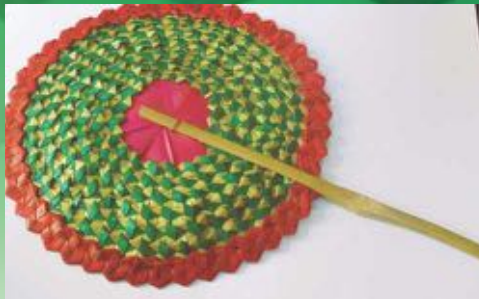


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*Rhipidoglossum polydactylum* (Kraenzl.) Garay (Photo by Nodza George)

Flower Basket (Photo by Taranisen Panda)

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## Research Article

# Butterfly Diversity at the Different Elevations along Crocker Range Park, in Malaysian Borneo

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## ABSTRACT

The butterfly diversity and assemblages at five substations located at the different elevations along the Crocker Range Park, ranging from 396m to 1,891m a.s.l. were documented in this study. The butterfly samplings were conducted from April to December 2019, involving 10 sampling sessions. The butterflies were sampled by using 20 baited traps and an aerial net at 20 sampling stations. A total of 727 individuals were sampled comprising 187 species. Nymphalidae was the dominant family at the five substations, which accounted for approximately 53% of the total species and 71% of the total individuals recorded, while *Ypthima pandocus* was the dominant species. The highest number of species was recorded at Keningau substation (965–1,062 m a.s.l.) but the highest number of individuals was recorded at Mahua substation (1060–1,249 m a.s.l.). Mount Alab substation recorded the least number of butterfly species and individuals. The overall pattern indicated that the butterfly species and abundance were relatively high at an elevation range of about 960–1,250 m a.s.l. Keningau substation was the most diverse area as shown by Shannon-Wiener Index ( $H' = 2.885$ ), followed by Inobong, Mahua and Ulu Kimanis substations. Ten endemic species were sampled in this study with most of these found at an elevation above 965 m a.s.l. The information obtained from this study would contribute to a better understanding of the elevational diversity pattern of tropical biota and also could serve as baseline data for conservation management at Crocker Range Park in facing threats on biodiversity, including global warming.

**Keywords:** Butterflies, diversity, assemblages, elevations, Crocker Range Park

## Introduction

Tropical butterflies have been widely used in ecological studies to assess tropical forest disturbance (e.g. Dumbrell & Hill, 2005, Hamer et al., 2005; De Vries et al., 1997). However, very little information is available on how these butterflies react to environmental changes such as an increase in temperature in tropical regions. Some studies reported that tropical organisms are particularly severely affected by global warming (Corlett, 2011). In temperate

regions, there is evidence that organisms are showing habitat shifts or expanding their range boundaries (Hughes et al., 2003; Root et al., 2003; Chin et al., 2009). Cooler areas in highlands would potentially be gradually occupied by lowland species as their refuge from increasing temperature. As documented in many studies, mountains serve as refuge for many lowland species during the Pleistocene (Tchouto et al., 2009; Corlett, 2012). In tropical rainforests, the highland area is demarcated at about 1,200m a.s.l (Saw, 2010).

Located on the island of Borneo, which is widely recognised as a biodiversity hotspot, Crocker Range Park (CRP) is the largest terrestrial park in Sabah and is situated in the northern part of the island. The CRP area consists of a wide range of habitats, from lowland to highland. The second highest mountain within this park, Mount Minduk Surong, is situated at 2,050m a.s.l. The park also harbours many species of flora and fauna (Majit et al., 2011). Therefore, it is a suitable area to carry out this study.

There are approximately 944 species of butterflies that have been recorded on Borneo (Otsuka, 1988; Otsuka, 2001), of which about 81 species are endemic (Aqidah et al., 2020). Most of the species can be found in Sabah. Despite the status as the largest terrestrial park in Sabah, no comprehensive information on the butterfly diversity and distribution at CRP have been documented and reported. Therefore, this study aimed to document the diversity pattern of butterflies along the different elevations of CRP. The objectives were to investigate the butterfly diversity and to compare the butterfly assemblages at different elevations at CRP.

## **Materials and methods**

### ***Study Area***

Crocker Range Park was established in 1984 and covers an area of 139,919 hectares (latitude 5°07' to 5°56'N and longitude 115°50' to 116°28'E) and falls under the management of Sabah Parks (Tuen et al., 2002). The area is 110km in length and 15km wide (Majit et al., 2011) and consists of several substations located at different elevations (**Figure 1**). In this study, five of the substations located at different elevations were selected as study sites namely Inobong substation (396–533ma.s.l), Ulu Kimanis substation (568–713ma.s.l), Keningau substation (Headquarters of Crocker Range Park) (965–1,062ma.s.l), Mahua substation (1,060–1,249ma.s.l) and Mount Alab substation (1,784–1,891ma.s.l) (**Figure 1**).

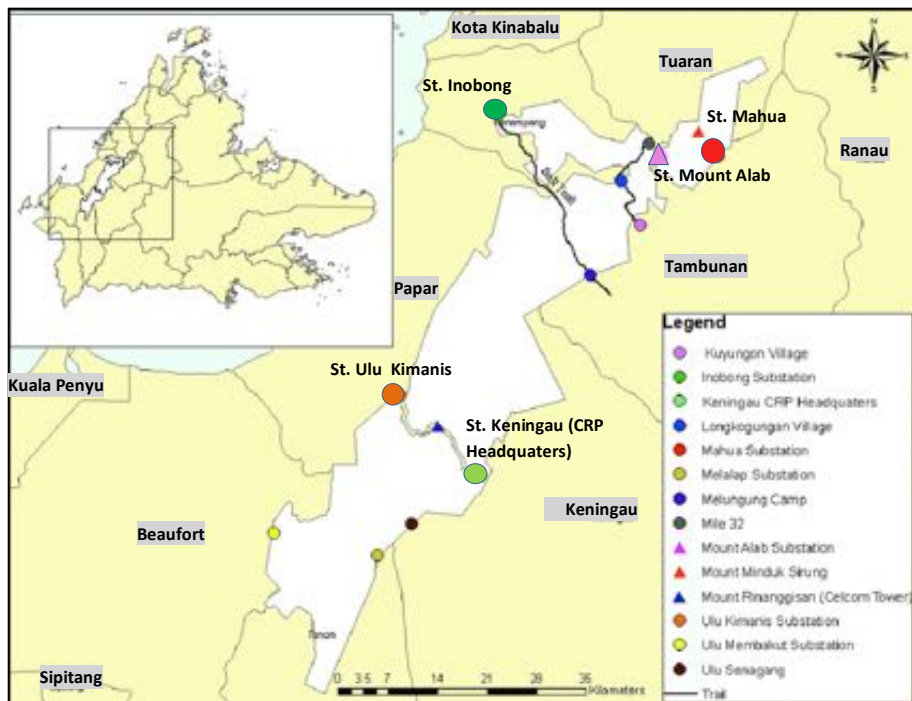


Figure 1. Map of Crocker Range Park and substations (modified from Suleiman et al., 2017)

### *Data collection*

Butterfly samplings were conducted in two cycles starting from April – December 2019. In each cycle, the five substations were visited once (Table 1). Each sampling session was conducted for 10 consecutive days, for a total of 100 sampling days. At each substation, a one kilometre transect was established and divided into 20 sampling stations. The sampling activity was started from 8.00 am to 3.00 pm. Butterflies were sampled by using baited traps and an aerial net.

#### *i. Baited traps technique*

Twenty traps were hung on the tree branches along a one kilometre line transect approximately at 50m intervals. The traps were placed at least one metre high from the forest floor (DeVries, 1988). Ten traps were baited with bananas and the other ten traps were baited with shrimp paste. These were placed alternately at the sampling stations. The traps were left overnight and checked daily. This technique was used to sample the fruit-feeding guild.

## ii. Aerial netting technique

The aerial netting technique was applied to sample butterflies that are not from the fruit-feeding guild and cannot be caught by the baited trap technique. An aerial net was used to catch butterflies within a five metre radius of each sampling station along the same transect used for the baited traps. Ten minutes were spent on searching butterflies at each station, which involved a one-person effort. This technique was used to sample butterflies that cannot be sampled using baited-traps.

**Table 1.** The sampling sessions during the two sampling cycles at the five substations.

Substation	First cycle	Second cycle
Inobong	1–11 April 2019	19–29 September 2019
Ulu Kimanis	19–29 April 2019	6–16 October 2019
Keningau	16–26 July 2019	5–14 December 2019
Mahua	7–17 May 2019	2–12 November 2019
Mount Alab	24 May–3 June 2019	17–27 November 2019

The specimens collected in the field were kept in triangular papers and brought to the Institute for Tropical Biology and Conservation (ITBC), Universiti Malaysia Sabah for the preservation and identification processes. The butterflies were identified based on Otsuka (1988).

## Data Analysis

The butterfly diversity was analysed using the Shannon-Wiener's Diversity Index ( $H'$ ), Simpson's Evenness Index ( $D$ ) and Margalef's Index ( $D_{mg}$ ) (Magurran, 2004). The analyses were performed by using the Paleontological Statistics Software (PAST) (Hammer et al., 2001).

Jaccard's coefficient index ( $C_j$ ) was used to measure butterfly species similarity between the substations.

Jaccard Similarity index:

$(C_j) = j / (a+b-j)$ , where  $j$  = the number of species present at both sites

$a$  = the number of species present in site A

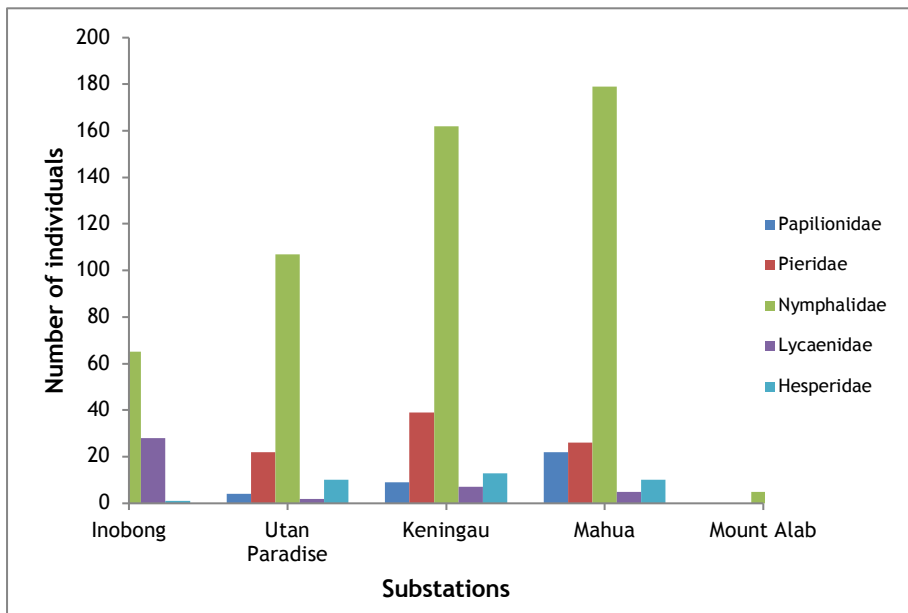
$b$  = the number of species present in site B

The Jaccard's index is equal to zero for two sites that are completely different and is equal to one for two completely similar sites (Jaccard, 1912).

## Results and Discussions

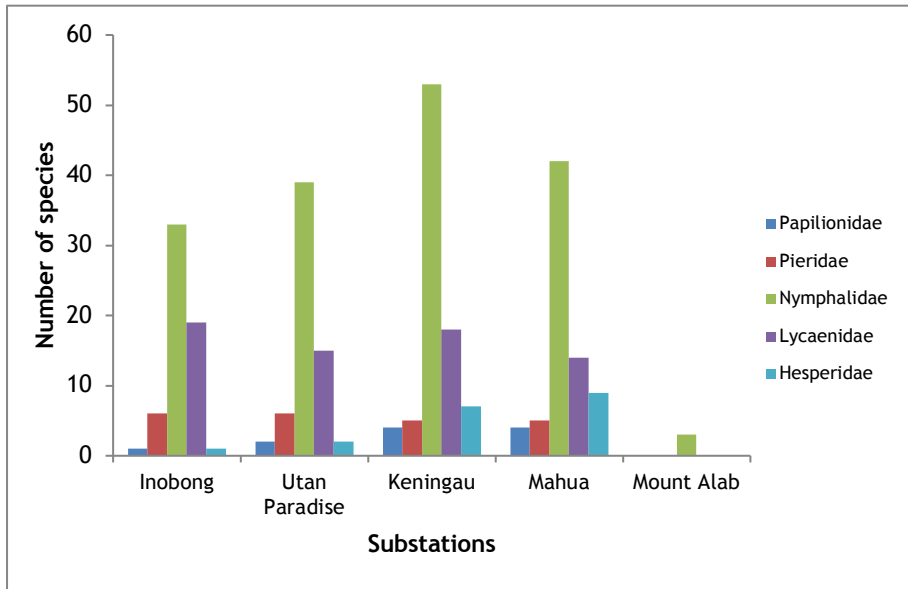
This study focused on the pattern of butterfly diversity and assemblages along the different elevations in the rainforest of Borneo. Butterflies are sensitive towards subtle changes in their environment which make them an excellent bio-indicator to assess environmental changes (Ismail et al., 2018).

Most of the studies on butterflies in Borneo were conducted in lowland forests and very little information is available on their diversity at higher elevations. In this study, a total of 727 individuals of butterflies belonging to 187 species from five families (Appendix) were sampled. All of the five families of butterflies (Papilionidae, Pieridae, Nymphalidae, Lycaenidae and Hesperidae) were recorded from each substation, except for Mount Alab substation which recorded only Nymphalidae (Figure 2 and Figure 3). Nymphalidae was the dominant family in terms of species richness and the number of individuals at all the substations compared to other families. As reported by Tabadeput et al., (2008), this family is distributed worldwide and can live in various types of habitats.



