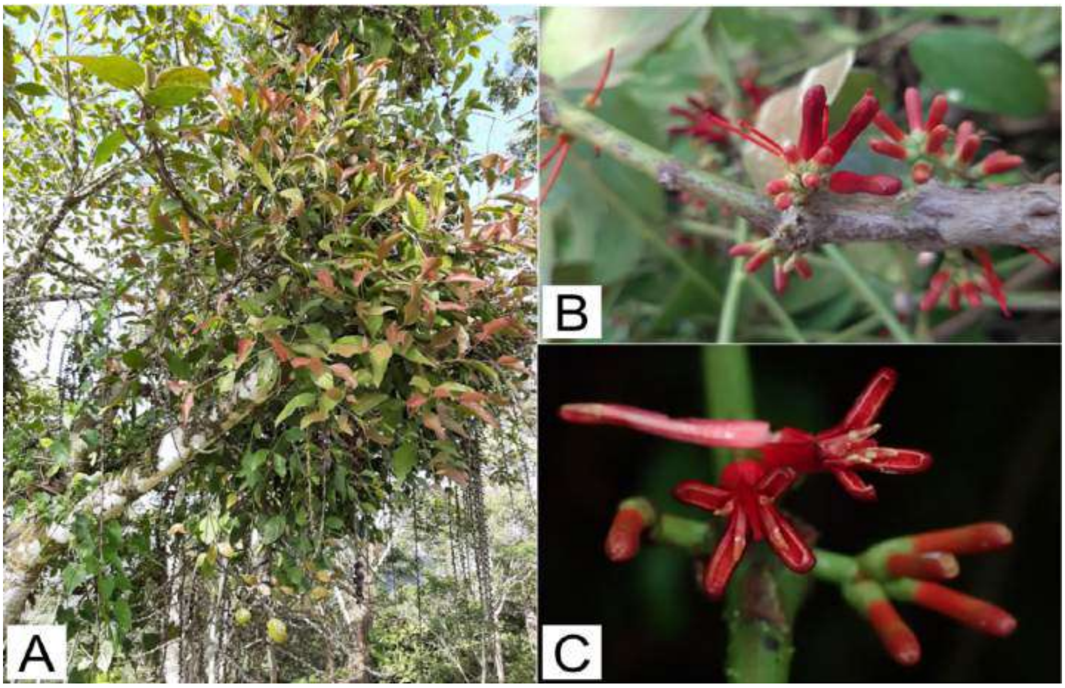

Figure 3: *Amyema seriata*. **A –** Habit; **B –** Inflorescence; **C –** Close up view of the inflorescence.

(iii) Decaisnina ovatifolia (Fig. 4)

Decaisnina ovatifolia has a solitary haustorium with erect stems attached. Leaves lanceolate. Inflorescence umbel with 3–4 red flowers. This species parasitizes on *Macaranga sinensis* Baill. Muell. Arg. and *Lithocarpus caudatifolius* (Merr.) Rehder. The species was also recorded in Bohol and Lanao del Norte, which is endemic to the Philippines (Pelser et al., 2011).

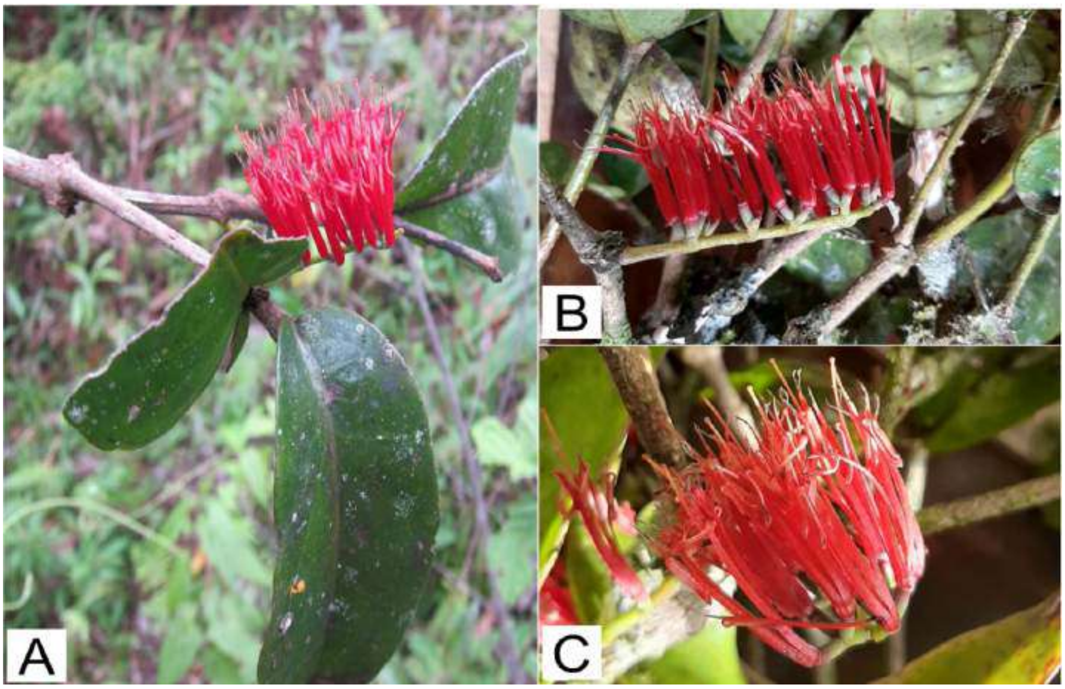

Figure 4: *Decaisnina ovatifolia*. **A –** Habit; **B –** Inflorescence; **C –** Close up view of the inflorescence.

Holoparasitic plants

(iv) Balanophora papuana (Fig. 5)

Balanophora papuana was first described by Schlechter in year 1913 based on two specimens of New Guinea, Schlechter 18250 from Finisterre Gebirges 1300 m asl, collected in September 1908 which was a male plant and Schlechter 18602 from Bismark, 1400 m asl, collected in November 1908 which was a female plant (Schlechter, 1913; Damayanto & Raistiwi, 2019). In the Philippines, *B. papuana* was reported parasitizing on roots of *Ficus* spp. (Amoroso & Semitara, 1999). This species is a dioecious plant with red-orange reproductive parts. In Marilog Dirstrict, this species was also recorded parasitizing on the roots of *Ficus* sp. and *L. caudatifolius*.

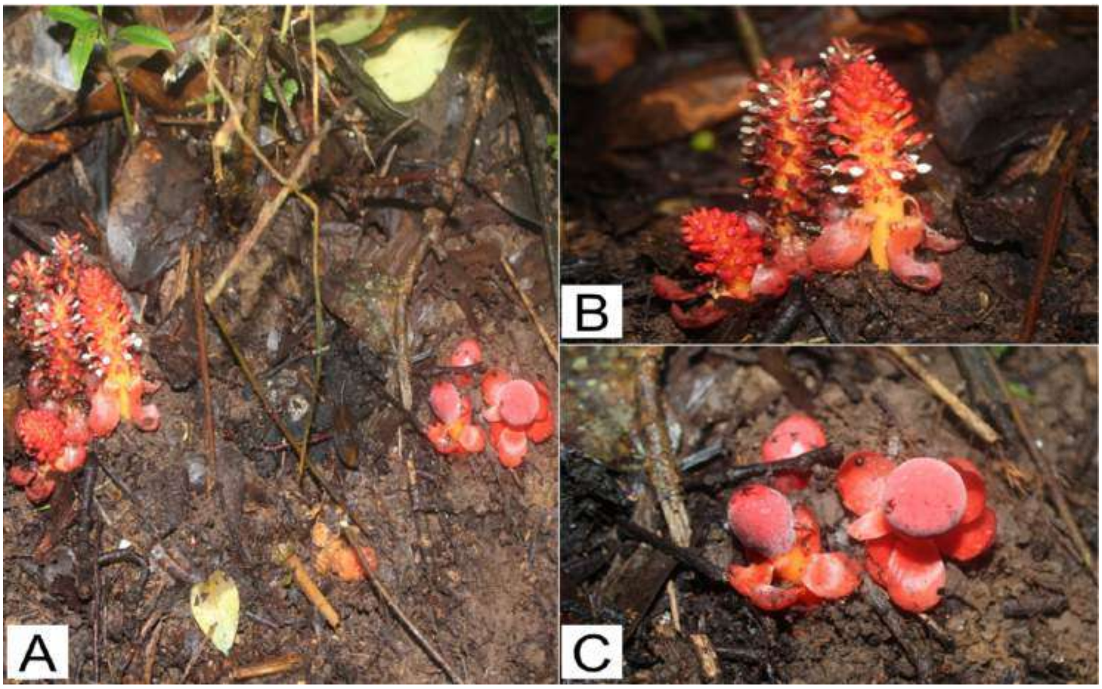

Figure 5: *Balanophora papuana*. **A –** Habit; **B –** Male inflorescence; **C –** Female inflorescence.

(v) Christisonia schortechinii (Fig. 6)

Christisonia scortechinii was recorded parasitizing on the roots of bamboo species and is almost covered by leaf litter at 1,421 m asl. Only two populations of this species were recorded in the mountain site which was covered by *Lithocarpus* leaf litter. The individuals of the first population were also near the inflorescence of the ginger species – *Etlingera philippinensis* (Ridl.) R.M.Sm. (Zingiberaceae) in which the inflorescence of *E. philippinensis* are borne adjacent to its main rhizome at ca. 1 m (Fig. 6A). Meanwhile, the second population was found at *ca.* 200 m away from the first population (Fig. 6B & C). The bamboo species serving as the host for this species was identical to the one previously reported by Mendez et al. (2018) in Cinchona Forest Reserve, Bukidnon, Philippines.

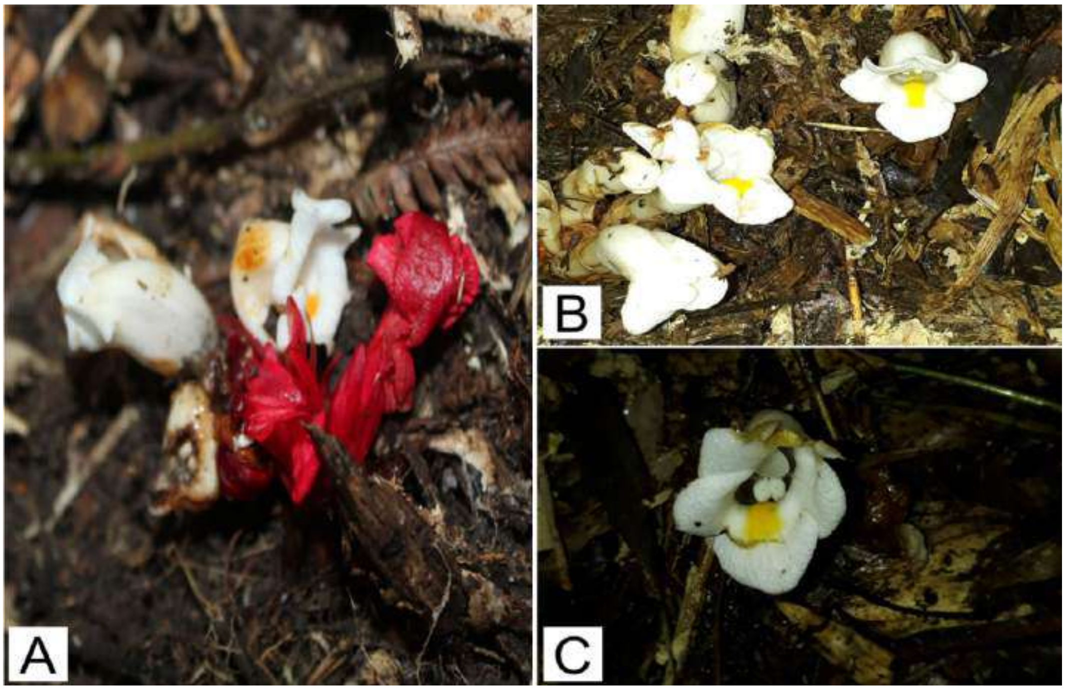

Figure 6: *Christisonia schortechinii.* **A –** Habit of *C. scortechinii* (white flowers) with inflorescence of *Etlingera philippinesis* (red flowers); **B –** Anterior view of the flower; **C –** Inflorescence.

(vi) Mitrastemon yamamotoi (Fig. 7)

Mitrastemon yamamotoi grows mainly on the roots of *Lithocarpus* spp., *Ficus* spp., *M. sinensis*, *Palaquium philippense* (Perr.) C.B.Rob., and *Tetrastigma* sp. as reported by Amoroso et al. (2018a). This species was found along the trail near the summit of Mount Malambo in Barangay Datu Salumay. It is quite unique that a dense population of this species was found parasitizing on these host plants under the shades of their canopy.

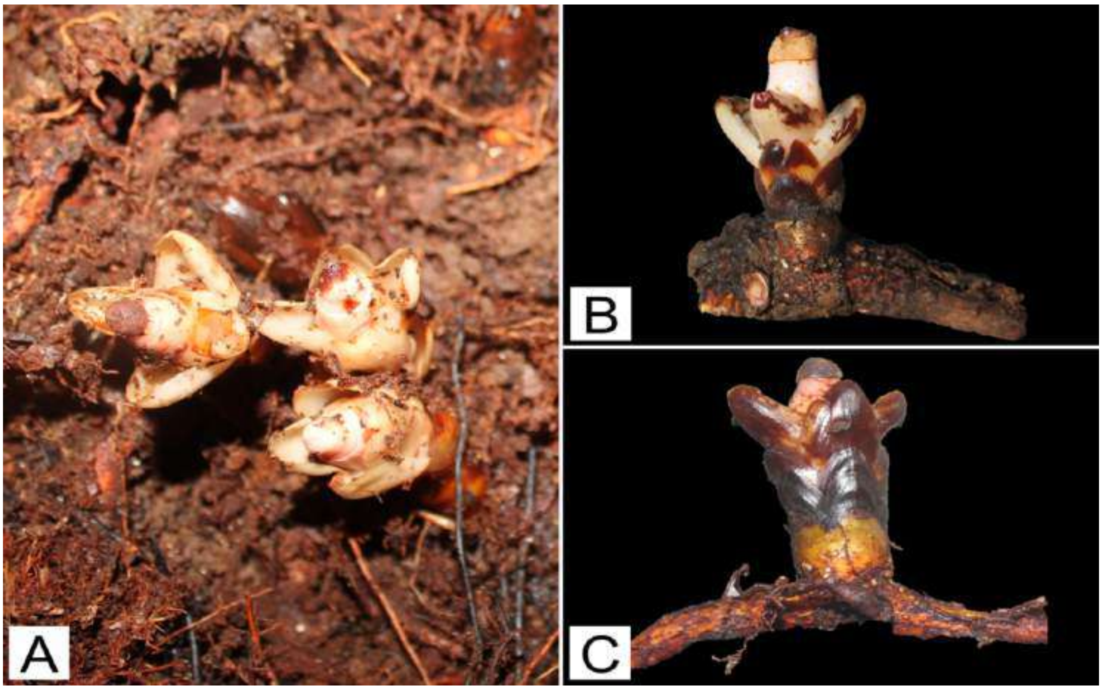

Figure 7: *Mitrastemon yamamotoi*. **A –** Habit; **B–C** Flower (lateral view).

Assessment of conservation status and endemism

Out of the six species collected, none of them were identified as threatened parasitic plants, and these species are not included in the IUCN (2023) list. However, three of these, *A. curanii, A. seriata,* and *D. ovatifolia* which are all from the Loranthaceae family, are endemic to the Philippines. Meanwhile, the other three species are native to the Philippines, namely, *B. papuana*, *C. scortechinii*, and *M. yamamotoi* (Table 3).

Table 3: Assessment of Conservation Status and Endemism Parasitic Plants in Marilog Forest Reserve, Southern Philippines.

No.	Species	Status
1	*Amyema curranii* (Merr.) Danser	Endemic to the Philippines
2	*Amyema seriata* (Merr.) Barlow	Endemic to the Philippines
3	*Balanophora papuana* Schltr.	Native to the Philippines
4	*Christisonia schortechinii* Prain	Native to the Philippines
5	*Decaisnina ovatifolia* (Merr.) Barlow	Endemic to the Philippines
6	*Mitrastemon yamamotoi* Makino	Native to the Philippines

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study provides valuable insights into the world of parasitic plants within Marilog Forest Reserve, Southern Philippines. The documentation of six species, representing five genera from five different families, is a noteworthy contribution, particularly within a specific geographic region of the country. Given the limited attention parasitic plants have received in the Philippines, this paper serves to enhance our understanding of these understudied species. It is highly recommended that future field expeditions are conducted within these sites and their neighbouring areas. These expeditions should focus on comprehensive data collection, including phenology and host plant associations, as well as the creation of voucher specimens for further research and conservation efforts. This work is a significant step towards expanding our knowledge of parasitic plants in the Philippines.

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Research Article

Species Composition and Assessment of Sphingid Moths (Heterocera, Sphingidae) in Mount Malimumu, Pantaron Mountain Range, Mindanao, Philippines

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ABSTRACT

High diversity of sphingid moth species can be found in tropical rainforests. Mount Malimumu of Pantaron Mountain Range, Mindanao, Philippines, has a vast rainforest, which is a Key Biodiversity Area. However, no records of sphingid moths have been known to occur in Mount Malimumu of the Pantaron Mountain Range. Thus, this study aimed to determine species composition and assess the local status of sphingid moths of Mount Malimumu. A 3 × 4 m white silk cloth and 250 V light bulbs were used for light trap sampling. Specimens collected were brought to the University Museum of Central Mindanao University. A total of 20 species distributed to 10 genera in 4 tribes was recorded. At least 8 species are endemic to the Philippines, i.e., *Ambulyx bakeri, Ambulyx. staudengeri, Ambulyx johnsoni, Amplypterus panopus mindanaoensis, Meganoton rufescens philippinensis, Acosmeryx socrates, Cechenena transpacifica* and *Theretra manilae.* Uncontrolled anthropogenic activities around the Mount Malimumu of Pantaron Mountain Range such as slash and burn, conversion of forest land to farmlands and overcollection of host plants may threaten these species.

Keywords: Pantaron Mountain Range; Key Biodiversity Area; ecological status; Philippine endemic.

INTRODUCTION

Sphingid moths commonly known as hawkmoths or sphinx moths, belong to the family Sphingidae and have a medium to large body size (Mohagan et al., 2019). This lepidopteran group is widely distributed and inventoried in every continent (Kitching & Cadiou, 2000; Yen et al., 2003). Global distribution of sphingid moths have been known from South Asia to the Sundaic region. This family has been documented from Cambodia, Java, Laos, Nepal, Northeast India, Peninsular Malaysia, the Ryukyu Archipelago, Southern China, Sumatra, Taiwan, Thailand, the Andaman Islands, Vietnam, Borneo and the Philippines (Pittaway & Kitching, 2008; Leong & Rozario, 2009). About 1,350 species of sphingid moths have been recorded around the world (except Antarctica) and their peak diversities are located mostly in tropical rainforests (Kitching & Cadiou, 2000; Karger et al., 2013). Furthermore, about 122 (9%) sphingid moth species have been documented in the Philippines (Hogenes & Treadaway, 1998).

Mount Malimumu is one the highest peaks of Pantaron Range located at Barangay Magkalungay, Municipality of San Fernando, Bukidnon, Mindanao, Philippines. The Pantaron Range is a major fragment of the central Cordillera of Mindanao Island (Gronemeyer et al., 2014). Research on biodiversity has been launched, which was spearheaded by the Center for Biodiversity Research and Extension in Mindanao (CEBREM) of Central Mindanao University, to include Pantaron Range as one of the NIPAS or National Integrated Protected Area System Act.

There are few studies of sphingid moths in terms of taxonomy and conservation, locally and internationally. Some researchers mostly focus their attention on the bio-pesticide and pest control of these highly destructive insects (Tigvattananont & Bumroongsook, 2016). While the overall diversity of sphingid moths in the Philippines is known (Hogenes & Treadaway, 1998), it is important to study the biodiversity at more local levels because of varying anthropogenic threats. A number of studies have investigated the diversity of sphingids elsewhere on Mindanao Island (Nuneza et al., 2016; Mohagan et al., 2019; Suelo et al., 2020 & 2023), but the sphingids of Mount Malimumu, Pantaron Range of the central highlands remain unreported. Thus, this study aims to determine the species composition and local status of sphingid moths of Mount Malimumu.

MATERIALS AND METHODS

Entry protocol

Necessary permits were obtained from the different community/agencies from the local people and Gratuitous Permit from the Department of Environment and Natural Resources.

Sampling stations, collection and preservation

The study was carried out in barangay Magkalungay, municipality of San Fernando, province of Bukidnon, on the island of Mindanao in southern Philippines (Fig. 1). The study was conducted from 21 to 28 October 2020. With the use of 3 × 4 m white silk cloth and 250 V light bulbs, light trap sampling was established from 6:00 pm to 2:00 am (Fig. 2). The sampling stations were done in a slope-to-ridge type location for the light to penetrate the deep parts of the forest. Vegetation type and habitat composition were documented during the sampling period.

Triangular-shaped glassine papers and mothballs were used to preserve the specimens. Specimens collected were brought to the University Museum of Central Mindanao University for permanent mounting.

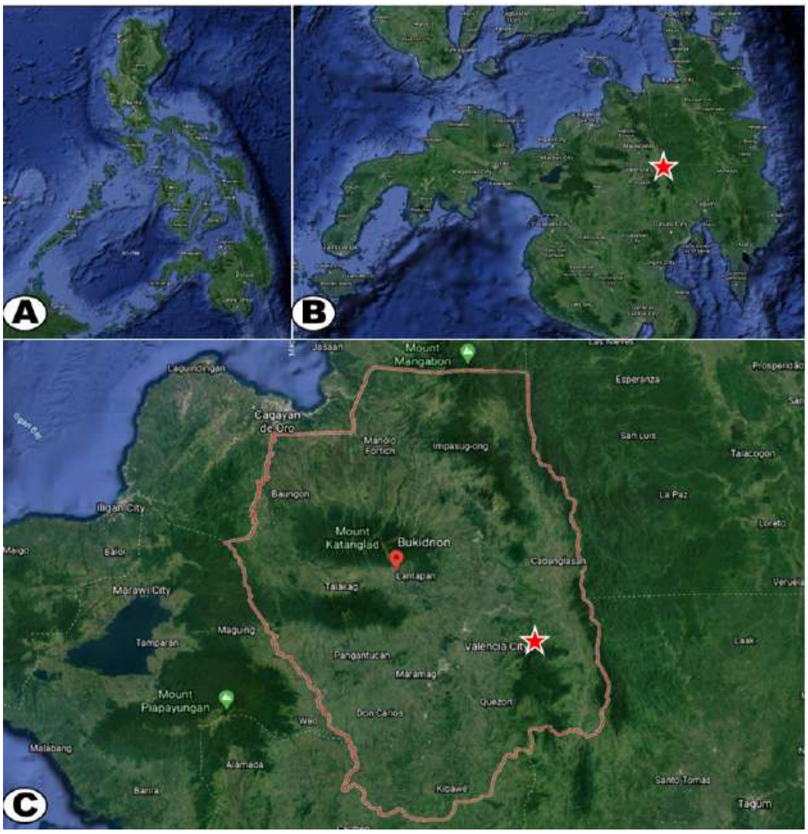

Figure 1: Study Site of Mount Malimumu, A) Map of the Philippines, B) Map of Mindanao, C) Map of Bukidnon showing the study station (red stars).

Figure 2: Light trap sampling, A) Researchers installing the light trap, B) Installed light trap, C) Close-up view of the light trap.

Taxonomic and conservation status

Identification, classification and status of the specimens were carried out using the checklist of Hogenes and Treadaway (1998) at the Central Mindanao University Museum's Zoology Section. Other published taxonomic papers such as Haber and Frankie (1987), Ghorpadé et al. (2013), Karger et al. (2013), Rafi et al. (2014), Nuneza et al. (2016), Mohagan et al. (2018; 2019) and Suelo et al. (2020; 2023) were also used.

RESULTS AND DISCUSSION

Species composition

Mount Malimumu of Pantaron Mountain Range has a total of 20 species distributed to 10 genera in 4 recorded tribes (Table 1). These species include *Acosmeryx socrates* Boisduval (1875)*, Acosmeryx anceus subdentata* (Rothschild & Jordan 1903)*, Ambulyx bakeri* (Clark 1929)*, Ambulyx tattinum uichancoi* (Clark 1938)*, Ambulyx staudengeri* (Rothschild 1894)*, Ambulyx johnsoni* (Clark 1917)*, Acherontia lachensis* (Fabricius 1798), *Agrius convolvuli* (Linnaeus 1758)*, Amplypterus panopus mindanaoensis* (Inoue 1996)*, Amplypterus panopus panopus* (Cramer 1779)*, Cechenena helops helops* (Walker 1856)*, Cechenena transpacifica* (Clark 1923)*, Marumba amboenicus luzoni* (Clark 1935)*, Megacorma obliqua obliqua* (Walker 1856)*, Meganoton rufescens philippinensis* (Clark 1938)*, Pergesa actea* (Cramer 1779)*, Psilogramma menephron menephron* (Cramer 1780)*, Theretra nesssus* (Drury 1773)*, Theretra manilae* (Clark 1922) and *Theretra rhesus* (Boisduval 1875).

Table 1: Species composition of Sphingidae moths in Mount Malimumu, Pantaron Mountain Range, Mindanao, Philippines.

Subfamily	Tribe	Genus	Species	No. of individuals recorded
Sphinginae	Smerinthini	*Ambulyx*	*Ambulyx backeri* (Clark 1929)	6
			Ambulyx johnsoni (Clark 1917)	2
			Ambulyx staudengeri Rothschild 1894	3
			Ambulyx tattina uiochancoi (Clark 1938)	1
		Amplypterus	*Amplypterus panopus mindanensis* Inoue 1996	6
			Amplypterus panopus panopus (Cramer 1779)	5
		Marumba	*Marumba amboinicus luzonica* Clark 1935	1
	Sphingini	*Acherontia*	*Acherontia lachesis* (Fabricius 1798)	2
		Agrius	*Agrius convulvoli* (Linnaeus 1758)	1
		Meganoton	*Meganoton rufescens philippinensis* Clark 1938	1
		Psilogramma	*Psilogramma menephron menephron* (Cramer 1780)	3
		Megacorma	*Megacorma obliqua obliqua* (Walker 1856)	1

Macroglossinae	Macroglossini	*Acosmeryx*	*Acosmeryx anceus subdentata* Rothschild & Jordan 1903	5
			Acosmeryx socrates (1875)	5
		Pergesa	*Pergesa actea* (Cramer 1779)	1
	Choerocampini	*Cechenena*	*Cechenena helops helops* (Walker 1856)	2
			Cechenena transpacifica (Clark 1923)	1
		Theretra	*Theretra manilae* Clark 1922	9
			Theretra nessus (Drury 1773)	1
			Theretra rhesus (Boisduval 1875)	4

There are a total of 122 annotated list of hawkmoth species in the Philippines and about 62 recorded species in Mindanao (Hogenes & Treadaway, 1998). In comparison, the species collected in Mount Malimumu is about 17.09% of the total recorded Philippine species and 32.25% of the overall species recorded in Mindanao. Compared to studies conducted elsewhere in the Philippines, the 20 species obtained in this study is close in number to the 22 species recorded by Mohagan et al. (2018) from Mount Hamiguitan and Busay Garden, Marilog District, Davao City, but more than that recorded by Nuneza et al. (2016) in Bega Watershed, Agusan del Sur (only 1 species), Mohagan et al. (2019) in Mount Hamiguitan (8 species), and Suelo et al. (2020; 2023) in Mount Kitanglad with 13 and 7 species recorded respectively.

Assessment

At least 8 species were Philippine endemic namely*, Ambulyx bakeri, Ambulyx staudengeri, Ambulyx johnsoni, Amplypterus panopus mindanaoensis, Meganoton rufescens philippinensis, Acosmeryx socrates, Cechenena transpacifica* and *Theretra manilae.* Forty percent of the species in the area are endemic to the Philippines.

The endemism of hawkmoths in Mount Malimumu is much higher compared to the data gathered by Mohagan et al. (2019) in the two proposed expansion sites of Mount Hamiguitan with only 3 endemic species recorded in the area.

Mount Malimumu has a secondary forest vegetation type, supporting the study of Shuize & Fielder (2003) that there was no direct significance between old-growth or primary forest and secondary forest in diversity and hawkmoths. The sampling season at Mount Malimumu was rainy and wet with a temperature of about 17°–22°C, which may be a result of its elevation of about 1,223 m asl because it was a lot cooler compared to the average temperature in San Fernando, Bukidnon, which are in the lowlands (385 m asl) and record temperature of 19°–29°C (Department of Science and Technology, 2024 onwards), a direct correlation to its elevation; the higher the elevation, the cooler the area. Mohagan et al. (2019) also stated that temperature and weather cannot affect the occurrence of hawkmoths during light trapping because they are furfuraceous and crepuscular species, meaning they can withstand heavy rainfall.

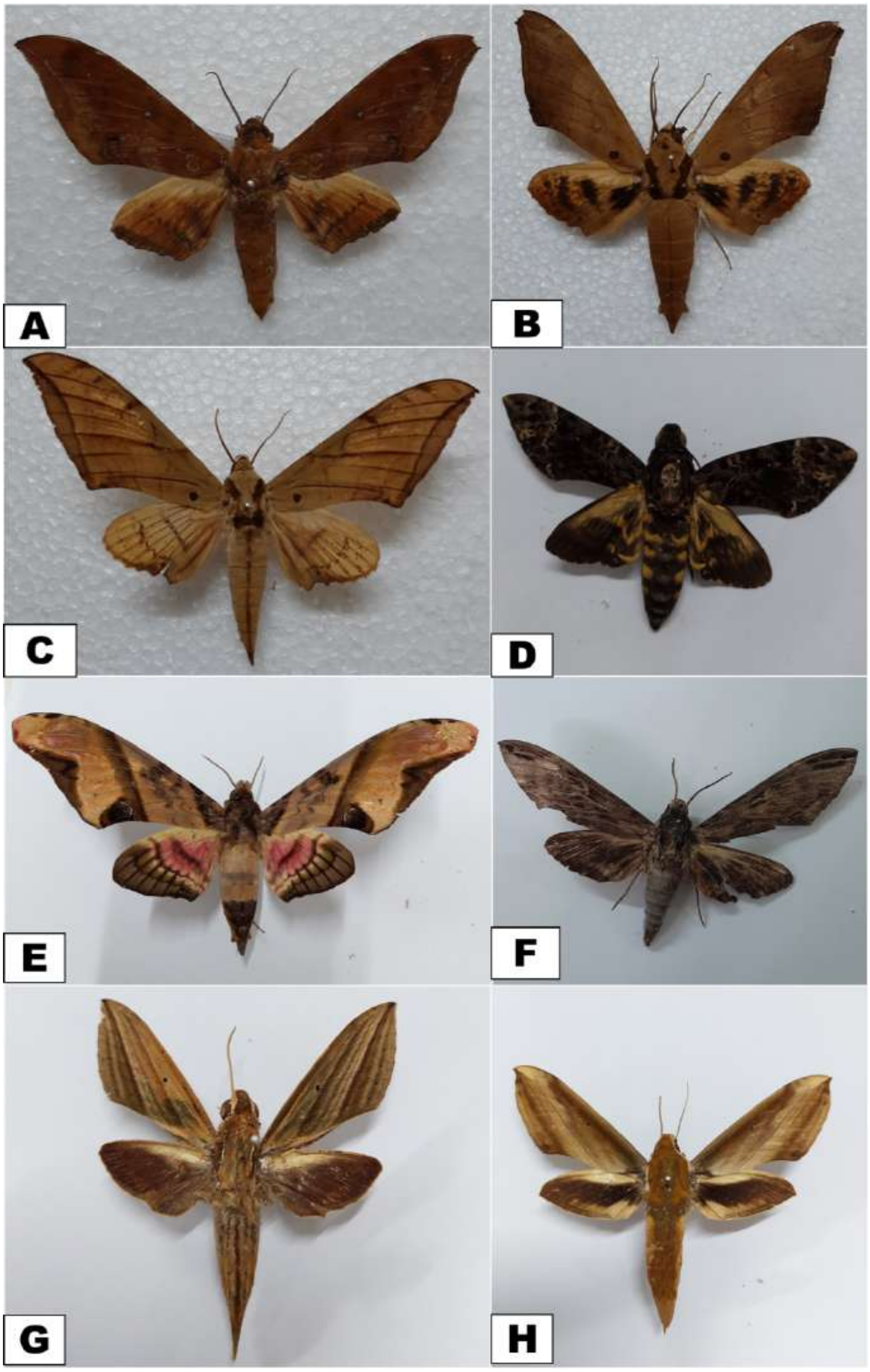

Figure 3: Some endemic species of sphingid moths in Mount Malimumu, Pantaron Mountain Range, **A)** *Ambulyx backer*, **B)** *Ambulyx staudengeri,* **C)** *Ambulyx johnsonii,* **D)** *Acherontia lachensis,* **E)** *Amplypterus panopus minandensis,* **F)** *Meganoton rufescens philippinensis,* **G)**, *Theretra manilae* and **H)** *Theretra nessus*

CONCLUSIONS

Mount Malimumu of Pantaron Mountain Range is home to 20 species of hawkmoths, with 8 Philippine endemic species. Hawkmoth species constitute 40 per cent of the species in the area. The ongoing widespread anthropogenic activities around the Mount Malimumu of Pantaron Mountain Range such as slash and burn method, conversion of forest lands to farmlands and over collection of host plants may threaten these species. Thus, it is recommended that the protection of the forest reserve and surrounding areas be strictly imposed for the protection and conservation of these enigmatic species and their habitat.