**Figure 2.** The number of individuals according to the families at each substation.





**Figure 3.** The number of species according to the families at each substation.

In this study, the overall pattern showed that the butterfly species richness and abundance peak at higher elevations (Keningau and Mahua substations) compared to lower elevations (Inobong and Ulu Kimanis substations) and sharply decrease at the highest elevation. As shown in **Table 2**, Keningau and Mahua substations recorded the highest number of butterfly species (87 and 74 species, respectively) and also the highest number of individuals (220 and 242 individuals, respectively). These results indicate that most of the species distributed at the elevations range from about 950–1,250m a.s.l. The least number of species and individuals were sampled at Mount Alab substation which is the highest elevation in this study, where only five individuals from three species were recorded (**Table 2**). Zainol (2017) also reported a low number of butterfly species and individuals at this substation. Inobong substation which is located at the lowest elevation in this study, recorded the second lowest number of species and individuals.

As shown in many studies, species richness and abundance of organisms decline with increasing elevation (Tattersfield et al., 2001; Zhang et al., 2016). On the other hand, the 'humped-shape' pattern of altitudinal diversity was reported and discussed in several studies (Guo et al., 2013). The environment at a higher elevation with low temperature, high humidity, cloudy and less sunlight, less

plant species and compact forest canopy could affect the butterfly abundance (Ismail et al., 2018). In contrast, a study conducted at Gunung Ledang in Peninsular Malaysia found that the number of butterfly species and abundance were high at an altitude of 400m, but showed a sharp decrease with increasing altitude (Ismail et al., 2018). The difference in the butterfly diversity pattern could be explained by the distribution of their food resources and breeding habitat (Jemal & Getu, 2018).

**Table 2.** The number of species and individuals sampled at each substation

Substation (Elevation, m)	No. of species	No. of individual
Inobong (396–533)	59	105
Ulu Kimanis (568–713)	63	145
Keningau (965–1062)	87	230
Mahua (1,060–1,249)	74	242
Mount Alab (1,784–1,891)	3	5

Based on the result, three species were dominant at CRP namely *Ypthima pandocus* (49 individuals), *Ragadia makuta* (43 individuals) and *Kallima limborgi* (42 individuals). They all belong to the family Nymphalidae. *Ypthima pandocus* and *Ragadia makuta* were found at all the substations except Mount Alab, whereas *Kallima limborgi* was found only at Keningau and Mahua substations, but not at the other study sites. *Ypthima pandocus* was the most abundant species and most of the individuals were found at the Ulu Kimanis substation. This species is known as an edge species and is frequently found at the forest edge and is fond of high light intensity. *Ragadia makuta* and *Kallima limborgi* are categorized as forest species and can only be found in shady areas in forests (Corbet & Pendlebury, 1992). Both species were found most abundant at higher elevations (Mahua substation). This result could explain the habitat preference of the butterfly species at CRP.

In the family Lycaenidae, *Eooxylides tharis* was the dominant species with 18 individuals, and this species was found at all the study sites except Mount Alab. Most of the species was caught at Keningau substation. On the other hand, *Hasora schoenherr* was the dominant species in the family Hesperidae with 14 individuals sampled, most of them were found at Mahua substation. All the species caught from the family Pieridae and Papilionidae were represented by less than 10 individuals.

Interestingly, 10 species that are endemic to Borneo were recorded in this study. Most of them were sampled at elevations above 965m a.s.l, except *Stibichiona schoenbergi* which was also sampled at a lower elevation, while

*Tanaecia orphne* was sampled only at an elevation of 568–713ma.s.l (Ulu Kimanis substation). Two out of three species sampled at Mount Alab are endemic, namely *Elymnias pellucida* and *Lethe perimede*. There were a total of 91 species (48.7%) collected as singletons along the different elevations at CRP. Most of the singletons were sampled at Keningau substation (30 species, 16%), followed by Ulu Kimanis substation (22 species) and Mahua substation (21 species) both of which accounted for about 11.7% respectively. Ismail et al. (2018) also reported that high a proportion (49%) of singletons were recorded in their study. Some of the species are considered rare including *Agathasa calydonia* and *Rhinopalpa polynice* (Corbet & Pendlebury, 1992). This finding highlighted the conservation values of butterfly assemblages at different elevations at CRP.

Based on the diversity indices, the Shannon-Wiener index showed that three substations (Keningau > Inobong > Mahua) were moderately diverse. The similar pattern was shown in the Simpson evenness index (Keningau > Inobong > Mahua). While the Margalef richness index showed a different pattern (Inobong > Ulu Kimanis > Mahua) (Table 3). In general, all the three diversity indices showed very little difference among the substations, except for Mount Alab. Some studies reported a significant difference in tropical butterfly diversity at different elevations (Jemal & Getu, 2018; Ismail et al., 2018).

**Table 3.** The value of Shannon-Wiener, Simpson and Margalef indices at the substations

Substation	Shannon-Wiener Index	Simpson's Evenness Index	Margalef's Index
Inobong	2.826	0.9358	3.971
Utan Paradise	2.645	0.9134	3.917
Keningau	2.885	0.9393	3.586
Mahua	2.812	0.9316	3.753
Mount Alab	1.332	0.72	1.864

Based on Jaccard's Similarity index, the Keningau and Mahua substations shared the highest species similarity, while the lowest similarity was found between Mahua and Mount Alab substations. Due to very few species recorded at Mount Alab, no similarity was found between Mount Alab and other substations (Inobong, Ulu Kimanis and Keningau). Based on the values, it can be concluded that the species similarity between the substations was relatively low, less than 0.25 (Table 4).

**Table 4.** Jaccard's similarity index between the substations.

	Inobong	Ulu Kimanis	Keningau	Mahua	Mount Alab
Inobong	-	0.196	0.20	0.187	0
Ulu Kimanis	0.196	-	0.155	0.142	0
Keningau	0.20	0.155	-	0.22	0
Mahua	0.187	0.142	0.22	-	0.013
Mount Alab	0	0	0	0.013	-

There were only 8 species (4.28%) out of the total 187 species that were found at all substations, except Mount Alab. The species are *Mycalesis orseis*, *Ragadia makuta*, *Ypthima fasciata*, *Ypthima pandocus*, *Zeuxidia aurelius*, *Zeuxidia doubledayi*, *Eooxylides tharis* and *Jamides pura*. These species are generally not confined to undisturbed habitats (Corbet & Pendlebury, 1992).

It can be suggested that the butterfly assemblages were different at each of the substations, thus might reflect the habitat suitability at different elevations of CRP. Some studies suggested that ectothermic organisms such as invertebrates could be vastly affected altitudinally by changes in abiotic factors such as temperature, humidity and wind speed, along with biotic factors (vegetation and competitions) (Levanoni et al., 2011). At CRP, Mahua and Keningau substations have a cooler and more humid environment compared to other substations, but there is not much difference in terms of canopy coverage. Chen et al. (2009) reported that upward elevation shifts of moth species on Mount Kinabalu are consistent of being responses to climate change observed in the region, either as a direct physiological response to climate or as a consequence of altered interactions with other species. Highlands in tropical regions and their surrounding lowland habitats represent some of the most important locations in the world to maintain biodiversity in face of climate change (Chen et al., 2009).

## Conclusion

From this study, information regarding butterfly diversity and assemblages at different elevations at CRP were obtained. A more comprehensive butterfly checklist of CRP was established, which includes the conservation value of the species as well as their distribution at CRP. The information would be useful as baseline data and reference for future research. More importantly, the information could be used as a monitoring tool in assessing the impact of environmental changes, including global warming, on biodiversity. There is an urgent need to monitor how tropical biota is responding to the changes in their environment, not only by anthropogenic disturbance but also climatic factors such as global warming. By documenting sufficient information on their

diversity and assemblages, we could gain a better understanding of the impact on tropical biodiversity. Therefore, conservation management could be planned and implemented with effective approaches.

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## Research Article

# Enhancing Community Commitment in Conservation through Participatory Approach

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## ABSTRACT

Kg. Tudan is a village situated in the buffer zone of Crocker Range Biosphere Reserve (CRBR). About 402 people live in Kg. Tudan, and majority are farmers. However, the steep terrain and infertility of the soil has affected the livelihood of communities, making them dependent on the surrounding forest to support their living. The Sabah State Government, Japan International Cooperation Agency (JICA), and Universiti Malaysia Sabah (UMS) implemented a project in Kg. Tudan, Tuaran, Sabah from 1<sup>st</sup> July, 2013 until 30<sup>th</sup> June 2017, under a programme called "Sustainable Development for Biodiversity and Ecosystem Conservation" (SDBEC). The project's main focus was to develop sustainable livelihoods through a participatory approach and the concept of living in harmony with the environment. After the project ended, a qualitative study was conducted to evaluate the effectiveness of the SDBEC project implementation in Kg. Tudan and its implication towards the local communities' conservation awareness. Thirty-three villagers of Kg. Tudan were interviewed. The data was analysed using Leximancer software with results illustrated in the form of a conceptual map. The study's findings indicated that the knowledge and commitment of the community in Kg. Tudan on conservation was enhanced through a participatory approach. The study also identified that the community of Kg. Tudan required capacity building and integration of sustainable livelihoods with community-based natural resource management. We recommended for ministries and NGOs engaged in environmental and biodiversity conservation to direct more efforts



towards developing sustainable practices that facilitate local communities' participation in preserving natural resources.

**Keywords:** participatory approach, capacity building, conservation, natural resources, sustainable livelihoods

## Introduction

The creation of protected areas usually exacerbates poverty due to the closure of some sites they require as natural capital for livelihood (Adams et al., 2004). Therefore, Da Fonseca et al. (2005) have stressed that in managing these protected areas (PAs), we should focus on the overall matrix in which the region is embedded within and to not neglect what is happening outside the PAs, because what happens at the exterior will influence the interior of PAs. Many local communities are depending on forests for their livelihood (IUCN, 2012). Displacement in PAs establishment can impoverish the people through landlessness, joblessness, homelessness, marginalisation, food insecurity, increased morbidity and mortality. Apart from these, there is loss of access to common property and social disarticulation (Cernea & Schmidt-Soltau, 2006; Borrini-Feyerabend et al., 2004). Strict protection of PAs incapable of micromanaging biodiversity conservation across the human-influenced landscape accelerates this new approach that allows some human activities within the PAs (Barrow & Murphree, 1998). An emerging understanding of adaptive management recognises the right and livelihoods of local communities living nearby PAs (Borrini-Feyerabend et al., 2004).

The conservation objective is difficult to achieve without considering the voice of local communities in such areas. The local community must actively participate in distribution of power (Arnstein, 1969). One of these benefits of allowing local community participation in PAs management is recognition of their autonomy. Autonomy is the ability to attribute the events caused in one's life to internal causes rather than external causes, such as own skills and preferences. Autonomous motivation also enhance the individual's emotional, physical and psychological well-being (Decaro & Stokes, 2008). Emotion has a crucial role in decision-making; thus, it is essential in public participation, especially when interpreting and summarising complex information and motivating people towards action (Vining & Tyler, 1999). Participation also helps in enhancing motivation for cooperation through the recognition of stakeholder's choices and the inclusion of individual and cultural identity (Decaro & Stokes, 2008). Most participation in biodiversity conservation induced aspects benefit human interests, such as the requirement for open space, aesthetics, and clean water, consistent with human-centred interests (Stokes et al., 2009). However,

participation persuaded by heteronomous motivation will not last long, and it is very costly to maintain (Decaro & Stokes, 2008).

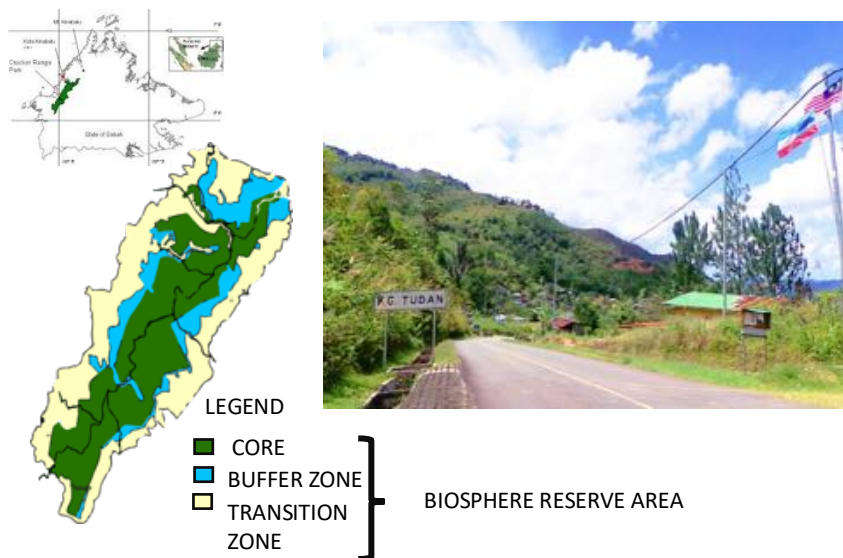
The participation process has three stages: the decision to participate, the initial participation, and to sustain participation. The desire to participate in conservation is influenced by (a) the existence of opportunity, (b) whether the opportunity fits with their interest and (c) motivation (West & Pateman, 2016). The Strategic Plan for Biodiversity 2011–2020 and Aichi Biodiversity Target include two targets that directly address the importance of protected areas: Target 5 and Target 11. In contrast, Target 14 deals with biodiversity services that contribute to the people's health and livelihoods (Secretariat of the Convention of Biological Diversity, 2013). This strategic plan will be reviewed at next CBD conference of parties meeting in Kunming, China.

Therefore, in a conservation project, the local community must be involved in the whole process of the initiation, design and implementation (Brooks et al., 2013). Participatory Rural Appraisal Training (PRA) is progressive learning with the local community to investigate and evaluate constraints and opportunities and make decisions on development projects (Chambers, 1994; Alam & Ihsan, 2012). PRA approaches were used intensively in participatory appraisal and planning for natural resources management, agriculture, poverty and social programmes and health and food security (Chambers, 1994). The advantages of PRA are as follows: (1) allow local communities to present their development priorities and ideas to be incorporated in the development plan; (2) the facilitators involved in PRA will be more motivated, and the government workers can understand the priority of other workers and local communities; and (3) establish better cooperation between communities, government agencies and other facilitators. However, there are also several limitations of PRA such as (1) PRA process prolongs the implementation of development; (2) specific individuals can take advantage of PRA to bring forth their problems; (3) most of the time, local communities expectations are too high, and it is difficult to meet their expectations; (4) misunderstandings between agencies; (5) domination of a specific sector of communities in the consultation and planning process and marginalisation of less or uneducated people, and (6) failure to consider the strata of society such as gender and social status (Mohd Yusof et al., 2012). To ensure the success of PRA, we have to distinguish between the different types of local community participation, the segregation of data produced in group interviews, and the knowledge and social competencies of the local community (Campbell & Vainio-Mattila, 2003).

## Methodology

### *Study Area*

Kg. Tudan, Tuaran District in the state of Sabah is located at 5°55'45''N, 116°19'53''E on steep terrain of western slope of Crocker Range. The area of the village is about 540 hectares. The village is accessible by a 3.8 km sealed road off the Penampang-Tambunan highway at kilometre 27 (**Figure 1**). Kg. Tudan is located in Crocker Range Biosphere Reserve (CRBR) which was designated as a Biosphere Reserve under the Man and Biosphere Reserve programme in June 2014 by UNESCO (core zone: 144,492ha, the buffer zone: 60,313 ha, the transition zone: 145,779ha) (**Figure 2A**). Under the zoning system of CRBR, Kg. Tudan is within the buffer zone of CRBR, which mainly follows the water catchment areas proposed by the state government. The village area also borders the core zone (consisting of Crocker Range Park and Crocker Range Forest Reserve) of CRBR (**Figure 2B**).



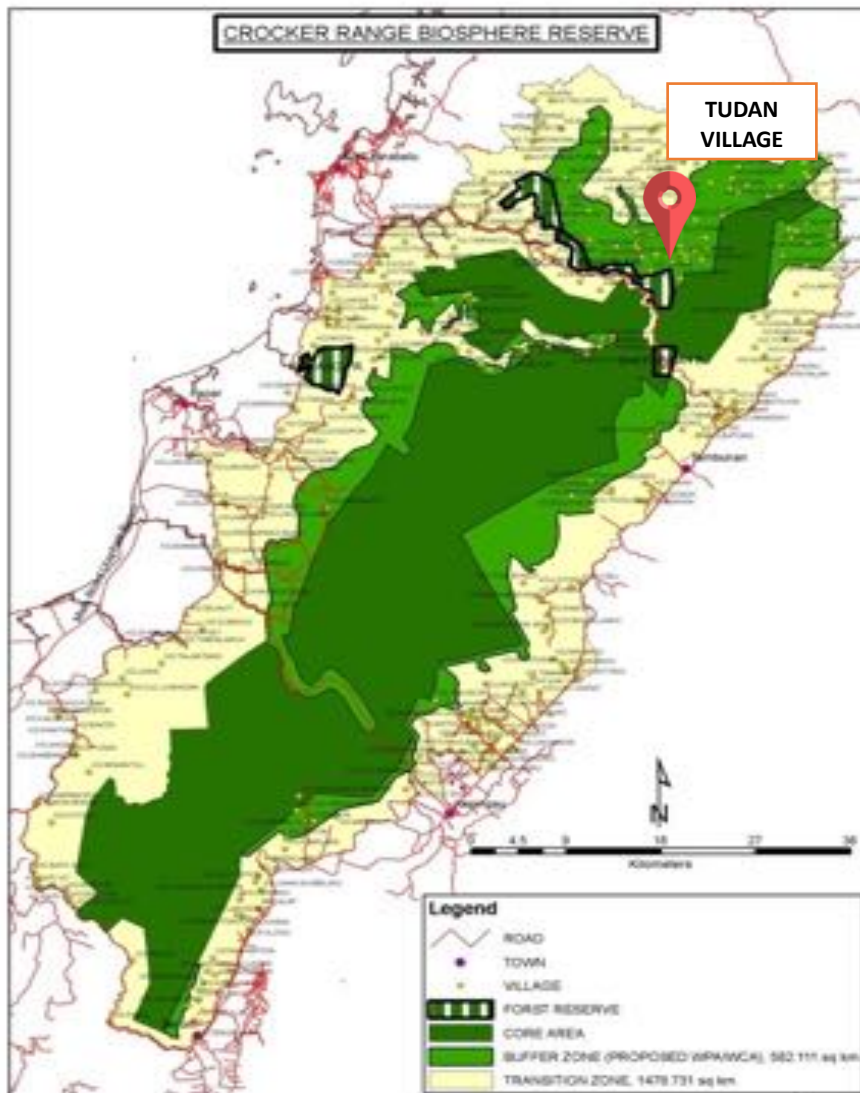
**Figure 1.** Map of Kg. Tudan, Kiulu and photo of the village

Generally, the landscape of Kg. Tudan consists of farmland, secondary forests and settlement areas. Secondary forests are areas left under long fallow, and the farmland is currently in use, and it is left under fallow in the future. Based on the landscape, the land use of Kg. Tudan's acreage can be classified as follows: (1) agriculture land (260.59 hectares); (2) secondary forest (189.90

hectares); and (3) settlement area (30.33 hectares) (Ere Consulting Group, 2015). Suzuki et al. (2015) found Kg. Tudan's soil to be low in nutrient content such as nitrogen, phosphorus, calcium, potassium, and cation exchange capacities (CEC) with low base saturation. By factoring the slope gradients, they concluded that only about 150 hectares or 27 per cent of Kg. Tudan is suitable for agriculture activities, with 130 hectares for medium to long-term crops and about 20 hectares for short-term crops (Jetony et al., 2021).

Kg. Tudan has a population of 402 persons (as of 2018) with 42 households. However, the number of 'permanent residents and occupied houses is less as many adults work in larger towns such as Tambunan and Penampang. These residents only come back to the village on weekends. At the same time, youngsters have also moved out to seek jobs elsewhere, including Peninsular Malaysia (Fifty et al., 2017). The agriculture sector is the primary source of livelihood for a majority of households in Kg. Tudan. As opposed to paid employment, the income from agriculture can vary substantially for each family based on crops harvested each week and sales generated at markets. Previous findings show that the agriculture activities in Kg. Tudan are based on the Satoyama concept, where recycling and traditional use of resources are within the carrying capacity (Dublin et al., 2014). As a typical village, most of the income generated each month as cash from sales is collected daily, much of which is spent immediately on necessary expenses. For the 25 households solely involved in agriculture, the data collected showed an average income of just over RM400 per month for each household which was substantially less than what was spent. Cash income was primarily derived from the sale of crops and supplemented from time to time with the sale of livestock such as domesticated pigs, and the income derived from forest-based products such as bushmeat, jungle durian, bee-keeping etc. (**Figure 3**) (Fifty et al., 2017).

A project under Sustainable Development for Biodiversity and Ecosystem Conservation in Sabah Malaysia (SDBEC) was implemented in Kg. Tudan, Tuaran. SDBEC is a technical cooperation project between the Sabah State Government, Japan International Cooperation Agency (JICA), and Universiti Malaysia Sabah (UMS), which started in July 2013 and ended in June 2017 (Jetony et al., 2021). The programme's main focus was to develop sustainable livelihood and other capacities for villagers in selected villages. These selected villages will then become models for living in harmony with the environment. The programme's primary objectives were to enhance the local communities' capacity and livelihood and improve their knowledge and awareness of the need to live in harmony with the environment.



**Figure 2A.** The location of Kg. Tudan in Crocker Range Biosphere Reserve (Source: Kementerian Air, Tanah dan Sumber Asli, 2019).



Figure 2B. The Google map of location of Kg. Tudan within CRBR.

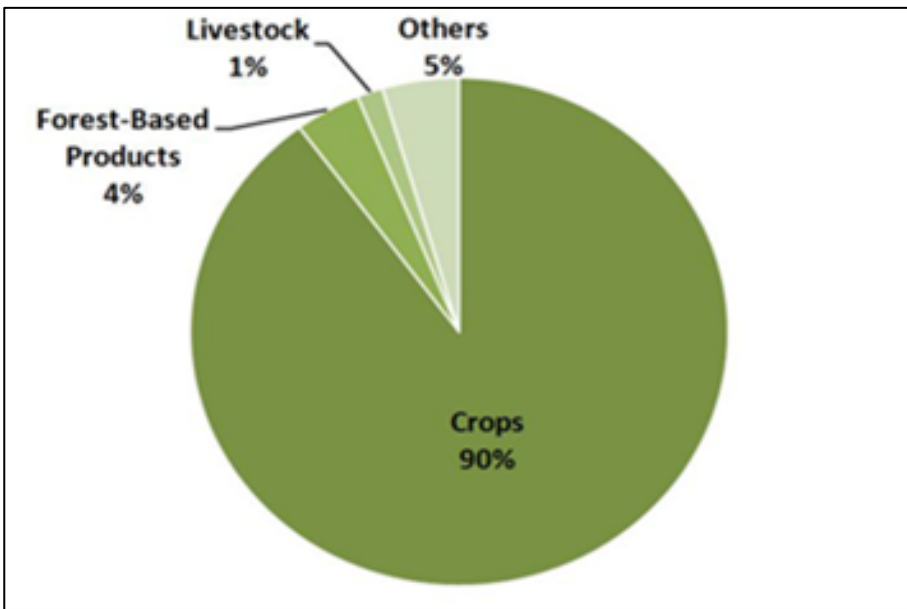


Figure 3. Sources of income for households involved in agriculture (Source: Fiffy et al., 2017).

The programme is aligned with the characteristics and objectives of UNESCO's Man and the Biosphere (MAB) programme in CRBR with the following criteria. Among the factors for consideration in selecting project areas for SDBEC are: important biodiversity; project has good potential to succeed; the villagers' willingness to participate in the programme; accessible; no similar ongoing programme in the site (SDBEC, 2013). Kg. Tudan was selected as one of two villages to implement the above project because it fulfils the above criteria. Under the SDBEC project, they carried out many activities. Among these activities were: (1) baseline survey; (2) Participatory Three-Dimensional Modelling (P3DM); (3) River Environmental Education Programme; (4) Participatory Rural Appraisal (PRA); (5) making compost and charcoal; (6) bee-keeping; (7) mulberry planting and juice and jam; and (8) strategies and action plans for Kg. Tudan (Ere Consulting group, 2015). The researcher was involved directly both as a project manager and chairman of the SDBEC Management Committee. Therefore, this study is to investigate the effectiveness of SDBEC in enhancing community commitment in conservation activities.

### ***Data Collection and Analysis***

A total of 33 data were collected from the field using a questionnaire and an in-depth interview. An ethic committee was formed to evaluate the question items used to interview and distribute to respondents. The ethic committee comprised the Sabah Biodiversity Council and the village head of Kg. Tudan. All respondents were provided with an informed consent form prior to the interview and questionnaire survey. Some secondary data from previous studies, namely by Suzuki et al. (2015), and Fiffy et al. (2017), were used to complement data collected.

As qualitative research generates a significant volume of rich data, especially with 33 in-depth interview transcripts. The data sets were subject to analysis via Leximancer; a software programme that facilitates thematic content analysis techniques (Loosemore & Galea, 2008). It provides the framework for discussing the identified themes, concepts and patterns that are the basis for all qualitative research analysis (Berg, 2001). The software is a proprietary mathematically-based text mining and text analytic tool that can visually identify the true meaning from text and visually display the extracted information. In addition to quantifying and coding text segments, Leximancer develops a thesaurus of words around a set of initial seed words. By incorporating the words' proximity in the transcripts, Leximancer displays the data in a "concept map" (Loosemore & Galea, 2008).

In the Leximancer concept map, the themes are represented by the circles, with the size and depth of colour used to indicate the theme's dominance. The overlap between the circles signifies these themes' co-occurrence in the data and the theme name derived from the most significant concept within the theme circle. Overlapping theme circles indicate a close association between concept groups. Leximancer was used to generate a "first-pass" visualisation of the survey from all qualitative or "free text" survey questions to identify connectivity or co-occurrence responses. Each theme circle comprises one or more concepts represented as nodes on the visualisation. Each themed circle's size denotes its relative importance; for example, the larger the circle, the greater the degree of concept interconnectedness.

This study investigated the community's commitment through their capacity requirements on conservation and their understanding of conservation activities. By analysing the capacity and their understanding of the environment, we can qualitatively correlate it with their eagerness to protect the environment surrounding them.

## Results and Discussion

**Figure 4** presents the "local" theme with 100% connectivity to be one of the main features of the community's capacity requirement. The main concept associated with "local" includes "conservation", "biodiversity", "protection", "manage", "habitat", "enforcement", and "action". All these are sub-notions of conservation of natural resources. Thus, the theme "community" appeared during the analysis with 35% connectivity. "Trust", "participation" and "information" are the concepts of importance for the theme "community".