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Research Article

Species Evenness and Diversity of Soil Invertebrates at Different Agricultural Lands and a Forest Reserve at Kota Belud, Sabah

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ABSTRACT

Increasing agricultural activities can lead to changes in soil ecosystems, potentially impacting soil invertebrate communities as they are highly responsive to soil disturbances. The composition of soil invertebrates within each agricultural habitat provides insights into how these communities respond to their environmental conditions. This study aimed to investigate the evenness and diversity of soil invertebrates in agricultural areas of Kota Belud, Sabah. Soil samples were collected from three different agricultural sites, a rubber plantation, an oil palm plantation, and a paddy field, as well as a forest reserve as a control site. Pitfall traps and Berlese-Tullgren funnels were used to collect soil invertebrates. A total of 180 soil samples and 474 individuals of soil invertebrates were collected and analyzed. Physicochemical analyses, including moisture content, soil texture, pH, soil nutrient content, and organic matter, were conducted on all soil samples to assess their influence on the composition of soil invertebrates. Results indicated that Julida, Coleoptera, Gastropoda and Araneae were frequently associated with high organic matter, pH, moisture content and phosphorus. On the other hand, Haplotaxida appeared to be more sensitive to potassium levels. Notably, the Blattodea, Orthoptera, Hymenoptera, Isopoda, Polydesmida and Diptera, were found to be abundant in areas with lower organic matter and pH in the control site, i.e., forest reserve area. These findings underscore the significance of soil layer activities in influencing the presence and survival of soil invertebrates. Given their vital role in sustaining life, prioritizing the enhancement of soil invertebrate populations is crucial, particularly in Sabah, a prominent food crop-producing state in Malaysia.

Keywords: Soil invertebrates; agriculture physicochemical; Sabah; evenness; diversity.

INTRODUCTION

Soil invertebrates are the communities that are responsible for a variety of functions in soil ecosystems both above and below ground; earthworms, termites, and ants are examples of “ecosystem engineers” that change the soil’s physical composition and movement of nutrients, meanwhile the microarthropods, known as “litter transformers”, break up decaying litter to increase the number of bacteria that can access it (Coleman et al., 1999). Despite the growing spotlight on these soil fauna, other factors, such as the acceleration of agricultural productivity, are given far greater consideration (Maqtan et al., 2021). The abundance of soil fauna gives positive results on the soil structure and fertility, e.g., by improving aeration, drainage, biological pest management, soil mixing, specific cropping, transportation of microorganisms and enhancement of food and space conditions for microorganisms and higher plants (Hill, 1985).

To assess soil health, it is crucial to take into consideration both biological and physical-chemical soil characteristics and to see the influence of these indicators on the abundance and diversity of the soil fauna groups (Cardoso et al., 2013).

Therefore, the focus of the current research was to study the diversity of soil invertebrates by showing the relationship of their diversity with the physicochemical properties of the agricultural soil located in Kota Belud, Sabah. By taking a forest reserve as the control site, we can compare the difference in the effect of the environmental variables (soil organic matter, soil moisture content, pH, soil nutrients, soil texture) on the diversity of soil invertebrates among different agricultural activities.

MATERIAL AND METHODS

Study location

Extensive soil sampling was carried out on a wide range of agricultural lands and a forest reserve at Kota Belud, Sabah. Three study areas located at Kampung Sarang (6°34'14.9"N 116°33'03.6"E), Kampung Timbang (6° 29' 31.9986"N, 116.5495937E) and Kampung Dudar (6° 35' 22.3074"N 116° 33' 22.824"E) represent agricultural areas and each agricultural area has a different type of plantation, such as rubber, paddy, and oil palm respectively (Figure 1). Meanwhile, the forest reserve is located at Kampung Ulu Kukut and is gazetted under the Forests (Amendment) Enactment 2014, as a Class 1 forest reserve. We observed that, in terms of their soil type, the forest reserve soil predominantly, consisted of a mixture of sandy clay loam meanwhile rubber plantation was in sandy loam soil, oil palm plantation was clay loam soil and paddy was cultivated in sandy clay loam soil.

Figure 1: Locations of soil sampling at different plantation types and a forest reserve in Kota Belud, Sabah. Base map modified from Google Maps.

Sampling method
Two sampling methods were applied to collect samples of the soil invertebrates, namely pitfall trap for mobile invertebrates and modified Berlese Tullgren funnels for soil micro invertebrates where soil macroinvertebrates were heat-extracted from the soil samples (Tuf, 2015). In each sampling plot, a quadrant of 30m × 30m was set up and applied in this study. Each quadrant was divided into 5 line transects and each line transect consisted of 3 pitfall traps with measurements of 10 cm depth and 10 cm diameter. The distance for each trap was 10 meters (Figure 2). A total of 15 pitfall traps were installed at each plot using a soil auger. Extraction of invertebrates inside the 10 cm soil cores was directly made using the hand-collection method. Extracted invertebrates were kept inside a specimen bottle with 70% alcohol solution for preservation. After the hand-collecting method was performed, the soil samples were then put into Berlese Tullgren funnels for 3 days to extract the remaining macroinvertebrates. The same 10 cm soil core sample that was taken from each plot was transferred to the laboratory for soil physicochemical analysis.

Three replicates of soil sampling were performed at each site, as per the methodology outlined by Arshad and Martin (2002). Specifically, from each study site, 15 pitfall traps were excavated, and only 9 soil cores were collected from these traps making a total of 27 soil samples (N=27) for each study site. This procedure yielded a sufficient total of 108 soil samples, which were subsequently transferred to the laboratory for further physicochemical analysis.

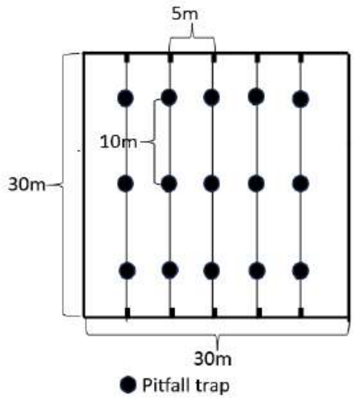

Figure 2: 30m × 30m quadrant used in this study.

Soil invertebrate identification
Soil invertebrates were identified in the laboratory through meticulous examination employing a laboratory stereo microscope, following an established identification key (Saxena & Rao, 2015). Each soil invertebrate specimen was systematically identified to the Order level. Subsequently, the abundance of each Order was recorded by aggregating the total number of individuals within each Order.

Soil physicochemical parameters
Soil moisture content
In this study, 10g of air-dried soil samples were taken and sieved before being placed in an oven at 105°C for 24 hours. Soil samples were cooled in a desiccator before being weighed. The percentage of moisture content (MC) in the soil was recorded using the formula below (Shukla et al., 2014):

$$\text{MC \%} = \frac{(\text{Weight of air – dried soil + porcelain basin}) - (\text{Weight of oven-dried soil + porcelain basin})}{(\text{Weight of oven – dried soil + porcelain basin}) - \text{Weight of porcelain basin}} \times 100$$

Soil organic matter

In the analysis of organic matter in soil samples, the Loss of Ignition (LOI) method was conducted (Heire et al., 2001). Soil samples from the moisture content determination above were then put in the furnace at 500°C for 24 hours. After drying overnight, the samples were cooled in the desiccator before the weighing process. The percentage of organic matter in the soil was calculated by using the formula as stated according to Heire et al. (2001):

$$\text{Organic matter \%} = \frac{(\text{Weight of oven – dried soil + porcelain basin}) - (\text{Weight of mass of ignited soil + porcelain dish})}{(\text{Weight of oven – dried soil + porcelain basin}) - \text{weight of porcelain dish}} \times 100$$

Soil pH

In this study, the pH of soil samples was taken by first weighing 10g of air-dried soil, putting it into a beaker, then adding 25 ml of distilled water to the soil sample. The mixture was then stirred and left for 30 minutes. After 30 minutes, a pH meter was used to measure the pH. pH was measured 3 times for each sample, with the electrode washed with distilled water between each reading.

Percentage composition

Calculations were made to determine the percentage composition as well as the relative abundance of the observed individuals at each study site. Relative abundance is the percentage composition of each order recorded at each study site relative to the number of organisms in the area (Hamzah et al., 2020). Percentage composition was calculated by dividing the number of individuals for each order by the total number of abundance from each site (Bufebo et al., 2021).

$$\text{Percentage Composition} = \frac{\text{Number of individuals in each order}}{\text{Number of abundances of all orders from each site}} \times 100$$

Biological indices

The diversity of soil invertebrates was calculated using PAST 4.03 software (Hammer et al., 2001). Four diversity indices were computed namely Shannon-Wiener, Evenness, Simpson and Menhinick's indices to compare the diversity of soil invertebrates in the forest reserve and the three different agricultural areas.

Shannon-Wiener Index (Shannon and Wiener, 1949)

Species diversity was calculated using a formula by Shannon and Wiener (1949) as below:

$$H' = - \Sigma \, Pi(\ln Pi)$$

Where;
H' = Shannon-Wiener Diversity Index
Pi = Proportion of grand total of abundance recorded by each taxon

Evenness

$$E = H/Hmax\ ,\ Hmax = \ln(N)$$

Where;
E= evenness
H= Shannon-Wiener Diversity Index
Hmax = Natural Logarithm of the number of specific categories (Number of samples in the study area)

Species Richness

In this study, species richness was calculated based on the taxa collected from each study area. Species richness is simply defined as the total number of species in an assemblage of a sample (Gotelli & Chao, 2013). Other than that, species richness was measured by computing Menhinick's index (Shah & Pandit, 2013) based on the data collected from all study sites.

Statistical analysis

The differences in the relative abundance of recorded soil invertebrates from each study site were statistically analysed using IBM SPSS Statistics software version 28. A normality test was conducted on the data and the value for Shapiro-Wilk showed that the data collected from each study site was normal ($P > 0.05$). Thus, a comparison of the differences in the relative abundance for each taxon between different study sites was calculated using one-way analyses of variance (ANOVA) followed by Tukey's significant difference (HSD) posthoc tests. Finally, a canonical-correlation analysis (CCA) was conducted by using PAST software (4.03) to see the relationship between the soil physicochemical parameters and the abundance of soil invertebrates at each study site.

RESULTS AND DISCUSSION

Relative abundance and percentage composition of soil invertebrates

A total of 496 individuals were collected composed of 17 Orders throughout the sampling period (Table 1). The highest abundance of soil invertebrates was recorded from the forest reserve (234 individuals, 16 Orders), followed by rubber plantation (110 individuals, 12 Orders), oil palm plantation (86 individuals, 6 Orders) and paddy field (65 individuals, 8 Orders) (Table 2). Altogether, the forest reserve was the most order-rich site, while the least number of Orders were found at oil palm plantations (44 Hymenoptera, 17 Haplotaxida, 5 Coleoptera, 12 Blattodea, 2 Orthoptera). Haplotaxida, Coleoptera and Araneae were found in both agricultural sites and the forest reserve. Hymenoptera recorded the highest abundance in the forest reserve with 96 individuals (40.85%) and 44 individuals (51.16%) in the oil palm plantation. Followed by Haplotaxida which showed the highest abundance in the rubber plantation with 66 individuals (60%), and Coleoptera showing the highest abundance in the paddy field. The relative abundance of Hymenoptera, Haplotaxida and Isopoda lead to the highest number of abundances among the study sites showing their superiority over other organisms (Table 1). However, there was no significant difference in the relative abundances among the study sites ($P > 0.05$).

Our findings indicate that the oil palm plantation typically lacks species that are more common in the forest reserve (Table 1). In the oil palm plantation as opposed to the forest reserve, large-bodied species and those from higher trophic levels were significantly rarer, similar to finding of Senior et al. (2013). In addition, less disturbance (Class 1) and better soil cover are the fundamental characteristics of a forest reserve, which improve the circumstances for the existence of soil organisms.

Table 1: Abundance and percentage composition of soil invertebrates collected from different study sites in Kota Belud, Sabah.

Taxon	Forest Reserve	%	Rubber Plantation	%	Oil Palm Plantation	%	Paddy Field	%	Total
Hymenoptera	96	40.85	11	10.00	44	51.16	0	0.00	
Haplotaxida	32	13.62	66	60.00	17	19.77	13	20.00	
Isopoda	28	0.12	9	8.18	0	0.00	5	7.69	
Coleoptera	21	8.94	4	3.64	6	6.98	32	49.23	
Araneae	15	6.38	7	6.36	5	5.81	8	12.31	
Blattodea	11	4.68	1	0.91	12	13.95	0	0.00	
Othoptera	7	2.98	1	0.91	2	2.33	0	0.00	
Gastropoda	4	1.70	2	1.82	0	0.00	2	3.08	
Julida	2	0.85	1	0.91	0	0.00	1	1.54	
Geophilomorpha	2	0.85	1	0.91	0	0.00	3	4.62	
Polydesmida	2	0.85	0	0.00	0	0.00	0	0.00	
Protura	2	0.85	0	0.00	0	0.00	0	0.00	
Diptera	6	2.55	0	0.00	0	0.00	0	0.00	
Glomerida	1	0.43	0	0.00	0	0.00	0	0.00	
Hemiptera	5	2.13	6	5.45	0	0.00	1	1.54	
Archaeognatha	1	0.43	0	0.00	0	0.00	0	0.00	
Collembola	0	0.00	1	0.91	0	0.00	0	0.00	
Total nr. Ab.	235		110		86		65		496

Diversity of soil invertebrates

The community of soil invertebrates in agricultural areas was less diverse and showed lower species richness (Order level) than the forest reserve (Table2.) Shannon-wiener diversity differs among sites (Table 2) where the highest diversity was recorded in the forest reserve (H'= 1.984). The values of Simpson, Evenness and Menhinick indices were again highest in the forest reserve (0.7843, 0.4546 and 1.042 respectively) while they were lower in the rubber plantation (0.5927, 0.5457 and 0.6928 respectively), oil palm plantation (0.5927, 0.5457 and 0.6928, respectively) and paddy field (0.6649, 0.5679 and 0.889, respectively) (Table 2.). Species richness was highest in the forest reserve (16 Orders) and lowest at the oil palm plantation (6 Orders) (Table 2).

The forest reserve had more species diversity (Order level), explaining the fact that the conversion of forests into agricultural areas negatively affects soil invertebrate species diversity (Gibson et al., 2011). Diversity has been shown to gradually decline as land use in the system became more intensive (Flynn et al., 2009). The abundance and diversity of soil invertebrates are believed to fluctuate depending on residue inputs and soil management practices. Other studies have shown that monoculture agriculture has lower diversity compared to agroforestry (Sisay & Ketema, 2015).

Table 2: Mean (SD) of relative abundance of soil invertebrates and values of diversity parameters from each study site in Kota Belud, Sabah.

		Forest Reserve	**Rubber Plantation**	**Oil Palm Plantation**	**Paddy Field**	**P**
Mean (SD)		14.69 (23.81)	9.17 (18.24)	14.33 (15.50)	8.86 (11.10)	0.844
Diversity Parameters	Richness	16	12	6	7	
	Shannon	1.984	1.483	1.186	1.38	
	Simpson	0.7843	0.6142	0.5927	0.6649	
	Evenness	0.4546	0.367	0.5457	0.5679	
	Menhinick	1.042	1.144	0.6928	0.889	

The mean relative abundance of soil invertebrates across the studied sites was highest at a value of 14.69 (23.81) in the forest reserve area (Table 2) However, these differences were not statistically significant (P = 0.844), suggesting relatively consistent abundance levels across the different land-use types. Nevertheless, the analysis of diversity parameters revealed variations in species richness and diversity indices among the study sites. The groups of soil invertebrates living in the forest reserve may have benefited from better quality of litter such as decomposed leaves from trees that may have encouraged the presence of ants (Hymenoptera). The availability of surface on the soil may influence the occurrence of litter-invertebrates (Burghouts et al., 1992).

Physicochemical characteristics of soil

The soil physicochemical parameters were measured and showed significant differences among study sites (Table 3). Soil physicochemical characteristics influenced the abundance and diversity of soil invertebrate communities including soil moisture content, pH, and organic matter. The forest reserve had higher soil moisture content compared to two agricultural areas yet there was no significant difference in soil moisture content of the forest reserve and the paddy field which were 21.41% and 20.00% respectively. This was due to the type of soil in the paddy field which was sandy loam and its watery characteristics; greyish colour and subangular blocky structure reflected flooding and high organic matter (Dou et al., 2016). In Table 3, the paddy field recorded the highest organic matter which was 7.00 %. Organic matter content may also be impacted by crop species and soil types (Zhou et al., 2014). All study sites had acidic soil where the forest reserve had a pH of 4.90, the rubber plantation a pH of 4.44, the oil palm plantation a pH of 4.48 and the paddy field a pH of 5.54.

The level of organic matter produced affects the soil water and pH with significant effects on the community of soil invertebrates. The forest reserve and the paddy field showed normal moisture content level, while the rubber plantation and the oil palm plantation recorded lower soil moisture content level that showed the soil was drier compared to the forest reserve and paddy field. Other studies showed that soil moisture content in an oil palm plantation is lower than in a secondary forest (Ngau et al., 2022). The conversion of forests into monoculture plantations affects the soil quality including soil moisture content and soil organic matter (Nguyen et al., 2020). The natural habitat in which the soil invertebrates dwell may be impacted by an ecological imbalance caused by any significant changes to the environmental variables (Bufebo et al., 2021).

Table 3: Physicochemical properties of soil in different agricultural activities and a forest reserve, in Kota Belud, Sabah. All data are represented in Mean ± SD (N=27) followed by different letters that showed significant differences at P < 0.05.

	Forest Reserve	Rubber Plantation	Oil Palm Plantation	Paddy Field
Moisture content %	21.41 ± 7.95^{a}	8.03 ± 4.57^{b}	6.26 ± 2.98^{b}	20.00 ± 10.37^{a}
pH	4.90 ± 0.86^{b}	4.44 ± 0.48^{b}	4.48 ± 0.49^{b}	5.54 ± 0.59^{a}
Organic matter %	5.08 ± 1.43^{b}	4.22 ± 1.63^{b}	4.25 ± 1.23^{b}	7.00 ± 2.63^{a}

Relationship of soil physicochemical characteristics and abundance of soil invertebrates

CCA is a multivariate analysis, an efficient way to relate the distribution of soil invertebrates with soil physicochemical parameters (soil organic matter, soil moisture content and pH). The CCA plot (Figure 3) revealed that the soil physicochemical influenced the abundance and distribution of soil invertebrates and the relationship between the presence of soil invertebrates with soil organic matter, moisture content and pH. Based on the CCA, Coleoptera and Geophilomorpha were abundant in the paddy field, where most of the Coleoptera were composed of ground beetles (Family: Carabidae). In high-yielding rice types, these insect pests and their natural opponents were more prevalent (Hussain Khan, 2013). In Figure 3, both Coleoptera and Geophilomorpha were associated with high soil moisture content and pH, but lower organic matter. Meanwhile, Glomerida, Archaeognatha and Polydesmida seem to be more sensitive to higher soil organic matter as well as both Hymenoptera and Orthoptera. Isopoda is located between higher organic matter and moisture content and more likely a lower pH. Next, Hemiptera seems to be more sensitive towards these three variables (organic matter, moisture content and pH). Lastly, Collembola located far from the point of three variables revealed this taxon seems to be not affected by these 3 environmental variables.

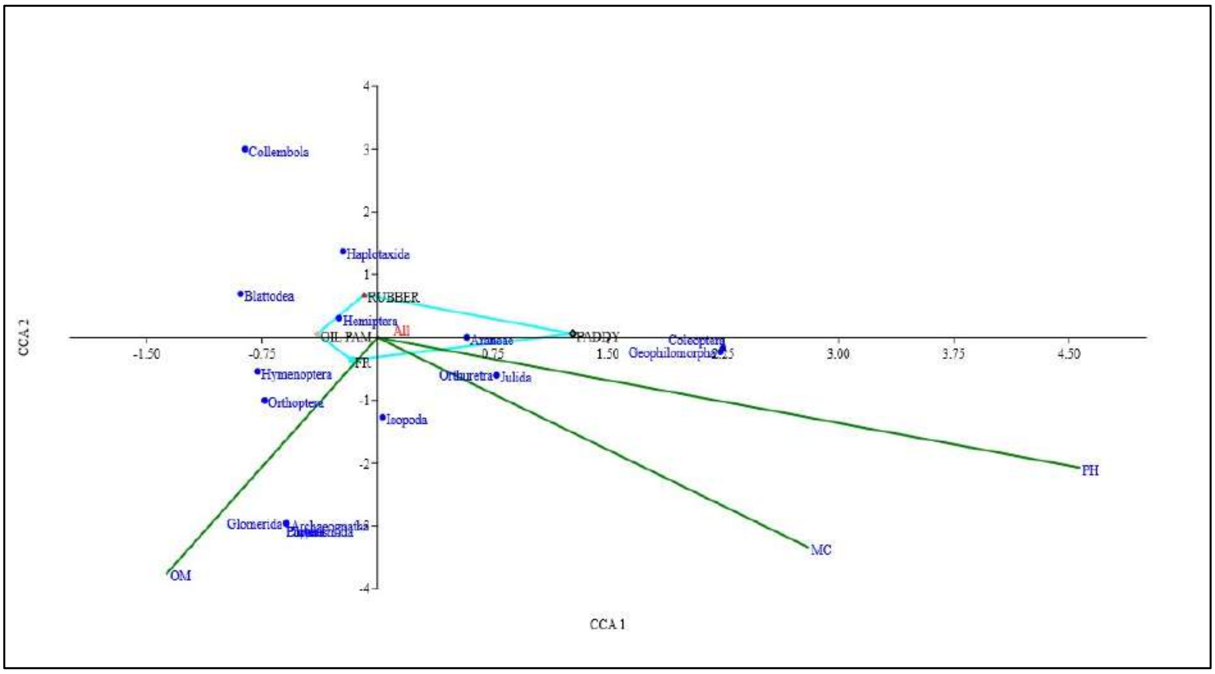

Figure 3: Canonical Correspondence Analysis (CCA) plot for the abundance of soil invertebrates in the different study sites in Kota Belud, Sabah.

CONCLUSION

The abundance of soil invertebrates depends on land use practice. Observing the results of this study, it is important to consider the activity in the soil layer that affects the existence and survival of soil invertebrates. Given their importance in maintaining life, proper attention should be paid to enhance the presence of soil invertebrates especially in Sabah, which is one of the food crop-producing states in Malaysia. This study is significant in that it adds baseline information to researchers and parties involved.

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Research Article

Suitability of the Former Mamut Copper Mine for Nature-based Tourism: A Preliminary Visual Assessment of Site Conditions

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ABSTRACT

The Former Mamut Copper Mine (MCM) is a historical landmark rich in both aesthetic and cultural values, but from a scientific perspective, its potential for nature-based tourism remains unexplored. A visual rapid assessment was conducted at MCM, to examine its suitability for nature-based tourism, focusing on site accessibility, hazard levels, scenic visibility, and scenic beauty. Twenty locations with high aesthetic and/or historical values were visually investigated, based on the four specified criteria, utilizing a three-point rating score system. The assessment emphasized a moderate suitability level for nature-based tourism at MCM (Score = 2.337), with significant variations in suitability level observed between the 20 locations ($\chi^2 = 32.36$, $p = 0.021$). Twelve locations showing higher scores in site accessibility, hazard level, and scenic visibility were determined to be more suitable for nature-based tourism, when compared to the other eight locations. Both the Principal Component Analysis and Spearman's Correlation test highlighted the significant influences of hazard level ($\rho = 0.685$, $p < 0.001$), accessibility ($\rho = 0.652$, $p = 0.002$), and scenic visibility ($\rho = 0.685$, $p < 0.001$) on the suitability of MCM for nature-based tourism, in comparison to site scenic beauty ($\rho = 0.203$, $p = 0.381$). These findings provide new insights highlighting the high suitability of MCM for nature-based tourism. Additionally, this research uncovers safety concerns, rendering public access and the carrying out of recreational activities at MCM, currently infeasible. Management authorities are urged to prioritize efforts that reduce hazard levels and enhance the quality and accessibility of local attractions in future.

Keywords: Former Mamut Copper Mine; nature-based tourism; site suitability; tourism potential; visual assessment.

INTRODUCTION

Sabah in Malaysian Borneo, known as the "Land Below the Wind", is renowned for its diverse landscapes and natural resources, and also its rich historical and cultural heritages (Tay & Chan, 2014; Augustine & Dolinting, 2016). These tangible and intangible resources are introduced as core tourism products by local communities, attracting a significant influx of international and domestic tourists to Sabah, thus fuelling the growth of the local tourism sector since 2010 (Danting et al., 2018). Presently, different alternative tourism practices are available throughout the state, yet nature-based tourism remains a focal point for tourists, because it offers tourists the opportunity to access diverse local landscapes and natural resources in natural settings (Zain et al., 2015; Talib, 2020). This trend has led to a surge in the promotion of nature-based tourism attractions, both domestically and internationally, resulting in the establishment of numerous new tourist destinations throughout Sabah in recent years (Augustine & Dolinting, 2016; Halim et al., 2018; Lim et al., 2022).

The suitability of a location for nature-based tourism is dictated by various aspects that significantly impact a tourist's satisfaction, including the quality of available tourism products or services, as well as the existing site's cultural and aesthetic values, scenic visibility, hazard level, and accessibility (Feng et al., 2010; Tay & Chan, 2014; Lim et al., 2019; Hasmat et al., 2020). These aspects can collectively dictate tourists' perception of a destination during their visit and, subsequently, its suitability for tourism (Zain et al., 2015). However, previous research has mostly focused on the mere presence or absence of specific elements, rather than their existing conditions, which consequently determines the suitability of a location for nature-based tourism (Zulhazman et al., 2004; Hasmat et al., 2020; Fiffy et al., 2021). This study intended to address this gap, focusing on the Former Mamut Copper Mine (MCM) as the chosen experimental site. Presently, this abandoned mine is a historical landmark that holds significant aesthetic and cultural values, yet its potential for nature-based tourism remains unexplored from the scientific standpoint. Transforming former copper mines into tourist destinations can help in funding the long-term conservation and restoration efforts of local landscapes and biodiversity, plus benefit the surrounding communities (Buonincontri et al., 2021; Gillette & Boyd, 2024). This initiative can help create job opportunities, such as work as tour guides or rangers for the sites, and offer accommodations and dining options for tourists, thereby fostering economic growth in the region (Rudd & Davis, 1998; Dimitrovski & Senić, 2019).

Consequently, many former copper mines across the globe are being marketed as tourist attractions, including the Bor Copper Mine in Serbia, the Kennecott Copper Mine in the United States of America, and the Falun Copper Mine in Sweden (Rudd & Davis, 1998; Pashkevich, 2017; Dimitrovski & Senić, 2019). These continual efforts are geared towards delivering lasting ecological, social, and economic benefits to nearby communities, while assisting management authorities in the long-term sustainable management, restoration, and conservation of the given areas (Armis & Kanegae, 2021; Buonincontri et al., 2021; Gillette & Boyd, 2024). This suggests that promoting MCM as a new nature-based tourism destination in Sabah can bring benefits to both site management authorities and neighbouring communities, underscoring the importance of determining its potential for nature-based tourism. However, there is limited understanding on how accessibility, scenic beauty, scenic visibility, and hazard level impact its suitability for nature-based tourism, and information on these subjects is currently limited (Musta et al., 2019; Adnan, 2021). In order to address these research gaps, a preliminary visual assessment was carried out at MCM. This research intended not only to investigate its suitability, but also to validate the factors that distinctly influenced its suitability, for nature-based tourism. The anticipated outcome of this research was to provide scientific insights to assist management authorities in making decisions regarding the long-term sustainable utilization and management of this old abandoned mine.

Study site

Covering about 2,112 ha of rugged montane terrain, MCM is characterized by numerous steep-sided valleys. This abandoned mine is located on the southeastern slope of Kinabalu Park in Ranau, Sabah, with an elevation ranging from 1,300 m to 1,600 m above sea level. Operating for 24 years from 1975 to 1999, the mining activities have left this area largely barren, occupied by patches of grasses and shrubs, while some sections host either native lower montane forest species or pioneer montane plant species (Saibeh et al., 2016). The former large open-pit quarry is inundated with water throughout the year, forming a large pit lake covering an area of approximately 50.0 ha and measuring over 100.0 m deep. The water in this lake is highly acidic, with a pH ranging from 3.6 to 3.9. The study site experiences a sub-tropical climate, with annual precipitation sometimes exceeding 4500.0 mm (monthly precipitation: 250.0 mm to 400.0 mm), and local annual temperature ranges from 15°C to 28°C (Saibeh et al., 2016; Cleophas et al., 2022). Around the large pit lake (Mamut Lake), a total of 20 locations were identified for their high aesthetic and/or historical values, and each of these locations were given a name reflecting their core attraction (Figure 1).

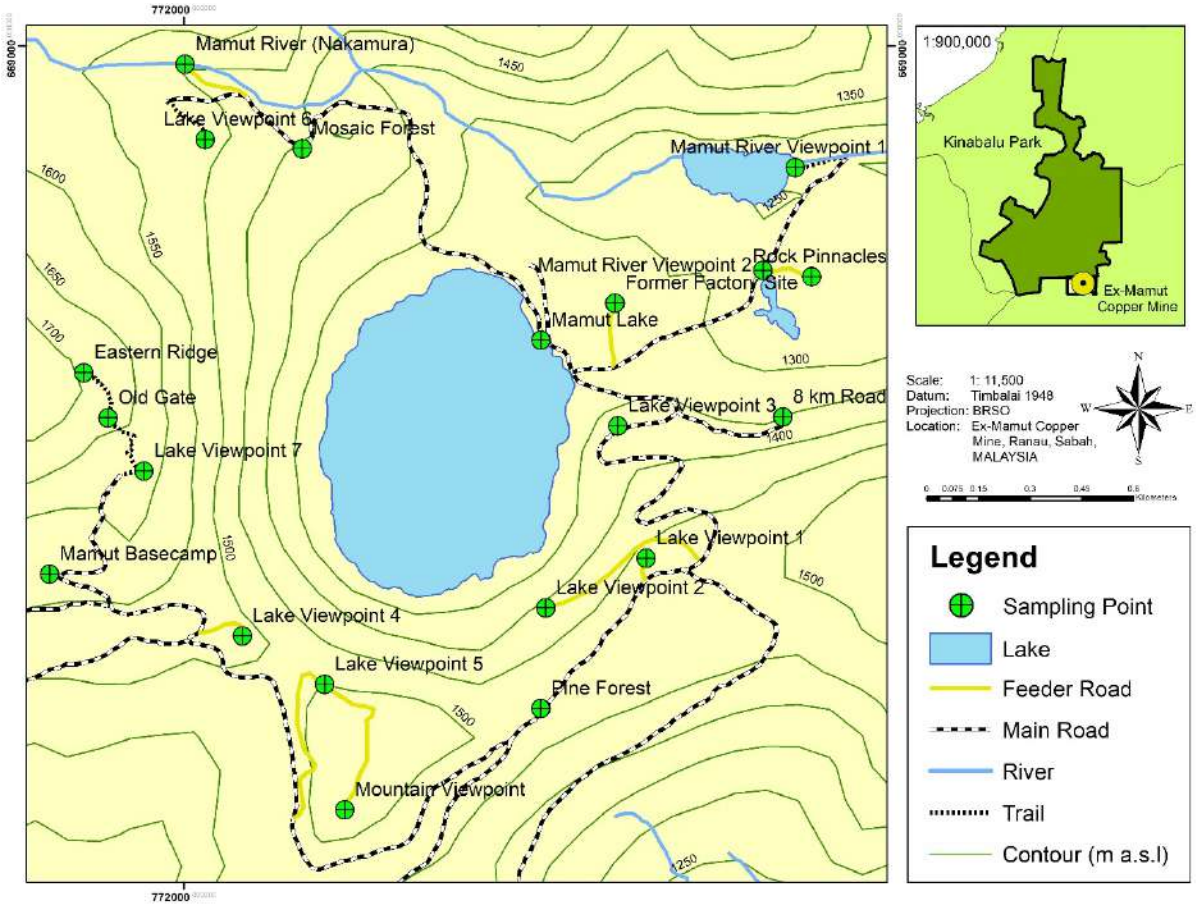

Figure 1: Locations of the 20 sampling points established across the Former Mamut Copper Mine in Ranau, Sabah, Malaysia Borneo

METHODOLOGY

This study was conducted at MCM over a five-day period, from 20th September till 24th September 2022, during the Mamut Scientific Expedition 2022, organized by Sabah Parks. A Garmin GPSMAPS 64s handheld global navigation satellite system receiver was used to record the coordinates of each of the 20 sampling points. The collected coordinates were then compiled and presented in two distinct coordinate systems, which were the World Geodetic System 1984 (WGS84) and Borneo Rectified Skew Orthomorphic (BRSO) (Table 1). The site conditions at each of the 20 sampling points were visually examined at field, focusing on factors such as 1) scenic visibility; 2) scenic beauty; 3) accessibility, and; 4) hazard level, which shaped the site suitability level for nature-based tourism, as suggested by the findings of several relevant past research (e.g., Zulhazman et al., 2004; Feng et al., 2010; Tay & Chan, 2014; Musta et al., 2019; Lim et al., 2022). Given that the only infrastructure remaining at MCM comprised pathways established prior to the mine closure (before 1999), this research exclusively assessed the conditions of the vehicle or human pathways that led to the respective sampling points.

Table 1: Coordinates and designated names of the 20 sampling points with high aesthetic and/or historical value, at the Former Mamut Copper Mine in Ranau, Sabah, Malaysia Borneo.