It indicates trust and information are crucial for eliciting the local community's participation in conservation because values influence participation. Values are indicators for feelings and emotions, which help interpret and organise information and summarise complex information that can be the source for motivation (Vinning & Tyler, 1999). People need information, and they need to assess the programme for them to commit to the activities. Therefore, one way for them to learn is through participation in the project. In the interview, "deforestation" is also a concern for respondents. It shows that "monitoring" with 17% connectivity must ensure "deforestation" problems are curbed. However, respondents stated that they require the capacity to monitor.



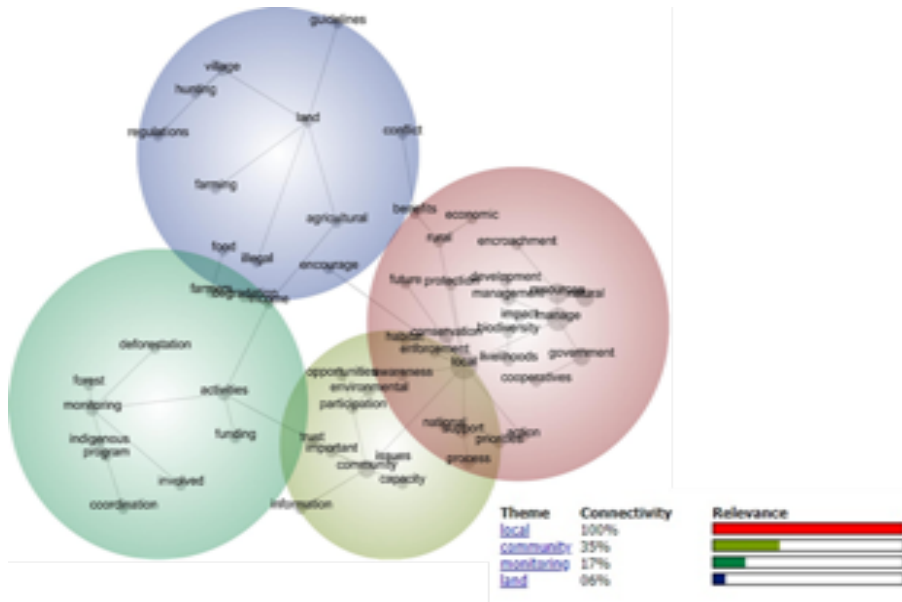
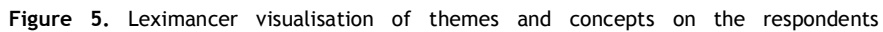


Figure 4. Concept map of capacity requirement of local community in Kg. Tudan

Figure 5 shows the Leximancer conceptual map related to respondent conservation perspectives. There are nine (9) themes that emerged from the data set. The themes are “conservation”, “community”, “local”, “information”, “work”, “forest”, “guide”, “financial” and “leadership”. The themes show that “conservation” is the highest connectivity with respondents' perspectives at 100%. It indicates that the respondents have a good comprehension of conservation. The respondents also stated that conservation is always associated with the community because almost all rural communities in Sabah live adjacent to forested areas. However, most of the respondents agreed that conservation is important for the community (92%) as it brings development. The respondents also revealed that locals (72 %), especially youth, are always keen to participate in conservation activities, probably because they are looking for new knowledge and employment opportunities. They also believe that conservation can generate new knowledge through the dissemination of information (18%), especially of the forest (3%) and giving more work prospects (4%), opportunity to be involved in guiding the conservation project (3%), financial aid for the community and leadership (2%), and capacity development (1%).



Their significantly good understanding of conservation could have contributed to SDBEC activities conducted through a participatory method in the village. Participation in the baseline study under SDBEC has enabled the community to get accurate and reliable information about their village. Participation in “three-dimension modelling”, formulation of strategies and formulation of action plans allowed them to visualise and realise the constraints and opportunities for development in the village. The participatory activities have reinforced their understanding as biodiversity protection is crucial for their survival due to the challenging steep terrain and infertile soil of Kg. Tudan. Bee-keeping, mulberry, and juice making activities have no disturbance to the environment and do not depend on soil fertility. These activities are generally in harmony with the environment. Participating in the formulation of strategies and action plans for the village has a very significant positive impact, thus enhancing commitment to managing their village sustainably. The community shared vision for their village development plan with an interlinked goal, thus

consolidating their efforts in achieving multiple objectives of the programme (UNU-IAS & IGES (eds.), 2016). Participation in the River Environmental Education Programme has allowed them to connect their development strategies in protecting the river system. Kg. Tudan is part of the CRP water catchment area. In Figure 5, one of the concepts is related to the watershed. This information indicates a community connected to watershed protection due to the knowledge that they have obtained previously. Participation in training can change people's behaviour if they apply skills locally and in a productive manner (Metha & Heinen, 2001).

## **Conclusion**

In conclusion, there is a strong indication that activities under SDBEC in Kg. Tudan conducted through a participatory approach managed to enhance the community's understanding and commitment. The community wanted not only to participate in the SDBEC but also in the management of the PA which is part of their socio-culture and economic domain. The participatory approach is the most feasible way of engaging the Kg. Tudan community. Hands-on practical learning and participatory peer-to-peer learning is the best option for the population residing in Kg. Tudan, as 67% of the population possess low literacy skills. They may have never been to school, or they may have only achieved primary-level schooling. It is multidimensional and multi-level, involving both natures with scale, uncertainty and multiple stability domains. Besides, human societies are influenced by values, perceptions, knowledge systems, traditions, rules, and diverging societal interest that guide their thinking and actions (Salasfsky et al. 2002; Berkes 2004). However, the study has shown that even with the local community's low education level, we can enhance their behaviour towards conservation and indirectly enhance their commitment to protecting and conserving biodiversity through a participatory approach. Therefore, an appropriate strategy is needed. To assist the local communities in the whole process of initiation, implementation, and monitoring, it must be based on their capabilities. If an intervention to implement a project does not consider the local communities' capacity to participate, the project will fail (Tiwari, 2006).

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## Research Article

# Preliminary Conservation Checklist of Orchid of Gashaka Gumti National Park, Nigeria

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## ABSTRACT

A preliminary checklist of the Orchidaceae of Gashaka Gumti National Park (GGNP) is presented, based on previous reports on the flora of GGNP and recent botanical explorations. The results from this study recorded 80 orchid species in 38 genera. The collection consists of 84% epiphytes and 16% terrestrial orchids. The genera with highest number of species are the *Bulbophyllum* (16 species) and *Polystachya* (11 species), while *Habenaria* is the richest terrestrial genus with five species. *Afropectinariella gabonensis*, *Bulbophyllum renkinianum* and *Rhipidoglossum polydactylum* are newly recorded for Nigeria. Other noteworthy taxa include *Angraecopsis elliptica*, *Bulbophyllum calvum*, *Holothrix aphylla* and *Tridactyle tridentata* which are some of the rarest orchid species in Nigeria. The highest species number was recorded in the lowland forest of the southern sector, particularly Kwano, with 42 species, followed by 29 species in the montane forest, five species in the savanna woodland, and four taxa in the lowland gallery forest. Our study identified recent transhumance to the enclave of the park (leading to grazing), and illegal logging of *Pterocarpus erinaceous* and *Afzelia africana* as the major noticeable threats to the continued existence of orchid species in GGNP. Therefore, further inventories with rigorous techniques such as the tree climbing technique are warranted for an exhaustive inventory and effective conservation of the orchid flora of GGNP.

**Keywords:** Chabbal Hendu, Kwano, Montane vegetation, Gumti sector, Orchidaceae

## Introduction

Nigeria flora has been better explored and documented than most other West African countries, particularly the Orchidaceae (Sanford, 1971). Of the 401 species of orchids recorded in West Africa, 157 species are recorded in Nigeria (Sanford, 1969a). After that, Segerbäck (1983) recorded 104 species. Recently there has been comparatively less botanical research on orchids in Nigeria. This lack of interest could be due to a lack of experts working in the group or due to



the morphological complexity of orchids. Consequently, this has halted the utilization of orchid species for scientific and aesthetic research in the country (Folorunso & Jayeola, 2009). Eventhough research on orchids from Nigeria spans well over several decades, certain areas of the country's botanical areas were less explored or not sampled. Also, there is no accurate checklist nor conservation notes on the orchids of Nigeria hence the published investigations of Summerhayes (1968), Sanford (1968, 1969 & 1971), are used as the only reference guides for the orchids of the country. With exception to the reports of Chapman & Chapman (2001), Akinsoji (1996, 2005 & 2016) and Umar et al. (2019), the available reports on orchids of Nigeria do not mention the orchids of Gashaka Gumti National Park (GGNP) thus this area remains poorly documented and the orchid there are less known.

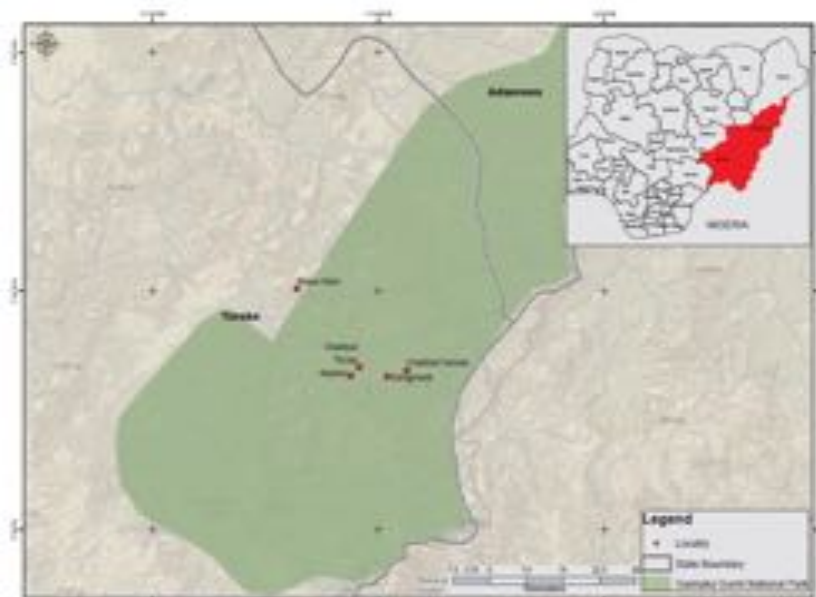
In recent times, orchid in GGNP is threatened by disturbances resulting from several anthropogenic activities leading to habitat loss. Floristic studies are the primary means by which the plant diversity of an area can be understood (Harris et al., 2012). Obtaining information on distribution patterns of threatened species like orchids and compiling these in the form of a checklist is pivotal for conservation planning (Shaheen et al., 2016). Also, providing an updated checklist will help contribute to forthcoming research into taxonomy and other aspects of Nigerian orchids (Lehnebach, 2003). Therefore, the study aimed to provide a comprehensive preliminary account of Orchidaceae occurring in GGNP, to inform conservation strategies and management decisions for better management of the national park flora diversity.

## **Material and Methods**

### ***Vegetation types of the study area***

Gashaka-Gumti National Park (6,731 km<sup>2</sup>; 06° 55' N, 11° 13'E) is the largest protected area within Nigeria, established through a federal decree in 1991 by the merging of two previously existing forest reserves (Sommer & Ross, 2011; Gumnior & Sommer, 2011). This park is managed by Nigeria National Park Service (NPS) Authority, under the Federal Ministry of Environment of Nigeria. The Park consists of two sectors: the northern sector, which is completely flat, and the southern sector, which is mountainous. The southern sector (Gashaka) varies in elevation from 300 m to 2,467 m and consists of mixed rugged terrain, with steep slopes, deep plunging valleys, precipititious escarpments, and swiftly flowing rivers (Sommer & Ross 2011; Dunn, 1993). The highest mountain in Nigeria, Chabbal Wadde with a height of 2,647 m above sea level is located in the Southeast of the park adjoining the Nigerian-Cameroon border (Sommer

& Gumnior, 2011). The climatic conditions of the park support forest vegetation, with a maximum annual rainfall of 1,977 mm, mean minimum temperature of 20.9 °C, and mean maximum temperature of 31.9 °C (Mucunguzi, 2007; Malhi et al., 2013). Although the soils within the park have not been systematically examined, Dunn (1999) and Chapman & Chapman (2001) reported humic ferrisols and lithosols as the soil types occurring at higher altitudes. Ferruginous tropical soils on crystalline acid rocks are found at lower elevation and alluvial soils in broad river valleys (Dunn, 1993). The park consists of seven habitat types: lowland gallery forest, riverine or riparian forest, montane forest, derived savanna, southern and northern Guinea savanna, and montane grassland. Access to this park by local communities is illegal. Therefore, hunting, fishing, timber, firewood, and other non-timber products' collection is illegal. Seasonal bush fires in the savanna area are common especially from December to January. Sometimes this fire enters some forests causing damage especially to the understorey species of plants. See **Figure 1** for map of study area.



**Figure 1.** Map of GGNP showing main sampling areas.

For this study, orchids were collected from the following vegetation types within GGNP:

***The Lowland Forest: Kwano (7°20'N, 11°35'E)***

The vegetation at Kwano consists of a mosaic of derived savanna in the old human settlement and a block of typical rainforest down Mayo Ngiti, which is probably the only remnant intact rainforest in northeastern Nigeria, with little or no human interference. The emergent trees grow up to a height of about 35–40 m, forming large buttresses as in *Ceiba pentandra* (L.) Gaertn. (Malvaceae) *Entandrophragma angolense* (Welw.) C.D.C. (Meliaceae), *Spondias microcarpa* A. Rich. (Anacardiaceae) and *Khaya grandifoliola* C.D.C. (Meliaceae). Further east, towards the Selbe footpath, there exist several gallery forests characterized by several lianas such as *Acacia pentagona* (Schumach.) Hook. f. (Fabaceae), *Landolphia owariensis* P. Beauv. (Apocynaceae), *Chasmanthera dependens* Hochst. (Menispermaceae) *Psychotria vogeliana* (Benth.) (Rubiaceae) amongst others. The transition between the lowland forest and the montane forest is around Tonga, an old settlement that extends to a hill called “Hitler” by the locals. A mosaic of rainforest exists here with most vegetation completely open and dominated by the invasive species *Chromolaena odorata* L (Asteraceae) forming a thicket at the edge of the forest.

***Submontane and montane area (1,500–2,000 m asl)***

**Chabbal Hendu:** 7°21'N, 11°44'E, 2,000m asl

The montane and submontane areas visited are Chabbal Ta'ale (7°22'N, 11°36'E), Chabbal Hendu (7°22'N, 11°36'E), 7°21'N, and 11°44'E) and Gangirwal located at (7°21'N, 11°41'E).

True montane vegetation in Nigeria is only found at Chabbal Hendu (Akinsoji, 1996; 2005), and Gangirwal which is an extension of Chabbal Hendu (CH), 32 km to the north (Chapman & Chapman, 2001). These are the two highest peaks in Nigeria, located at an altitude ranging from 2,000 to 2,467 m asl. Most forest at CH is located along streams, maintaining constant water year-round. The forest is characterized by luxuriant vegetation, strewn with ferns and orchid species. While the vegetation of Gangirwal is similar to that of CH, most of the forest is located at the escarpment and therefore is not easily accessible. The Nigeria-Cameroon frontier passes through the highest part of Gangirwal (Chapman & Chapman, 2001; Ezukanma et al., 2017). The montane vegetation is characterized Grasslands in Gangirwal by elevated montane grassland and a typical montane forest along the valleys dominated by *Loudetia simplex* (Nees) C. E. Hubbard, (Poaceae) *Elionurus argenteus* Nees (Poaceae) and *Rhytachne*

*rottboellioides* Desv. (Poaceae) (Chapman & Chapman, 2001). Trees here do not form a canopy, with the emergent species not exceeding 35 m in height. They include *Entandrophragma angolense* C.D.C. (Meliaceae) and *Newtonia buchananii* (Baker) G.C.C. Gilbert & Boutique (Fabaceae), with *Pouteria altissima* (A. Chev.) Baehni, (Fabaceae) as the most commonly distributed (Chapman et al., 2004). Other tree species are *Albizia gummifera* C.A.Sm. (Fabaceae), *Ixora foliosa* Hiern, (Rubiaceae) *Warneckea acutifolia* (De Wild.) Jacq.-Fél., (Melastomataceae) *Pterygota mildbraedii* Engl., (Sterculiaceae) *Prunus africana* (Hook.f.) Kalkman, (Rosaceae) *Schefflera abyssinica* Harms, (Araliaceae) and *Syzygium guineense* subsp. *bamendae* F. White in the family Fabaceae, (see Chapman & Chapman, 2001 for a complete description of Northeastern Nigerian montane forest). Although farming activities seem to be completely absent from some locations (e.g., Gangirwal), vegetation deterioration is at an alarming rate due to fire damage from seasonal bush burning and cattle grazing, which is now posing a major concern for the continual existence of local plant diversity. These often result in erosion surfaces on the underlying basement complex (Chapman & Chapman 2001). In turn, CH is probably the largest grazing enclave within GGNP. Cattle graze the montane grass throughout the year and graze along the streams in the dry seasons. This is the most obvious threat to the montane forest. Cattle grazing is very evident everywhere on montane vegetation with most of the vegetation trampled, thereby reducing the rate of regeneration (Chapman & Chapman, 2001). Slash-and-burn farming is also expected to increase due to the migration of people into the montane region.

For the submontane vegetation, we surveyed Mayo Sabere (920 m asl), Filinga (1,200 m asl), and Ta'ale (1,400 m asl). Ta'ale is about 3–4 km further east of Kwano (Gashaka Biodiversity Project Camp). It is the continuous extension of Chabbal Hindu enclave located at altitudes of 1,650 m asl. The vegetation here is predominantly grassland, with scattered trees that do not form a canopy. The emergent trees do not exceed 25 m; they include *Combretum molle* R.Br. ex G. Don, (Combretaceae), *Entada abyssinica* Steud. ex A. Rich. (Fabaceae), *Psorospermum febrifugum* Spach (Hypericaceae), *Syzygium guineense*, (Wild.) D.C. (Fabaceae), *Croton macrostachyus* Hochst. ex Del. (Combretaceae) and *Harungana madagascariensis* Lam. ex Poir. (Hypericaceae). The vegetation here is progressively experiencing slash-and-burn farming from its inhabitants. To the north of Chabbal Ta'ale, there is the rocky former settlement of Tonga Hill, which is the transition between lowland forest and montane forest. There is human settlement here; this area is

experiencing slash-and-burn farming, with rapid expansion and a bush fallow farming system.

### ***Savanna woodland (500m) (7°25'N, 11°31'E)***

This vegetation type is predominantly distributed at the lowest elevations of the park. It is dominated by tall coarse grasses reaching a height of about 2 m (*Andropogon gayanus* Kunth family Poaceae) and some trees. *Uapaca togoensis* Pax (Euphorbiaceae) seems to be the dominant woody species. Other abundant tree species include *Azelia africana* Sm., *Annona senegalensis* Pers (Annonaceae), *Crossopteryx febrifuga* Rubiaceae (G.Don) Benth, *Daniellia oliveri* (Rolfe) Hutch. & Dalziel, (Fabaceae) *Nauclea latifolia* Sm (Rubiaceae), *Piliostigma thonningii* (Schum.) Milne-Redhead (Fabaceae), *Prosopis africana* (Guill. & Pen.) Taub., (Fabaceae), *Parkia biglobosa* (Jacq.) Benth (Fabaceae), *Vitellaria paradoxa* C. F. Gaertn (Sapotaceae) and *Terminalia* spp (Combretaceae), while *Brachystegia eurycoma* Harms (Fabaceae) is the dominant tree along the rivers. This vegetation is considerably degraded and affected by humans. For example, *Pterocarpus erinaceus* Poir (Fabaceae) and *Azelia africana* Pers. are often pollarded to feed cattle and their bark is collected for medicinal purposes (Akinsoji, 1996 & Sommer & Ross, 2011).

### ***Sample collection and identification***

Several field campaigns took place between 2012 and 2019, during which living plants and herbarium specimens were collected. Identification of the samples was carried out using detailed comparison with herbarium reference specimens from the National Herbarium Yaoundé (YA) and Forest Herbarium Ibadan (FHI). The keys from Hutchinson and Dalziel (1954), Summerhayes (1968), Szlachetko & Olszewski (2001a, 2001b) were used for identification. We deposited samples at the Lagos University Herbarium (LUH), at the University of Lagos. Plants that were not fertile at the time of collection were cultivated and monitored in the shade house in the University of Lagos until they produced flowers, which enabled accurate identification following Sanford (1970a) and Stévant et al., (2010).

## **Results and Discussion**

### ***Distribution and floristic composition of the orchid flora in GGNP***

Results from the survey within the study area recorded a total of 80 taxa belonging to 37 genera (Table 1). *Bulbophyllum* Thouars (16 taxa) and *Polystachya* Hook. (11 taxa) are the most species-rich genera of epiphytic

orchids in GGNP while *Habenaria* represents the richest terrestrial genus with five species. These number of species recorded from GGNP alone represents about 26% of the combined orchid flora of Nigeria recorded by Sanford (1968, 1969a & 1971) approximately 157 species, Segerbäck (1983) illustrating 104 species, and Govaerts et al. (2018) 305 species compiled from the World Checklist of Monocots of the Royal Botanic Gardens Kew (Droissart et al., 2019). Of all the species recorded, three represent new national records, *Afropectinariella gabonensis* (Summerh.) M. Simo & Stévant, *Bulbophyllum renkinianum* Laurent (De Wild.), and *Rhipidoglossum polydactylum* (Kraenzl.) Garay. In addition, four of the rarest orchid species in the country, viz. *Angraecopsis elliptica* Summerh, *Bulbophyllum calvum* Summerh, *Holothrix aphylla* (Forssk.) Rchb.f., and *Tridactyle tridentata* Schltr., were recollected after 58 years from the same vegetation belt. The highest number of species was recorded in the lowland forest (42 species), followed by the montane forest (29 species), the savanna woodland (five species) and the lowland gallery forest (four species) (Figure 2). With regard to growth form, most orchids (84 %) were epiphytic, while only 16 % were terrestrial, recorded mostly in the savanna woodland of the study area.

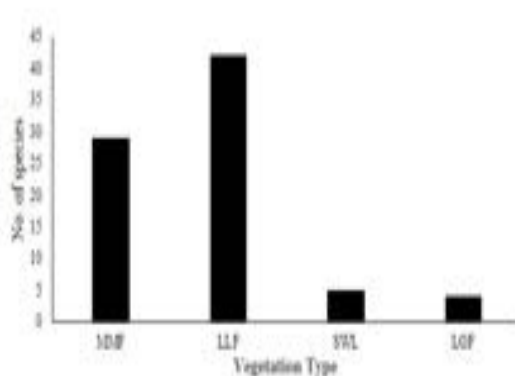


Figure 2. Distribution of orchids of GGNP in different vegetation types.

The majority of the species recorded in GGNP are epiphytic (84 %), which agrees with the general pattern found in most tropical orchid floras (Swartz & Dixon 2009, Zhang et al., 2016). The epiphytic orchids with most species belonged to *Bulbophyllum* and *Polystachya*, which are the two most-species epiphytic orchid genera in tropical Africa. Species richness in these genera is consistent with the

report of Kurzweil (1992) on the orchid flora of Mt Mulanje in Malawi, Szlachetko (2008), in his study of the Orchidaceae of Ivory Coast, and Simo et al., (2009), who recorded the highest number of species from these genera from Mbam Mikon hills in Cameroon. We recorded the highest number of species (45 taxa) from the lowland forest around Kwano. This area is highly protected, with minimal anthropogenic interference. In the northern sector of GGNP, we recorded four taxa only. This area is dominated by savanna woodland and have been highly impacted by cattle grazing and consistent annual fire burning (Gumnior & Sommer, 2011). This comparative high species richness in the mountainous region of GGNP is possibly due to the heterogeneity of mountains habitats, which provide different habitats for the survival of several species and climatic variables (temperature and precipitation) provide a good potential explanation for orchid species richness along the mountainous areas (Acharya et al., 2011 & Zhang et al., 2016). However, orchid species richness could be influenced by the level of habitat disturbance, climatic conditions such as temperature, moisture availability, humidity, precipitation (length of the dry season), and amount of rainfall and availability of undisturbed sites (Blanchard & Runkle 2006, Sanford 1970a).

### ***Conservation threats***

Undoubtedly, orchids are among the most threatened flowering plants (Zhao et al. 2021). Major threats highlighted to be facing orchids include habitat destruction, unsustainable harvest for horticulture, food, or medicine and exacerbated by climate change (IUCN/SSC Orchid Group 1996; Swarts & Dixon 2009; Zhang et al., 2015; Kull et al., 2016; Fay 2018). These result from deforestation, logging, fire, road construction, and the expansion of forest plantations and agricultural activities (Simo et al., 2009; Gale et al., 2018). In this study area, despite being a protected area; the orchid species here are jeopardized by illegal recent transhumance into the enclave of GGNP, which leads to illegal grazing in the national park. This livestock grazing negatively affects plant growth, community structure, ecosystem functioning, and services especially in the grasslands (Kirk et al., 2019; Rahmanian et al., 2019), and changes the floristic composition and the structure of herbaceous orchids species (Sonne et al., 2014). During grazing, most herdsmen looped branches of *Afzelia africana* Sm, where the leaves are used as forage for livestock. Similarly, several species are illegally harvested as fuel woods especially the *Uapca togoensis* Pax which is regarded as a softwood by the locals. This selective logging activities result in significant changes in forest structure, composition and function. It affects the epiphytic orchids by reducing the available habitat for the species and also alters the microclimate in the forest thereby leading to

species loss (Padmawathe et al., 2004). Together, these activities are leading to sharp forest fragmentation and habitat loss. In turn, one of the major noticeable threats, especially in the savanna woodland of the park and the lowland gallery forest vegetation, is phorophyte destruction around the enclave of GGNP, the greatly been decimated species of *Pterocarpus erinaceus*, *Azelia africana* have and locally extinct at the buffer zones of the park. Recently, interest has been shifted to logging of *Erythrophloeum suaveolens* (Guill. & Perr.) Brenan (Fabaceae) *Brachystegia eurycoma* Harms (Fabaceae), *Pseudospondias microcarpa* (A. Rich.) Engl., (Fabaceae) and several other large trees for timber exportation. To this day almost all the proximate ancestral vegetation in GGNP has been destroyed through livestock grazing and incessant logging. Regrettably, attention has been shifted away from the national park, and illegal logging has taken place in the park. Most of the species logged are phorophytes for orchids in the study area. For example, *Azelia africana* Sm and *Uapca togoensis* Pax have been reported as one of the phorophytes for epiphytic orchids and other epiphytes in the park (Akinsoji 2016). These activities are capable of causing drastic changes in the forest structure and subsequently affect the growth and diversity of the epiphytic species in particular (Jalal, 2012). Cattle grazing has a significant detrimental impact on the growth and survival of terrestrial plants (Narantsetseg et al., 2018), a result of trampling. Often, the cattle are capable of eating not only young flowering buds but also whole orchid plants (Jalal, 2012). Grazing and trampling, together, can influence the composition of the diversity of the terrestrial orchid.