Sampling Point	BRSO[1]		WGS84[2]	
	Latitude	Longitude	Latitude	Longitude
Pine Forest	667069.6 m	773045.7 m	N 6 01.196	E 116 39.497
Lake Viewpoint 1	667510.2 m	773341.8 m	N 6 01.431	E 116 39.664
Lake Viewpoint 2	667365.7 m	773052.7 m	N 6 01.354	E 116 39.505
Lake Viewpoint 3	667894.4 m	773264.2 m	N 6 01.640	E 116 39.620
Former Factory Site	668250.4 m	773250.1 m	N 6 01.833	E 116 39.617
Mamut River Viewpoint 1	668645.2 m	773778.8 m	N 6 02.045	E 116 39.902
Rock Pinnacles	668335.0 m	773828.2 m	N 6 01.873	E 116 39.927
Mamut River Viewpoint 2	668349.1 m	773680.1 m	N 6 01.883	E 116 39.850
Lake Viewpoint 4	667291.7 m	772171.6 m	N 6 01.313	E 116 39.028
Mamut Basecamp	667460.9 m	771618.2 m	N 6 01.411	E 116 38.726
Mountain Viewpoint	666780.6 m	772467.6 m	N 6 01.038	E 116 39.188
Lake Viewpoint 5	667147.2 m	772407.7 m	N 6 01.235	E 116 39.157
Mosaic Forest	668701.6 m	772340.7 m	N 6 02.079	E 116 39.127
Lake Viewpoint 6	668729.8 m	772065.8 m	N 6 02.094	E 116 38.974
Mamut River (Nakamura)	668941.3 m	772005.9 m	N 6 02.213	E 116 38.943
Mamut Lake	668144.7 m	773042.2 m	N 6 01.776	E 116 39.500
8 km Road	667922.6 m	773740.1 m	N 6 01.653	E 116 39.880
Lake Viewpoint 7	667763.1 m	771889.6 m	N 6 01.573	E 116 38.875
Old Gate	667922.6 m	771776.8 m	N 6 01.657	E 116 38.819
Eastern Ridge	668053.0 m	771706.3 m	N 6 01.728	E 116 38.782

*Note: [1]BRSO=Borneo Rectified Skew Orthomorphic (m=meter).
[2]WGS84=World Geodetic System 1984 (N=North, and; E=East).

In this study, the four assessment criteria were analysed quantitatively by using a three-point rating score system (Table 2). The accessibility of each sampling point was determined by averaging the rating scores for 1) its proximity to the nearest road/trail; 2) the condition of the nearest road/trail surface, and; 3) the steepness of the nearest road/ trail (Zulhazman et al., 2004; Lim et al., 2019, 2022). Additionally, the scenic beauty surrounding each sampling point was verified by averaging the rating scores for seven scenic features, such as: 1) water body; 2) atmospheric phenomenon; 3) vegetation; 4) mountain massif; 5) tourist environment; 6) folk culture, and; 7) fauna. These seven scenic features were rated through the system applied by Feng et al. (2010). Henceforth, the suitability level of each sampling point for nature-based tourism was represented by the mean value of rating scores for accessibility, hazard level, scenic visibility, and scenic beauty with the ratings as followed: A) 1 = Low suitability; B) 2 = Medium suitability, and; C) 3 = High suitability (Lim et al. 2022).

Table 2: The assessment criteria applied in examining the suitability levels of the 20 sampling points for nature-based tourism at Former Mamut Copper Mine in Ranau, Sabah, Malaysia Borneo.

Assessment Criterion	Description	Rating System
Scenic Visibility	Visibility of the scenery presented at the surrounding of a sampling point to the visitors.	1=Low (0–33%); 2=Moderate (33–67%); 3=High (67–100%)
Scenic Beauty	Qualities of the scenic features that are viewable to visitors, namely the vegetation (VE), water body (WB), fauna (FA), folk culture (FC), tourist environment (TE), mountain massif (MM), and atmospheric phenomenon (AP), presented at the surrounding of a sampling point. The quality of each scenic feature is rated by utilizing the rating system proposed by Feng et al. (2010)	1=Low; 2=Moderate; 3=High (Calculated as the mean value of the rating scores for seven scenic features that define the site scenic beauty)
Accessibility	Accessibility of a sampling point to visitors, which is dictated by its proximity to the nearest road/ trail, as well as the surface condition and the steepness of the nearest road/trail.	1=Low (0–33%); 2=Moderate (33–67%); 3=High (67–100%) (Calculated as the mean value of the rating scores for three factors that define the site accessibility)
	Proximity to the nearest road/trail (PR)	1=Far (>300m); 2=Moderate (150–300m); 3=Near (<150m)
	Surface condition of the nearest road/trail (SC)	1=Poor; 2=Moderate; 3=Good (Dependent to the type of ground cover and surface smoothness of the road/trail-in-question)
	Steepness of the nearest road/ trail (ST)	1=Steep (>30º); 2=Moderate (15 –30º); 3=Gentle (<15º)
Hazard Level	Level of risk posted to the visitors at a sampling point.	1=High risk; 2=Moderate risk; 3=Low risk (Dependent to the diversity and severity of hazard presented at the site-in-question)

This study utilized the statistical software PAST ver. 3.25 (Hammer et al., 2001) for all descriptive and inferential analyses, using a 95.0% confidence interval ($p = 0.05$). The gathered data was examined for normal distribution, and the outcome indicated that it was non-normally distributed (Shapiro-Wilk test: $p<0.05$). Therefore, the non-parametric Kruskal-Wallis test was applied to evaluate the variability in suitability levels among the 20 established sampling points. The clustering pattern of the given sampling points, based on site suitability level, was validated through Principal Component Analysis (PCA). Additionally, the non-parametric Spearman's Correlation test was utilized to examine the correlations between site hazard level, accessibility, scenic visibility, scenic beauty, and the suitability level for nature-based tourism at each point in this research.

RESULTS

The 20 sampling points were found to be highly accessible to visitors (Score>2.0), with five areas receiving the highest rating scores for accessibility in this research (Lake Viewpoint 1, Lake Viewpoint 4, Lake Viewpoint 7, Mamut Lake, and Pine Forest; Score = 3.0) (Table 3). These areas were positioned close to pathways (<100m) constructed along gentle slopes (<15°) with smooth surfaces covered in gravel, rocks, or moss. Contrarily, the remaining 15 locations were verified to either having moderate or good accessibility, based on the three defining factors (Table 2). The hiking trail leading Mamut River Viewpoint 1 to the vehicle road was inundated with algae-filled water. Parts of the vehicle roads leading to Lake Viewpoint 2, Lake Viewpoint 6, 8 km Road, and Mosaic Forest were severely eroded and very rough, and the routes to Lake Viewpoint 3 and Eastern Ridge were notably steep (>30°) and lengthy (>300m), respectively. In other words, the pathways connecting these seven areas to the vehicle road were considered poor in various aspects (Score = 1.0), designating Lake Viewpoint 2, Lake Viewpoint 3, Lake Viewpoint 6, and Mamut River Viewpoint 1 as the least accessible locations to visitors in this research (Score = 2.0) (Table 3).

The scenery visibility at the Old Gate and Eastern Ridge were validated to be the lowest (<33.3%; Score = 1.0) (Table 3). Subsequently, four locations were verified to possess moderate scenery visibility (Lake Viewpoint 7, Mamut River (Nakamura), 8 km Road, and Mamut River Viewpoint 1; 33.3%<Scenic Visibility<66.7%; Score = 2.0), whereas the remaining 14 locations offered high scenery visibility to visitors (>66.7%; Score = 3.0) (Table 3). The scenic beauty ratings across the 20 sampling points were generally moderate, with the Old Gate and Eastern Ridge receiving the lowest rating scores (1.714), whereas eight sampling points ascertained the highest rating scores (2.286) (Table 3). This result stemmed from the similarity in rating scores across the seven scenic features defining scenic beauty between these 20 sampling points (Table 4). A majority of these sampling points provided good scenic views of Mount Kinabalu and the surrounding mountain massif, which were frequently accompanied by lenticular clouds, mist, and expanse of cloud cover (MM and AP: Score = 3.0). At Mamut River (Nakamura) and Lake Viewpoint 7, visitors could observe the highly aesthetic-looking mountain ranges veiled in mist and enveloped by expanse of clouds, contrasting with the Old Gate and Eastern Ridge offering restricted views of local atmospheric phenomena and mountain massif to visitors (MM and AP: Score = 1.0). Furthermore, these 20 sampling points hosted limited diversity and density of local vegetation and were rarely visited by local wildlife, thus resulting in generally low to moderate scenic views of local fauna and vegetation across these 20 sampling points (Table 4).

Table 3: Mean rating scores computed for the four assessment criteria and suitability level that aim to represent the overall conditions of various aspects of the Former Mamut Copper Mine in Ranau, Sabah, for nature-based tourism.

Sampling Point	Rating Score				
	Scenic Visibility	Scenic Beauty	Accessibility	Hazard Level	Suitability Level
Pine Forest	3.000	2.000	3.000	3.000	2.750
Lake Viewpoint 1	3.000	2.286	3.000	3.000	2.822
Lake Viewpoint 2	3.000	2.000	2.000	1.000	2.000
Lake Viewpoint 3	3.000	2.286	2.000	2.000	2.322
Former Factory Site	3.000	2.286	2.667	1.000	2.238
Mamut River Viewpoint 1	2.000	2.143	2.000	1.000	1.786
Rock Pinnacles	3.000	1.857	2.667	2.000	2.381
Mamut River Viewpoint 2	3.000	2.286	2.667	2.000	2.488
Lake Viewpoint 4	3.000	2.286	3.000	2.000	2.572
Mamut Basecamp	3.000	2.000	2.667	3.000	2.667
Mountain Viewpoint	3.000	2.000	2.667	3.000	2.667

Lake Viewpoint 5	3.000	2.000	2.667	2.000	2.417
Mosaic Forest	3.000	2.286	2.333	2.000	2.405
Lake Viewpoint 6	3.000	2.000	2.000	2.000	2.286
Mamut River (Nakamura)	2.000	2.000	2.333	3.000	2.333
Mamut Lake	3.000	2.286	3.000	2.000	2.572
8 km Road	2.000	2.143	2.333	2.000	2.119
Lake Viewpoint 7	2.000	2.286	3.000	2.000	2.322
Old Gate	1.000	1.714	2.667	2.000	1.845
Eastern Ridge	1.000	1.714	2.333	2.000	1.762
Overall Condition	2.600 (Good)	2.100 (Moderate)	2.550 (Good)	2.100 (Moderate)	2.337 (Moderate)

Mamut River (Nakamura), Lake Viewpoint 7, and Eastern Ridge were the only regions providing good scenic views of the pristine highland forest (Score = 3.0) (Table 4). However, sighting of wildlife was scarce at Mamut River (Nakamura) (Score = 1.0), unlike the other two locations with higher probability of sighting local wildlife, especially the Pig-tailed Macaque (*Macaca nemestrina*) (Score = 2.0). Numerous bird species could be easily observed at Mountain Viewpoint and Lake Viewpoint 7, where moderate scenic views of the local vegetation (grassy and bushy, with several trees in the vicinity) were present at these two locations (Score = 2.0).

Table 4: Rating scores of the seven scenic features and three factors used to represent site scenic beauty and accessibility, respectively, at the Former Mamut Copper Mine in Ranau, Sabah.

Sampling Point	**Scenic Beauty[1]**							**Accessibility[2]**		
	WB	**MM**	**TE**	**VE**	**FA**	**FC**	**AP**	**SC**	**PR**	**ST**
Pine Forest	1	3	3	2	1	1	3	3	3	3
Lake Viewpoint 1	2	3	3	2	1	2	3	3	3	3
Lake Viewpoint 2	2	3	1	1	1	3	3	1	3	2
Lake Viewpoint 3	2	3	2	2	1	3	3	2	3	1
Former Factory Site	2	3	2	2	1	3	3	2	3	3
Mamut River Viewpoint 1	2	3	2	2	1	2	3	1	2	3
Rock Pinnacles	1	3	2	1	1	2	3	2	3	3
Mamut River Viewpoint 2	2	3	2	2	1	3	3	2	3	3
Lake Viewpoint 4	2	3	2	2	1	3	3	3	3	3
Mamut Basecamp	1	3	2	2	1	2	3	2	3	3
Mountain Viewpoint	1	3	2	2	2	1	3	2	3	3
Lake Viewpoint 5	2	3	1	1	1	3	3	2	3	3
Mosaic Forest	2	3	2	2	1	3	3	1	3	3
Lake Viewpoint 6	2	3	2	2	1	3	3	1	2	3
Mamut River (Nakamura)	3	2	2	3	1	1	2	2	2	3
Mamut Lake	2	3	2	2	1	3	3	3	3	3
8 km Road	1	3	2	2	1	3	3	1	3	3
Lake Viewpoint 7	2	3	2	2	2	3	2	3	3	3
Old Gate	1	1	3	2	2	2	1	3	2	3
Eastern Ridge	1	1	3	2	2	2	1	3	1	3

*Note: [1]Scenic Beauty: WB=Water Body; MM=Mountain Massif; TE=Tourist Environment; VE=Vegetation; FA=Fauna; FC=Folk Culture, and; AP=Atmospheric Phenomenon.
[2]Accessibility: SC=Surface Condition of the Nearest Road/Trail; PR=Proximity to the Nearest Road/Trail, and; ST=Steepness of the Nearest Road/Trail.

The large pit lake of MCM could be observed from over half of the 20 sampling points, whether from a distance or up close. However, the scenic qualities of the lake visible from these locations were reduced by its contaminated water (Score = 2.0). Conversely, the upstream of the Mamut River retained its cleanliness and was surrounded by dense native vegetation, providing visitors at the Mamut River (Nakamura) a good scenic view (Score = 3.0) (Table 4). Water bodies were hardly visible at the remaining seven sampling points (Score = 1.0), although water puddles were evident in the vicinity of Mountain Viewpoint. Over half of the 20 sampling points offered visitors good scenic views of local folk cultural elements (Score = 3.0), along with moderately attractive tourist environments (Score = 2.0) (Table 4). The folk cultural elements showcased at MCM included waste rock dumpsites, abandoned mining infrastructure, and a large pit lake. Therefore, the remaining nine areas offered visitors either moderate or poor scenic views of these elements. Lake Viewpoint 2 and Lake Viewpoint 5 were found to have poor tourist environments (Score = 1.0), whereas the remaining four sites, including Pine Forest, Lake Viewpoint 1, Old Gate, and Eastern Ridge, were validated to provide good tourist environments (Score = 3.0) (Table 4).

Various hazards were identified across the 20 sampling points examined in this research. Specifically, hazards identified at the Mamut River Viewpoint 1, Former Factory Site, and Lake Viewpoint 2, include the potential for landslides, flash floods, rugged rocky pathways, falling rocks, sulphuric scents, slippery surfaces, wildlife attacks, contaminated water, and erosion were verified to pose high risks to visitors (Score = 1.0) (Table 3). Visitors at Mamut Basecamp, Pine Forest, Mountain Viewpoint, Lake Viewpoint 1, and Mamut River (Nakamura) were likely to face comparable hazards, albeit with low hazard levels (Score = 3.0) (Table 3). Regarding the remaining 12 areas with moderate hazard levels (Score = 2.0), the risks of falling deadwood and tree branches were only observed at Old Gate and Eastern Ridge (low risks). Moreover, similar hazards like rocky pathways, sulphuric scents, erosion, and landslides could potentially happen at these 12 areas (low risks). Essentially, MCM was verified with high accessibility (Score = 2.55) and scenic visibility (Score = 2.6), along with moderate scenic beauty and hazard level (Score= 2.1), resulting in its moderate suitability for nature-based tourism (Score = 2.337) (Table 3).

According to the data presented in Table 3, Mamut Lake, Mamut Basecamp, Mountain Viewpoint, Lake Viewpoint 1, Lake Viewpoint 5, and Pine Forest exhibited significantly higher levels of suitability (Score>2.5) compared to some other locations (1.5<Score<2.5), particularly Old Gate (Score = 1.845), Eastern Ridge (Score = 1.762), and Mamut River Viewpoint 1 (Score = 1.786; $\chi^2 = 32.36$, $p = 0.021$). The outcomes of the PCA analysis revealed that 89.01% of the total variance was explained by the first two principal components (PC), indicating the existence of two distinct clusters, as depicted in a scatter plot (Figure 2). Within one of the two clusters, 12 locations were validated with high rating scores for accessibility, hazard level, scenic visibility, and suitability level, all exhibiting positive values for PC1. On the contrary, the other eight sites comprising the other cluster displayed contrasting characteristics. The results from Spearman's Correlation test further indicated a significant positive correlation between the suitability level of a sampling point and accessibility ($\rho = 0.652$, $p = 0.002$), scenic visibility ($\rho = 0.685$, $p<0.001$), and hazard level ($\rho = 0.685$, $p<0.001$). Nonetheless, there was no significant correlation between the suitability level of a sampling point and scenic beauty ($\rho = 0.203$, $p = 0.381$), thereby aligning with the results of the PCA analysis. In summary, these three factors predominantly shaped the suitability level of a sampling point at MCM for nature-based tourism.

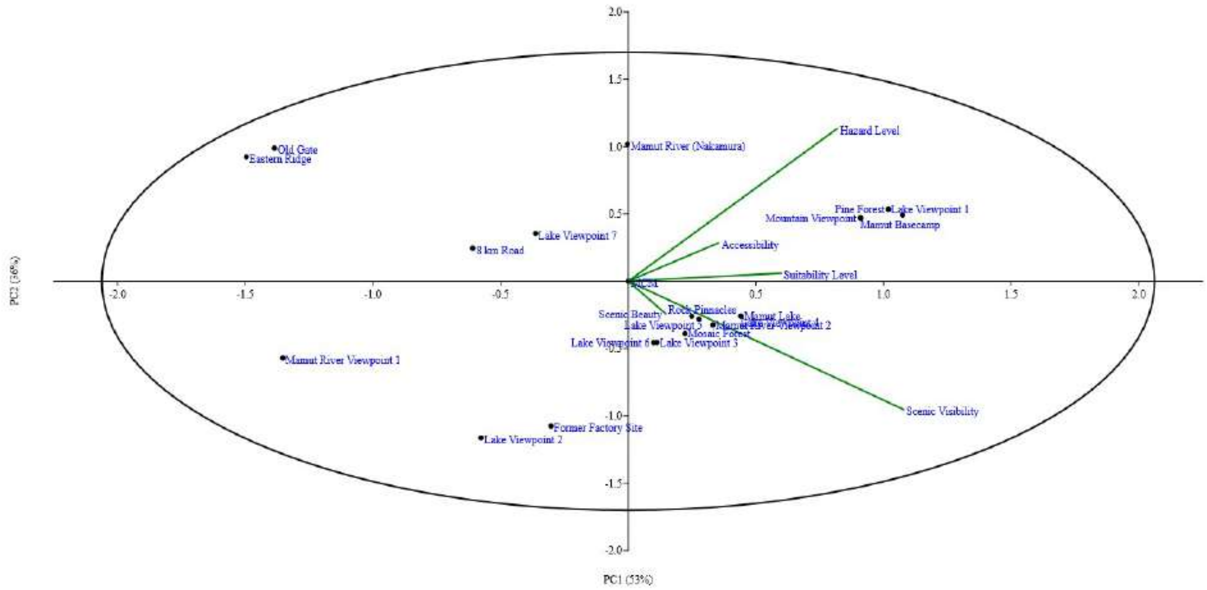

Figure 2: A scatter plot depicting the scenic visibility, scenic beauty, accessibility, hazard level, and suitability levels of the 20 established sampling points across the Former Mamut Copper Mine.

DISCUSSION

The results of this study indicated notable differences in the suitability levels for nature-based tourism among the 20 established sampling points. Owning to their high aesthetic and/or historical values, the qualities of the seven scenic features visible at these 20 locations exhibited a general similarity. Consequently, the scenic beauty among these 20 locations also exhibited a general similarity, falling within a moderate range (1.5<Score<2.5). The environments at these 20 locations had undergone changes from their original state, due to habitat degradation caused by past mining operations. Furthermore, these areas ceased to be affected by human disturbance following the decommissioning of the local mining operation in 1999 (Low et al., 2020; Adnan, 2021). Hence, the scenic views focusing on the atmospheric phenomenon and mountain massif, namely the lenticular cloud that regularly formed around the peaks of Mount Kinabalu, maintained high qualities at the majority of the 20 established sampling points. Conversely, the waste rock dumpsites, abandoned mining infrastructure, and large pit lake were visible only at certain areas, offering unfavourable tourist environments and limited scenic views of the local vegetation and fauna. This is because Mount Kinabalu being situated at a distance and at a higher elevation than the whole MCM area, unlike the tourist environments, vegetation, water bodies, fauna, and folk cultures found within the vicinity of this abandoned mine (Saibeh et al., 2016; van der Ent & Edraki, 2016; Cleophas et al., 2022).

Previous research emphasized diverse landscapes and distinctive natural features, which were rare and authentic to a particular area, and highly suitable for promotion as local flagship attractions (Zulhazman et al., 2004; Hasmat et al., 2020; Fiffy et al., 2021). The highly aesthetic Mount Kinabalu and lenticular cloud were readily viewable to visitors from various sites within MCM. Moreover, the abandoned mining infrastructure, waste rock dumpsites, and large pit lake were inherent to MCM's identity. Henceforth, these unique and authentic natural and manmade features were well-suited to be introduced as local flagship attractions. All of the sampling points were located within the post-mining environment of MCM, hence emphasizing the absence of significant differences in surrounding environmental conditions throughout these areas. This scenario could contribute to similarities in the scenic beauty qualities of the different accessible scenic features observed by visitors across these 20 locations (Feng et al., 2010; Zain et al., 2015; Talib, 2020). On the other hand, there were generally diverse levels of accessibility, scenic visibility, and hazard levels among these locations. The differences in these three factors and the similarity in scenic beauty qualities between these 20 locations would ultimately result in the insignificant influence of scenic beauty on the suitability of a sampling point within MCM for nature-based tourism, as emphasized by the results of both the PCA analysis and Spearman's Correlation test conducted in this research (Li et al., 2018; Alsaqr, 2021; Wang et al., 2021).

The site accessibilities and scenic visibilities were generally high (Score>2.5), while the site hazard levels were predominantly moderate (Score = 2.0), resulting in the identification of 14 locations as moderately suitable and 6 locations as highly suitable, for nature-based tourism. The visibility of the scenery surrounding an area to visitors is dictated by the characteristics of the local vegetation (size, structure, density, and composition) and topography (slope steepness, aspect, and position) (Lang et al., 2021; Courbin et al., 2022). The perceived scenic quality by visitors may differ when viewing a particular scenery from different locations with different topographic and vegetation conditions (Lim et al., 2019, 2022). A change in the visible scenic beauty can impact the satisfaction experienced by visitors when engaging in recreational activities at the location (Feng et al., 2010; Zain et al., 2015). As a result, a dense forest situated along a slope area is likely to provide visitors with lower scenic visibility, visible scenic beauty, and satisfaction, while an open area located on a plateau, valley, or ridge region is likely to offer the opposite experience to visitors (Bayliss et al., 2014; Talib, 2020; Lang et al., 2021; Courbin et al., 2022). This phenomenon helps in clarifying the significantly positive correlation between site scenic visibilities and suitability levels across the 20 sampling points evaluated in this study.

Since 1999, environmental degradation and pollution resulting from the past mining activities have not been properly addressed, and the maintenance of the hiking trails and vehicle roads leading to the 20 sampling points have been neglected (van der Ent & Edraki, 2016; Musta et al., 2019; Low et al., 2020; Adnan, 2021). The combined factors of pathway length, gradient, and surface condition determine the difficulty level in accessing an area, thereby impacting the motivation for visitors to visit the given location for recreational purposes (Bayliss et al., 2014; Lim et al., 2019, 2022). Moreover, this research validated a wide variety of hazards, with each posing varying risks to visitors across the 20 established sampling points. Among these hazards, the sulphuric scent and contaminated water were verified as the major local hazards, contrasting with the other hazards categorized as minor local concerns. These two hazards can have adverse effects on human health, with the severity of their impact dictated by both the proximities and the duration of exposure of visitors to their sources (Barbusiński et al., 2021). The presence of the hydrogen sulphide (H_2S), which is a toxic gas mostly originating from the large pit lake at MCM, was indicated by the smell of sulphur. Different sources of contaminated water, such as the large pit lake, puddles, and several smaller pit lakes within the MCM, were identified in this study. Past results denoted that these waters contained elevated levels of copper and sulphur minerals, and also a variety of bacterial species and trace elements (van der Ent & Edraki, 2016; Low et al., 2020). In summary, visitors are at risk of encountering both major and minor hazards, which may detrimentally impact their sense of security and overall satisfaction, both physically and mentally, when engaging in recreational activities within the MCM area (Tay & Chan, 2014; Augustine & Dolinting, 2016).

The results of PCA indicated that a cluster of twelve sampling points, characterized by moderate to high site accessibility, scenic beauty, and scenic visibility, and also low to moderate site hazard levels, exhibited positive values for the PC1. Six of these 12 locations (Mamut Lake, Pine Forest, Mountain Viewpoint, Lake Viewpoint 1, Lake Viewpoint 4, and Mamut Basecamp) were validated as highly suitable candidates for promotion as the main attraction sites at MCM (Score>2.5), despite their moderate site scenic beauty (1.5<Score<2.5). The results of this study highlight that the visitors' perceptions of security and convenience while accessing a particular feature in its entirety, either physically or scenically, could serve as primary motivations for engaging in recreational activities, in contrast over the importance of scenic beauty (Zulhazman et al., 2004; Hasmat et al., 2020; Fiffy et al., 2021). Furthermore, visitors typically show strong preference for safe environments that offer convenient access to flagship attractions, regardless of the area's scenic beauty being of low quality, and vice versa (Feng et al., 2010; Bayliss et al., 2014; Talib, 2020; Lim et al., 2022). Consequently, these twelve sampling points, distinguished by their high aesthetic and/or historical significance, were instrumental in shaping the moderate suitability level of MCM for nature-based tourism, thereby contributing to the existing body of tourism research at this abandoned mine.

The establishment of a successful nature-based tourism destination greatly relies on the engagement of multiple stakeholders, comprising local communities, government agencies, site managers, private organizations, environmental groups, and potential destination visitors (Tay & Chan, 2014; Danting et al., 2018). This trend is evident in the renowned Falun Copper Mine, Bor Copper Mine, and Kennecott Copper Mine, all of which have been successfully introduced as tourism destinations in recent years (Rudd & Davis, 1998; Pashkevich, 2017; Dimitrovski & Senić, 2019). Their achievement in establishing long-term sustainable management as tourism destinations is the result of ongoing environmental remediation efforts that prioritize restoring site ecosystems, mitigating site environmental impacts, and ensuring the safety of visitors and neighbouring communities, with support from involved stakeholders (Armis & Kanegae, 2021; Buonincontri et al., 2021; Gillette & Boyd, 2024). As for nearby communities, this tourist activity could significantly influence their livelihoods, either positively or negatively, contingent towards the long-term sustainability of local site management (Rudd & Davis, 1998; Buonincontri et al., 2021; Gillette & Boyd, 2024). Consequently, specific adjacent communities may oppose the conversion of MCM into a recreational site, out of concern about exacerbating the local environment's ongoing recovery from past mining activities, and its potential adverse impacts on their long-term livelihoods (Pashkevich, 2017; Musta et al., 2019). Since researchers have yet to thoroughly investigate the perception of local communities regarding the promotion of MCM for nature-based tourism, further research should be conducted to address this subject, before proceeding with its promotion for nature-based tourism in the future.

The findings of this research simply aid management authorities in validating the existing availabilities and qualities of tourism attractions (supplies) in the vicinity of MCM. As this area remains closed to public access at present, this research did not investigate the expectations (demands) of visitors who may visit this abandoned mine. The expectations and preferences of visitors seeking first hand experiences with certain attractions at a destination are referred to as visitors' demands, and these highly-anticipated attractions may not always be the local flagship attractions (Arbieu et al., 2017; Lim et al., 2022). The failure to meet expectations and preferences of visitors can negatively influence their levels of satisfaction and subsequently, the perceived image of the given destination (Zain et al., 2015; Kubo et al., 2019). This emphasizes that the true feasibility of MCM for nature-based tourism is determined by the compatibility between local tourism supplies and visitors' demands (Arbieu et al., 2017; Kubo et al., 2019). Particular stakeholders may also envision alternative uses for MCM, such as fully preserving this abandoned mine for restoring the local environment and biodiversity. This could potentially impact public access to this area for recreational purposes in the future (Pashkevich, 2017). However, neither of these subjects have been thoroughly evaluated in previous or the present study, underscoring the necessity for additional research on these topics at MCM.

The absence of basic facilities like signboards, restaurants/cafés, trash bins, and resting areas, along with poorly maintained pathways leading to the attraction sites, continue to be the shortcomings that have prevented MCM from being ready to be promoted as a nature-based tourism destination at this moment. The given facilities and infrastructures are essential for the operation of a tourist destination site (Augustine & Dolinting, 2016; Halim et al., 2018). It is crucial for the management authority to ensure the proper establishment of these amenities, not only at the 12 sampling points with high levels of suitability for nature-based tourism, but also across the entirety of MCM. Additionally, numerous hazards were presented throughout MCM, with some posing prominent risks to visitors. This may undermine their sense of security when engaging in recreational activities at local attraction sites (Zulhazman et al., 2004; Hasmat et al., 2020; Fiffy et al., 2021). Therefore, it is crucial for the management authority to effectively manage and mitigate local hazards, particularly the sulphuric scent and contaminated water, to reduce local hazard levels and elevate visitor satisfaction in this region (Lim et al., 2019, 2022). In other words, further initiatives are required to improve local basic infrastructure and facilities, grasp the perceptions of all involved stakeholders regarding the possibility of promoting MCM as a nature-based tourism destination, restore the local ecosystem, and mitigate local hazards, prior to permitting public access for recreational purposes in future at this abandoned mine. Most importantly, these endeavours should be underpinned by scientific evaluations and robust evidence before suggesting feasible recreational activities to be promote at MCM, stressing the significance of safety measures for its potential as a future nature-based tourism destination.

CONCLUSION

The findings of this study affirms that MCM holds moderate suitability for nature-based tourism. Among the 20 established sampling points, 12 locations were identified as suitable for promotion as local core attraction sites, attributed to the moderate to high site accessibility and scenic visibility, along with moderate to low site hazard level. This abandoned mine boasts both natural (Mount Kinabalu and lenticular cloud) and manmade (large pit lake, waste rock dumpsites, and abandoned mining infrastructure) features, all of which are suitable to be promoted as local flagship attractions. However, the accessibility of these potential attractions to visitors, whether in terms of physical access or scenic views, differs from one site to another. Moreover, the suitability of each established sampling point for nature-based tourism is determined to be majorly defined by accessibility, hazard level, and scenic visibility, instead of scenic beauty. This highlights the significance of these three aspects in determining the potential of MCM for promotion as a nature-based tourism destination. However, the findings of this research merely validate the existing availabilities and qualities of features in the vicinity of MCM with potential to be marketed as local tourism attractions. Moreover, the present study neglects to address stakeholder perceptions on the potential of MCM for nature-based tourism, plus the alignment of visitor demand with local tourism supplies, which are acknowledged as crucial determinants in determining the feasibility of marketing a location as a nature-based tourism destination. The limitations of this study, along with inadequate essential infrastructures and facilities, ongoing ecosystem recovery from past mining activities, and the presence of numerous hazards posing risks to visitor safety, render the opening of MCM for public recreational use unviable at present time. Additional research is needed to overcome the limitations of this study and address the shortcomings at MCM. This will help establish robust scientific evidence that can support management authorities and other stakeholders in decision-making regarding the planning and management of MCM. Consequently, this will enhance its suitability for nature-based tourism, thereby increasing the feasibility of opening it to public recreational access in the future.

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Research Article

Assessment of Antioxidant and Hepatoprotective Effects of *Kappaphycus alvarezii* (Doty) Doty ex Silva Against Carbon Tetrachloride-Induced Liver Injury in Rats

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ABSTRACT

Kappaphycus alvarezii (Doty) Doty ex Silva, commonly known as red algae, holds economic significance as a primary source of κ-carrageenan, which exhibits promising medicinal and therapeutic properties. This study aims to assess the antioxidant potential as well as hepatoprotective activity of the ethanolic extract of *K. alvarezii* (EEKA). The assays utilised to determine the antioxidant properties of EEKA were total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. In addition, the ability of EEKA to ameliorate experimentally induced liver injury in Sprague-Dawley rats caused by carbon tetrachloride (CCl_4) was evaluated. The biochemical assays involved measuring liver marker enzymes (AST and ALT) in serum as well as determining the levels of reduced glutathione (GSH), lipid peroxidation (LPO) via malondialdehyde, catalase (CAT), and glutathione S-transferase (GST) in liver homogenates. The TPC and DPPH radical scavenging activity of EEKA demonstrated relatively low antioxidant properties compared to standard references. However, CCl_4-induced groups exhibited significantly increased levels of AST and ALT, along with depletion of antioxidant status (GSH, CAT, and GST) indicated by LPO. Pretreatment with EEKA resulted in slightly decreased liver marker enzyme activity and LPO, coupled with an increase in antioxidant status. These findings suggest that EEKA contains active principles capable of counteracting the hepatotoxic effects induced by CCl_4.

Keywords: *Kappaphycus alvarezii*; antioxidant; hepatoprotective; carbon tetrachloride; oxidative stress.

INTRODUCTION

Seaweed is a type of macroalgae that includes both cultivated and wild types. It is divided into three groups: brown algae (Phaeophyta), green algae (Chlorophyta), and red algae (Rhodophyta) (Araujo & Peteiro, 2021). Brown and red algae are frequently utilised as food sources, being rich in carbohydrates and low in fat, akin to vegetables like lettuce and celery, and serving as effective roughage agents (Peñalver et al., 2020). Aware et al. (2022) reported that 100 g of algae provides ample daily human vitamin requirements, including vitamins A, B2, and B12, and a significant portion of vitamin C. Natural seaweed compounds or extracts offer milder effects compared to synthetic drugs like butylated hydroxyanisole and butylated hydroxytoluene. Around 500 species of seaweed have been utilised by humans for agricultural, culinary, and chemical purposes. Notably, red seaweeds such as *Kappaphycus*, *Eucheuma*, *Gracilaria*, as well as brown kelps such as *Laminaria* and *Undaria*, are cultivated in aquaculture for human consumption or gelling compound extraction (Leandro et al., 2020). Significantly, seaweed production saw a notable surge from 2000 onwards, reaching 174,100 tonnes in 2018, indicating an average annual growth from 16,100 tonnes in 2000 (FAO, 2020).