In recent years, gold mining has rapidly increased across the southern sector of the park, especially along the riverbank, which contributes to deforestation in some locations. Most of the forest loss within the riverbank of the park is caused by artisanal miners. The mining activities leave in their wake extensive soil erosion, and rivers and streams full of soil from the mining sites. This gold mining usually results in the felling of trees, mostly *Brachystegia eurycoma* which are the dominant species of the riverbank. This species is an important phorophyte of many orchid species in this study area (Akinsoji 2005). Considering all these threats, the very survival of some of the orchid species in this area are in peril, given the fact that protected areas are no longer spared from anthropogenic activities.





**Plate 1:** A-J= *Aerangis biloba*, *Tridactyle tridentata*, *Bulbophyllum falcatum* var. *bufo*, *B. lupulinum*, *B. intertextum*, *Calypstrochilum christyanum*, *Diaphananthe vesicata*, *Cyrtorchis arcuata*, *Cyrtorchis aschersonii*



**Plate 2:** J-R= *Habenaria zambesina*, *Eulophia cucullata*, *Habenaria malacophylla*, *Plectrelminthus caudatus*, *Eulophia horsfallii*, *Eulophia cristata*, *Tridactyle anthomaniaca*, *Rhipidoglossum polydactylum*, *Polystachya laxiflora*

## Conclusion

With the number of species recorded and the new taxa first recorded for Nigeria in this study, it is possible that knowledge of the orchid flora of GGNP is far from complete. We can ascertain this study area is a haven for orchid diversity in Nigeria given the number of species recorded. Unfortunately, however, there are looming threats facing this area, with the montane vegetation appearing to be more vulnerable, predominantly from grazing and constant annual burning by inhabitants of the enclave. Therefore, further inventories are warranted to further our knowledge of the regional orchid flora and guarantee its conservation.

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**Table 1.** List of the orchid taxa collected at GGNP during the survey, including their habitats.

S/N	Species	Habit	Savanna	Lowland gallery forest	Lowland rainforest	Montane forest
1	<i>Aerangis biloba</i> (Lindl.) Schltr.	E	X	X	X	
2	<i>Aerangis kotschyana</i> (Rchb.f.) Schltr.	E			X	
10	<i>Afropectinariella gabonensis</i> * (Summerh.) M. Simo & Stévant	E				X
11	<i>Afropectinariella pungens</i> (Schltr.) M. Simo & Stévant	E			X	
3	<i>Ancistrohynchus capitatus</i> (Lindl.) Summerh.	E		X	X	
4	<i>Ancistrohynchus clandestinus</i> (Lindl.) Schltr.	E		X	X	
5	<i>Ancistrohynchus recurvus</i> Finet	E			X	
6	<i>Angraecopsis elliptica</i> Summerh.	E				X
18	<i>B. falcatum</i> var. <i>velutinum</i> (Lindl.) J.J.Verm.	E			X	
12	<i>Bolusiella zenkeri</i> (Kraenzl.) Schltr.	E		X		
15	<i>Bulbophyllum calvum</i> Summerh.	E				X
16	<i>Bulbophyllum cochleatum</i> Lindl.	E			X	
26	<i>Bulbophyllum cochleatum</i> var. <i>tenuicaule</i> (Lindl.) J.J.Verm.	E				X
17	<i>Bulbophyllum falcatum</i> Var. <i>falcatum</i> (Lindl.) Rchb.f.	E				
14	<i>Bulbophyllum falcatum</i> var. <i>bufo</i> (Lindl.) Govaerts	E		X		
13	<i>Bulbophyllum imbricatum</i> Lindl.,	E				X
19	<i>Bulbophyllum intertextum</i> Lindl.	E			X	
20	<i>Bulbophyllum lupulinum</i> Lindl.	E		X	X	
21	<i>Bulbophyllum oreonastes</i> Rchb.f.	E			X	X
22	<i>Bulbophyllum oxychilum</i> Schltr.	E		X		X
23	<i>Bulbophyllum pumilum</i> (Sw.) Lindl.	E			X	X
24	<i>Bulbophyllum renkinianum</i> * (Laurent) De Wild.	E			X	
25	<i>Bulbophyllum schimperianum</i> Kraenzl.	E			X	
27	<i>Bulbophyllum</i> sp	E	X		X	

28	<i>Bulbophyllum winkleri</i> Schltr.	E				X	
29	<i>Calyptrochilum christyanum</i> (Rchb.f.) Summerh.	E	X		X	X	X
30	<i>Calyptrochilum emarginatum</i> (Afzel. ex Sw.) Schltr.	E	X		X	X	
33	<i>Corymborkis corymbis</i> Thouars	E				X	
35	<i>Cyrtorchis arcuata</i> (Lindl.) Schltr.	E			X	X	
36	<i>Cyrtorchis aschersonii</i> (Kraenzl.) Schltr.	E				X	
37	<i>Cyrtorchis chailluana</i> (Hook.f.) Schltr.	E			X	X	
38	<i>Cyrtorchis ringens</i> (Rchb.f.) Summerh.	E					X
39	<i>Diaphananthe bidens</i> (Afzel. ex Sw.) Schltr.	E			X	X	
31	<i>Diaphananthe odoratissima</i> (Rchb. f.) P.J.Cribb & Carlsward	E			X	X	
40	<i>Diaphananthe pellucida</i> (Lindl.) Schltr.	E					X
32	<i>Diaphananthe vesicata</i> (Lindl.) P.J.Cribb & Carlsward	E				X	
41	<i>Disa equestris</i> Rchb.f.	T					X
42	<i>Disa welwitschii</i> Rchb.f.	T				X	
7	<i>Dolabrifolia aporoides</i> (Summerh.) M.Simo & Stévant	E					X
8	<i>Dolabrifolia disticha</i> (Lindl.) M. Simo & Stévant	E				X	
9	<i>Eichlerangraecum eichlerianum</i> (Kraenzl.) Szlach., Mytnik & Grochocka	E				X	
43	<i>Eulophia cristata</i> (Afzel. ex Sw.) Steud.	T	X				
44	<i>Eulophia cucullata</i> (Afzel. ex Sw.) Steud.	T	X				
45	<i>Eulophia horsfallii</i> (Batem.) Summerh.	T	X				
46	<i>Graphorkis lurida</i> (Sw.) O. Kuntze	T				X	
47	<i>Habenaria longirostris</i> Summerh.	T				X	
48	<i>Habenaria malacophylla</i> Rchb.f.	T			X	X	
49	<i>Habenaria mannii</i> Hook.f.	T				X	
50	<i>Habenaria procera</i> (Sw.) Lindl.	T				X	
51	<i>Habenaria zambesina</i> Rchb.f.	T				X	
52	<i>Holothrix aphylla</i> (Forssk.) Rchb.f.,	T					X
53	<i>Liparis nervosa</i> (Thunb.) Lindl.	T					X
54	<i>Malaxis chevalieri</i> Summerh.,	T					X
55	<i>Nervilia</i> sp.	T					X
56	<i>Oeceoclades maculata</i> (Lindl.) Lindl	T				X	
57	<i>Plectrelminthus caudatus</i> (Lindl.) Summerh.	E	X			X	
58	<i>Podangis dactyloceras</i> (Rchb. f.) Schltr.	E				X	
59	<i>Podangis rhipsalisocia</i> (Rchb.f.) P.J.Cribb & Carlsward	E					X



60	<i>Polystachya alpina</i> Lindl.	E				X
61	<i>Polystachya bennettiana</i> Rchb.f.	E				X
62	<i>Polystachya cooperi</i> Summerh.	E				X
63	<i>Polystachya dolichophyla</i> Schltr.	E		X	X	X
64	<i>Polystachya elegans</i> Rchb.f.	E				X
65	<i>Polystachya laxiflora</i> Lindl.	E			X	X
66	<i>Polystachya modesta</i> Rchb.f.	E		X		X
67	<i>Polystachya odorata</i> Lindl.	E		X	X	X
68	<i>Polystachya paniculata</i> (Sw.) Rolfe	E				X
69	<i>Polystachya</i> sp.	E	X	X		
70	<i>Polystachya tessellata</i> Lindl.	E			X	X
71	<i>Rangaeria rhipsalisocia</i> (Rchb.f.) Summerh.	E			X	X
34	<i>Rhipidoglossum brachyceras</i> (Summerh.) Farminhão & Stévant	E				X
72	<i>Rhipidoglossum kamerunense</i> (Schltr.) Garay	E				X
73	<i>Rhipidoglossum polydactylum</i> * (Kraenzl.) Garay	E				X
74	<i>Solenangis clavata</i> (Rolfe) Schltr.	T				X
75	<i>Stolzia</i> sp.	E	X		X	
78	<i>Tridactyle tridactylites</i> (Rolfe) Schltr.	E	X		X	
76	<i>Tridactyle anthomaniaca</i> (Rchb.f.) Summerh.	E	X		X	
77	<i>Tridactyle bicaudata</i> (Lindl.) Schltr.	E			X	X
79	<i>Tridactyle tridentata</i> * (Harv.) Schltr.	E	X		X	
80	<i>Vanilla</i> sp.	T			X	

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The species marked with an asterisk (\*) are being reported for the first time from the park.

## Research Article

# Bamboo: A Source of Multiple Uses for Adoption as an Alternative Livelihood in Odisha, India

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## ABSTRACT

Despite becoming one of the most valuable resources, the role of bamboo in livelihoods and rural development is poorly understood. The present study documents the indigenous utilization pattern of bamboo (*Bambusa vulgaris* Schrad ex Wendl.) and its significance in the social, cultural and religious life of the people of Bhadrak district, Odisha, India. An exploratory assessment was made from 71 informants through field surveys, literature consultations and key informant interviews. Plant parts such as culms are used for various purposes including construction, handicraft, fencing, musical instruments, as well as in rituals and ceremonies. Leaves are mostly used as fodder. This plant has been instrumental for indigenous people by providing them substantial livelihood through their own indigenous wisdom, from collection to the processing of products. Proper training with modern technology, financial assistance to develop infrastructure, as well as proper marketing of products will encourage more earning opportunities among rural people of the said district.

**Keywords:** *Bambusa vulgaris*, Bhadrak district, indigenous knowledge, phytoresources, rituals.

## Introduction

Communities around the world possess knowledge, based on centuries of experience, adapted to their environment and communicated from one generation to another. This knowledge manifests in various forms and is acquiring greater significance in the present-day context. The use of plant resources through indigenous knowledge of local people represents the foundation for human societies (Alcorn, 1995). Since ancient times,

phytoresources have been used to support human existence and development, and the collection and utilization of plant resources have played a key role in promoting human civilization and economic development. Bamboo is one such plant resource that has been linked with mankind for ages and has been an indispensable part of almost every civilization (Atanda, 2015). Bamboos is botanically considered a group of tall arborescent, perennial, evergreen, woody grass in the family Poaceae (Goyal et al., 2013). It is dominant in tropical and subtropical regions, especially in eastern and southern Asia, and South and Central America (Jiang et al., 2007; Lobovikov, 2007). The World Checklist of Bamboos and Rattans describes a total of 1,642 bamboo species belonging to 123 genera worldwide (Vorontsova et al., 2016). India supports about 136 bamboo species under 23 genera, which are found naturally and under cultivation, or both (Hore, 1998; Basumatary et al., 2017; FSI, 2017). Bamboo is extensively grown in diverse geographic, soil and climatic conditions of Madhya Pradesh, Maharashtra, Arunachal Pradesh, Telengana, Andhra Pradesh, Manipur, Karnataka, Assam, Chhattisgarh and Odisha. (Garbyal et al., 2008; Shukla & Joshi, 2000). *Dendrocalamus strictus* (Roxb.) Nees is the most widely distributed species and predominantly found in dry deciduous forests all over India. *D. sikkimensis* Gamble ex Oliv. is abundant in eastern India. *Bambusa bambos* (L.) Voss [Syn. *Bambusa arundinacea* (Retz) Willd.] is pantropical and flourishes in the moist mixed deciduous forests of Odisha, West Bengal, Assam, and in all states of Deccan.

In Odisha, there are 13 bamboo species, among which five are common. *Dendrocalamus stricus* (Salia) and *Bambusa bambos* (L.) Voss [Syn. *Bambusa arundinacea* (Retz) Willd.] (Kantabaunsa) are predominant in forests. *Bambusa vulgaris* Schrad ex Wendl. (Badi baunsa or Golden bamboo), *B. nutans* Wall. ex Murno (Sundar kani), and *B. tulda* Roxb. (Balangi), are mostly encountered in village areas (Bansal, 2016). *Bambusa vulgaris* is the preferred species for planting in homestead lands either in pure stands or mixed with other plants. Although bamboos have played essential roles for both subsistence and emerging economies through commercial products, many of which are profoundly linked in local cultures, scientific studies on this grass species are sporadic in India (Ghosh, 2008; Singh, 2008; Bhandari et al., 2015; Basumatary et al., 2017; Sharma et al., 2018; Chaurasia, 2019; Shukla & Joshi, 2020). In Odisha, it is not properly looked into (Bansal, 2016) and reports from Bhadrak district are still lacking. Hence this study was carried out in Bhadrak district of Odisha, India, to document the multifarious uses of thorn-less bamboo (*Bambusa vulgaris* Schrad ex Wendl.) and attempts to assess its significant role for artisans as a supplement to livelihood support.

## Methodology

### *Study site*

Bhadrak district (20° 43'–21° 13' N and 86° 6'–87° E) is located in northeast Odisha and covers an area of 2,505 km<sup>2</sup>, with a population of 1.507 million (2011 Census). It borders the Balasore district in the north, Jajpur in the south, Bay of Bengal and Kendrapara district in the east and Koenjhar in the west (**Figure 1**). The district has 1,311 villages with schedule caste population of 334,896 (22.23%) (Census, 2011). The district accounts for 1.61% of the state's territory and shares 3.62% of the state's population. The climate of the district is warm and humid. The maximum and minimum temperatures range from 37.4°C to 17.7°C, respectively, and the annual average rainfall is approximately 1,428mm (Anonymous, 2019), of which about 71% occurs in the monsoon season. The varying intensities of cyclones, drought and flood are the characteristics of the district.

### *Data collection*

The method employed in this study was designed with the purpose of providing baseline information on the traditional use of *Bambusa vulgaris* in the local system, through literature surveys and field visits to various areas (seven blocks of the district i.e., Basudevpur, Bhadrak, Bhandaripokhari, Bonth, Chandbali, Dhamnagar and Tihidi) from 2018 to 2020 in Bhadrak district, Odisha, India. Several field investigations were conducted using ethnobotanical methods (Martin, 1995). Documentation of the community indigenous knowledge system of bamboo artifacts was done through informant interviews, unstructured questionnaires, and direct observations. Specific questions asked during the field study include a) use of bamboo in day-to-day life, b) traditional craft of bamboo and c) socio-cultural significance of bamboo. A total of 71 artisans (44 male and 27 female) with an age group range of between 35–80 years were interviewed (**Table1**). Detailed information with reference to species used, areas and mode of collection, time spent for collection of the raw material, and general condition (poor, good, better) of the resource at the site were recorded. An inventory of different bamboo products, their local names, and their uses were also noted. The quantity of raw material used for making different products, designs used, time taken for making these items, and mode of selling of the product were also investigated. Discussions were held with the artisans to get their perception and views about possible ways and means for bamboo resource management and conservation, the demand and supply status of the resource, and the community demand for the products. In addition to records, personal observations were made on uses, and social and religious rituals were attended to study the actual uses of bamboo or its products. Visits to local

markets were undertaken to gather information on bamboo and bamboo products sold.

## Results and Discussion

The link between plant and human societies has resulted in several uses of plants that were developed through trial and error as well as by the imaginative minds of indigenous people the world over (Albuquerque et al., 2009; Dahlberg & Trygger, 2009). Plant diversity and associated traditional knowledge have been emphasized since the Rio Convention on Biological Diversity (CBD, 1992). The ethnic communities of India produce a wide range of products using available raw materials with indigenous processing methods. Correspondingly, bamboo multifarious products and uses by the people of Bhadrak district are one such typical use of the plant. In the present study, some areas of application of bamboo (*Bambusa vulgaris*) are depicted in **Table 2** and **Figures 2-5**. The culms and leaves were the most used plant part (**Figure 2a**). To harvest bamboo, a thick cast iron knife was used. Then it was split into half, the diaphragm removed, and strips of any width or thickness served to prepare a variety of products, used, and traded by rural artisans. The majority of permanent houses in the region are largely constructed out of bamboo. Bamboo is used to make all parts of the house from structural walls and columns to woven roofs to doors and windows (**Figure 2b and c**). In the studied area, bamboo was used in combination with other natural materials like wood and clay. Various authors affirm that bamboo is a suitable material for use in housing (Basumatary et al., 2015; Nurdiah, 2016; Selvan & Tripathi, 2017; Jose et al., 2021). According to villagers, houses constructed using bamboo as raw material are comfortable in hot and humid climates. This study shows that bamboo is used for building schools, shops, shelters for livestock, poultry farms, foot-bridges, scaffolding, as staircase and winnowing of sand during construction work (**Figure 2d-i**). It is also used for diverse purposes such as transportation, fencing of crop fields and homestead land, support to creeper plants, walking sticks, in afforestation programmes, ladder, incense sticks and many others as represented in **Figure 2j-o and 3a-h**. Bamboo, as a multi-facet substance, is widely used in several parts of the world (Borah, 2008; Liese et al., 2015; Ede et al., 2018; Panda et al. 2018; Chaurasia, 2019; Daramola et al., 2019).

The bamboo artisans at the study site are mostly Scheduled Castes and Scheduled Tribes, and economically backward. A majority are either daily wage labourers or marginal farmers, and making handicraft is their secondary occupation. The matured culms were used by artisans (**Figure 3i and j**) to make

a variety of handicraft products including small-baskets for sowing rice, big-baskets for fodder collection and carrying manure, winnowing trays (winnowing food grains), round-basket for winnowing of parched paddy colloquially called *mudhi* and *khai*, small-baskets with a handle for the offering of fruits and flowers to deities during different festivals, hand fans and many others as depicted in **Figure 3k-o** and **4a-j**. The present result substantiates the findings of Patel (2005), Singh (2008), Teron & Borthakur (2012), Darabant et al. (2016), Maulana et al. (2017), Sharma et al. (2018) and Nongkynrih et al. (2019). In our study, leaves were widely used as fodder for domestic animals. Bhandari et al. (2015) and Bhardwaj et al. (2018) opined that the leaves of *Bambusa vulgaris* can be exploited as forage, to reduce fodder scarcity and feed ruminants and meet their nutritional requirements. In the present investigation, bamboo was used for the preparation of various types of fishing devices (**Figure 4k-o**). The present result corroborates the findings of Niyonkuru & Lalèyè (2010) and Nath et al. (2015). In addition to fishing crafts and gears, bamboo was also used for the traditional fish drying process (**Figure 5a-b**). The utilization of bamboo for the fish drying process is also reported elsewhere (Sharma et al., 2013; Jhakar 2020).

The flute, a bamboo-made musical instrument is widely used in the study area (**Figure 5c**). The use of bamboo for preparing folk musical instruments is also reported (Patel, 2005; Cottingham, 2014; Kang et al., 2017; Liana et al., 2017). Additionally, another bamboo-made musical instrument locally called *kendara* was also used by a particular group of people in the district (**Figure 5d**). It was observed that dry leaves, dead and distorted culms in the clump, and branches were used as fuel for cooking. Previous studies suggest that bamboo can provide an alternative renewable energy source for cooking (Singh, 2008; Lucas, 2013; Hossain et al., 2015). The current investigation recorded the utilization of bamboo for various traditional socio-religious purposes, for instance, in the Raja festival, preparation of cottage for Jangnya, Hindu marriage rituals and Dola Purnima (**Figure 5e-h**). The present result draws support from the studies of Deb (2015). It is noteworthy to mention that in Hindu religious beliefs and socio-cultural practices, in the villages in India as well as in the Bhadrak district, a dead body is placed on a bier made of bamboo poles and carried on the shoulders of close male relatives to the funeral ground. In the current study, the young shoot (branch) was used as a tooth stick. The present result is similar to the findings of Vasishth et al. (2008) and Hounnankpon et al. (2017). During the study, it was revealed that both males and females were engaged in the production of bamboo items. It is noteworthy to mention that males exclusively engaged in harvesting and splitting of bamboo which needs more physical

labour, expertise, skill and tenacity. Females assist in all possible stages of handicraft making. Currently, bamboo handicraft art has suffered setback due to modernization that has brought in plastic, aluminum and iron ore products which are used to assist in various household chores. It is however noteworthy to comment that traditional bamboo handicraft art has survived and is still relevant to people's needs, in spite of modernization. The study revealed that most of the products were sold for Rs (Rupee). 20-100 and artisans earn a meager Rs. 200-300 per day as return due to lack of proper exposure, sponsorship and marketing of their products. Moreover, it provides livelihood to rural people in remote areas and also aids in women empowerment as most of the grassroot artisans are women. The present result is in agreement with Nath & Das (2008), Nath et al. (2009) and Lobovikov et al. (2012). In the studied area, bamboo is helping i.e., facilitate poverty mitigation and elimination among poor people (Rao et al., 1987; Lobovikov et al., 2009). The evidence is higher contribution of bamboo to the household economy in poorer households (Hogarth et al., 2013). Another important aspect of this indigenous craft is that illiterate artisans involved in craft making are in the unorganized rural sector and have no cooperative society for proper marketing and are also not aware of the laws concerning modern trade and commerce to protect their designs and technologies. This has resulted in their traditional knowledge, skills and practices in crafting such items with intricate designs and novelty now becoming vulnerable. Eventhough there are some training programmes on-going to train rural artisans in bamboo craft by the Central as well as State governments (Odisha Bamboo Development Agency and Directorate of Handicrafts & Cottage Industries, Odisha) in Odisha, due to lack of awareness, the artisans are unable to access such facilities to modernize their bamboo handicraft techniques.

## Conclusion

Woody bamboo grass is observed to be invaluable for a specific community -- those who have age-old expertise in processing and utilizing different parts of this plant to produce various household articles. This has resulted in the plant providing livelihood to these people. However, it is a matter of concern that many local men go to urban areas to make more income, leaving women at home to look after the children and to do housework. It is suggested that bamboo is an excellent pro-poor resource, especially in remote areas with limited off-farm income opportunities. Proper training with modern technology, financial assistance to develop infrastructure, as well as proper marketing of products will boost earning opportunities among rural people of the said district. In the aspect of women empowerment in the bamboo sector, the government should

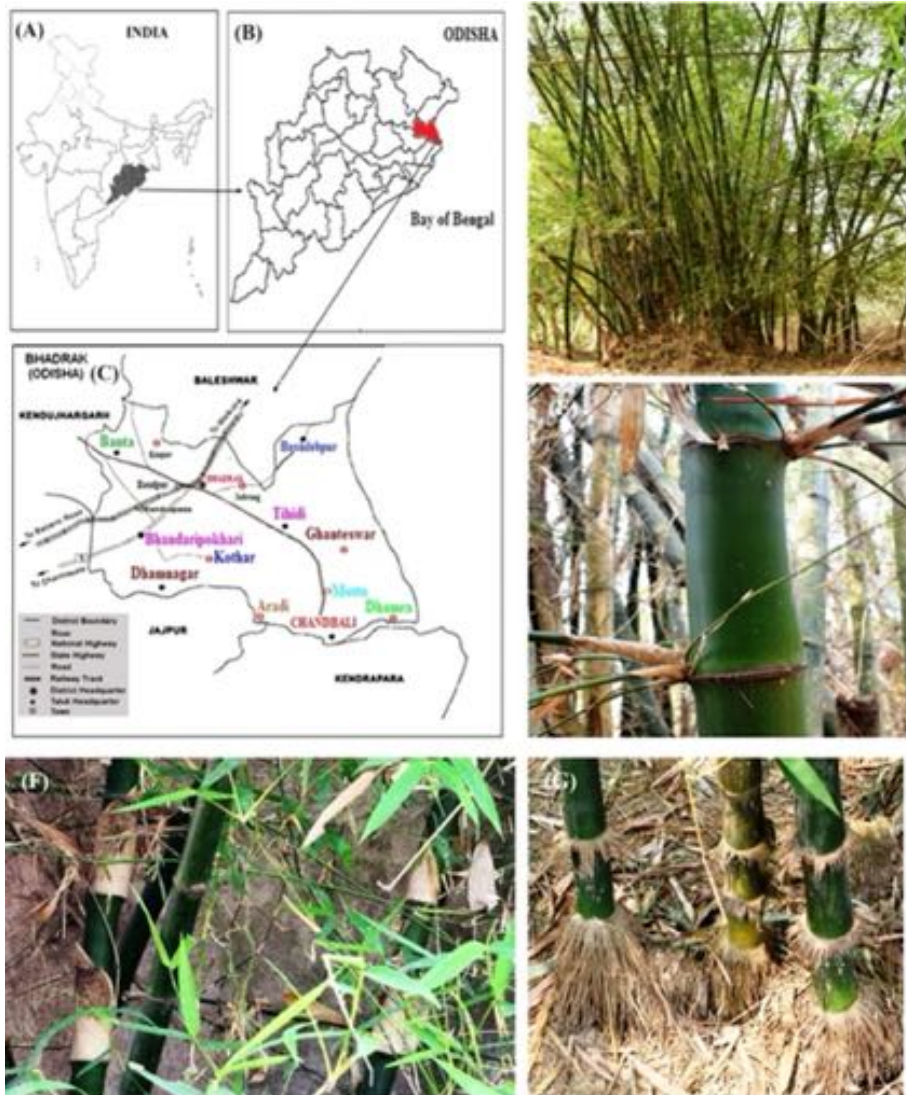
take necessary steps for the improvement of weaving skills among women. We envision that the development of traditional handicrafts can be combined well with poverty alleviation in the context of a government-targeted poverty alleviation programme.

**Table 1.** List of respondents, their gender and age, parts of bamboo used and traditional bamboo products manufactured by bamboo artisans in Bhadrak district, Odisha.