Kappaphycus, a unicellular red alga belonging to the genus Rhodophyta, is a marine macroalgae within the Rhodophyceae class. Specifically, *Kappaphycus alvarezii* (Doty) Doty ex Silva, previously referred to as *Eucheuma cottonii*, is categorised under the order Gigartinales, which comprises significant phycocolloid producers primarily found in the families Furcellariaceae, Gigartinaceae, and Solieriaceae (Rupert et al., 2022; Jalal et al., 2023). Notably, certain members of Gigartinales are highly sought after commercially due to their unique properties. *K. alvarezii* has been extensively employed due to its notable nutritional attributes. This extensive analysis focuses on the most recent publications concerning the pharmacological and phytochemical properties of *K. alvarezii*, which demonstrate its prospective utility in the nutraceutical, cosmeceutical, and pharmaceutical sectors. *K. alvarezii* is rich in various phytochemical constituents, such as lectins, phenolics, flavonoids, alkaloids, terpenoids, phytosterols, and the polysaccharide carrageenan, all of which potentially contribute to its pharmacological activity. *K. alvarezii* has been shown to possess antioxidant anti-inflammatory, antibacterial, antiviral, antifungal, anticancer, and antidiabetic properties in both in vivo and in vitro studies (Jalal et al., 2023). Because of its phytochemical and pharmacological attributes, *K. alvarezii* holds economic importance as a primary producer of κ-carrageenan (Bulboa Contador et al., 2020). Research has highlighted the potential of *K. alvarezii* in protecting against liver disease, attributed to its rich antioxidant content, particularly phenolics. These antioxidants have shown promise in shielding the liver from oxidative stress, a critical factor in liver cirrhosis (Sekar & Chandramohan, 2008; Shihab et al., 2023).

Despite this, there is still a scarcity of studies examining the antioxidant activity of *K. alvarezii* concerning oxidative stress and antioxidant enzymes in hepatic tissues. Bridging the gap between acknowledging antioxidant activity and demonstrating its therapeutic impact, particularly in liver health, necessitates further investigation. Thus, the aim of this study is to assess the antioxidant properties of the ethanolic extract of *K. alvarezii* (EEKA) and their potential impact on liver disease, particularly through experimentation involving carbon tetrachloride (CCl_4)-induced liver damage in rats. The current study seeks to address the knowledge deficit by conducting a thorough investigation into the potential therapeutic advantages of EEKA in mitigating oxidative stress-induced liver disease induced by CCl_4. The objective of this undertaking is for a scholarly contribution towards the realm of enhanced liver disease treatment modalities.

MATERIALS AND METHODS

Laboratory supplies

Ethanol, ascorbic acid, gallic acid, sodium carbonate (Na_2CO_3), Folin-Ciocalteu reagent, CCl_4, 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, 2,4-dinitrophenylhydrazine (DNPH), α-ketoglutarate, L-aspartate, L-alanine, and other biochemical assays were obtained from Sigma-

Aldrich (Burlington, MO, USA). The chemicals and reagents that were used have been selected for their exceptional purity.

The equipment used in this study were obtained from the Biotechnology Research Institute, Universiti Malaysia Sabah, Sabah, Malaysia. It included a freezer (Thermo Fisher Scientific, Waltham, MA, USA), rotary evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland), Soxhlet extractor (Bionics Scientific, Delhi, India), freeze dryer (Labconco, Kansas City, MO, USA), spectrophotometer (PerkinElmer, Waltham, MA, USA), refrigerated centrifuge (Beckman Coulter, Brea, CA, USA), and homogeniser (Kinematica AG, Malters, Switzerland).

Sample preparation

Whole *K. alvarezii* samples (Figure 1) were collected in July 2012 from the Mini Estate Sejahtera of Sebatik Island in Semporna, Sabah, Malaysia. Their identification and authentication were done by Assoc. Prof. Dr. Wilson Thau Lym Yong from the Biotechnology Research Institute, Universiti Malaysia Sabah. The samples (voucher no.: GS004) were placed in the plant tissue culture laboratory at the same institute. Upon collection, the samples underwent washing with tap water to eliminate salt, epiphytes, and debris (Sivasankari et al., 2006). Subsequently, they were rinsed in distilled water. Following the cleaning process, the raw seaweeds were shade-dried for 72 h.

Figure 1: Sample of *K. alvarezii* obtained from the Mini Estate Sejahtera on Sebatik Island.

Sample extraction

Extraction followed the traditional method outlined by Jinoni et al. (2024) with slight alteration. The extraction process involved the use of a Soxhlet extractor, followed by the removal of ethanol solvent using a rotary evaporator, and freeze-drying to eliminate water content and to obtain the crude extract. Prior to extraction, the samples were homogenised into fine powder using a conventional blender and stored at –20 °C. Approximately 60 g of powdered samples were weighed and transferred into a beaker. Soxhlet ethanolic extraction was performed using 20 g of the sample in 100 mL of 95% ethanol at 78 °C. The rotary evaporator was employed to concentrate the resulting extract. Subsequently, the extracts were transferred into containers, securely sealed, and preserved in a freezer at –80 °C for a duration of 48 h. To facilitate lyophilisation, the tubes were sealed with parafilm, punctured with small holes using a toothpick, and subjected to freeze-drying at –80 °C for 48 h. The resulting powdered outcome was stored in room temperature until further analysis.

Total phenolic content

The determination of total phenolic content (TPC) followed a protocol similar to that described by Awang et al. (2023) with some modifications. Gallic acid concentrations (10–200 µg/mL) were prepared from a 1 mg/mL stock. Folin-Ciocalteu reagent (1.5 mL) was added, followed by incubation at room temperature for 5 min. Na_2CO_3 solution (1.5 mL) was then added, and the mixture was left in the dark for 90 min. Absorbances were measured at 720 nm, and results were expressed as mg GAE/g dry extract.

DPPH assay

The DPPH method, as outlined by Jinoni et al. (2024) with slight modifications, was used to assess the free radical scavenging activity of EEKA. Stock solutions of the sample (5 mg/mL) were prepared, from which eight concentrations (10–2400 µg/mL) were obtained. Each tube contained 2.7 mL of DPPH solution. After vortexing and incubating for 60 min at room temperature, absorbances at 517 nm were measured using a spectrophotometer. Ascorbic acid served as the positive control. The DPPH scavenging capacity of the extract was determined using Equation (1).

$$\text{DPPH free radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

where $A_{control}$ represents the absorbance of the solution without the extract, and A_{sample} represents the absorbance of the extract mixed with the DPPH solution. The results were converted to IC_{50} values (representing the sample concentration necessary to impede 50% of DPPH radicals) through the process of extrapolating the regression analysis.

Experimental procedure

The animal experiment was carried out in adherence to the ethical guidelines of the university and federal laws governing animal experimentation. The Animal Ethics Committee granted approval for the study under protocol number UMS/IP7.5/M3/4-2012. For the investigation, male Sprague-Dawley rats aged 7 to 8 weeks, with a body weight ranging from 150 to 250 g, were chosen based on their good health. Before commencing the experiments, each animal was subjected to a period of acclimation to laboratory conditions. The animals were accommodated in a regulated setting within the animal housing facility of Biotechnology Research Institute, Universiti Malaysia Sabah. Throughout the study, the rats were provided with access to tap water and rodent chows to ensure their well-being and nutritional needs were met.

The experimental animals were categorised into four groups (n = 6), as indicated in Table 1. In Group I, animals were administered normal saline for a period of 14 days. Group II received normal saline for 14 days, followed by the administration of CCl_4 in corn oil (1:1) on the 13th and 14th days. Group III and Group IV were administered EEKA at doses of 150 mg/kg b.wt. and 300 mg/kg b.wt., respectively, for 14 consecutive days, followed by the administration of CCl_4 in corn oil (1:1) on the 13th and 14th days. All administrations were carried out orally using oral gavage via force feeding.

Table 1: Summary of experimental procedure.

Group	Treatment	Period
I	Normal saline, p.o., normal group	14 days
II	Normal saline, p.o. + CCl_4 (1.0 mg/kg b.wt.), p.o., control group	14 days
III	EEKA (150 mg/kg b.wt.), p.o. + CCl_4 (1.0 mg/kg b.wt.), p.o., pretreatment	14 days
IV	EEKA (300 mg/kg b.wt.), p.o. + CCl_4 (1.0 mg/kg b.wt.), p.o., pretreatment	14 days

Each animal that had been treated was sacrificed 24 h after the final administration of EEKA or hepatotoxin inducers. Following the induction of anaesthesia with mild ether, the subject was decapitated. Plasma samples were isolated from blood samples extracted via cardiac puncture into tubes coated with lithium heparin. Following the excision, connective tissue was removed from the liver, and it was cleansed with saline to eliminate any potential blood contamination. Subsequently, the liver tissue was stored in a freezer at –80°C for biochemical analyses to assess the function of hepatic antioxidant enzymes.

Estimation of serum ALT and AST

Blood serum, collected through centrifugation for 15 min at 1,500 rpm and kept at –20 °C for ALT and AST analysis, underwent separate enzymatic assays. For ALT, 0.5 mL of 200 mM L-alanine and 2 mM α-ketoglutarate were combined, incubated at 37 °C for 10 min. Afterward, 0.1 mL of serum was introduced, volume adjusted using sodium phosphate buffer to 1.0 mL, and left to incubate for 30 min. For AST, a similar process was followed using 200 mM L-aspartate and 2 mM α-ketoglutarate with a 60-min incubation. Afterward, 1 mM DNPH was added, incubated for 20 min, and the resulting colour change was measured after 30 min at 510 nm (Reitman & Frankel, 1957).

Preparation of post-mitochondrial supernatant

The tissue fractionations utilised in this study were prepared in accordance with standard procedure, which was modified from the approach outlined by Mohandas et al. (1984) and as described by Iqbal et al. (1999). About 1.17% KCl (w/v) was added to a chilled phosphate buffer (0.1 M, pH 7.4) in which the liver tissue was pulverised with a homogeniser. After centrifugation in a refrigerated centrifuge at 2,000 rpm at 4 °C for 10 min, the remnants of the cell nucleus were separated from the liver homogenate. In order to obtain the post-mitochondrial supernatant (PMS), a 30-minute centrifugation at 10,000 rpm and 4 °C was performed. The reduced glutathione (GSH), lipid peroxidation (LPO), catalase (CAT), and glutathione S-transferase (GST) activities were all determined using this PMS.

Estimation of hepatic enzyme activities

The procedure utilised to measure GSH was that of Jollow et al. (1974). At 412 nm, the absorbance was ascertained utilising a spectrophotometer. The outcomes were quantified and expressed in μmol reduced GSH/g tissue. The procedure for determining the malondialdehyde (MDA) content, which serves as an indicator of LPO, was delineated by Iqbal et al. (1999): the utilisation of thiobarbituric acid reacting substances (TBARS). At 535 nm, a spectrophotometer was utilised to determine the optical density of the supernatant, which directly correlated with the amount of MDA generated in each sample. The findings were measured as nmol MDA formed/g tissue. The CAT activity was measured in accordance with the procedure outlined by Thanebal et al. (2021) and Claiborne (1985). At 240 nm, the changes in absorbance of the solution in the reaction mixture were detected with a spectrophotometer, and the activity was computed as nmol H_2O_2 consumed/min/mg protein. The GST activity was determined utilising the methodology outlined by Habig et al. (1974) and Athar & Iqbal (1998). The absorbance was quantified utilising a spectrophotometer at 340 nm, and the catalytic activity was determined as nmol CDNB conjugate formed/min/mg protein.

Statistical analysis

The results were presented using mean ± standard deviation (SD). To facilitate comparisons, a one-way analysis of variance (ANOVA) was employed, followed by the application of Tukey's Honestly Significant Difference (HSD) test. Data analysis was conducted using IBM SPSS Statistics v. 17.0. Establishing a significance level of *p*-value < 0.05 was performed.

RESULTS

Effect of EEKA on TPC and DPPH radical scavenging activity

The TPC in EEKA was found to be 18.92 ± 0.31 mg GAE/g dry extract, indicating relatively low concentrations of TPC. Additionally, the DPPH assay of EEKA was evaluated at concentrations ranging from 150 to 5000 µg/mL. The IC_{50} values for DPPH radical scavenging activity were determined as follows: ascorbic acid (75.00 ± 0.84 µg/mL) and EEKA (4150.00 ± 0.41 µg/mL), indicating lower antioxidant properties.

Effects of EEKA on serum ALT and AST

The assessment of liver structural integrity involves determining the activities of aminotransferases (ALT and AST). In comparison to the normal group, the serum levels of AST and ALT increased significantly ($p < 0.05$) by 53% and 68%, respectively, in the control group, as shown in Figure 2. For 150 mg/kg b.wt., no significant differences ($p > 0.05$) occurred compared to CCl_4, with only a 7% decrease for AST and a 4% decrease for ALT, respectively. However, EEKA pretreatment at 300 mg/kg b.wt. significantly ($p < 0.05$) inhibited increase in serum ALT and AST induced by CCl_4 by 14% and 25%, respectively.

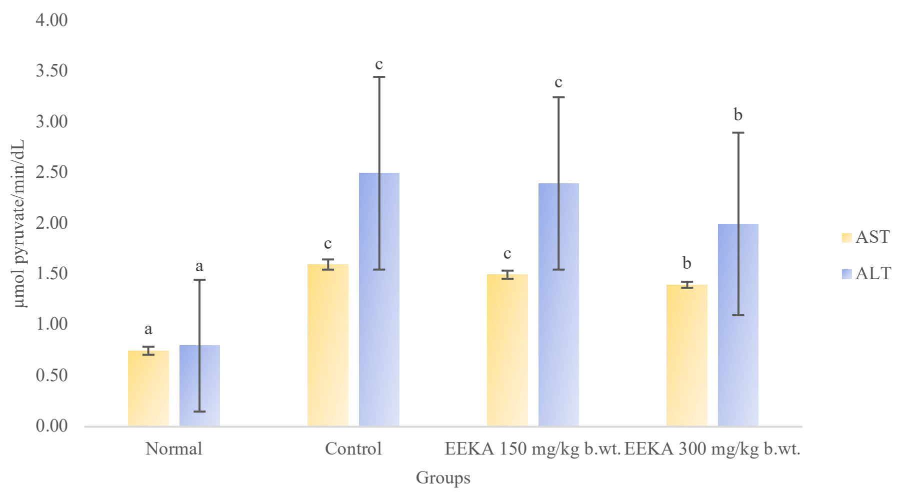

Figure 2: Effects of EEKA on serum ALT and AST levels. Each bar depicts the mean ± SD (n = 6). Distinct letters (within a bar) signify statistically significant variations (one-way ANOVA, Tukey's HSD test, $p < 0.05$).

Effects of EEKA on GSH activity

The GSH content in liver tissue was significantly ($p < 0.05$) reduced by 56% in the control group, as illustrated in Figure 3. This indicates the presence of oxidative stress in comparison to the normal group. On the contrary, administering CCl_4 followed by pretreatment with EEKA at doses of 150 and 300 mg/kg b.wt. provided significantly ($p < 0.05$) protection against GSH depletion, with reductions of 50% and 69%, respectively.

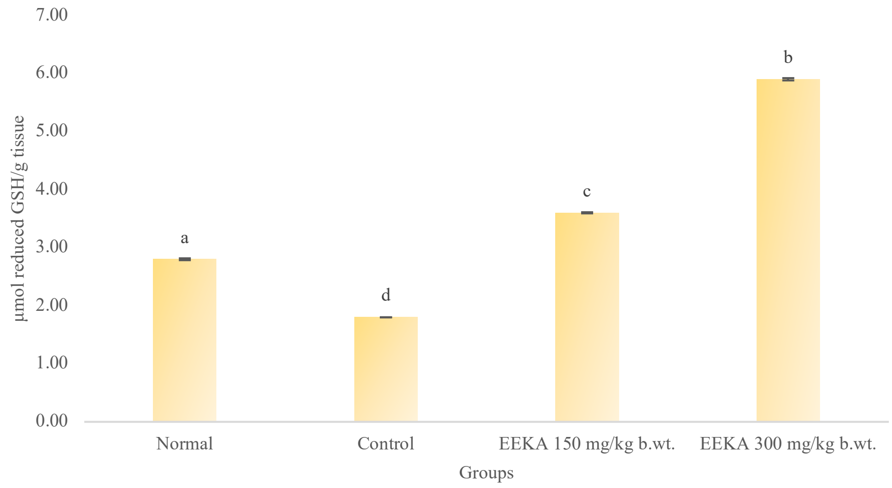

Figure 3: Effects of EEKA on GSH levels. Each bar depicts the mean ± SD (n = 6). Distinct letters (within a bar) signify statistically significant variations (one-way ANOVA, Tukey's HSD test, $p < 0.05$).

Effects of EEKA on LPO activity

Utilising MDA and TBARS reactivity with TBA, which produced a pink chromophore, the TBARS level in liver tissue was determined. The levels of TBARS (expressed as MDA) in the liver homogenate of rats subjected to CCl_4 treatment exhibited a substantial ($p < 0.05$) increase of 4% compared to the normal group, as detailed in Figure 4. However, EEKA at doses of 150 and 300 mg/kg b.wt. significantly ($p < 0.05$) decreased TBARS levels in comparison to the control group by 25% and 43%, respectively.

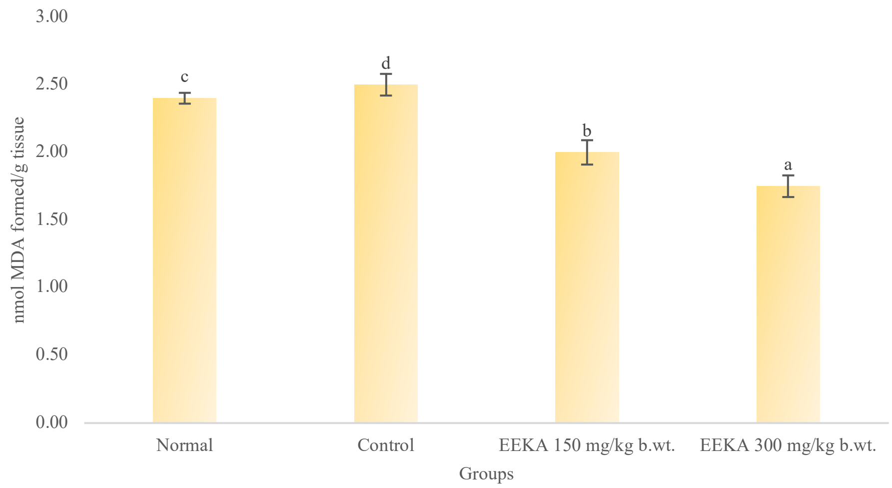

Figure 4: Effect of EEKA on LPO levels. Each bar depicts the mean ± SD (n = 6). Distinct letters (within a bar) signify statistically significant variations (one-way ANOVA, Tukey's HSD test, $p < 0.05$).

Effects of EEKA on CAT activity

Figure 5 illustrates the CAT activity in control and experimental groups of rats. The CAT enzyme activity showed a substantial ($p < 0.05$) seven-fold reduction after CCl_4 administration compared to the normal group. In contrast, pretreatment of rats with EEKA (at doses of 150 and 300 mg/kg b.wt.) significantly ($p < 0.05$) increased CAT activity by 58% and 77%, respectively, in comparison to rats treated solely with CCl_4. This indicates that EEKA pretreatment of rats could potentially restore the antioxidant capacity depleted by CCl_4.

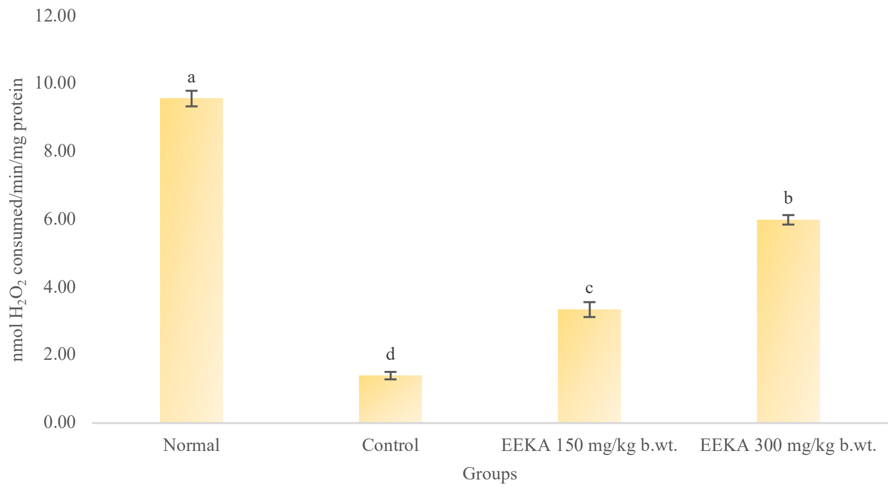

Figure 5: Effect of EEKA on CAT levels. Each bar depicts the mean ± SD (n = 6). Distinct letters (within a bar) signify statistically significant variations (one-way ANOVA, Tukey's HSD test, $p < 0.05$).

Effects of EEKA on GST activity

As shown in Figure 6, the GST activity of liver tissue homogenates from all experimental groups was quantified. The GST activity demonstrated a substantial ($p < 0.05$) decrease of 54% in the control group in comparison to the normal group. This signifies the presence of oxidative stress in hepatic tissues and the depletion of antioxidant reserves. Conversely, in the pretreatment groups (150 and 300 mg/kg b.wt.) with EEKA prior to CCl_4 intoxication, GST activity was significantly increased ($p < 0.05$) by 17% and 42%, respectively, compared to the control group.

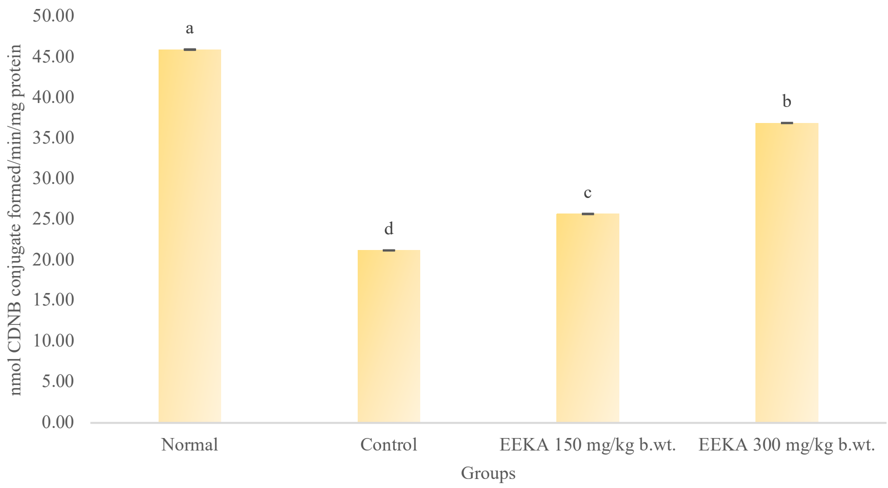

Figure 6: Effect of EEKA on GST levels. Each bar depicts the mean ± SD (n = 6). Distinct letters (within a bar) signify statistically significant variations (one-way ANOVA, Tukey's HSD test, $p < 0.05$).

DISCUSSION

K. alvarezii is a red tropical seaweed that holds significant economic value due to high demand for its cell wall polysaccharides, specifically κ-carrageenan (Lim et al., 2017; Rupert et al., 2022). Carrageenan is composed of sulphate half-esters affixed to the sugar unit of linear polysaccharide chains, which impart water solubility, the ability to form a highly viscous solution, and stability over a broad pH range. Each of its three general forms (κ, λ, and ι) possesses distinct gelling characteristics. Ethanol, with a low boiling point of 69 °C, is commonly used as a solvent in extraction processes because it helps prevent damage or overheating of the chemical compounds (Miazek et al., 2017). The selection of an extraction technique is substantially influenced by the nature of the plant and its components (Lefebvre et al., 2021). The Soxhlet extractor, a laboratory apparatus designed for compound extraction from solid materials, is utilised in the extraction process. Subsequently, the ethanol solvent was delicately extracted from the sample via rotary evaporation using a rotary evaporator, yielding the unrefined extract. Consequently, the ethanol extraction method using a Soxhlet apparatus is preferred over water extraction due to its effectiveness.

The EEKA, evaluated for its TPC and antioxidant properties using the DPPH radical scavenging assay, shows potential for commercialisation in human health consumption. The Folin-Ciocalteu method was used to determine the TPC in this investigation. The method involves the reduction of metal oxides by phenolic acids, resulting in a blue solution with maximal absorption at 765 nm (Blainski et al., 2013; Pérez et al., 2023). Phenolic compounds, known for their antioxidant activities and potent chain-breaking antioxidant properties (Losada-Barreiro et al., 2022), exhibit physiological functions and hepatoprotective effects. These effects are attributed to their redox characteristics (Al Mamari, 2021; Shi et al., 2022), essential for neutralising free radicals, decomposing peroxides, and suppressing singlet and triplet oxygen. Similarly, DPPH is a valuable reagent for assessing the scavenging ability of compounds against free radicals. In alcoholic solutions, it undergoes reduction to its non-radical form DPPH-H, in the presence of hydrogen-donating antioxidants. Characterised by maximal absorbance at 517 nm, DPPH is widely used for evaluating the radical scavenging capacity of various compounds. The formation of the non-radical form of DPPH-H is indicated by a visual change from purple to yellow during the reduction of alcoholic DPPH solution (Gulcin, 2020).

The investigation revealed that EEKA exhibits varying degrees of scavenging ability against DPPH radicals, depending on its concentration. The DPPH radical scavenging activity of EEKA was confirmed by its IC_{50} value, which represents the concentration of an antioxidant required to achieve a 50% reduction in the initial concentration of DPPH radicals (Gulcin, 2020). A lesser IC_{50} value signifies a more potent capacity of sample to function as a DPPH scavenger. In this study, an IC_{50} value of 4150.00 ± 0.41 µg/mL was determined, indicating weaker antioxidant activity compared to the positive control (75.00 ± 0.84 µg/mL). While phenolic compounds constitute a significant group, other antioxidant secondary metabolites such as flavonoids and tannins also play a crucial role in combating oxidative stress and safeguarding liver health (Yogi & Mishra, 2017; Vun-Sang & Iqbal, 2023).

CCl_4 has long been recognised as a hepatotoxin and is known to cause liver damage (Thanebal et al., 2021). Its main route of entry into water is through industrial waste, stemming from its use in chlorofluorocarbons synthesis (Unsal et al., 2020). The accumulation of adipose tissue in the liver and centrilobular necrosis are both associated with CCl_4. Numerous studies have shown that hepatotoxicity induced by CCl_4 is catalysed by cytochrome P-450 in the endoplasmic reticulum of hepatocytes, making it a common model for hepatotoxicity examination (Li et al., 2015). CCl_4 undergoes biotransformation at the endoplasmic reticulum-based cytochrome P-450 system, resulting in the production of the trichloromethyl free radical (CCl_3•). When cellular lipids and proteins react with CCl_3• in the presence of oxygen, trichloromethyl peroxyl radical is generated. Consequently, cell mortality occurs as a result of trichloromethyl peroxyl radicals initiating LPO and disrupting Ca^{2+} homeostasis (Unsal et al., 2020).

Free radicals are produced under specific environmental conditions and as part of normal cellular processes in the body. These molecules lack an electron, making them electrically charged. To stabilise this charge, free radicals seek to either acquire or donate an electron from or to neighbouring molecules. Antioxidants counteract these molecules by neutralising them before they initiate the chain reaction leading to oxidative damage (Gulcin, 2020; Chaudhary et al., 2023; Vun-Sang et al., 2023). To ascertain the capacity of EEKA to alleviate oxidative stress and damage caused by free radicals, its proposed antioxidant properties were evaluated using a rat model of CCl_4-induced hepatotoxicity in an experimental setup. Thus, the evaluation of hepatic health can be accomplished by estimating the activities of serum AST and ALT, enzymes predominantly localised in the cytoplasm and present significantly in the cytosol of hepatocytes (Li et al., 2015). EEKA found in seaweed may have acted to protect the plasma cellular membrane of hepatocytes from being breached by reactive metabolites generated by CCl_4 exposure. This prevention action could have reduced hepatocyte injury and consequently decreased AST and ALT leakage due to cell destruction. The diminished expression of these transaminases in rats treated with EEKA following toxin exposure may explain this phenomenon (Wang et al., 2017).

Pathological oxidative stress can lead to cellular deformity (Gulcin, 2020). The level of GSH significantly influences tissue vulnerability to oxidative injury and hepatic depletion. Tripeptides, prevalent in the liver, act as non-enzymatic biological antioxidants with properties on both intracellular and extracellular surfaces (Iqbal et al., 2020; Vun-Sang et al., 2022). GSH, a significant nonprotein sulphydryl compound, performs various biological functions, including preserving the reduced form of membrane protein-sulphydryl groups. GSH levels were substantially lower in rats exposed to CCl_4 compared to those induced with CCl_4 and treated with EEKA. This disparity suggests that EEKA treatment of CCl_4-induced rats may increase susceptibility to CCl_4. One primary hypothesis suggests that the free radical derivatives of CCl_4 contribute to the formation of lipid peroxidases, leading to hepatopathy. Therefore, effective protection against CCl_4-induced toxicity requires significant antioxidant activity or prevention of free radical generation (Vun-Sang et al., 2022). The current investigation revealed a notable increase in MDA level in the group exclusively treated with CCl_4, indicating elevated levels of LPO causing tissue damage and compromising the ability of antioxidant defence systems to counter excessive free radical production (Iqbal et al., 2020; Thanebal et al., 2021; Vun-Sang et al., 2022; Iqbal et al., 2023; Shah et al., 2023). EEKA treatment considerably inhibited LPO, indicating its antioxidant activity.

Within the body, an effective defence mechanism exists to prevent and neutralise damage caused by free radicals. Oxidative enzymes, including CAT, play a crucial role in this defence mechanism. CAT, classified as a phase 2 enzyme, serves as a non-enzymatic antioxidant and acts as the initial line of defence against reactive oxygen species. It is widely distributed across all animal tissues, with the liver demonstrating the highest activity levels (Iqbal et al., 2020; Thanebal et al., 2021). The primary function of CAT is to convert active oxygen molecules into non-toxic compounds. Reduced CAT activities in the liver were observed in the CCl_4-treated groups, which can lead to various detrimental consequences due to the accumulation of superoxide radicals and hydrogen peroxide (H_2O_2). (Vun-Sang et al., 2022; Iqbal et al., 2023; Shah et al., 2023) CAT primarily protects tissues from highly reactive hydroxyl radicals and decomposes H_2O_2. Interestingly, in the EEKA pretreated group, hepatic CAT activity in CCl_4-intoxicated rats significantly increased. Phase 2 enzymes play a crucial role in detoxifying xenobiotics, providing the body with defence mechanisms against potential environmental hazards (Iqbal et al., 2020; Vun-Sang et al., 2022; Iqbal et al., 2023; Shah et al., 2023). GST, an innate antioxidant defence mechanism found in living tissues, is a key player in this process. A reduction in GST activities is associated with an accumulation of highly reactive radicals, leading to detrimental consequences such as cellular membrane impairment and malfunction (Iqbal et al., 2023; Shah et al., 2023). CCl_4 administration results in the production of peroxyl radicals, leading to the inactivation of GST enzymes and a significant decrease in GST activity in exposed rats. Based on the results, EEKA demonstrates the potential to maintain or enhance phase 2 enzymes, including GST.

Prior research has conclusively established that antioxidative enzymes serve as the principal defence mechanism against ROS and other free radicals. This research aims to assess the antioxidant potential of EEKA against ROS, using a rat hepatotoxicity model induced by CCl_4 intoxication. The investigation evaluates the levels of ALT and AST in serum, as well as GSH, LPO, CAT, and GST in hepatic tissues. This study enhances understanding of how *K. alvarezii* administration may reduce oxidative damage in the liver. Additionally, this research suggests that EEKA could serve as a viable nutritional alternative to current pharmacological methods for reducing hepatotoxicity.

Relying solely on TPC and DPPH assays may limit the comprehensive assessment of antioxidant potential in EEKA, as these assays provide valuable but incomplete insights into antioxidant activity. They may not identify the specific compounds that contribute significantly to antioxidant activity, crucial for understanding the oxidative stress mechanism. Therefore, additional assays such as ferric reducing antioxidant power and oxygen radical absorbance capacity should be emphasised for EEKA to further delineate its ability to mitigate oxidative stress, while high-performance liquid chromatography can elucidate the contribution of other compound groups to its antioxidant properties. Additionally, the absence of mandatory histopathological analysis represents a significant limitation. Such analysis offers insights into hepatic tissue integrity and complements biochemical analyses, which are essential in animal protocols for evaluating mechanisms or evidence of tissue inflammation induced by substances such as CCl_4. Future recommendations would encompass a broader range of antioxidant assays and histopathological examinations to provide a more comprehensive understanding hepatoprotective potential of EEKA and its impact on hepatic health.

CONCLUSIONS

In summary, the findings suggest that EEKA has the potential to augment or sustain the functionality of hepatic antioxidant enzymes, offering protection against liver injury induced by CCl_4. This protective effect of EEKA can be ascribed to its capacity to function as a scavenger of free radicals, intercepting those involved in CCl_4 metabolism via microsomal enzymes. Incorporating antioxidant-rich marine resources such as *K. alvarezii* into a regular diet may help restore balance, given their multifaceted effects. Hence, based on the results, EEKA exhibits potential as a hepatoprotective agent, likely due to its antioxidant compounds.

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Research Article

Non-Native Plants Provide Nectar and Host Plant Resources to Native Butterflies

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ABSTRACT

Urban restoration projects often aim to completely remove non-native plant species from the restored landscape and replace them with native plant species. However, this is unrealistic as early successional plants in urban settings comprise predominantly of non-native plant species. In this study, we investigate whether native butterfly species showed a preference on using native versus non-native plant species in their nectar sourcing and caterpillar host plant choice in two urban gardens at Monash University Malaysia. Native butterflies significantly preferred non-native plant species as nectar food plants, suggesting butterflies are generalists in nectar sourcing. Native butterflies showed no preference towards non-native plants as caterpillar host plants. However, six native butterfly species in our study (*Zizula hylax*, *Hypolycaena erylus*, *Chliaria othona*, *Junonia orithya*, *Yppthima* spp., and *Eurema hecabe*) can use non-native plants as their caterpillar host plants. This demonstrates the usefulness of these non-native plant species in an urban garden by maintaining native butterfly biodiversity. This study indicates a paradigm shift is required among urban ecologists to recognize that not all non-native plants have negative impacts on biodiversity. For more effective urban restoration planning and management, further research emphasising how native insects use non-native plant species is required to maximise biodiversity and ecosystem services restoration.

Keywords: non-native species; nectar food plants; caterpillar host plants; *Zizula hylax*; *Lantana camara*; *Acraea terpsicore*.

INTRODUCTION

In developing countries, urbanisation is a major threat to biodiversity, disrupting the balance and ecological interaction between species (McDonald et al., 2008). By 2050, 68% of the world's population will inhabit urban areas (Simkin et al., 2022). Most of this urban expansion occurs in areas of high biodiversity, putting tremendous pressure on global biodiversity (Simkin et al., 2022). Thus, it is crucial to emphasise the preservation of biodiversity in urban areas as much as possible, to minimise the impact of urbanisation through restoration projects guided by scientific principles (Lepczyk et al., 2017).

Cities are natural laboratories for testing ecological theories and uncovering new community patterns (Rivkin et al., 2019). This is because newly cleared pockets of areas in the urban landscape undergo different stages of succession, colonisation, and community assembly sorting between species, all within a significantly altered ecosystem (Rebele, 1994). As most existing community ecological theories were derived from research conducted in natural, non-urban systems (Wainwright et al., 2018), the direct application of these theories in the context of urban community and biodiversity planning may not be a matter of direct translation (Aronso et al., 2017).

In most contexts, urban restoration projects aim to restore the biotic communities of profoundly altered landscapes to their pre-urbanised state (Gobster, 2007). These restoration processes are often achieved through re-vegetation using a diverse assemblage of native plant species (Miyawaki, 2004). However, native plant species obtained from relatively undisturbed habitats (i.e., climax species) are often better adapted to habitats at later ecological succession stages (Chazdon et al., 2007). They are, therefore, unsuitable for newly disturbed or substantially altered urban landscapes. For instance, native plant species from pristine environments will most likely have the characteristics of slow growth, low intrinsic rate of population increase, and production of few offspring with long generation intervals (Guariguata & Ostertag, 2001). On the other hand, cleared or highly disturbed landscapes in the urban environment may be more suited for colonisers or generalists with characteristics of rapid development, high intrinsic rate of population increases, and the production of numerous offspring with short generation intervals (Guariguata & Ostertag, 2001).

Urban plant communities are composed of a few anthropogenically selected plant species, such as herbs, edible plants, and aesthetically pleasing bushes that have been introduced globally, the majority of which are non-native species (Jasmani et al., 2017). Despite the loss of genetic diversity, many of these non-native plants are thriving in urbanised conditions. These plants can influence local plant composition and diversity, e.g., through competition, underscoring the need to comprehend how communities and biodiversity can be sustained in the presence of non-native species in novel environments (Kowarik, 2011).

Consequently, the choice of plant species in urban biodiversity projects should not be based on their origin (native or non-native) but on suitable life history characteristics (Faeth et al., 2011). For urban green spaces, depending on the disturbance level (i.e., temperature, light intensity, soil integrity, etc.), plant species with the characteristics and life history of pioneers and generalist species can be gradually introduced to facilitate succession stages (Sullivan et al., 2019).

This study investigates how native butterflies utilise native and non-native plant species in two garden plots at Monash University Malaysia. We surveyed the plants used by butterflies at the garden plots. Butterflies visit plants for two resources: nectar source (nectar food plants) and to lay their eggs for butterfly larval development (caterpillar host plants). Butterflies are generalists when it comes to nectar feeding. Hence, we expect butterflies will not show preference regarding plant origin in food plant visitations. However, butterfly development on host plants is derived from evolutionary-ecological processes (Janz et al., 2001). As such, we can expect butterflies to visit native host plants.

METHODOLOGY

We set up two study plots to investigate the interactions between pollinators (we used butterflies as our pollinator species) and urban plant communities. Both plots were located at Monash University Malaysia (N3°3’47.52”, E101°36’1.26”). The first plot was a garden patch (15.77 m^2), designated to be set up as a butterfly garden for a campus biodiversity initiative (Fig. 1A). This patch was wholly cleared of its original landscaping vegetation (*Leucophyllum frutescens*) and replaced with a mixture of native and non-native plant species in April 2018. In the first garden plot, some of the plants, such as *Arundina graminifolia*, *Spathoglottis plicata*, *Bidens pilosa*, *Passiflora foetida*, *Passiflora suberosa*, and *Stachytarpheta indica*, were harvested directly from a nearby location. Other non-native plants (*Lantana camara* and *Ixora* cultivar) commonly used in landscaping projects and public parks in the state of Selangor were obtained from nurseries in Sunway City. Other species (i.e., *Orthosiphon stamineus*, *Antigonon leptopus*, *Torenia* sp., *Angelonia* sp., and a *Citrus* cultivar), commonly found in private gardens, were donated. The remaining plant species (i.e., *Mimosa pudica*, *Tridax procumbens*, *Ageratum conyzoides*, *Emilia sonchifolia*, and *Desmodium triflorum*) were primarily weeds.

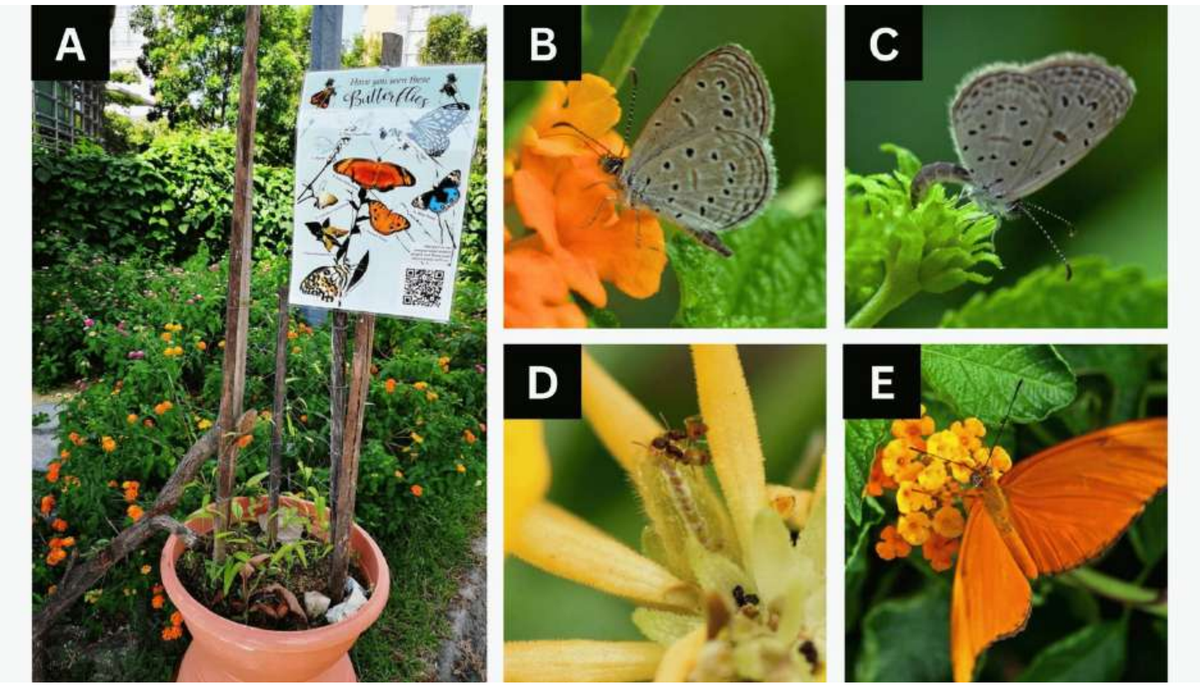

Figure 1: (A) Monash University Butterfly Garden. (B) *Zizula hylax* adult taking nectar from *Lantana camara* flowers. (C) *Zizula hylax* adult ovipositing on *Lantana camara* flower buds. (D) *Zizula hylax* larva feeding on *Lantana camara* flowers while tended to by ants. (E) *Dryas iulia* adult taking nectar from *Lantana camara* flowers.

Plants were identified with the help of the pl@ntNet plant identification website (Affouard et al., 2023). The second plot (129.49 m^2) is an established garden that consists of a mixture of native and non-native herbs, maintained by a campus gardener, and serves as an additional data point for our butterfly plant visitation observation. Hence, we did not identify the plant species at this garden plot. These two gardens had minimal maintenance to allow natural succession and pollinator-plant interactions.

We conducted butterfly observations for two time periods: (1) 11:00 to 11:30 hr and (ii) 15:00 to 15:30 hr for 29 days in April 2019, one year after the garden plots were established. These two time periods correspond to the peaks of butterfly activity. Butterfly species visiting plants inside the garden plots were photographed during observation. These were subsequently identified to species levels using the identification guide 'The Butterflies of the Malay Peninsula (5th Edition)' (Corbet & Pendlebury, 2020). The interaction between butterflies, potential food, and host plants was also recorded. The plant species where the butterfly was observed feeding nectar from the flowers was classified as a nectar food plant. In comparison, the plant where the butterfly was seen laying eggs or caterpillars feeding on the leaves was classified as a caterpillar host plant.

To assess the native butterflies' preference for native versus non-native nectar food plants, we compared the number of native butterfly species visiting native nectar food plants, to the number of native butterfly species visiting non-native nectar food plants. Similarly, to assess the native butterflies' preference for native versus non-native caterpillar host plants, we compared the number of native butterfly species utilising native caterpillar host plants, to the number of native butterfly species utilising non-native caterpillar host plants. Due to the uneven number of native and non-native nectar food- and caterpillar host plants, our data points do not meet assumptions required for parametric testing. Hence, we decided to conduct two independent non-parametric comparison tests (Wilcoxon Signed Rank), one on native butterfly preference on nectar food plants, and the second test on native butterfly preference on caterpillar host plants. These statistical analyses were carried out in JASP (2022) statistical software.

RESULTS AND DISCUSSION

Nineteen butterfly species visited the two garden plots over 29 days of observation, with two non-native species (*Acraea terpsicore* and *Dryas iulia*) recorded (Table 1). The 17 native butterfly species significantly preferred non-native plants for nectar sources (Wilcoxon Signed Rank Test; $Z = 2.953$, $p < 0.05$). Butterflies are generalists in nectar sourcing, responding to the composition of flowers with nectar sources in that landscape (Jain et al., 2016).

Table 1: Butterfly species and their food and host plant visitations over 29 days at garden plots at Monash University Malaysia. Nineteen butterfly species were recorded, with two non-native species (*Acraea terpsicore* and *Dryas iulia*).

Butterfly species	Status	Native food plant	Non-native food plant	Native host plant	Non-native host plant
Zizula hylax	Native	*Emilia sonchifolia, Desmodium triflorum*	*Lantana camara, Ageratum conyzoides, Tridax procumbens,*	*Mimosa pudica*	*Lantana camara*

			Antigonon leptopus		
Acytolepis puspa	Native	*Emilia sonchifolia, Ixora javanica, Desmodium triflorum*	*Lantana camara, Ageratum conyzoides, Tridax procumbens, Antigonon leptopus*		
Hypolycaena erylus	Native	*Ixora javanica, Desmodium triflorum*	*Tridax procumbens, Caesalpinia pulcherrima, Antigonon leptopus*	*Ixora javanica*	*Khaya senegalensis*
Arhopala centaurus	Native		*Bidens pilosa, Antigonon leptopus*	*Syzygium grande*	
Chliaria othona	Native	*Ixora javanica*		*Dendrobium crumenatum*	*Phalaenopsis hybrid*
Ideopsis vulgaris	Native	*Vitex negundo*	*Lantana camara, Tridax procumbens, Bidens pilosa, Caesalpinia pulcherrima, Antigonon leptopus*		
Danaus chrysippus	Native	*Ixora javanica, Vitex negundo*	*Lantana camara, Tridax procumbens, Bidens pilosa, Caesalpinia pulcherrima, Antigonon leptopus*		
Junonia iphita	Native		*Lantana camara, Bidens pilosa*	*Ruellia repens*	
Junonia almana	Native	*Ixora javanica*	*Lantana camara, Tridax procumbens, Bidens Pilosa*	*Ruellia repens*	
Junonia orithya	Native		*Tridax procumbens*	*Asystasia gangetica*	*Angelonia spp.*
Acraea terpsicore	Non-native	*Ixora javanica, Vitex negundo*	*Lantana camara, Tridax procumbens, Bidens pilosa*		*Passiflora foetida, Passiflora suberosa*
Hypolimnas bollina	Native	*Vitex negundo*	*Lantana camara, Bidens pilosa*	*Aystasia gangetica*	
Yppthima spp.	Native	*Emilia sonchifolia*	*Tridax procumbens*	*Tylophora flexuosa*	*Axonopus compressus*
Dryas iulia	Non-native				
Papillio demoleus	Native	*Ixora javanica, Vitex negundo*	*Lantana camara,*	*Calamondin cultivar*	

			Caesalpinia pulcherrima		
Papillio polytes	Native	*Ixora javanica, Vitex negundo*	*Caesalpinia pulcherrima*	*Calamondin cultivar, Murraya koenigii*	
Appias libythea	Native	*Emilia sonchifolia*	*Lantana camara, Ageratum conyzoides, Tridax procumbens, Bidens Pilosa*	*Cleome rutidosperma*	
Eurema hecabe	Native	*Vitex negundo*	*Ageratum conyzoides, Tridax procumbens, Caesalpinia pulcherrima*		*Caesalpinia pulcherrima*
Leptosia nina	Native		*Ageratum conyzoides*	*Cleome rutidosperma*	

The native butterfly species showed no preference for whether the caterpillar host plants were native or non-native (Wilcoxon Signed Rank Test; $Z = 2.132$, $p = 0.025$). Caterpillar host plant choice depends on a narrower set of parameters, especially their co-evolutionary history (Janz et al., 2001). As expected, caterpillar host plant choices were restricted to one or two species, unlike the nectar food plant preferences. Six native butterflies (*Zizula hylax*, *Hypolycaena erylus*, *Chliaria othona*, *Junonia orithya*, *Yppthima* spp., and *Eurema hecabe*) were able to lay eggs on non-native host plants, demonstrating these non-native caterpillar host plants (*Lantana camara*, *Khaya senegalensis*, *Phalaenopsis* hybrid, *Angelonia* spp., *Axonopus compressus*, and *Caesalpinia pulcherrima*) serve a beneficial role in the local butterfly community (Table 1, Fig. 1B-D).

Unsurprisingly, we detected a significant preference for butterfly visitations on non-native flowers in heavily altered urban landscapes with higher proportions of non-native vegetation. The non-native butterfly *Acraea terpsicore* visited flowers of both native and non-native plants for nectar sources. *Dryas iulia*, the second non-native butterfly, was actively flying and interfering with other butterflies but not alighting on any flowers during our survey period (personal observation, Fig. 1E). In addition, the non-native butterfly *Acraea terpsicore* lays eggs on non-native host plants (*Passiflora foetida* and *P. suberosa*), suggesting that this non-native butterfly species was likely introduced with their host plants (Abang et al., 2016). *Acraea terpsicore* introduction is believed to be a result of the invasive population extending to Peninsular Malaysia from Southern Thailand (Burg, 2018).

CONCLUSION

Ultimately, the insistence on using native floral species exclusively in urban rehabilitation is misguided and the common perception that all non-native species are damaging is not always true (Richardson & Ricciardi, 2013) but rather, context-dependent (Boltovskoy et al., 2018). Instead of directing limited resources to remove established exotics, it is more important to first evaluate the role of these non-native plant species play in maintaining or interfering with local diversity and providing ecosystem services. Additionally, studies on the "merits" of non-native species are few and far between (e.g., the potential emergence of new ecosystem services). Still, they must be conducted to better understand their roles in urban ecosystems (Schlaepfer, 2018). This study showed that most non-native plant species in urban gardens can serve as nectar sources for native butterflies and some even as caterpillar host plants for the native butterfly species. We believe that urban biodiversity management can be improved if we carry out more studies such as this one, incorporating other insect-plant interactions such as native insect herbivores, and plant parasites to understand what roles non-native plant species play in an urban community.

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Research Article

Diversity of Pteridophytes in Different Vegetation Types of Mount Musuan, Bukidnon, Philippines

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ABSTRACT

Mount Musuan is one of the Long-Term Ecological Research (LTER) sites in the Philippines. As part of continuous monitoring of pteridophyte distribution and ecological sensitivity, this paper investigated pteridophyte diversity on the established vegetation types of Mount Musuan as basis for conservation management. Two 20 m × 20 m plots were established per vegetation type—Natural Forest, Mixed/Biro, Thailand Shower, and Teak which recorded 38 pteridophyte species, from 28 genera, and 18 families. Highest diversity index was recorded in Mixed/Biro vegetation (H'=1.18), followed by Teak vegetation (H'=1.006), Thailand Shower (H'=0.886), and Natural Forest (H'=0.85). Pteridaceae had the highest number of species (6), Thelypteridaceae (4), Nephrolepidaceae (3) and Lygodiaceae (3). *Sphaeropteris glauca* is an endangered species and a new record for Mount Musuan, and *Angiopteris evecta* as other threatened species. The presence of these species highlights the importance of botanical exploration in Mount Musuan as well as the need for conservation management to protect its remaining biodiversity.

Keywords: diversity; LTER; vegetation type; species richness; pteridophytes.

INTRODUCTION

The Philippines has diverse floral species with a high endemism level, and recent classification shows that the Philippine flora have an estimated number of 1,100 species of ferns and lycophytes, widely distributed among 154 genera and 34 families. Ferns and lycophytes, collectively referred to as pteridophytes, are a group of vascular plants that do not produce seeds and reproduce through spores (Myers et al., 2000; Smith et al., 2006; Pelser et al., 2024; Singh, 2024).

Mount Musuan is one of the Long-Term Ecological Research (LTER) sites in the Philippines, located in Bukidnon, Northern Mindanao (Kim et al., 2018) and is in a decade-long monitoring, targeted by research programs by Amoroso et al., 2013 and 2015b. It is known for its rich biological resources, allowing the growth of pteridophytes and gymnosperms along with different plant species.

Mount Musuan also has the highest litter turnover among the three LTER sites in Mindanao, viz., Mount Apo, Mount Hamiguitan and Mount Musuan, correlating forest litter as an important aspect of healthy ecosystems (Acma et al., 2018). It is also a dormant volcano, and it is known that volcanoes greatly affect landscape evolution which can generate predictable biodiversity processes and usually possess outstanding levels of richness and endemism (Sanin et al., 2024). Pteridophytes located in Mount Musuan are significant due to their high number of species (Amoroso, 2007). However, habitat destruction such as forest fires, environmental pollution as neighbouring places undergo heavy infrastructure development, presence of invasive species, and over-exploitation for recreational activities and ornamental purposes have been observed in Mount Musuan, posing major threats to its biodiversity (Dadang et al., 2020; Khapugin et al., 2020; Paquit et al. 2023). Many pteridophyte species have been investigated extensively, and it was discovered that a substantial number of these are becoming rare and endangered, particularly in Mindanao, Philippines (Coritico & Amoroso, 2020).

This study builds on the preliminary pteridophyte data from the earlier studies (Amoroso et al., 2013; 2015b) and aims to continually assess species diversity, composition, distribution, species importance value, and assessment of dominant species in the established vegetation types of Mount Musuan based on the classification of Paquit et al. (2023) for future pteridophyte flora conservation management plan.

MATERIALS AND METHODS

Entry protocol

A gratuitous permit was obtained from the Department of Environment and Natural Resources with the GP number R10 2023-140, allowing researchers to collect pteridophyte flora from Mount Musuan, LTER Site, Bukidnon, Philippines.

Study site

The duration of the study was from February-March 2023, at Mount Musuan situated at 7° 52' 37.62639" N, 125° 4' 11.0422" E, with the highest altitude of 600 m asl. This site is one of the target research sites for the third LTER program following previous studies conducted in 2013 and 2015 (Amoroso et al. 2013; 2015b), hereafter referred to as ‘LTER 1’ and ‘LTER 2’, respectively. The sampling sites were according to the vegetation types recognised by Paquit et al. (2023). The four vegetation types are classified as, (1) Natural Forest (7°52’57” N 125°03’56” E, 372-383 m asl); (2) Mixed/Biro (7°5242 N 125°04’04” E, 491–499 m asl); (3) Thailand Shower (7°52’46” N 125°04’05” E, 471–474 m asl); and (4) Teak (7°52’38” N 125°04’06 E, 528-535 m asl). Natural Forest vegetation type is classified as a forest composed of indigenous trees, with a tree canopy cover of more than 10 percent (CBD, 2023). Mixed/Biro, on the other hand, is characterised by a combination of different trees as well as the Biro tree (*Rhus* sp.), as the dominant species which can be observed in the area. Thailand Shower vegetation type comprises the Thailand Shower tree (*Cassia siamea* Lam.) dominant in the area. Lastly, Teak (*Tectona grandis* L.) vegetation type is characterised by the presence of the towering Teak trees planted in the area (Figure 1).

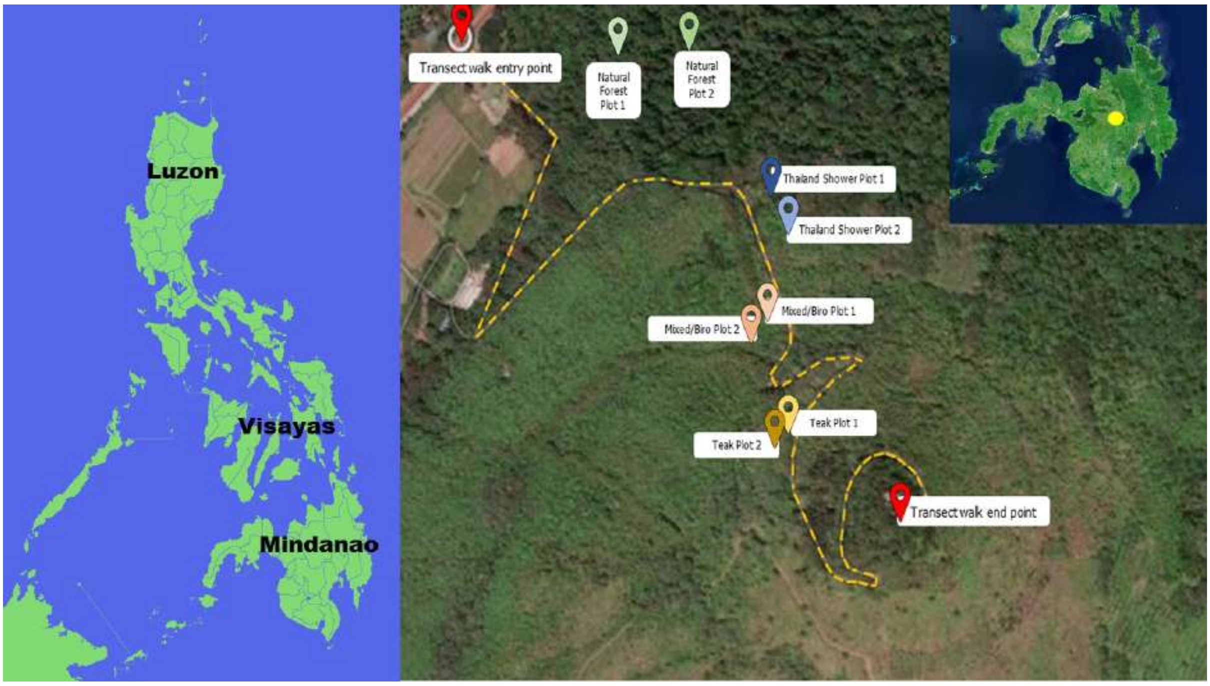

Figure 1: Repeated transect walk map for different vegetation types in Mount Musuan LTER Site, Bukidnon, Philippines.

Sampling procedure and data gathering

Two 20 m × 20 m sampling plots were established at a 50 m interval in elevation and 20 m width on both sides of the trail were marked and established in each of the four vegetation types for the computation of diversity indices. The sampling plots were established objectively based on the spatial distribution of pteridophyte species across different vegetation types. Repeated transect walks and opportunistic sampling was also employed in the study to document the pteridophyte species found outside the established plots for a more comprehensive list of Mount Musuan pteridophytes. All pteridophyte species were recorded, including epiphytic species.

Specimen collection, herbarium voucher processing and identification

Shear and trimming cutter were used in the collection of at least 4 fertile fronds of each pteridophyte flora for the voucher specimens. Specimens were processed in the field using the wet method (Hodge, 1947). All voucher specimens were deposited at the Central Mindanao University Herbarium (CMUH). The specimens were identified and examined by the experts in this study, Co's Digital flora, and Copeland's Fern flora of the Philippines (1958–1961). Initial identification was done by the authors.

The conservation status of the species recorded were listed following the national list of threatened Philippine plants and their categories (DAO, 2017), which in turn largely follows the criteria of the International Union for Conservation of Nature (2023). An additional category established by the national list is 'Other Threatened Species', which refers to 'a species, subspecies, varieties, or other infraspecific categories that is not critically endangered, endangered nor vulnerable but is under threat from adverse factors, such as overcollection throughout its range and is likely to move to the vulnerable category in the near future' (DAO, 2017). The information from the national list serves as a basis for governmental agencies that set environmental policy (Protected Area Management Board [PAMB], Department of

Environment and Natural Resources [DENR] and Local Government Units [LGUs]) for monitoring and protecting threatened species, both within the sanctuary and beyond.

Computation of diversity indices

The computation of the species importance value for the plots established in each vegetation types was done by utilising the formula of Brower et al. (1997) in Microsoft Excel, and the diversity of the pteridophyte flora was calculated using the Shannon-Weiner Index of Diversity, in BioDiversity pro software (McAleece et al., 1997). The species importance value of Importance Value Index (IVI) in each vegetation types was estimated as IVI = RA+RD+ RF, where RA is relative abundance calculated as the number of individuals per species, RD is relative dominance defined as the basal area per species and RF is relative frequency (Curtis, 1959; Mishra, 1968). To statistically test whether there are significant differences in species diversity and the altitudinal differences in each vegetation types, ANOVA (Analysis of Variance) was used. Hence, giving confidence to the quantitative results on the diversity.

Species similarity

BioDiversity pro software (McAleece et al., 1997) was used to determine the similarity of the species composition of the different sampling sites using the Bray-Curtis cluster analysis.

Occurrence of species in different vegetation types

Published literature (Amoroso, 2007; Pielser et al., 2011; Coritico & Amoroso, 2020), data from research projects conducted between 2013 and 2015, i.e. LTER 1 and LTER 2 (Amoroso et al., 2013; 2015b), and fieldwork conducted during the present study, i.e., ‘LTER 3’ using Garmin Global Positioning System (GPS) were used to record the occurrence of species in different vegetation types.

RESULTS

Diversity

Results revealed that among the four established sampling sites, Mixed/Biro has the highest diversity index of H’=1.18. Followed by Teak, H’=1.006, Thailand shower, H’=0.886, and the vegetation type with the lowest diversity is the Natural Forest with H’=0.85 index of diversity (Table 1).

Table 1: Shannon-Weiner Diversity Index of Mount Musuan per vegetation type.

Index	Natural Forest	Mixed Biro	Thailand Shower	Teak
Shannon H' Log Base 10.	0.85	1.18	0.886	1.006
Shannon Hmax Log Base 10.	0.954	1.301	0.954	1.079
Shannon J'	0.891	0.907	0.929	0.933

The ANOVA indicated that there was no significant difference among the diversity of pteridophytes in different vegetation types (Table 2).

Table 2: Analysis of variance (ANOVA) of species diversity and the altitudinal differences in each vegetation type for pteridophytes of Mount Musuan.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.097676	3	0.032559	2.605189	0.124026	4.066181
Within Groups	0.099981	8	0.012498			
Total	**0.197658**	**11**				

Species composition

A total of 35 species of ferns and 3 species of lycophytes belonging to 18 families and 28 genera (Fig. 2, Table 3) were recorded in Mount Musuan, Bukidnon, Philippines. Pteridaceae recorded the highest number of species (6), followed by Thelypteridaceae (4), Nephrolepidaceae and Lygodiaceae (3), Dennstaedttiaceae, Polypodiaceae, Tectariaceae, Dryopteridaceae and Selaginellaceae (2), and families Marattiaceae, Lomariopsidacae, Gleicheniaceae, Hymenophyllaceae, Cyatheaceae, Lycopodiaceae, Ophioglossaceae, Lindsaeaceae and Pteridryaceae with one species each (Fig. 2, Table 3).

Table 3: Checklist of pteridophytes in Mount Musuan, Bukidnon, Philippines from 2013 to 2023.

FAMILY/SPECIES	Amoroso et al. 2013	Amoroso et al. 2015b	Present 2023
I. CYATHEACEAE			
1. *Sphaeropteris glauca* (Blume) R.M. Tryon			/
II. DENNSTAEDTIACEAE			
2. *Microlepia* sp.			/
3. *Hypolepis tenuifolia* (G.Forst.) Bernh.			/
III. DRYOPTERIDACEAE			
4. *Bolbitis heteroclita (*C.Presl) Ching	/	/	/
5. *Bolbitis rhizophylla* (Kaulf.) Hennipman	/	/	/
IV. GLEICHENIACEAE			
6.*Dicranopteris linearis (*Burm.f.) Underw.			/
V. HYMENOPHYLLACEAE			
7. *Hymenophyllum javanicum* Spreng.			/
VI. LINDSAEACEAE			
8. *Odontosoria chinensis* (L.) J.Sm.			
VII. LOMARIOPSIDACEAE			
9. *Cyclopeltis crenata* (Fée) C.Chr.			/
VIII. LYCOPODIACEAE			
10. *Palhinhaea cernua* (L.) Vasc. & Franco			
IX. LYGODIACEAE			
11. *Lygodium circinnatum* (Burm.f.) Sw.	/	/	/
12. *Lygodium flexuosum* (L.) Sw.			/
13. *Lygodium japonicum (*Thunb.) Sw.			
X. MARATTIACEAE			
14. *Angiopteris evecta* (G.Forst.) Hoffm.	/	/	/
XI. NEPHROLIPEDACEAE			
15. *Nephrolepis biserrata* (Sw.) Schott			/
16. *Nephrolepis cordifolia* (L.) C.Presl			/
17. *Nephrolepis hirsutula* (G.Forst.) C.Presl.	/	/	/
XII. OPHIOGLOSSACEAE			
18. *Ophioglossum reticulatum* L.			/
XIII. POLYPODIACEAE			
19. *Selliguea* sp.			
20. *Phymatosorus scolopendria* (Burm.f.) Pic.Serm.			/
21. *Aglaomorpha quercifolia* L.			

22. *Microsorum punctatum* (L.) Copel.			
23. *Pyrrosia longifolia* (Burm.f.) C. V. Morton	/		/
XIV. PTERIDACEAE			
24. *Adiantum philippense* L.			/
25. *Hypolepis tenuifolia* (G. Forst) Bernh.			/
26. *Pityrogramma calomelanos (*L.) Link			/
27. *Pteris ensiformis* Burm.f.	/	/	/
28. *Pteris tripartita* Sw.	/	/	/
29. *Pteris cretica* L.			
XV. PTERIDYACEAE			
30. *Pteridrys microthecia (Fée)* C.Chr. & Ching			/
XVI. SELAGINELLACEAE			
31. *Selaginella* sp. 2	/		
32. *Selaginella usterii* Hieron.	/		/
33. *Selaginella gastrophylla* Warb.			/
XVII. THYLEPTERIDACEAE			
34. *Christella dentata* (Forssk.) Brownsey & Jermy	/		/
35. *Macrothelypteris torresiana* (Gaud.) Ching			/
36. *Pronephrium xiphioides* (Christ) Holtt.			/
37. *Sphaerostephanos unitus* (L.) Holttum			/
XVIII. TECTARIACEAE			
38. *Tectaria polymorpha* (Wall. ex. Hook.) Copel.			/

Figure 2: Habit. **A.** *Lygodium japonicum* (Thunb.) Sw.; **B.** *Pteris ensiformis* M.G. Price; **C.** *Sphaeropteris glauca* (Blume) R.M. Tryon; **D.** *Pronephrium xiphoides* (Christ) Holttum; **E.** *Tectaria polymorpha* (Wall. ex Hook) Copel.; **F.** *Hymenophyllum javanicum* Spreng.; **G.** *Adiantum philippense* L.; **H.** *Cyclopeltis crenata* (Fee) C.Chr.; **I.** *Angiopteris evecta* (G.Forst) Hoffm.; **J.** *Bolbitis heteroclita* (C.Presl) Ching; **K.** *Ophioglossum reticulatum* Hook.; **L**. *Aglaomorpha quercifolia*.(L.) J.Sm.

Species distribution and similarity across vegetation types

Of all the four vegetation types, Mixed/Biro (491–499 m asl) recorded the highest distributed number of species recorded with 20; this is followed by Teak vegetation (528–535 m asl) with 14, Thailand Shower (471–474 m asl) with 11, and Natural Forest (372–383 m asl) with 10 species of ferns and lycophytes.

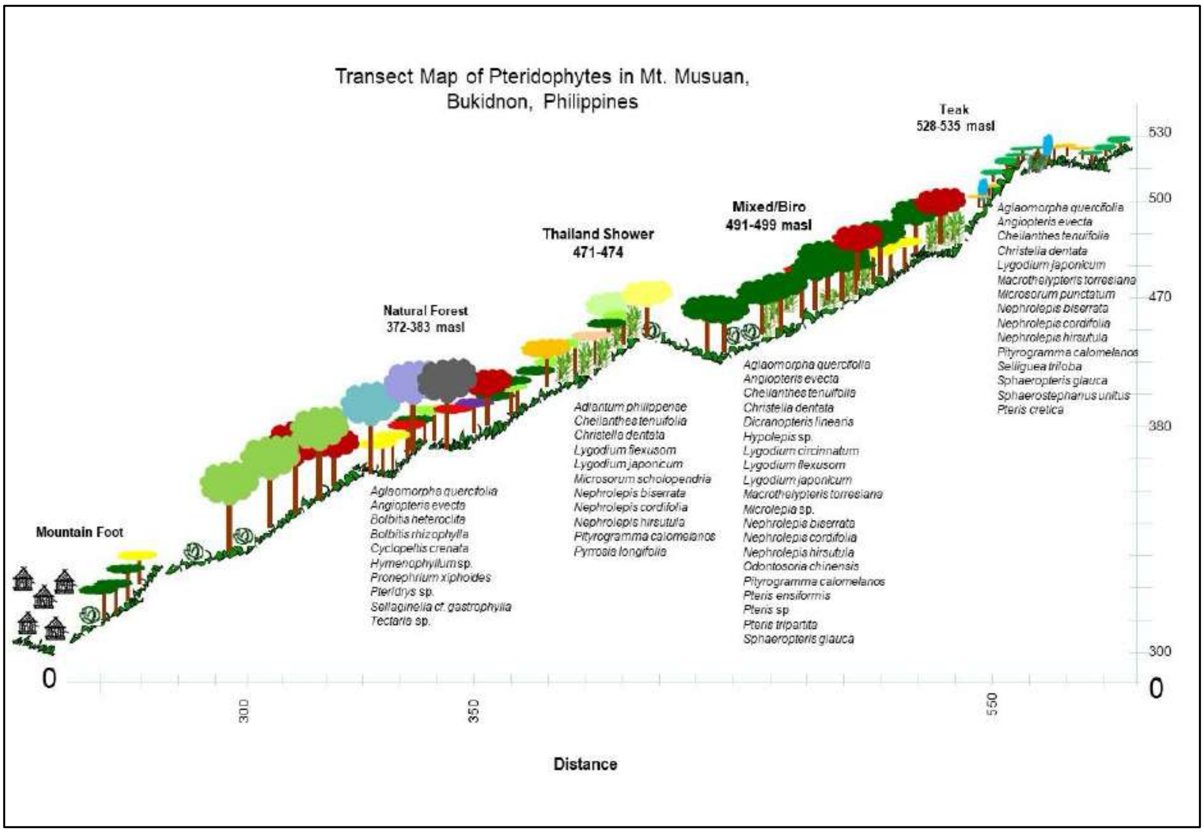

Figure 3: Pteridophyte species occurrence across four vegetation types in Mount Musuan, Bukidnon, Philippines.

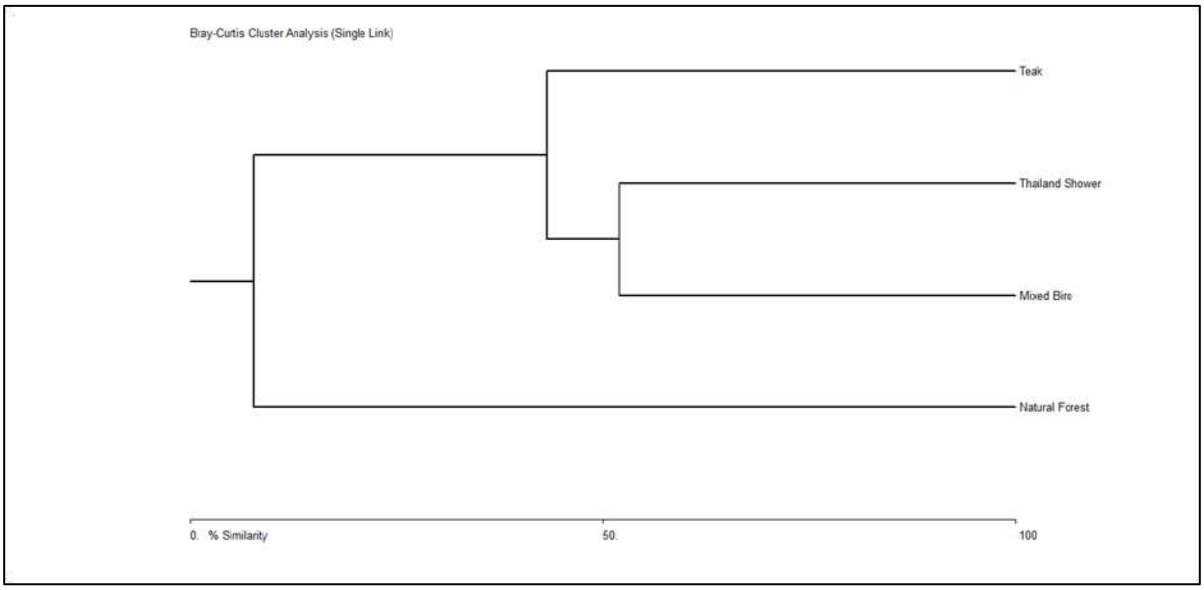

Figure 4: Bray-Curtis species similarity index of pteridophyte species across four vegetation types in Mount Musuan.

Similar species clustered in the vegetations Mixed/Biro, Thailand Shower, and Teak vegetation types (Fig. 4). Some species belonging on these vegetation types include *Adiantum philippense* (Thailand Shower), *Dicranopteris linearis* (Mixed/Biro), *Hypolepis tenuifolia* (Mixed/Biro), *Microlepia* sp. (Mixed/Biro), *Microsorum scolopendria* (Thailand Shower), *Odontosoria chinensis* (Mixed/Biro), *Selliguea triloba* (Teak), and *Pteris cretica* (Teak).

Species importance value

In determining the dominant species in an area, whilst providing an overall estimate of the influence of the species in the community, it is important to compute for the species importance value (SIV). The species listed for the importance value were gathered from the plots established in each sampling sites for each vegetation type (Table 3). Species with high species importance value in Mount Musuan-Natural Forest vegetation includes *Bolbitis rhizophylla* (73.44%), followed by *Bolbitis heteroclita* (66.34%), *Pteridrys microthecia* (42.25%), *Selaginella usterii* (30.03%) and *Aglaomorpha quercifolia* with 29.62% SIV. Mixed/Biro vegetation type recorded high Species importance value of *Nephrolepis biserrata* (51.58%), *Christella dentata* (36.04%), *Nephrolepis cordifolia* (27.90%), *Nephrolepis hirsutula* (20.66%), and *Lygodium circinnatum* (19.05%). The Thailand Shower vegetation type also recorded *Nephrolepis cordifolia* (68.82%), *Nephrolepis biserrata* (52.84%), *Christella dentata* (43.53%), *Nephrolepis hirsutula* (29.87%), and *Microsorum scolopendria* (28.19%). Lastly, Teak vegetation recorded species with high importance value, i.e., *Hypolepis tenuifolia* (63.99%), *Sphaerostephanus hirsutus* (45.60%), *Pityrogramma calomelanos* (30.70%), *Sphaeropteris glauca* (28.55%), and *Nephrolepis biserrata* (22.70%).

Table 4: Species Importance Values (SIV) of pteridophytes at Mount Musuan, Bukidnon, Philippines.

Vegetation Types	Species	SIV	Rank
Natural Forest	*Bolbitis rhizophylla*	73.44	1st
	Bolbitis heteroclita	66.34	2nd
	Pteridrys microthecia	42.26	3rd
	Selaginella wildernovi	30.03	4th
	Aglaomorpha quercifolia	29.62	5th
Mixed/Biro	*Nephrolepis biserrata*	51.8	1st
	Christella dentata	36.41	2nd
	Nephrolepis cordifolia	27.89	3rd
	Nephrolepis hirsutula	20.66	4th
	Lygodium circinnatum	19.05	5th
Thailand Shower	*Nephrolepis cordifolia*	68.82	1st
	Nephrolepis biserrata	52.84	2nd
	Christella dentata	43.54	3rd
	Nephrolepis hirsutula	29.87	4th
	Microsorum scolopendria	28.19	5th
	Hypolepis tenuifolia	64.00	1st
Teak	*Sphaerostephanos hirtus*	54.61	2nd
	Pityrogramma calomelanos	30.7	3rd
	Sphaeropteris glauca	28.56	4th
	Nephrolepis biserrata	22.70	5th

Trend of pteridophyte richness from 2013, 2015 and 2023

The diagram highlights the pteridophyte species richness between the monitoring of Amoroso et al. in 2013 (LTER 1), 2015b (LTER 2), and 2023 (LTER 3), respectively (Figure 5). Results show that the same nine (9) pteridophyte species are recorded for LTER 1, 2, and 3, namely, *Angiopteris evecta, Bolbitis heteroclita, Bolbitis rhizophylla, Lygodium circinnatum,* *Nephrolepis hirsutula, Pteris ensiformis, Pteris tripartita, Pyrrossia longifolia* and *Selaginella usterii.* Additionally, from 2015 (LTER 2) to 2023 (LTER 3), more pteridophytes (9) are recorded, namely, *Aglaomorpha quercifolia, Cyclopeltis crenata, Hymenophyllum* sp., *Hypolepis tenuifolia, Microlepia* sp., *Nephrolepis cordifolia, Pronephrium xiphoides,* and *Pteris cretica.* Notably, two identified species are recorded exclusively in 2015, namely, *Asplenium nidus* and *Huperzia serrata,* and recent assessment (LTER 3) shows no record for these species in Mount Musuan. Furthermore, an additional of 19 species are recorded for LTER 3, namely, *Adiantum philipppense, Hypolepis tenuifolia, Dicranopteris linearis, Lygodium flexusom, Lygodium japonicum, Macrothelypteris torresiana, Microsorum scolopendria, Microsorum punctatum, Odontosoria chinensis*, *Ophiglossum reticulatum, Palhinhaea cernua, Pityrogramma calomelanos, Pteridrys microthecia, Pteris cretica, Selaginella* cf. *gastrophhylla, Selliguea triloba, Sphaeropteris glauca Sphaerostephanus hirsutus,* and *Tectaria polymorpha*. As observed in this study, the increase of species composition from 2013 to 2023 can be explained by the increase in sampling area and the identification of the different vegetation types covering Mount Musuan.

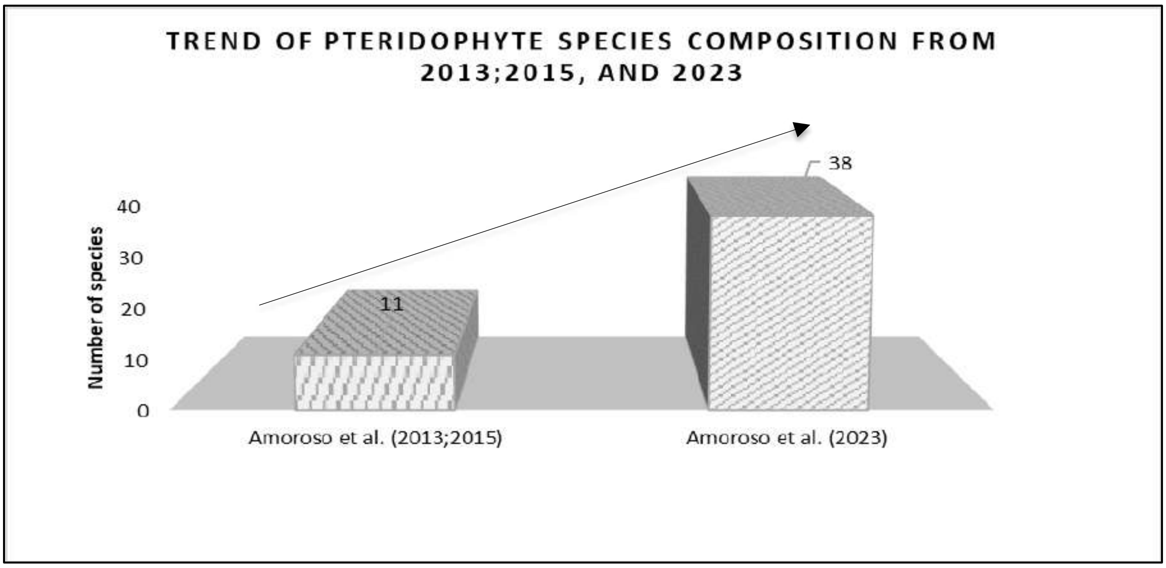

Figure 5: Graph showing increasing trend of species richness from 2013, 2015 and 2023.

Conservation status based on DENR-DAO (2017) and IUCN (2023)

To monitor the conservation status of pteridophyte flora across the three LTER projects, a table was generated based on DENR-DAO (2017) and IUCN (2023) (Table 4). *Asplenium nidus* is categorised as a vulnerable species and was documented in LTER 2 but is no records for LTER 1 and 3. *Angiopteris evecta*, categorised as other threatened species, was recorded for all LTER programs. Notably, the endangered fern species, *Sphaeropteris glauca*, is a new record for Mount Musuan in LTER 3 (2023), implying no records for LTER 1 and 2 for this endangered species.

Table 4: Conservation status of pteridophyte species in Mount Musuan based on DENR-DAO (2017) and IUCN list (2023).

LTER PROJECTS	CONSERVATION STATUS		
	VULNERABLE	OTHER THREATENED SPECIES	Endangered
LTER 1 (2013)	-	*Angiopteris evecta* (Forst.) Hoffm.	-
LTER 2 (2015)	*Asplenium nidus* L.	*Angiopteris evecta* (Forst.) Hoffm.	-
LTER 3 (2023)	-	*Angiopteris evecta* (Forst.) Hoffm	*Sphaeropteris glauca* (Blume) R.M.Tryon

DISCUSSION

The diversity of pteridophytes in Mount Musuan has already been in a decade-long monitoring from 2013 to 2023 by Amoroso et al. (2013; 2015b). Recent results show that Mount Musuan has a low index of pteridophyte diversity as Fernando et al. (2008) defined sampling sites with <1.99 index of diversity is classified as low species diversity. However, occurrence of the unique fern *Ophioglossum reticulatum* L. in Mount Musuan, only observed during the wet season, and newly recorded endangered species *Sphaeropteris glauca,* indicate the importance of Mount Musuan as a suitable environment for these significant ferns to thrive.

Mount Musuan has four types of vegetation (Paquit et al., 2023), among these, Mixed/Biro has the highest diversity index with the most unique soil type observed by the presence of rocks, which provide an appropriate substrate for epiphytic ferns to attach (Delos Angeles et al., 2020). Meanwhile, the Natural Forest vegetation type was recorded to have the lowest diversity index due to the presence of the invasive understory plant *Donax canniformis* (G.Forst) K. Schum. which dominates the natural forest, disabling proper sunlight absorption for pteridophyte species. This species cohabitation where competition occurs is one of the factors, along with microclimate, area sampled, and elevation gradient that influence pteridophyte diversity (Kessler, 2010).

Human activities in Mount Musuan can also affect species composition, where activities that can be observed include, but are not limited to, tourism, deforestation, and conversion of forests for various specific purposes.

Implications of distribution

The highest number (20) of pteridophytes in Mixed/Biro vegetation type can be explained by the presence of tall trees enclosed in large canopies, this canopy layer composition hosts a wide variety of understory plants, which represents the largest component of biodiversity in most forest ecosystems and plays a key role in forest functioning (Mestre et al., 2017). The Mixed/Biro vegetation is also considered to be the least disturbed of all the plots, located near a steep cliff with the presence of rocks—where lithophytic pteridophytes thrive. Ecological study shows that epiphytic and lithophytic ferns survive in different ecology types and pteridophytes in general, can tolerate dry soil environments and direct sunlight (Suksathan, 1998; Perida et al., 2023). Furthermore, species distribution of the aforementioned species is consistent with their ecology in that they occupy a variety of habitats—these pteridophytes thrive in moist environment that can still be penetrated by sunlight, intolerant of shade, opportunists of open, disturbed areas (Hennipman et al., 1990; Khwaiphan & Boonkerd, 2008; Brownsey and Perrie, 2018; Russell et al. 1998; McGlone et al., 2005).

The Natural Forest vegetation type, on the other hand, has the lowest number of distributed species with 10. Although this vegetation type is ideal for some pteridophytes, the low number of species distribution can be explained by the dominating presence of the understory plant—specifically *Donax canniformis* (G. Forst.) K. Schum., a native understory plant that is common in low and medium elevation secondary forests, especially along streams (Pelser et al., 2011). Notably, hygrophilous ferns in this study such as *Hymenophyllum javanicum* Spreng., *Bolbitis rhizophylla* (Kaulf.) Hennipman*, Bolbitis heteroclita* (C. Presl) Ching*, Pronephrium xiphoides* (Christ) Holtt*, Cyclopeltis crenata* Fée*,* and *Tectaria polymorpha,* (Wall ex. Hook) Copel are found in the Natural Forest vegetation type where the presence of a small stream and large canopy cover are observed. This suitable habitat is where most moist-loving pteridophytes thrive and greatly affect the species richness of pteridophytes in Mount Musuan (Amoroso et al., 2015a; 2016).

Aglaomorpha quercifolia is the most widely distributed species across the four vegetation types. Ecological information of this species shows that *A. quercifolia* thrives on dry rocks on hillsides in light shade or at the edge of forests, and are fairly common at low altitudes (Chayamarit and Balslev, 2019)—which is consistent with most of the vegetation type characteristics in this study.

CONCLUSION

This study revealed that Mount Musuan has a low index of pteridophyte diversity (38 species, 28 genera, 18 families). Diversity in different vegetation types showed that Mixed/Biro vegetation recorded the highest index of diversity with H'=1.18, Teak vegetation with H'=1.006, Thailand Shower with H'=0.886, and Natural Forest with H'=0.85. *Bolbitis rhizophylla, Bolbitis heteroclita, Pteridrys microthecia, Selaginella usterii,* and *Aglaomorpha quercifolia* are recorded to have the highest species importance value, thus, identified as the dominant species in the different vegetation types. *Sphaeropteris glauca,* a new record in Mount Musuan, is categorised as Endangered species, meanwhile, *Angiopteris evecta* as Other Threatened Species; *Aglaomorpha quercifolia* is also classified as an Economically Important Species. The findings of this study provide necessary information for the potential drafting of scientific-based policies for the protection and conservation of pteridophyte species in Mount Musuan.

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Research Article

An Inventory of Bats (Chiroptera) in Mount Musuan, Maramag, Bukidnon, Philippines Conducted Between 2013 and 2023

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ABSTRACT

Bats play a crucial role in the ecosystem. However, their richness and abundance is declining, especially in tropical countries like the Philippines. This study was conducted to monitor and compare the species richness, abundance and status of bats in Mount Musuan, Maramag Bukidnon over 3 sampling periods between 2013 and 2023. Three sampling sites were established in the study area. Only the mist netting strategy was applied to capture bats, which were identified through morphometric measurements and field guide. The IUCN Red List was referred to determine the conservation statuses of the recorded bats. A total of 11 species belonging to 4 families were recorded: Megadermatidae, Pteropodidae, Rhinolophidae and Vespertillionidae. The highest number of individuals captured were *Ptenochirus jagori* (186), *Cynopterus brachyotis* (113) and *Miniopterus schreibersi* (53). Of the bats collected, 82% (9) were classified as Least Concern, 9% (1) as Endangered and 9% (1) as Near Threatened. Out of 11 species, 7 (64%) species were Philippine endemic species. This study contributes important information to the existing knowledge of bats in the region, highlighting their abundance and significance in local wildlife. The results can be used to inform conservation strategies of these species.

Keywords: Bats; endemism; Mindanao; Mount Musuan; monitoring.

INTRODUCTION

The Philippines is home to a rich variety of unique flora and fauna, with many species endemic to the archipelago (Cabras et al., 2017). Among these diverse fauna groups are bats. Bat diversity and richness in the Philippines has been well-documented and studied (Tanalgo and Hughes, 2018). Heaney et al. (2010) conducted comprehensive surveys of bat species in Luzon, identifying over 70 species, including several endemics, such as the Philippine pygmy fruit bat (*Haplonycteris fischeri*) and the endangered giant golden-crowned flying fox (*Acerodon jubatus*). Mildenstein et al. (2005) explored the habitat preferences of flying foxes in Subic Bay and found that these bats heavily rely on primary and secondary forest habitats for roosting and feeding, highlighting the impact of deforestation on their populations. Insectivorous species, such as those documented by Wiles et al. (2011), have been found to play a crucial role in controlling agricultural pests, yet the broader implications of this ecosystem service remain underexplored. However, some remote areas in the Philippines particularly in Southern Mindanao have yet to be explored and studied in detail (Tanalgo and Hughes, 2018).

Bats are essential to the ecosystem because they perform critical ecological functions such as insect pest control, pollination, and seed dispersal (Costa et al., 2018). Species of fruit bats, particularly those in the genus *Pteropus*, help pollinate native plants and crops such as durian (*Durio zibethinus*), bananas (*Musa* spp.) and the kapok tree (*Ceiba pentandra*) (Heaney et al., 2010). Many tropical fruits, including economically valuable crops, rely on bat pollination. These functions are vital for maintaining and restoring forests and promoting the growth of fruit trees that rely on bats. Additionally, bats act as bioindicators for ecosystem health, providing insights into environmental quality (Stahlschmidt and Bruhl, 2012). Because bats are sensitive to changes in habitat quality, pollution levels, and climate conditions, they are often among the first species to show signs of ecological stress (Heaney et al., 2010), particularly the Philippine cave-dwelling bat species like *Eonycteris robusta* that are vulnerable to habitat disturbances.

The major threats to bat populations in the Philippines include habitat loss and fragmentation, agricultural land conversion, hunting, cave exploitation, extractive industries, climate change, and logging (Tanalgo and Hughes, 2019). These threats have led to significant declines in bat populations, particularly for species that depend on intact forest habitats (Voigt and Kingston, 2016).

Mount Musuan, also known as Mount Kalayo, is a stratovolcano located in the province of Bukidnon on the island of Mindanao in the Philippines. With an elevation of 646 meters above sea level, this majestic mountain offers stunning views of the surrounding landscape, along with a unique blend of cultural and natural attractions. The mountain is renowned for its diverse flora and fauna, and provides a suitable habitat for various species of bats. It has also been identified as one of the Long-Term Ecological Research (LTER) sites in the Philippines. Due to its accessibility, Mount Musuan is a popular tourist destination, especially during Holy week. However, reports of small-scale logging in the lowlands raises concern about the potential impact on the richness and abundance of bat species. The bat population in Musuan, Bukidnon has been extensively studied and documented by Mohagan et al., (2009) and Mohagan and Baguhin (2011). However, no additional data on bats has been published since 2011. Their work highlighted significant differences in bat species richness between intact forest habitats and areas disturbed by human activities, underscoring the detrimental effects of deforestation and land conversion.

While these studies provided critical insights into bat diversity and the impacts of habitat degradation, the studies were conducted over a decade ago, during a time when the rate of deforestation and urban expansion in the Philippines was accelerating but not at its current pace. Since then, increased agricultural intensification, and climate shifts have likely affected bat populations in ways that were not fully captured by these earlier studies. This study which conducted over three samplings between 2013 and 2023, provides a comprehensive assessment of bat species present in Mount Musuan, a critical biodiversity site. The long-term nature of this study is essential for understanding the population trends of bats in response to ongoing environmental changes such as deforestation, agricultural expansion and climate change. Long term monitoring is necessary to detect gradual shifts in species richness and abundance that may not be apparent in shorter studies. The study hypothesises that bat species richness and abundance have decreased between 2013 and 2023, likely due to habitat loss, human disturbance, and climate-related changes.

MATERIALS AND METHODS

Sampling area

The study was conducted in Mount Musuan (7.87699˚N, 125.06966˚E), Maramag Bukidnon (Fig. 1). The site features a wet forest environment with ferns-dominated ground cover, and the soil is of mixed muddy and loamy textures. Sampling took place in 2013, 2015 and 2023. The significant gap between 2015 to 2023 was due to restrictions, particularly during the Covid-19 pandemic. In addition, these years were chosen to capture potential medium- and long-term changes in bat populations while accounting for logistical constraints. Specifically, 2013 and 2015 were selected as initial and intermediate assessment points to establish early trends, while the final sampling in 2023 aimed to capture the cumulative effects of environmental changes over the decade. Three sampling locations were selected: the base (forested area), middle (grassland) and peak (mixed grassland and pine tree plantation).

Sampling was done from March to November to collect data in the dry and wet seasons. The area experiences temperatures ranging from 27–29ºC with relative humidity of 80–100%. Mount Musuan experiences an average annual rainfall of approximately 2,500 mm, with the dry season from March to May and the wet season from June to November. The wet season typically brings typhoons and heavy rainfall, which can lead to temporary reductions in bat foraging activity due to adverse weather conditions. Conversely, the dry season offers more favourable weather, making it an ideal time for sampling.

Figure 1: Study site map. A) Philippines and Mindanao Island, Mount Musuan (red spot). B) Mount Musuan, Maramag, Bukidnon (©2023 Google, image).

Collection and identification of bats

Bats were collected using mist nets set at different levels: 0-5 meters for ground nets, 5-10 meters for subcanopy and above 10 meters for canopy nets. Bats were collected during both the dry and wet seasons. Captured bats were placed in a cloth bags and their morphometric measurements were recorded including head length, ear length, tail length, forearm length, tarsus length, and total body length. The bats were identified using the taxonomic guides by Ingle and Heaney (1992) and Ingle et al., (1999). Voucher specimens were collected and deposited at the Central Mindanao University Museum. Bats released were marked by clipping fur on the head following the standard protocol by Gannon et al, (2009). Bats were photographed by handling them within the shortest time possible as prolonged handling can induce stress, affecting their health and behaviour (Murray et al., 2016). They were also fed sugar syrup before release to help them regain energy and reduce stress.

The conservation statuses and distribution ranges of the identified bats were recorded following the IUCN Red List of Threatened Species (IUCN, 2023). This list was referenced to provide up-to-date information on the global conservation statuses of the species, including classifications such as Least Concern, Vulnerable, Endangered, and Critically Endangered. However, no formal IUCN assessment was conducted as part of this study. Instead, the IUCN Red List served as a reference point for reporting the conservation statuses of the species encountered during the sampling period.

RESULTS AND DISCUSSION

Species richness and abundance of bats

During the ten-year monitoring period from 2013 to 2023, a total of 11 bat species were recorded in Mount Musuan. These species belong to 4 families, with the majority from Pteropodidae (8), followed by Megadermatidae (1), Vespertilionidae (1) and Rhinolopidae (1) (Table 1). The 11 species collected during the sampling in Mount Musuan are *Megaderma spasma, Cynopterus brachyotis, Haplonycteris fischeri, Macroglossus minimus, Ptenochirus jagori, Ptenochirus minor, Acerodon jubatus, Miniopterus schreibersii*, *Eonycteris robusta, Eonycteris spelaea* and *Rhinolophus subrufus* (Fig. 2).

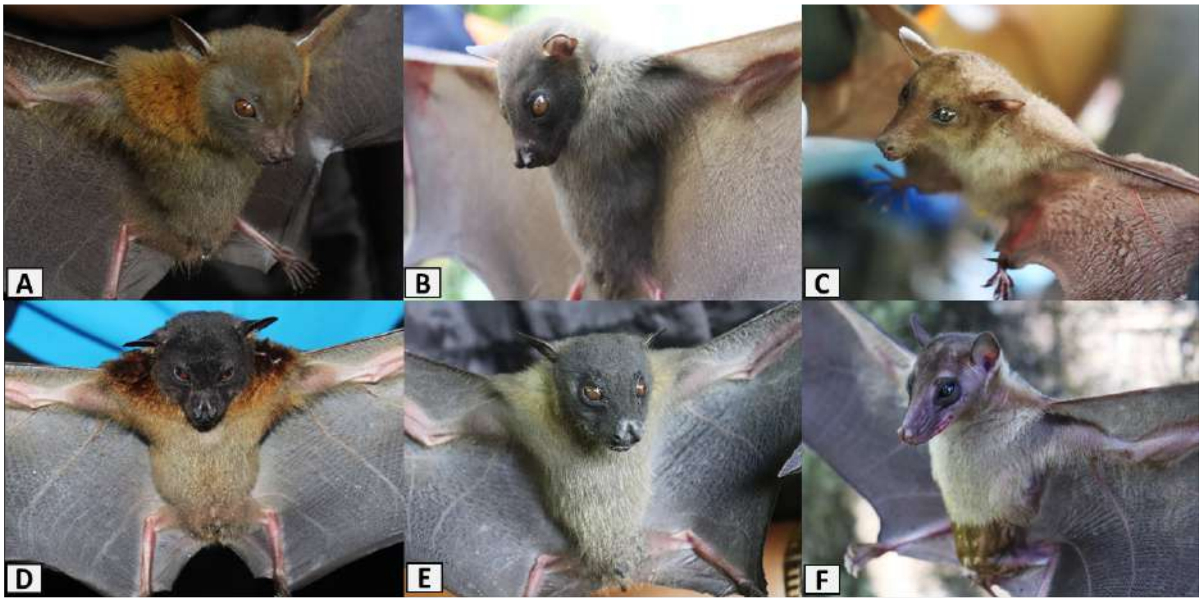

Figure 2: Bats species collected in Mount Musuan (a) *Cynopterus brachyoti*s (b) *Macroglossus minimus* (c) *Haplonycteris fisheri* (d) *Ptenochirus jagori* (e) *Ptenochirus minor* and (f) *Eonycteris spelaea*.

In 2013, only 5 bat species were collected, with *Ptenochirus jagori* having the highest number of individuals (186) followed by *Cynopterus brachyotis* (98) (Table 1). A decline in species was observed in 2015 with only 4 species recorded. Additionally, the number of individuals also decreased with *Cynopterus brachyotis* being the most abundant (113 individuals) followed by *Ptenochirus jagori* (42). The most recent study, conducted in 2023, recorded 10 bat species, but it also showed a decline in the number of individuals for species collected in previous years. *Miniopterus schreibersi* had the highest number of individuals (53) followed by *Cynopterus brachyotis* (48). These results align with findings from previous studies conducted in other parts of Mindanao and across the Philippines, which have also reported *Ptenochirus jagori* and *Cynopterus brachyotis* as common fruit bats in disturbed or secondary habitats. For instance, Achondo et al. (2014) recorded *Cynopterus brachyotis* as the dominant species in Mount Apo, while Salmoy et al. (2017) found *Ptenochirus jagori* to be highly abundant in Mount Hilong-hilong, which may suggest these species are particularly resilient to habitat disturbances in both lowland and montane forest areas.

This study recorded higher number of bat species per unit area compared to the previous studies. In comparison to the study by Achondo et al., (2014), which reported only 8 bat species collected in portions of Mount Apo Natural Park with an elevation of 2,954 meters and an area of approximately 54,000 hectares, and Kidapawan Cotabato City Ecotourism Park. Salmoy et al., (2017) collected 11 species in the mossy forest of Mount Hilong-hilong, (elevation of 2,367 meters and 24,500 hectares) located in Agusan del Sur. Additionally, Amoroso et al., (2019) collected 10 species in Mount Hamguitan (elevation: 1,620 meters, area: 1,942 hectares), and Relox et al., (2017) collected 9 species in Mount Kitanglad (elevation: 2,899 meters, area: 30,000 hectares). However, it is important to note that Mount Musuan with an elevation of 646 meters and a smaller area of approximately 1,600 hectares, exhibits lower elevations and size relative to the other study areas. As cited by Relox et al., (2017) the forest area is positively correlated with the species abundance and richness, as well as the diversity of endemic foliage inhabitants. Furthermore, the quality of the area, vegetation and the size of forest fragments are important factors in the distribution and abundance of bats (Relox et al., 2017). Despite its smaller surveyed area, the relatively high species richness observed in Mount Musuan suggests this habitat still supports diverse bat populations, likely due to the availability of food resources and suitable roosting sites.

According to Mildenstein et al., (2005), a decline in bat populations can indicate habitat destruction, particularly deforestation, and the degradation of cave ecosystems where many bats roost. Monitoring bat populations in agricultural landscapes can also provide insights into the impact of pesticide use and landscape changes on biodiversity (Lunney and Mathews, 2005). Conversely, thriving bat populations can indicate healthy ecosystems with abundant food sources, suitable roosting sites, and balanced predator-prey relationships (Heaney et al., 2010). The restoration of bat populations in areas where they had declined can often coincide with the recovery of overall biodiversity, as bats help rebuild plant communities through seed dispersal and pollination (Mildenstein et al., 2005).

Table 1: Bat species collected in Mount Musuan from 2013–2023

Classification	Species	Common Name	2013	2015	2023
			Number of Individuals		
Chiroptera					
Megadermatidae	1. *Megaderma spasma*	Lesser false vampire bat			1
Pteropodidae	2. *Acerodon jubatus*	Giant golden-crowed flying fox			2
	3. *Cynopterus brachyotis*	Common Shorted-nosed Fruit Bat	98	113	48
	4. *Eonycteris robusta*	Philippine Dawn bat	2	3	
	5. *Eonycteris spelaea*	Lesser Dawn Bat	1		1
	6. *Haplonycteris fischeri*	Philippine Pygmy Fruit Bat			3
	7. *Macroglossus minimus*	Daggered-toothed Long-nose Fruit Bat	7	2	5
	8. *Ptenochirus jagori*	Greater Musky Fruit Bat	186	42	36
	9. *Ptenochirus minor*	Lesser Musky Fruit Bat			17
Vespertelionidae	10. *Miniopterus schreibersii*	Schreiber's Bent wing Bat			53
Rhinolophidae	11. *Rhinolophus subrufus*	Philippine horseshoe Bat			3

Statuses of bats

According to the IUCN Red List (2024), 9 species (82%) are classified as Least Concern, 1 species (9%) as Near Threatened and 1 species (9%) as Endangered (Table 2). Seven (64%) of the bat species are endemic to the Philippines, while 4 (36%) are more widely distributed (IUCN, 2024). In comparison, the study by Nuñeza et al. (2015) recorded a total of 7 endemic species. Achondo et al. (2014) and Escarlos et al. (2019) both recorded 3 endemic species while Salmoy et al., (2017) recorded 4 endemic species. Large forest patches serve as habitats of more endemic species of fruit bats which exhibit strong site fidelity (Relox et al., 2017). Endemic species are particularly important for Philippine biodiversity conservation because they have adapted to specific ecological niches and contribute uniquely to their ecosystems (Mackie and Smith, 2016). Furthermore, as anthropogenic disturbances increase, endemic species may decline while non-endemic species may increase. This is consistent with the findings of Amoroso et al. (2019), who reported that endemism is affected by human activities such as hunting, logging, shifting cultivation and mining. In addition, many endemic bats rely on specific types of vegetation for foraging and roosting, making them less able to adapt to changing environmental conditions (Achondo et al., 2014).

A recent study showed that most of the bats collected were dominated by non-endemic and widespread species *Cynopterus brachyotis*. This may suggest that *C. brachyotis* can tolerate slightly disturbed areas, such as Mount Musuan. However, it was also observed that most endemic species were found in the more forested areas of Mount Musuan. These findings are consistent with the studies of Amoroso et al. (2019) and Salmoy et al. (2017). According to Relox et al. (2017), fragmented habitats support a diverse range of fruit bats including species that are highly tolerant of anthropogenic disturbances, unlike large, continuous forest, which harbour more endemic species that are sensitive to such disturbances.

Table 2: Conservation and Ecological Status of Bats in Mount Musuan, Maramag, Bukidnon.

Classification	Species	Conservation Status	Ecological Status
Chiroptera			
Megadernmatidae	1. *Megaderma spasma*	LC	Widespread
Pteropodidae	2. *Acerodon jubatus*	EN	PE
	3. *Cynopterus brachyotis*	LC	Widespread
	4. *Eonycteris robusta*	NT	PE
	5. *Eonycteris spelaea*	LC	PE
	6. *Haplonycteris fischeri*	LC	PE
	7. *Macroglossus minmus*	LC	Widespread
	8. *Ptenochirus jagori*	LC	PE
	9. *Ptenochirus minor*	LC	PE
Vesperlionidae	10. *Miniopterus schreibersi*	LC	Widespread
Rhinolophidae	11. *Rhinolophus subrufus*	LC	PE

Legend: PE-Philippine Endemic, LC-Least Concern, NT- Near Threatened, EN-endangered

CONCLUSION

The study revealed that Mount Musuan is home to 11 species of bats, of which 9 (82%) are Least Concern, 1 (9%) Endangered and 1 (9%) Near Threatened. Additionally, 7 (64%) species are Philippine endemic species. The increasing number of bats species suggests that suitable food sources and habitats are still present in Mount Musuan. However, anthropogenic disturbances are contributing to the decline in species abundance. While this study provides valuable insights, it is important to acknowledge its limitations, including the uneven dataset across the three sampling years and potential biases in sampling methods that may affect the interpretation of bat populations. This study recommends conducting a diversity analysis and employing additional collection methods such as harp traps, to enhance bat sampling. Furthermore, the use of bioacoustics in monitoring studies is highly recommended. In addition, future research should focus on investigating the habitat preferences of endemic bat species in Mount Musuan and assessing the impact of specific anthropogenic activities, such as agricultural expansion and urban development, on bat populations. Longitudinal studies could provide a clearer understanding of these dynamics and inform conservation management practices.

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Research Article

Tissue Culture Propagation of *Afzelia africana* – A Potential Candidate for Biofuel

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ABSTRACT

Afzelia africana is a medicinal and multipurpose tree that is under permanent pressure from its continuous exploitation for medicine, food and timber products. Adding to being under threat of extinction, its seeds are dormant and recalcitrant with a low rate of seed germination thus posing a challenge on propagation through conventional planting. This study, the first of its kind, reports an *in vitro* shoot regeneration protocol in *Afzelia africana*. *In vitro* propagation method is appropriate for propagating *A. africana* since it can boycott the use of seeds. Leaf, nodal, cotyledonary node (CN), shoot tip, stem and hypocotyl explants were cultured on Murashige and Skoog (MS) medium 1962 supplemented with different concentrations and combinations of plant growth regulators (PGR) and phloroglucinol. Two sets of experiments were done; (1) effect of PGRs and explant types on callus and shoot induction; (2) effect of PGRs on shoot multiplication and elongation from cotyledonary node explant. Both nodal explant and the cotyledonary node induced multiple shoots, 20% of nodal explants formed multiple (3) shoots on MS supplemented with 20mg/L BA and 40% of cotyledonary nodes formed multiple (10) shoots on MS supplemented with 15 mg/L BA and 15 mg/L PG. The response was better in cotyledonary explants, hence the shoot multiplication protocol was maximized using cotyledonary nodes where multiple (10.00 ± 0.00) shoots resulted from CN cultured on both MS supplemented with 15 mg/L BA,15 mg/L PG and 5 mg/L TDZ and MS supplemented with 15 mg/L BA, 15 mg/L PG and 10 mg/L TDZ. This study has paved the path for rapid regeneration of *A. africana* through *in vitro* propagation.

Keywords: African mahogany; multiple; shoots; *in vitro.*

INTRODUCTION

Forest trees are a major trap for Carbon IV Oxide; thus, they play a key role in protecting the earth against global warming. However, the rate of deforestation now is beyond reconciliation, calling for quick and consistent action from experts, policy makers as well as the public. Plant tissue culture method provides a means for mass clonal propagation of trees and serves as a tool for conserving their germplasm (Tefera, 2019). In tree species such as *Pongamia pinnata,* WPM supplemented with 30µM BAP and 1 mM phloroglucinol (PG) induced multiple shoots from nodal explants (Tan et al., 2018). Also, DCR medium supplemented with TDZ promoted bud breaking and adventitious bud formation in *Pinus pinaster* (Humánez et al., 2011).

Afzelia africana is known as African mahogany or African oak and it is a large deciduous tree in the family of Fabaceae. The seeds contain about 31% fat and may be a source of seed oil for domestic and industrial uses. The leaves of *A. africana* are consumed (Mefoh et al., 2013) and ruminants feed on the leaves as forage during the dry season (Oduguwa et al., 1997). Oedema can be treated with a mixture of the decoction of the leaves of *A. africana* with *Syzygium guineensis* leaves and *Xylopia* fruit (Orwa et al., 2009). The nitrogen rich leaves help improve the soil during mulching (Orwa et al., 2009).

Afzelia africana is propagated by seeds, and through budding and cuttings. However, seed predation by animals in the wild is usually high; thus, constraining natural regeneration (Amusa, 2010). The seeds of *Afzelia africana* are dormant and they become recalcitrant when stored (Orwa et al., 2009). Also, the rate of seed germination in the wild is low and its seedlings rarely develop into saplings. This is because the seeds of *A. africana* have hard seed coat, placing a mechanical barrier on the embryo; thus, limiting germination. Animals like birds and rodents feed on the seeds even while still on the parent plant. Indigenous people of the eastern part of Nigeria use the seed as an important condiment in meals, hence the seeds are heavily harvested when they are still on trees. Also, because *A. africana* is high grade timber, it is harvested for construction in some parts of Africa (Ajayi and Arowosoge, 2018). In addition to all these, the IUCN has listed *A. africana* as a threatened species (IUCN, 2024). Furthermore, the seeds of *A. africana* are used as an alternative to wheat flour. This has led to permanent pressure on its natural populations. As a result of this, conservation measures have been recommended (Amusa, 2010).

One of the numerous applications of plant tissue culture is germplasm conservation and transgenic plant production for crop improvement. Propagation of *A. africana* through tissue culture technique is appropriate and recommended because it bypasses the use of seeds with hard seed coat constraint as starting material for its propagation. In addition to its usefulness in propagation, it is only through the tissue culture approach that *A. africana* can be subjected to transgenic manipulations, which will be necessary to study the metabolic pathways involved in oil production, as well as characterize and modify the genes responsible for oil production in its seeds. Again, because *A. africana* seeds contain 31% of oil, it is a potential candidate for biofuel or oil production in the near future. Molecular studies to identify genes and mechanisms responsible for oil production, and thus allowing their modification for increased oil production, will only be possible through a transgenic approach. To date, there is paucity of research on the *in vitro* regeneration of *A. africana,* as an alternative means of propagating and conserving this potential biofuel plant. Hence this study was carried out to explore its regeneration *in vitro* and to establish a protocol for its mass propagation, conservation purposes and for future genetic engineering studies on it.

MATERIALS AND METHODS

Seed collection and culture media and conditions

Viable seeds were obtained from Obafemi Awolowo University, Nigeria (7° 32' N 4° 31' E). All the seeds collected were placed in a transparent jar containing water, and the viable seeds among them were identified as those that sank to the bottom of the water jar. These were sorted and kept safely in a well-labelled container for the study, while the others were removed. Freshly collected seeds of *A. africana* were disinfected properly by subjecting them to soaking in 100g/L mancozeb ($C_4H_6MnN_2S_4.C_4H_6N_2S_4Zn$) for 120 minutes, soaking in 100^{o}C water for 30 minutes and finally in 10% sulphuric acid for 30 minutes (Ogbimi et al., 2023). 5 seeds were used per treatment and the experiment was repeated. Murashige and Skoog (1962) medium supplemented with 3% w/v sucrose and 0.2% w/v phytagel prepared according to standard protocols was used throughout the study. Cultures were maintained in a growth room under a temperature of 25 ± 2 ⁰ C and 16/8 -hour photoperiod.

Callus and shoot induction

The responsiveness of *A. africana in vitro* was tested by culturing the leaf, node, cotyledonary node, shoot tip, stem, and hypocotyl explants on MS supplemented with different concentrations and combinations of plant growth regulators. Explants were collected from 4-week-old *in vitro* grown seedlings. The Percentage Response of Callus Induction (PCRI), Percentage Response of Shoot Initiation (PRSI), were calculated using the below formula, Morphology of Callus (MOC) was recorded by visual observation while number of shoots per explant (NSPE) was counted.

$$PCRI = \frac{Number\ of\ explants\ showing\ callus\ induction\ response\ on\ a\ given\ media\ combination}{Number\ of\ replicates\ for\ that\ media\ combination} \times 100$$

$$PSRI = \frac{Number\ of\ explants\ showing\ shoot\ initiation\ response\ on\ a\ given\ media\ combination}{Number\ of\ replicates\ for\ that\ media\ combination} \times 100$$

Shoot multiplication and elongation

In other to maximize the shoot initiation potential of cotyledonary node explants, cotyledonary nodes were obtained from *in vitro* grown seedlings of *A. africana.* They were cultured on MS media supplemented with different concentrations and combinations of plant growth regulators. Five explants were used per treatment and explants cultured on MS basal media alone was kept as the control. The cultures were maintained in a growth room at a temperature of 25 ± 2 ⁰C and 16 / 8 - hour photoperiod. The number of multiple shoots initiated were recorded. In the elongation experiment, multiple shoots initiated from cotyledonary nodes were transferred into elongation media (MS + 15 mg/L BA+ 15 mg/L PG) after which number of elongated shoots and length of shoots were recorded.

Statistical analysis

The number of shoots, the number of elongated shoots and the length of shoots recorded from the shoot multiplication and elongation experiment were subjected to one-way ANOVA using SAS version 9.2 and the means were separated using Duncan's multiple range test at 0.05 alpha level.

RESULTS

Effect of PGRs on callus induction and shoot initiation

Varying responses resulted from the culture of different explants on MS basal media alone and MS supplemented with different concentrations and combinations of plant growth regulators. Leaf explants was subjected to MS supplemented with 2,4-D (4,10) mg/L in combination with BA (0.1 to 1.0) mg/L. Nodal explants was subjected to MS supplemented with BA (1, 10, 15, 20 and 25) mg/L NAA (0.5 and 5) mg/L. Cotyledonary node was subjected to MS supplemented with BA (1, 3, 15, 20 and 25) mg/L, NAA (0.5 and 5) mg/L, IAA (5 and 10) mg/L and PG (5, 10 and 15) mg/L. Shoot tip explant was subjected to MS supplemented with BA (0.1, 2, 5, 15, 20 and 25) mg/L in combination with NAA (5 mg/L). Stem explant was subjected to MS supplemented with BA (2, 3, 5, 15 and 20) mg/L in combination with NAA (0.5 mg/L) and PG (15 mg/L), and hypocotyl explant was subjected to MS supplemented with BA (1, 5, 15 and 20) mg/L in combination with NAA (0.5 mg/L) and PG (10, 15 and 20) mg/L. Leaf explants cultured on MS basal media alone showed no response (Table 1). However, when the basal media was supplemented with varying concentrations of cytokinin and auxin, callus response was elicited with varying morphology observed (Table 1). Dark-brown compact callus resulted from leaf explants cultured on MS supplemented with 4 mg/L 2,4 – D and 0.1 mg/L BA (Fig. 1A, Table 1) with the highest callus response - 100%, and off-white embryogenic callus formed when leaf was cultured on MS supplemented with 4 mg/L 2,4 – D and 0.7 mg/L BA (Fig. 1B, Table 1) with 60% callus response.

Table 1: Response of leaf explants of *Afzelia africana* on MS supplemented with 2, 4 – D in combination with BA.

2,4 -D (mg/L)	BA (mg/L)	DOCF	PRCI (%)	MOC
0	0	NR	0	_
4	0	NR	0	_
4	0.1	++	100	White/dark brown, compact hard
4	0.2	++	60	White/brown, compact hard/soft
4	0.3	+	60	White/light brown, compact hard
4	0.4	+++	80	White/cream/light brown/dark brown, compact hard/soft
4	0.5	++	40	Cream/dark brown, compact hard/soft
4	0.6	NR	0	_
4	0.7	+	60	White/ friable
4	0.8	++	40	White/dark brown, compact hard
4	0.9	++	20	Light brown/compact soft
4	1	+++	60	Light brown/compact soft
10	0	NR	0	_
10	0.1	NR	0	_
10	0.2	+++	20	White/brown, compact hard
10	0.3	NR	NR	_
10	0.4	+	40	White/compact hard
10	0.5	NR	NR	_
10	0.6	+	30	White/compact hard
10	0.7	+	40	White/compact hard
10	0.8	NR	0	_
10	0.9	+	30	White/transparent soft
10	1	+	20	White/compact hard

Keys: 2, 4 – D – 2, 4 – Dichlorophenoxy acetic acid, BA – Benzyladenine, DOCF – Degree of callus formation, MOC – Morphology of callus, PRCI – Percentage response of callus induction, NR – No response

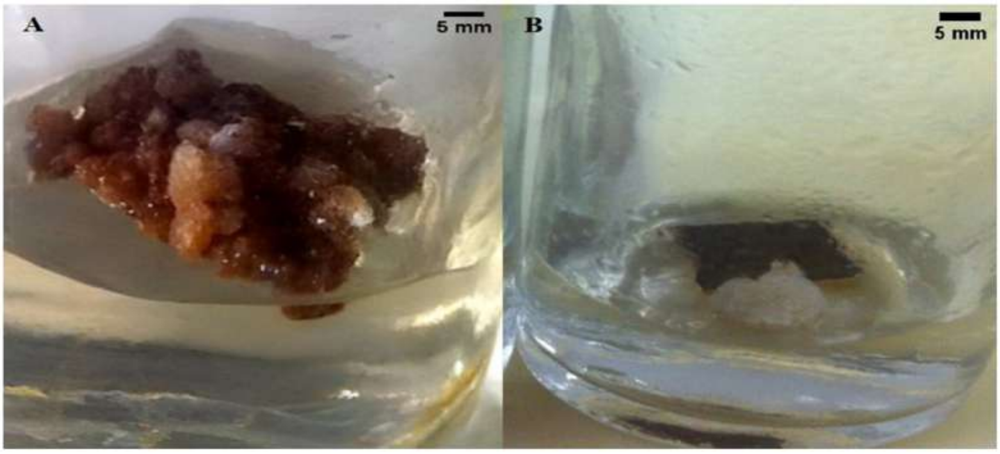

Figure 1: Leaf explant *of Afzelia africana* (A). Dark-brown compact callus on MS + 4 mg/L 2,4 – D + 0.1 mg/L BA. (B). Off-white embryogenic callus on MS + 4 mg/L 2,4 – D + 0.7 mg/L.

Nodal explants also did not show any visible response on MS basal media alone. However, 20% of nodal cultures responded by initiating a bud on MS supplemented with 1 mg/L BA (Fig. 2A, Table 2). The inclusion of NAA to the media – MS + 1 mg/L BA + 0.5 mg/L NAA suppressed shoot initiation and induced callus formation from 40% of nodal cultures. As the concentration of BA was further increased in the media, shoot initiation was inhibited (0%) and callus induction increased to 100% with massive callus production when media was MS + 10 mg/L BA + 0.5 mg/L NAA. On MS + 15 mg/L BA, only shoot initiation was stimulated with one shoot initiating from 50% of the cultures. 20% of the cultures formed 3 shoots on MS supplemented with 20mg/L BA (Fig. 2B, Table 2). The addition of auxin NAA (5 mg/L) to the media containing 20 mg/L BA inhibited shooting and enhanced the formation of friable callus (Fig. 2C, Table 2), while the addition of auxin IAA (5 mg/L) to the same media suppressed shooting (one thick shoot formed) and also enhanced the formation of compact callus (Fig. 2D, Table 2).

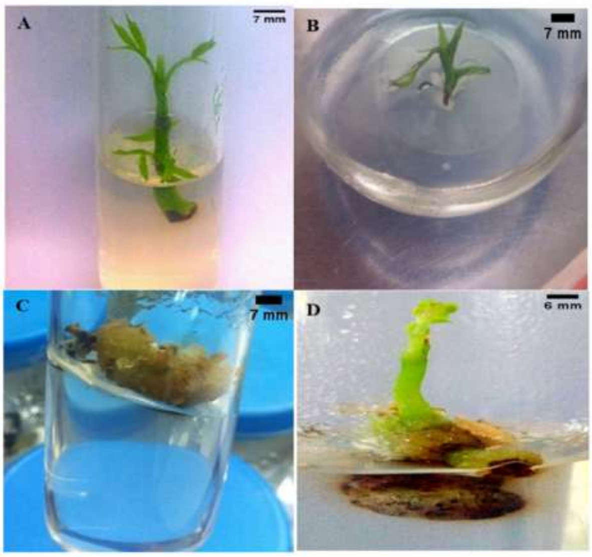

Figure 2: Nodal explant of *Afzelia africana* (A). Young developing bud formed on MS + 1 mg/L BA. (B). Multiple shoots formed on MS + 20 mg/L BA. (C). Friable callus formed on MS + 20 mg/L BA+ 5 mg/L NAA. (D). A thick shoot and basal callus formed on MS + 20 mg/L BA + 5 mg/L IAA.

Table 2: Effect of plant growth regulators on callus induction and shoot initiation from nodal explants of *Afzelia africana*.

BA (mg/L	NAA (mg/L)	IAA (mg/ L)	PRCI (%)/ DOCF	PRSI (%)/ NSPE
-	-	-	-	-
1	-	-	NR	20 / 1
1	0.5	-	40 / +	-
10	-	-	50 / +	40/ 1
10	0.5	-	100 / +++	-
15	-	-	100 / ++	50 / 2
15	5	-	25 / +	-
15	-	5	NR	NR
15	-	10	100 / +	-
20	-	-	20 / ++	20 / 3
20	5	-	25 / +	-
20	-	5	60 / +	40 / 1
20	-	10	75 / ++	50 / 2
-	-	20	50 / +	50 / 1
25	-	-	60 / +	100 / 1
25	-	5	100 / +	-
25	-	10	25 / +	75 / 2
-	-	25	100 / +	-

Keys: - PRCI – percentage response of callus induction, DOCF – degree of callus formation, PRSI – percentage response of shoot induction, NSPE – number of shoots per explants, NR – No response

Cotyledonary nodes of *A. africana*, similar to the response of leaf and nodal explants, also did not show any observable response on MS basal media alone. The addition of 1 mg/L BA to the MS media induced callusing response from 40% of the cultures (Table 3). However, when NAA was added to this media where callusing was initially observed – 0.5 mg/L NAA, the callusing response was inhibited and callus formed only from 20% of the cultures. At higher concentrations of BA; on MS + 15 mg/L BA, shooting response was first observed with two shoots initiating from 67% of the cultures. Shooting response was also inhibited on MS + 15 mg/L BA + 5 mg/L NAA, a shoot being induced from 33% of the cultures. On another media, shooting response was completely inhibited and callusing enhanced from 67% of the cultures, while on MS + 20 mg/L BA, number of shoots increased to three (Fig. 3A Table 3). Again, the addition of NAA to media containing high concentration of BA – MS + 15 mg/L BA+5 mg/L NAA inhibited shoot organogenesis but enhanced callus induction. Callus resulted from 67% of the cultures. The addition of another type of auxin to the media; MS + 15 mg/L BA+5 mg/L IAA, completely inhibited both the callusing and shooting response. Increasing the concentration of BA to 20 mg/L initiated more shoots – 3 from 20% of the cultures (Fig. 5A, Table 3). Cotyledonary node (CN) explants cultured on MS and 25 mg/L BA and 5 mg/L IAA formed friable callus from 75% of the cultures (Fig. 3B, Table 3). 10 shoots were formed from 40% of CN cultured on MS supplemented with 15 mg/L BA and 15 mg/L PG (Fig. 3C, Table 3). The number of shoots were observed to reduce at a higher concentration of PG - MS + 15 mg/L BA + 20 mg/L PG, 8 shoots were formed from 20% of the cultures.

Table 3: Effect of different plant growth regulators on shoot induction from cotyledonary node explants of *Afzelia africana.*

BA (mg/L)	NAA (mg/L)	IAA (mg/ L)	PG (mg/L)	PRCI(%)/ DOCF	MOC	PRSI (%)/ NSPE
-	-	-	-	-	-	-
1	-	-		40 / +	Cream, soft friable/light brown	0 / 0
1	0.5	-		20 / +	Green friable	0 / 0
3	-	-		40 / ++	Cream soft	0 / 0
15	-	-		NR	-	67 / 2
15	5	-		67 / +		0 / 0
15	-	5		NR	-	0 / 0
-	-	10		NR	-	0 / 0
20	-	-		67 / ++	Light brown, soft	20 / 3
20	5	-		75 / +	Cream friable	0 / 0
20	-	5		100 / +	Cream friable	0 / 0
25	-	-		NR	-	50 / 1
25	5	-		100 / ++	Cream, soft friable	100 / 3
25	-	5		75 / +	Cream friable	0 / 0
-	-	-	5	NR	-	20 / 1
-	-	-	20	NR	-	0 / 0
15	-	-	15	NR	-	40 / 10
15			20	NR	-	20 / 8

Keys: PRCI – Percentage Response of Callus Induction, DOCF – Degree of Callus Formation, PRSI – Percentage Response of Shoot Induction, MOC – Morphology of Callus, NSPE – Number of Shoots Per Explant, PG – Phloroglucinol, IAA – Indole Acetic Acid, NAA – Naphtalene Acetic Acid, BA – Benzyladenine, NR – No response

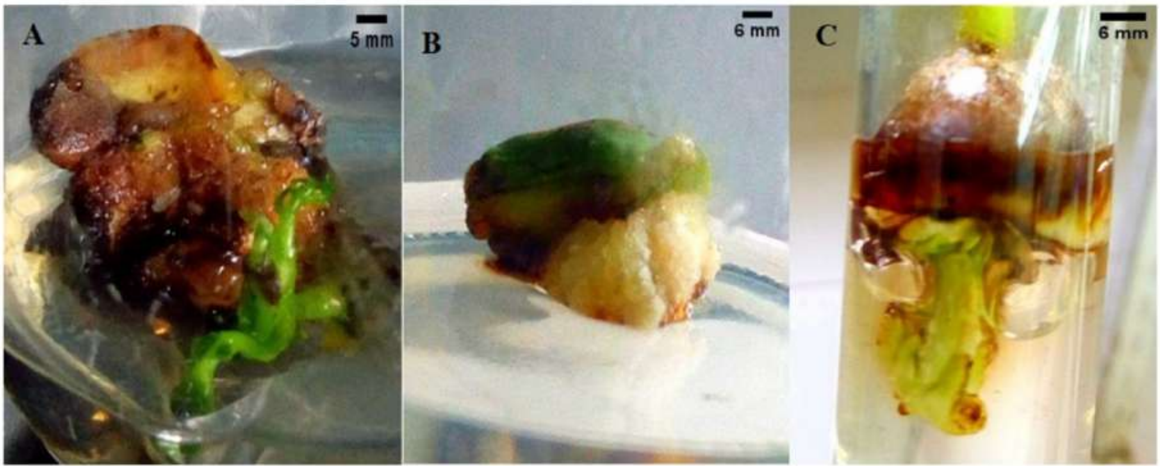

Figure 3: Cotyledonary node *of Afzelia africana* (A). Multiple shoots formed on MS + 20 mg/L BA. (B). Cream friable callus formed on MS + 25 mg/L BA+ 5 mg/L IAA. (C). Multiple buds formed on MS + 15 mg/L BA + 15 mg/L PG.

Shoot tip explants cultured on media with different plant growth regulators showed both shoot organogenesis and callogenesis. One shoot was initiated from shoot tip cultured on MS basal media alone (Fig. 4A, Table 4). At a very high concentration of BA supplemented media, 2 shoots were initiated from 40% of the cultures (Fig. 4B, Table 4). Also, soft cream callus resulted from 40% of cultures in MS media supplemented with 25 mg/L BA and this was the highest recorded.

Table 4: Organogenesis and callogenesis from shoot tip explants of *in vitro* grown seedlings of *Afzelia africana*.

BA (mg/L)	NAA (mg/L)	PRCI (%)/ DOCF	MOC	PRSI (%)/ NSPE
-	-	-	-	20/1
0.1	-	NR	-	20/1
2	-	NR	-	20/1
5	-	NR	-	20/1
15	-	NR	-	20/1
15	5	NR	cream, soft callus	20/1
20	-	20/+	cream, compact brown	20/1
25	-	20/+	cream, soft callus	40/2

Keys: BA – Benzyladenine, NAA – Naphtalene Acetic Acid, PRCI – Percentage Response of Callus Induction, MOC – Morphology of Callus, PRSI – Percentage Response of Shoot Induction, NR – No response

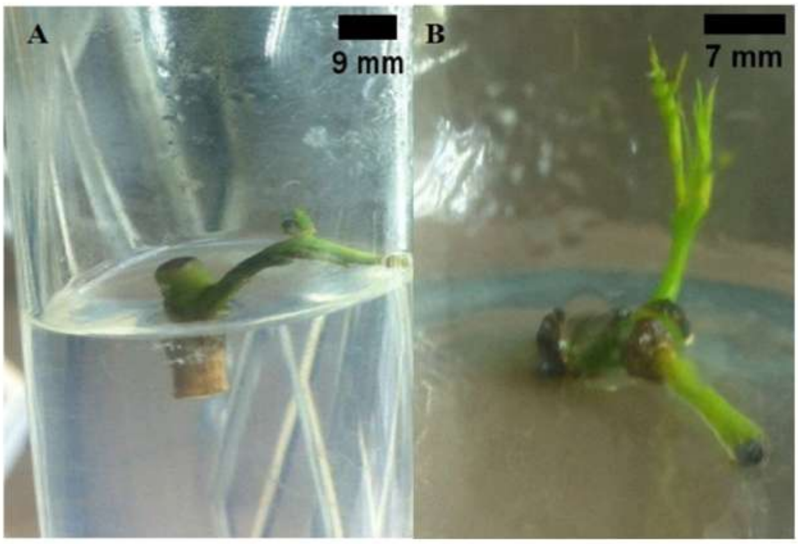

Figure 4: Shoot tip of *Afzelia africana* (A). Young developing buds formed on MS alone. (B). Two shoots formed on MS + 25 mg/L BA.

Stem (Table 5) and hypocotyl (Table 6) explants of *A. africana* were also responsive *in vitro* showing shoot initiation and callus formation, however multiple shoot formation was not recorded in these explant types.

Table 5: Organogenesis and callogenesis from stem explants of *in vitro* grown seedlings of *Afzelia africana.*

BA (mg/L	NAA (mg/L)	PG (mg/L)	PRCI (%)/ DOCF	MOC	PRSI (%)/ NSPE
-	-	-	NR	-	-
2	-		NR	-	-
3	-	-	40 / ++	Soft cream/Dark brown	20 / 2
5	0.5		NR	-	-
15		15	100 / ++	Off white/dark brown, compact	- / -
20	-		60 / ++	Creamy white, soft	20 / 1

Keys: BA – Benzyladenine, NAA – Naphtalene Acetic Acid, PG – Phloroglucinol, PRCI – Percentage Response of Callus Induction, MOC – Morphology of Callus, PRSI – Percentage Response of Shoot Induction, NR – No response

Table 6: Organogenesis and callogenesis from hypocotyl explants of *in vitro* grown seedlings of *Afzelia africana*.

BA (mg/L)	NAA (mg/L)	PG (mg/L)	PRCI(%)/ DOCF	MOC	PRSI (%)/ NSPE
-	-	-	NR	-	20 / 1
1	0.5	-	NR	-	NR
	-	10	NR		20 / 1
5	0.5		NR	-	NR
15	-	10	80 / +	Compact hard, dark	NR
15	-	15	80 / ++	Off white / cream,	20 / 1
15	-	20	20 / +	Soft cream, friable	NR
20	-	-	100 / +	Soft cream	NR

Keys: BA – Benzyladenine. NAA – Naphtalene acetic acid, PG – Phloroglucinol, PRCI – percentage response of callus induction, DOCF – degree of callus formation, MOC – morphology of callus, PRSI – percentage response of shoot initiation, NSPE – number of shoots per explants

Shoot multiplication and elongation from cotyledonary node of *Afzelia africana*

The preliminary experiment showed that CN explants were more responsive *in vitro*, hence shoot multiplication experiment was maximized using them. However, variations in shoot response were recorded due to different environmental conditions under which this replication and shoot maximization experiment occurred (Table 7). In the second experiment, cultures were subjected to a more humid environment and this accounted for the variation in response with respect to the preliminary experiment. Multiple shoots were formed from the culture of CN explant on MS + 15 mg/L BA + 15 mg/L PG (4.33 ± 0.33) (Fig. 5A, Table 7) and also on MS + 15 mg/L BA + 20 mg/L PG (4.00 ± 0.00) (Fig. 5B, Table 7). Significant multiplication of shoot was recorded on thidiazuron-containing media with 10.00 ± 0.00 shoots forming on both MS + 15 mg/L BA + 15 mg/L PG + 5 mg/L TDZ (Fig. 5C, Table 7) and MS + 15 mg/L BA + 15 mg/L PG + 10 mg/L TDZ (Fig. 7D, Table 7). The multiple shoots formed (Fig. 5C, Fig. 5D) were then transferred back to the media without thidiazuron (15 mg/L BA + 15 mg/L PG) for elongation which occurred within 22 days. (Fig. 5E, Table 8). The media containing equal concentration of BA and PG - 15 mg/L BA + 15 mg/L PG enhanced the longest shoots (5.63 ± 1.10), while the highest number of elongated shoots was formed on MS + 15 mg/L BA + 20 mg/L PG (2.33 ± 0.21).

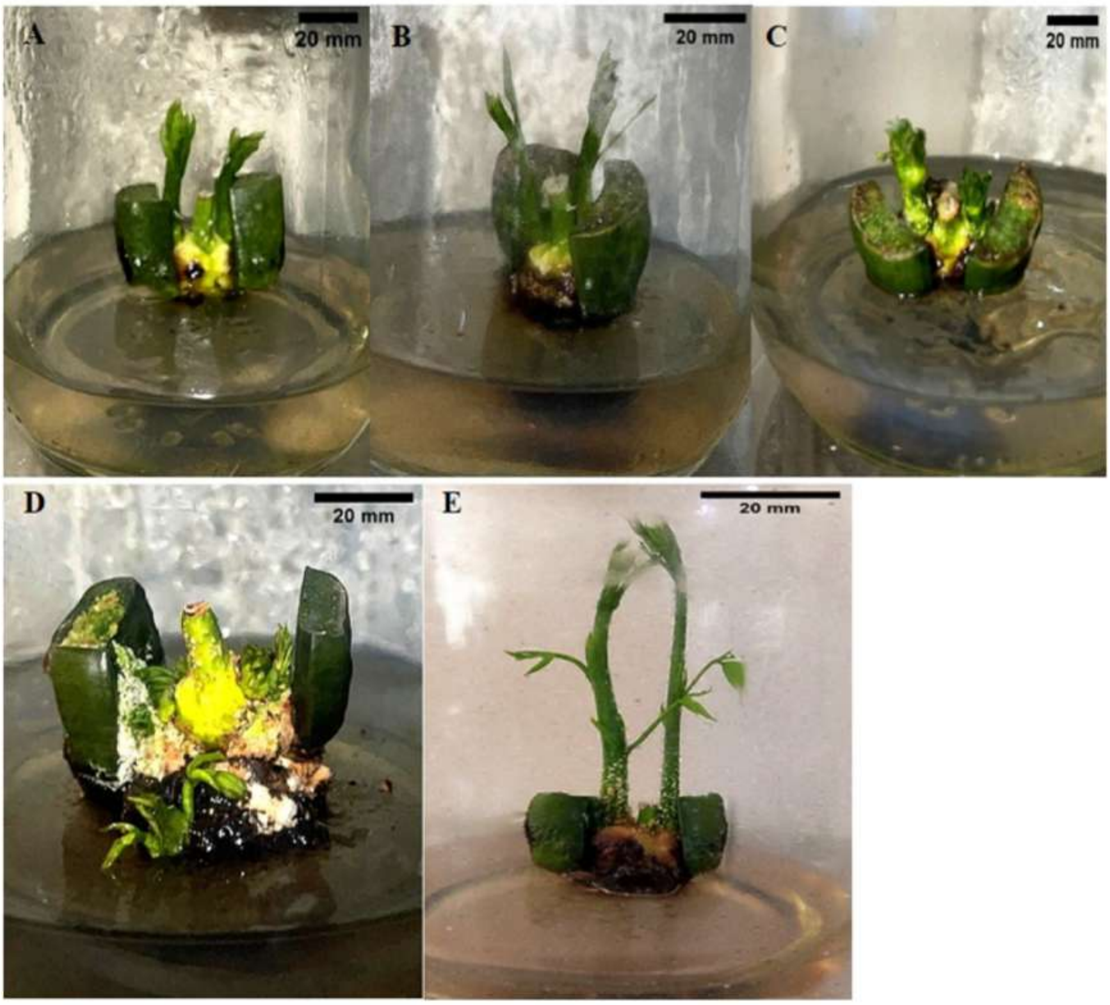

Figure 5: Shoot multiplication and elongation from cotyledonary node of *Afzelia africana* (A). Multiple shoots formed on MS + 15 mg/L BA + 15 mg/L PG (B). Multiple shoots formed on MS + 15 mg/L BA + 20 mg/L PG (C). Multiple shoots formed on MS + 15 mg/L BA + 15 mg/L PG + 5 mg/L TDZ (D). Multiple shoots formed on MS + 15 mg/L BA + 15 mg/L PG + 10 mg/L TDZ (E). Elongated shoots on MS + 15 mg/L BA + 15 mg/L PG

Table 7: Shoot multiplication from cotyledonary node of *Afzelia africana.*

Media combination	BA (mg/L)	PG (mg/L)	TDZ (mg/L)	Number of Shoot Initiated
1	-	-	-	0.00 ± 0.00^{c}
2	-	15	-	0.00 ± 0.00^{c}
3	15	15	-	4.33 ± 0.33^{b}
4	15	20	-	4.00 ± 0.00^{b}
5	15	15	5	10.00 ± 0.00^{a}
6	15	15	10	10.00 ± 0.00^{a}
7	15	15	15	0.00 ± 0.00^{c}

Means with different letter along the column are significantly different from each other at $p \leq 0.05$.
Keys: BA – Benzyladenine, PG – Phloroglucinol, TDZ – Thidiazuron

Table 8: Elongation of multiple shoots induced from cotyledonary node of *Afzelia africana*.

Media combination	BA (mg/L)	PG (mg/L)	Number of Shoots Transferred From TDZ-containing media	Number of Elongated Shoots	Length of Shoot (cm)
1	-	-	10.00 ± 0.00^a	0.00 ± 0.00^d	0.00 ± 0.00^e
2	-	15	10.00 ± 0.00^a	0.00 ± 0.00^d	0.00 ± 0.00^e
3	15	15	10.00 ± 0.00^a	2.00 ± 0.00^b	5.63 ± 1.10^a
4	15	20	10.00 ± 0.00^a	2.33 ± 0.21^a	3.48 ± 0.05^b

Means with different letter along the column are significantly different from each other at $p \leq 0.05$.
Keys: BA – Benzyladenine, PG – Phloroglucinol, TDZ – Thidiazuron

DISCUSSION

No response was observed from leaf explants cultured on MS media devoid of PGR, however on the inclusion of PGR, varying responses resulted. Raspor et al. (2021) reported that PGR especially auxins and cytokinins play a key role in the differentiation process in *in vitro* culture systems. Callus response was initiated from the leaf explants of *A. africana* when media was supplemented with a high concentration of auxin (2, 4–D) in combination with a low concentration of cytokinin. Usually, the addition of exogenous auxin to the media stimulates the synthesis of endogenous auxin for regeneration (Nic-Can and Loyola-Vargas, 2016) and in this case the callusing response. 2, 4–D alone or in combination with cytokinin has been widely used in callus cultures (Atta et al., 2009), where its concentration affects the callus culture and its morphology. Similar to previous findings that embryogenic or friable callus is produced from low concentrations of 2, 4 – D and inhibited by high concentration 2, 4-D, our finding showed the same observation where 4 mg/ L 2, 4 – D + 0.7 mg/L BA produced friable callus while 10 mg/ L 2, 4 – D + 0.9 mg/L BA produced compact callus. 2 mg/L 2, 4-D was reported to be optimal for embryogenic callus induction in Poaceae plants (Çabuk and Özgen, 2016). At lower concentrations of 2, 4-D, the percentage response of leaf explants to callus induction was high while at higher concentrations, the percentage response was low, which can be an inhibitory effect of PGR at higher concentrations. The best callus response (100%) was observed on MS + 4 mg/ L 2, 4 – D + 0.1 mg/L BA, and this concentration is optimal for callus induction from leaf explants of *A. africana*. In *Zigyphus jujuba*, Ye et al. (2012) reported that the best callus induction response (95%) was obtained from leaf explants cultured on MS + 2 mg/L 2, 4 – D + 0.5 mg/L BA.

Similarly, as varying responses resulted from the culture of leaf explants, nodal explant cultures also resulted in different responses. Observable responses did not occur from the culture of nodal explants on the basal media alone, however addition of BA (1 mg/L) initiated organogenesis with a shoot bud arising from 20% of the cultures. BA is a cytokinin, and cytokinins are known to initiate shoot development (Pernisova et al., 2009). In addition, BA is a widely used cytokinin for promoting cell division and organ differentiation (Phillips and Garda, 2019). The inclusion of exogenous cytokinin (BA) in the medium increased the concentration of cytokinin available, hence increasing the competence of nodal explants for organogenesis. Irina (2008) reported that the optimum level of BA needed for organogenesis is dependent on the concentration of endogenous cytokinin available in the plant species. The media containing either BA alone or in combination with IAA elicited shoot and callus response simultaneously while only callus resulted in media containing BA and NAA. A similar observation was reported by Zafarullah (2013) where IAA in addition to BA enhanced shooting while NAA in addition to BA inhibited it in *Chrysanthemum Indicum* L. Shoot initiation was inhibited from nodal explants on MS + 1 mg/L BA +0.5 mg /L NAA while this media enhanced callus induction from 40% of the cultures. Callus induction is usually favored in media containing equal (or almost equal) concentrations of cytokinin and auxin (Rahman et al., 2010), hence the observed callusing response with this media combination. A similar response of inhibition of shoot initiation was observed with MS + 10 mg/L BA + 0.5 mg/L NAA and MS + 15 mg/L BA + 5 mg/L NAA. Shoot organogenesis occurred in these media combinations when NAA was excluded, hence showing that NAA in the media suppressed the shoot-stimulating effect of BA. The optimal media for shoot initiation from nodal explant is MS + 20 mg/L BA where 3 shoots were simultaneously produced with an average-sized white friable and transparent callus. BA-containing media has been used to induce shoot emergence and development in Jerusalem artichoke (Abdalla et al., 2021; Kim et al., 2016).

Other explants apart from nodal explants such as shoot tip, stem, and hypocotyl explants also initiated shoots *in vitro*. However, cotyledonary node explants of *A. africana* responded best with multiple shoots production *in vitro*. Cotyledonary node explants cultured on MS + 25 mg/L BA + 5 mg/L NAA produced 3 shoots from 100% of cultures. On MS + 15 mg/L BA + 15 mg/L PG; 10 shoots were produced from 40 % of the cultures and finally on MS + 15 mg/L BA + 20 mg/L PG - 8 shoots from 20% of the cultures. PG-containing media was best for initiating the multiple shoots. In the initial part of the study, MS + 15 mg/L BA + 15 mg/L PG was optimal for multiple shoot initiation in *A. africana*. PG has growth-promoting properties and acts in synergism with auxin and cytokinin as well as acting like cytokinin and auxin (Teixeira da Silva et al., 2013). In the further experiment to maximize shoot multiplication protocol, a significant difference was recorded in the number of shoots initiated on MS + 15 mg/L BA + 15 mg/L PG when compared to what was recorded on it during the initial experiment. Environmental differences from varied humidity conditions can explain this variation in the result as both experiments were undertaken at different times. On all media tested, shoot multiplication was highest in MS + 15 mg/L BA + 15 mg/L PG + 5 mg/L TDZ and MS + 15 mg/L BA + 15 mg/L PG + 10 mg/L TDZ producing 10.00 ± 0.00 number of initiated shoots, which however did not elongate while they remained in the media containing TDZ. A similar response was observed in the continuous culture of explants of *Syzygium cumini* on TDZ-containing media, the multiple shoots induced did not elongate until they were transferred to a media without TDZ (Naaz et al., 2021). In this study also, shoots present on TDZ devoid media were shown to elongate significantly, hence multiple shoots produced on TDZ containing media were further transferred back to MS alone, MS supplemented with PG alone, and MS supplemented with BA and PG for elongation. MS media containing BA and PG was optimum for elongation and a significantly higher number of elongated shoots (2.33 ± 0.21) were produced on MS + 15 mg/L BA + 20 mg/L PG while on MS + 15 mg/L BA + 15 mg/L PG, the highest elongation was recorded with shoots growing up to 5.63 ± 1.10 cm. Usually, auxin function is promoted by PG (Petti, 2020), hence the PG in the media has enhanced endogenous auxins within the shoots resulting in stem elongation - a known functional role of auxin. In addition, Manokari et al. (2021) reported the role of PG not only in inducing new shoots but also in the development and elongation of the formed shoots.

CONCLUSIONS

This study has confirmed that regeneration of *A. africana* is feasible through *in vitro* propagation. In addition, it has provided a shoot multiplication and elongation protocol from cotyledonary node explants of *A. africana* for its massive propagation. MS + 15 mg/L BA + 15 mg/L PG + 5 mg/L TDZ and MS + 15 mg/L BA + 15 mg/L PG + 10 mg/L TDZ were optimal for the production of the highest number of multiple shoots. Hence, this media can be utilized for massive propagation of *A. africana*, and for transformation studies during genetic modification. Again, it can be useful for raising seedlings *in vitro* for afforestation, reforestation and ex-situ conservation purposes such as gene banks.

Genetic modification for crop improvement studies to increase the seed oil content of *A. africana* is recommended for future research. These studies will identify and characterize the genes and the metabolic pathways responsible for oil accumulation in seeds and attempt to upregulate these genes for higher expression resulting in higher oil accumulation. Plant tissue culture techniques remain the only means through which transformed cells can be grown into whole plants. In addition, through Plant Tissue Culture techniques, resulting transformed plants can be massively propagated using the protocol developed in this study. However, the protocol should be optimized to eliminate the effect of environmental factors. Further studies to root the shoots regenerated *in vitro* and acclimatize them in the field are ongoing. In conclusion, this study, which is the first of its kind, has provided information on how *A. africana* - a potential biofuel plant, can be regenerated through *in vitro* propagation methods, hence reducing the pressure on its wild stands as well as conserving its germplasm for future purposes.

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Short Note

Observation of Nest Predation by a Spotted Wood Kingfisher (*Actenoides lindsayi*) on a Yellow-breasted Fruit-dove (*Ramphiculus occipitalis*) Nest

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In nature, numerous ecological pressures exert selection on bird species as part of their survival (Martin, 1995). One of these pressures, described as applying strong selection on species coexistence, is nest predation (Ricklefs, 2000). It is recognized as a major factor, among other environmental pressures with a significant impact on population structure, because it directly affects the fledglings (Ricklefs, 1969) and can result in total nest failure (Nilsson, 1984). The structure and placement of the nest itself also influence the frequency of predation and interactions with other birds (Nilsson, 1984).

When studying nest predation, three key active elements are involved: offspring, adults, and predators. Understanding the role of each in their natural habitat is now vital in advancing avian studies (Ibáñez-Álamo et al., 2015). Technological advancements, such as the use of camera traps, have allowed researchers to observe the natural behaviour of certain species up close (Ibáñez-Álamo et al., 2015; O'Brien & Kinnaird, 2008). In light of this, this study presents a brief observation of a Spotted Wood Kingfisher (*Actenoides lindsayi*), a forest kingfisher that feeds on insects, small vertebrates, and invertebrates, predating on a Yellow-breasted Fruit-dove (*Ramphiculus occipitalis*) nestling. This kingfisher species is known to search for prey in the covered spaces in the understory (Pagaduan & Afuang, 2012). Both of these birds have a close association to forest habitats. They are currently listed as Least Concern but with decreasing population trend (Birdlife International 2018; Birdlife International 2024). Other observations regarding the behaviour of the latter and its parent are also included.

The study site is in Balinsasayao Twin Lakes Natural Park (BTLNP), Negros Oriental, Philippines—a protected area (Fig. 1). A single nest was discovered along a man-made trail during transect surveys intended for a larger scope of the study on May 3, 2024. It was situated around 2.5 to 3 meters above the ground on a branch and about 1.5 meters from the *Syzygium* sp. (Myrtaceae) stem. Because of this, only one camera trap was placed on the stem of the same tree, facing the direction of the nest at the same height. The stem was the only part where the camera trap could be securely placed. The camera trap used was a 20-megapixel, 1080p mini outdoor trail camera with infrared night vision (SuntekCam). It was left for four weeks, set to capture photos and videos with 30 seconds record time. Sensitivity of the camera capture was set to medium to prevent it from being triggered by leaf movement.

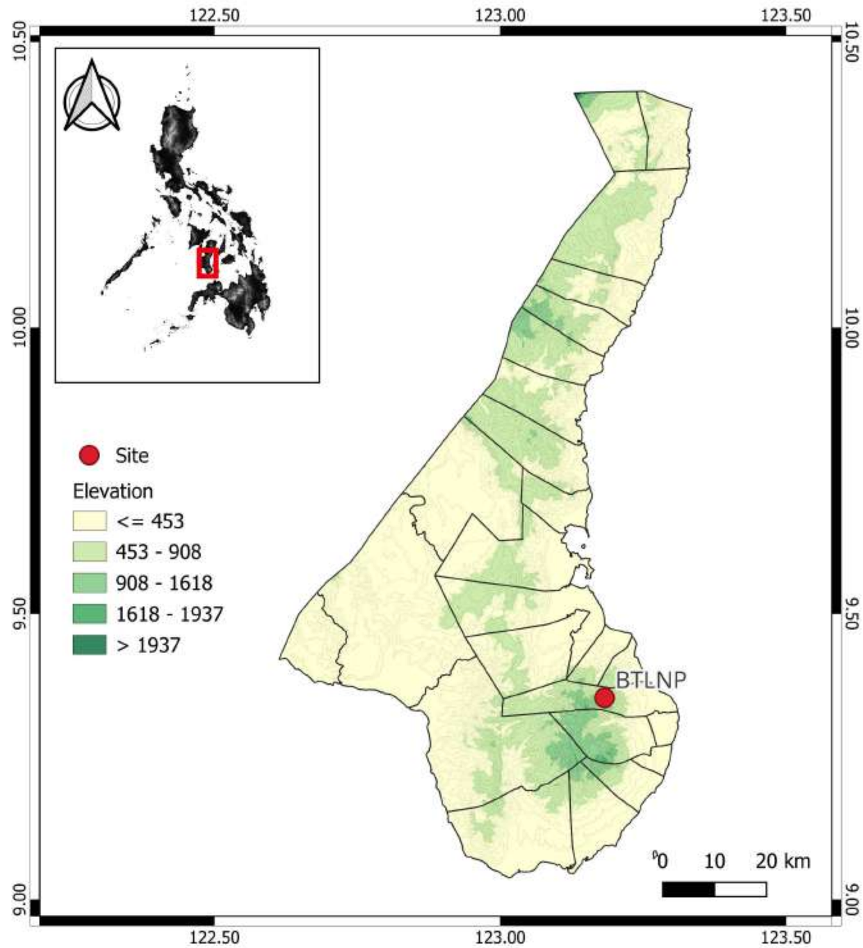

Figure 1: Location of Balinsasayao Twin Lakes Natural Park (BTLNP) in Negros Oriental, Philippines.

The result of camera trapping shows interaction of the three key players in nest predation involving the Yellow-breasted Fruit-Dove nestling and its parent, and the Spotted Wood Kingfisher, the predator. Before the predation happened, the parent dove could be seen not immediately perching on the nest but on nearby branches, apparently checking the surroundings. It did not directly perch in close contact with its nestling unless the nestling gave signals, such as shivering or making calls, indicated by bill movements (Fig. 2A). As the parent finally climbed onto the nest, it fluffed up its feathers (Fig. 2B) as a means of thermoregulating (Mota-Rojas et al., 2021) by increasing thickness of the plumage for better insulation. Through this, it may have shared its body heat as the nestling nestled under the parent since it was still covered with mostly down feathers. Unexpectedly, in one instance at night, which is expected to be colder, the parent was not present, but it may have just been away for a moment (Fig. 2C).

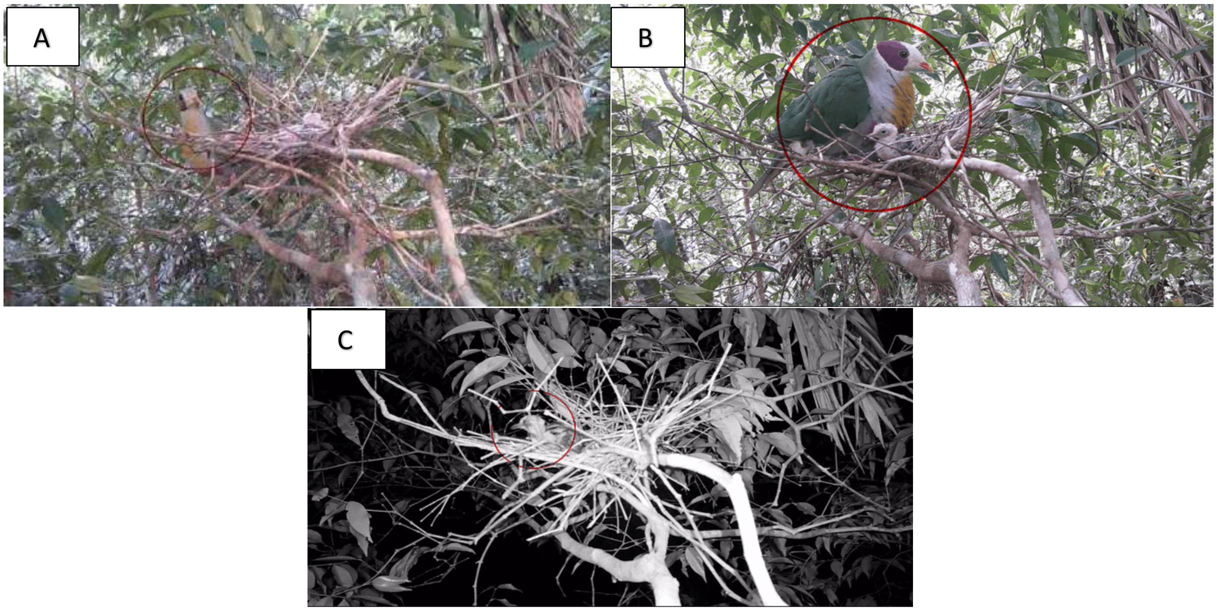

Figure 2: Camera trap images of a Yellow-breasted Fruit-dove nest. A) The parent approaching the nest. B) The nestling nestled near its parent. C) The nestling alone in the nest.

The exact age of the nestling could not be determined, but it was at the stage where it was mostly covered by down feathers and wobbled when it attempted to move. At this point of the nestling's developmental stage, it did not seem to be actively moving around the nest. It spent most of its time sitting whilst waiting for the arrival of its parent. According to a local guide, based on his past observations of nestlings of this species, once a nestling becomes quite mature, it would probably walk back and forth on the branch close to its nest (J. Zerna, personal communication, 2024).

The actual predation happened a day after the setting up of the camera trap. The kingfisher appeared to observe the surroundings first before attempting to prey on the nestling (Fig. 3A). It even left the tree to where the nest was, but returned after a moment. The kingfisher finally took on the nestling, biting its head and pulling it downwards, out from the nest (Fig. 3B). This method likely leveraged the nestling's weight for easier handling, rather than lifting it from above. However, the exact manner of eating the nestling was not captured in the footage. As the kingfisher was doing this, the parent was nowhere to be seen and no signs of retaliation was observed. Later that same day after the incident, the parent returned to the nest, did its usual routine as mentioned above before climbing onto the nest, and searched for the nestling, but unfortunately it was gone (Fig. 3C). In the subsequent frames captured by the camera, the nest was abandoned and neither of the birds returned (Fig. 3D).

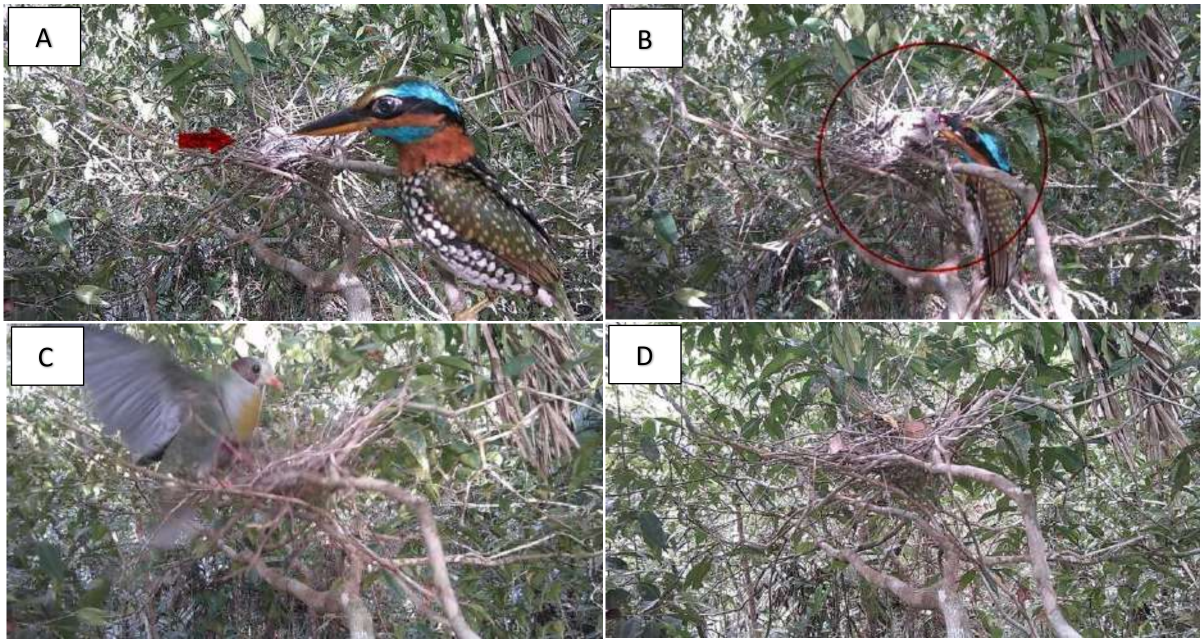

Figure 3: Camera trap images of a predatory event at a Yellow-breasted Fruit-dove nest. A) The arrival of a Spotted Wood Kingfisher. B) The actual predation on the dove nestling. C) The dove parent returning to its nest. D) The abandoned nest after predation.

The dove's nest appeared to be mostly made up of piles of twigs, with the nestling fully exposed on it. The nest was placed at a height that was relatively lower and seemingly obscured by the dense forest cover. It was almost within reach of potential poachers. This could be a form of defence strategy from certain predators, such as flying raptors in the area, by disrupting their search efficiency through reduced nest visibility (Horie & Takagi 2012; Kleindorfer et al., 2005). However, this may have introduced a trade-off by making the nest more noticeable to other predators dwelling below the canopy level, like the kingfisher (Pagaduan & Afuang, 2012). The openness of its nest does not even seem to provide much security for the young against predators. The nest predation observed may be one example of a density-dependent factor wherein likelihood of predation increases as the number of nests in an area increases (Caro, 2005). The frequency of predation is also related to how the nests are positioned, as well as their type and structure (Djomo et al, 2014; Nilsson, 1984). Such attacks on nestlings could possibly influence the population structure of a species (Ibáñez-Álamo et al., 2015). Additional attacks from other potential predators, such as raptors that exploit nestlings (Sazima & Hipolito, 2017), could exacerbate the effect. In relation to this, although this study lacks sufficient data to discuss the population of doves in the area, the detrimental effect of nest predation on them remains plausible but requires further study to be verified.

The use of a camera trap effectively captured the natural behaviour between the interaction of the species involved. However, this single nest observation poses limitations, such that, there may have been other interactions that had occurred beforehand. Other forms of interactions may have taken place around the nest as well. Therefore, placement of more camera traps to observe more nests interactions may provide holistic and comprehensive information on the behaviour of these birds. It can also be a practical method to assist in determining if other bird species could be predating on nestlings.

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Short Note

New Locality Record of the Lowland White-eye (*Zosterops meyeni*) in Dumaguete City, Negros Oriental, Philippines: A Potential Range Expansion?

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White-eyes are birds in the Old-World tropical regions belonging to family Zosteropidae, described to be a sister taxon to the babblers (Borghesio & Laiolo, 2004; Gill, 2020). This family exhibit strong dispersal capabilities that allowed them to colonise and adaptively radiate across many continents and into Afro- and Indo-Pacific tropical regions, where there is also a high endemicity of these species (Craig, 1990; Gill, 2020; van Balen, 2008). As a result, White-eyes have occupied various niches, as demonstrated by observations in different habitats, as well as having diverse feeding habits, according to other studies (see Borghesio & Laiolo; Cowles, 2019; Craig, 1990; Dutson, 2008; Mulwa, 2007; Vinciguerra et al., 2023). Because of this, different White-eye species can be seen in high mountainous areas, mid elevation, forest over limestones, agricultural and plantation area, among others.

The Lowland White-eyes (*Zosterops meyeni*) in particular, based on the most recent field guide on the Birds of the Philippines by Allen (2020), is a common resident generally found in Luzon and in nearby islands such as Mindoro, Batanes, Calayan, Lubang, and Leyte. However, it is notably absent in most parts of Visayas, including Negros Island, and there are no records at all from Mindanao Island. Similarly, the latest field guide by Jakosalem et al. (2019), which focuses on Central Visayas, has not listed this bird for Negros, Cebu, and Panay islands. This species commonly inhabits forests and forest edges, scrublands, gardens, areas with bamboos in the lowlands, and even in mangroves in coastal areas. However, some studies have also documented its presence in urban green spaces in Manila (Vallejo, 2009). While Jakosalem et al., (2019) did not list this bird for Negros, a more recent study conducted in Bacolod City, Negros Occidental by Mabugat et al. (2024) encountered this species during their 2022 assessment, marking its presence on the island.

In light of this, this paper presents the first record of the Lowland White-eye in Dumaguete City, Negros Oriental, to provide more support to the information that the encounter of this bird in the urban spaces in Bacolod City was not incidental.

Two individuals were seen during a preliminary survey on urban avian diversity near the coastal area at the port of Dumaguete City (9.312072, 123.309198) (Fig. 1), which is also close to Silliman University, a campus with relatively dense vegetation.

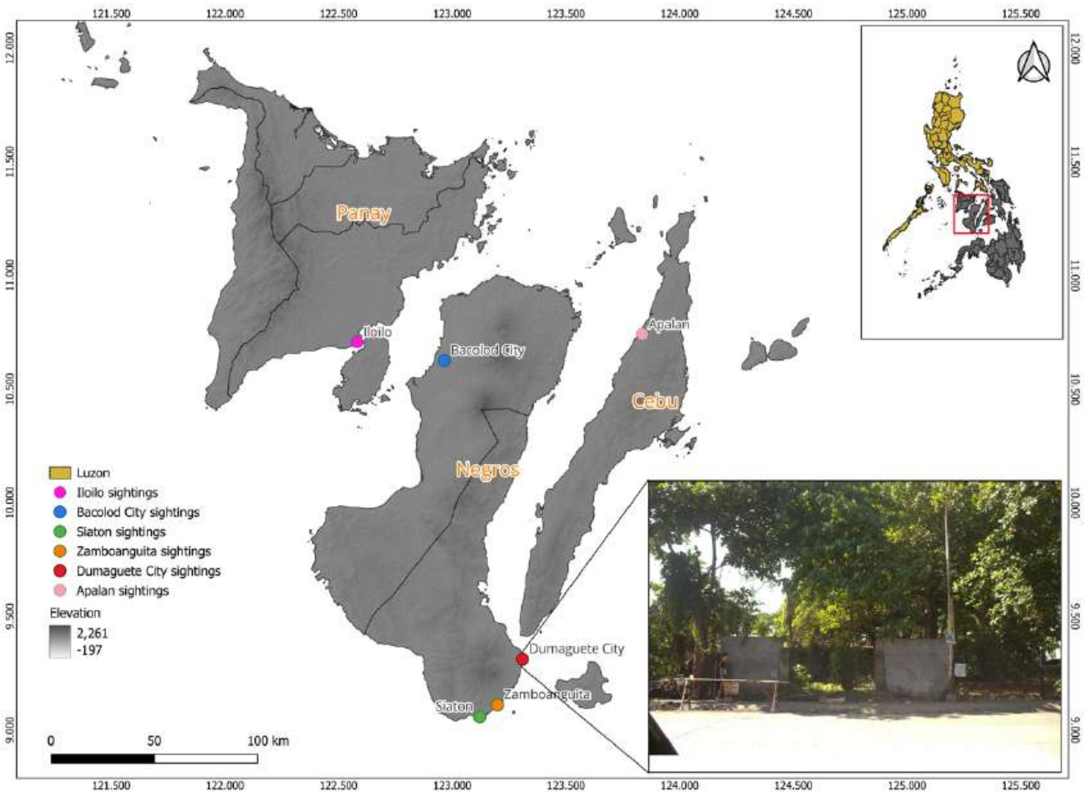

Figure 1: Locations of White-eye bird sightings in the Central Visayas and Western Visayas Islands (Negros, Cebu, Panay) of the Philippines. Top right inset shows an overview map of the Philippines. Bottom right inset shows the site where two Lowland White-eyes were seen at Dumaguete City, Negros.

Environmental parameters were measured during the time the birds were observed. The average sound level was 65.5 dB, average temperature was at 30.8 °C, and average humidity was 70.5 %. The location where they were observed is a mixture of vegetation cover, concrete, and other building debris (Fig. 1).

The bird was identified by its characteristic white ring around the eye, brighter yellow throat, and greyish underparts with no yellow stripe on its belly (Fig. 2), and in low elevation which is not a typical characteristic for other White-eyes. In comparison with the two White-eye species also known to occur in Negros, the Yellowish White-eye is recognizable by its predominantly yellowish and greenish colouration. Its underparts are entirely yellow and olive, with no presence of greyish or white plumage, making it clearly distinct from the two. In contrast, the Mountain White-eye, commonly found at elevation of 1000 m asl, has prominent white or greyish underparts, and a yellow stripe along its breast and belly.

Furthermore, the two Lowland White-eye individuals were seen feeding on fruits of the *Macaranga tanarius* (Fig. 2), locally known as binunga. It is a native and pioneer species that usually grows in disturbed areas and is frequently observed in regenerating forests (Galias & Cuevas 2018; Paclibar & Tadiosa, 2020). It is a small to medium-sized dioecious tree that has been widely used in reforestation programmes because of its resilience and fast-growing nature (Orwa et al., 2008).

Figure 2: Individuals of Lowland White-eye feeding on *Macaranga tanarius* at Dumaguete City, Negros Oriental, in the Philippines.

Given the conditions of where the birds were seen, they can possibly thrive in urban areas. In the past, this bird was not listed in Negros Island, suggesting that, given the superb dispersing capabilities of White-eyes (Vinciguerra et al., 2023; Borghesio & Laiolo), it enabled them to move into the island. In fact, aside from the records presented herein and the one in Bacolod City in 2022, an observation was also made available on the Ebird website in 2024 for the rural municipality of Zamboanguita (see Fig. 1, Burton, 2024). Another observation available on Ebird was in Tabobo Bay, Siaton on the same island where 4 individuals were seen moving around the mangrove area (Chafer, 2024). Many sightings of this bird in Negros and other islands of Panay and Cebu were in nearby coastal or estuarine areas where mangroves are present, which may suggest this bird also has preference to these kinds of habitats (C. Chafer, personal communication, November 21, 2024; Cornell Lab of Ornithology, 2024).

These encounters are so far very new, which could also suggest a recent dispersal of this bird. Based on more recent evidence shared on Ebird in 2023 and 2024 from the neighbouring provinces of Iloilo on Panay Island and Apalan on Cebu Island (Fig. 1, Tarrosa, 2023; Cabahug, 2023; Chafer, 2022), they might be currently expanding their range. With this, its dispersal pattern can be traced as apparently directed southwards of the country from its former distribution in Luzon. We do not have the data to explain the nomadic movement of this bird, but there are some plausible reasons for this behaviour. This bird could be responding according to the ephemeral availability of resources (Mueller et al., 2011), which opens up new potential food sources as they explore new areas; challenges against competition and predation (Smith et al., 2011), or to climate change and weather conditions (Sauter et al., 2010).

There are 141 recognized species of White-eyes in the world where majority of them are found in the archipelagos of Southeast Asia (Gill, 2020). Of these, there are five White-eye species known to occur in the Philippines, three of which are also found in Negros Island (Allen, 2020) with the addition of the newly listed Lowland White-eye. With the consistent records, Negros Island now has three White-eye species across high and low elevations: Lowland White-eye in the lower elevations, Yellowish White-eye (*Zosterops nigrorum*) in mid elevations, which could potentially overlap both elevations but does not seem to extend far beyond these ranges based on prior field observations; and the Mountain White-eye (*Zosterops japonicus*) at the higher elevations.

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