Respondent	Gender	Age	Use of plants	Bamboo Product for household use
Respondent 1	Female	75	Shoot	Fishing devices.
Respondent 2	Female	62	Shoot	Winnows, round winnows.
Respondent 3	Female	59	Shoot & branch	Small basket, small basket with handle.
Respondent 4	Female	48	Shoot & branch	Small round and flattened baskets.
Respondent 5	Female	57	Shoot & branch	Small round and flattened baskets.
Respondent 6	Male	80	Shoot & branch	Big basket, medium basket.
Respondent 7	Male	77	Shoot & branch	Big basket, medium basket.
Respondent 8	Male	64	Shoot & branch	Big basket, medium basket.
Respondent 9	Male	60	Shoot & branch	Big basket, medium basket.
Respondent 10	Male	71	Shoot & branch	Big basket, medium basket.
Respondent 11	Female	59	Shoot & branch	Medium basket, small medium basket.
Respondent 12	Female	35	Shoot	Food grain storage basket.
Respondent 13	Female	41	Shoot	Food grain storage basket.
Respondent 14	Female	58	Shoot & branch	Food grain storage basket.
Respondent 15	Male	45	Shoot & branch	Fishing devices, big basket.
Respondent 16	Male	72	Shoot & branch	Fishing devices, big basket, medium basket.
Respondent 17	Male	35	Shoot & branch	Fishing devices, medium basket.
Respondent 18	Male	41	Shoot & branch	Fishing devices, big basket.
Respondent 19	Male	65	Shoot & branch	Fishing devices, big basket, medium basket.
Respondent 20	Female	73	Shoot & branch	Round winnows, medium basket.
Respondent 21	Male	78	Shoot & branch	Big basket, medium basket, small basket.
Respondent 22	Male	71	Shoot & branch	Round winnows, big basket, medium basket.
Respondent 23	Male	64	Shoot & branch	Big basket, medium basket, small basket.
Respondent 24	Male	61	Shoot & branch	Big basket, medium basket, small basket.
Respondent 25	Male	56	Shoot & branch	Fishing devices, medium basket.
Respondent 26	Female	57	Shoot & branch	Small basket with handle, small round and flattened baskets.
Respondent 27	Female	37	Shoot & branch	Small basket with handle, small round and flattened baskets.
Respondent 28	Female	39	Shoot & branch	Small basket with handle, small round and flattened baskets.
Respondent 29	Female	46	Shoot & branch	Small basket with handle, small round and flattened baskets.
Respondent 30	Female	61	Shoot & branch	Small basket with handle, small round and flattened baskets.
Respondent 31	Female	73	Shoot & branch	Small basket with handle, small round and flattened baskets.
Respondent 32	Male	67	Shoot	Mat



Respondent 33	Male	47	Shoot	Mat
Respondent 34	Male	39	Shoot	Mat
Respondent 35	Male	42	Shoot & branch	Mat
Respondent 36	Male	64	Shoot & branch	Big basket, medium basket, small basket
Respondent 37	Male	70	Shoot & branch	Big basket, medium basket, small basket
Respondent 38	Male	56	Shoot & branch	Big basket, medium basket, small basket
Respondent 39	Male	70	Shoot	Mat
Respondent 40	Male	39	Shoot	Mat
Respondent 41	Male	47	Shoot	Mat
Respondent 42	Male	50	Shoot	Mat
Respondent 43	Female	48	Shoot & branch	Round winnows, small basket with handle.
Respondent 44	Female	67	Shoot & branch	Round winnows, small basket with handle.
Respondent 45	Female	36	Shoot & branch	Round winnows, small basket with handle.
Respondent 46	Female	43	Shoot & branch	Round winnows, small basket with handle.
Respondent 47	Female	59	Shoot & branch	Round winnows, small basket with handle.
Respondent 48	Male	60	Shoot & branch	Big basket, medium basket.
Respondent 49	Male	65	Shoot & branch	Big basket, medium basket.
Respondent 50	Male	43	Shoot & branch	Food grain storage basket.
Respondent 51	Male	49	Shoot & branch	Small round and flattened baskets.
Respondent 52	Male	50	Shoot	Winnows, round winnows.
Respondent 53	Male	53	Shoot & branch	Big basket, medium basket, small basket
Respondent 54	Female	69	Shoot & branch	Small basket with handle.
Respondent 55	Male	47	Shoot	Food grain storage basket
Respondent 56	Male	40	Shoot & branch	Small round and flattened baskets
Respondent 57	Male	76	Shoot	Mat
Respondent 58	Male	65	Shoot	Mat
Respondent 59	Male	53	Shoot	Mat
Respondent 60	Male	51	Shoot	Mat
Respondent 61	Male	48	Shoot	Mat
Respondent 62	Male	41	Shoot	Mat
Respondent 63	Male	64	Shoot	Mat
Respondent 64	Female	67	Shoot	Hand fan, food grain storage basket
Respondent 65	Female	50	Shoot	Hand fan, food grain storage basket
Respondent 66	Female	49	Shoot	Hand fan, small basket with handle.
Respondent 67	Female	54	Shoot	Hand fan, small basket with handle.
Respondent 68	Female	66	Shoot	Hand fan, small basket with handle.
Respondent 69	Male	63	Shoot & branch	Hat, big basket,
Respondent 70	Male	70	Shoot & branch	Big basket, medium basket, small basket.
Respondent 71	Male	61	Shoot & branch	Big basket, medium basket, small basket.



**Figure 1.** A. Location of Odisha state in the eastern region of India B. Map of Odisha state showing Bhadrak district and C. Study area showing different blocks of the Bhadrak district D. Clumps of *Bambusa vulgaris* Schrad ex Wendl E. Bamboo shoot showing node, internode and branch F. Culm sheath and leaves of *Bambusa vulgaris* G. Roots of *Bambusa vulgaris*.

**Table 2.** Multifarious uses of bamboo in Bhadrak district, Odisha.

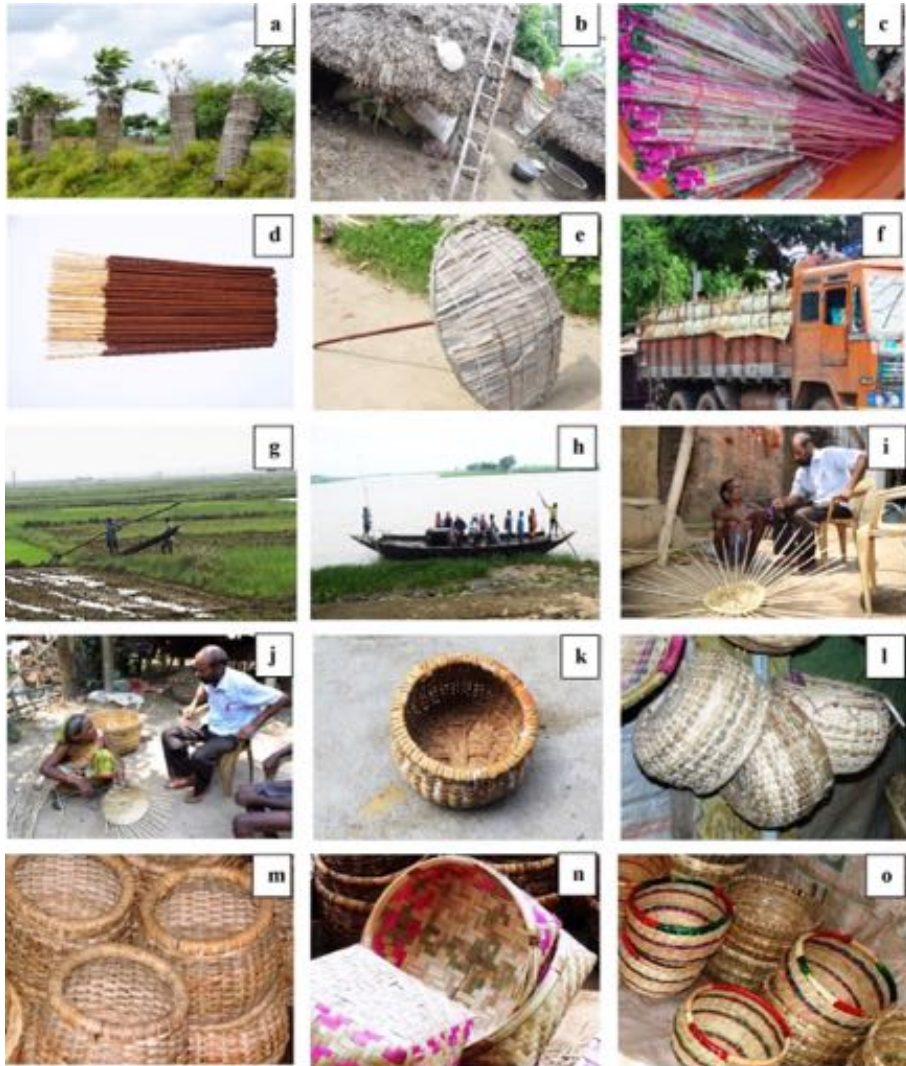
Use category	Local name	Specific uses
Construction for human shelter		Bamboo pole and splitting culms are used for construction of houses.
Sealing of house		Bamboo pole is used for sealing of house.
Poultry and cattle shelter		Bamboo pole and splitting culms used for poultry and cattle shelter.
Shed building (market, and other sales places)		Bamboo pole and splitting culms are used for shed building (market, and other sales places).
Construction in the field to observe crop		Bamboo pole and splitting culms is used for construction in the field to observe crops.
Foot bridge construction		Bamboo poles are used for foot bridge construction.
Bamboo pole Scaffolding		Bamboo poles are used for scaffolding.
Household goods-construction (stool, bench, shelf)		Strips of bamboo are used for house hold articles.
Bamboo pole		Carrying gods and goddess in various religious festivals such as Dola Purnima.
Bamboo pole		Used in Raja festival (June 13- June 15).
Fishing devices		To capture fish from pond, river, and rice fields.
Fish drying		Bamboo poles and strips are used for fish drying.
Ladder	Sidi	Used for climbing.
Bamboo bier		For transporting dead bodies to cremation ground.
Piece of bamboo		For mixing and stirring of paddy.
Hand fans	Binchana	Used rural and urban areas in summer season particularly during halt of electricity supply.
Solid lathis	Thenga	Used by the police.
Gate	Phataka	Main gate for the house.
Winnows	Kula	Winnowing of rice and other grains
Round winnows	Dala	Winnowing of parched paddy colloquially called 'mudhi' and 'khai'.
Big basket	Tola	For keeping hen.
Big basket	Jhanka	Fodder collection, carrying manure.
Medium basket	Tokei	For filtering rice water from cooked rice and collection of agricultural crops.
Medium round basket	Jhudi	For carrying manure and soil.
Small round basket	Chaluni	For sorting rice, wheat.
Small basket	Chota pachhia	For sowing rice in the field, keeping kitchen items as vegetables, collection of flower.
Small basket with handle		Used for carrying puja materials for deities.
Food grain storage basket	Bada tokei	For storage of food grain.
Small round and flattened baskets	Chota dala	Religious rites/worship.

Hat	Topi	Used as cap.
Mats	Chatei	Used in wall of rooms and protection to roadside plants.
Packing material		Used as packing material during transportation of goods.
Balance	Taraju	Used for weighing.
Walking sticks	Chhadi	Used by old age people for walking.
Bullock cart	Baladagadi	Used as part of bullock cart.
Small stick	Danga	To handle the cattle during ploughing and during driving of bullock cart.
Broom	Chanchuni	For collection of animal dung.
Mouth traps	Munha bandha	Mouth traps for ploughing animals (ox).
Ceremonial temporary construction	Mancha	Used to prepare pandals in ceremonies, for instance, Durga puja, marriage ceremony, meetings.
Hoardings		For information to public.
Boat	Danga	Used as pole in boat.
Rickshaw		Hoarding of rickshaw.
Agarbatii	Dhupakathi	Incense sticks.
Ice-cream		Ice-cream sticks.
Fencing	Bada	Fencing of home and farmland to protect the sapling from other animals.
Sealing of road		To block the road and contentment zone during Covid-19 pandemic.
Flag pole	Pataka Khunta	Flagpole for flags and cultural emblems
Musical instruments	Badyajantra	Flute.
Glass		Container for drinking.
Cup		Tea cup.
Pond protection		Fish pond protection.
Support for plants	Mancha	Stand and cover for creepers plants.
Firewood		Used for cooking.
Tooth stick	Danta kathi	Used as tooth brush.
Fodder	Pasukhadya	Fodder for domestic animals.



**Figure 2.** a. New shoot of *Bambusa vulgaris* Schrad ex Wendl b. bamboo used as structure base in mud house c. used as roofing material d-e. used in livestock shelter and poultry farm f. traditional bamboo foot bridge g. used in scaffolding h. stair case used during construction i. used for winnowing of sand j-k bamboo strip and basket used for transportation of fishes, fodder, manure, and rice-seedling l. used for protection to crop field m. used for protection of home garden n. support to creeper plants and o. walking stick.





**Figure 3.** a. Bamboo used for protection to plants in afforestation programme along roadside b. a ladder c. used in crackers d. incense sticks e. used as stick in umbrella f. used as packing material g. used for watering rice field h. bamboo pole (locally called *kata* used as device to start and halt the passenger boat i. and j. interview with artisan at work k-o. various handicraft product of baskets.



**Figure 4.** a. Bamboo handicraft product used for keeping vegetables b. round device generally utilized for carrying manure and other material used in construction work. c. Winnowing tray d. round winnowing tray e. *puja dala* with a handle f. flat *puja dala* usually used by the shop-keeper to sell *bhoga* to customers during various festivals g. hand fan h. Japanese fan i. round basket usually used by fisher man to keep fresh catch j. tea cup k-o. bamboo made fishing devices used by the fisher folk.



**Figure 5.** a-b. Bamboo used in fish drying process c. Bamboo made flute d. A musical instrument locally called *kendara* e. Bamboo made swing used in Raja festival f. Bamboo made open cottage used for Jangnya g. used in marriage ceremony h. used in carrying gods and goddess during Dola purnima.

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## Research Article

# The Chemotaxonomic Identification Using Structure Types of Secondary Metabolites and Their Bioactivities of Bornean *Litophyton arboreum*

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## ABSTRACT

The structure types and bioactivities of secondary metabolites derived from *Litophyton arboreum*, distributed in Sepanggar Bay, Sabah, Malaysia, were investigated as additional tools for establishing their species identification. As a result, a total of two secondary metabolites (alismol (1) and 10 $\alpha$ -methoxy-4B-hydroxy guaian-6-ene (2)) were isolated from Bornean soft coral *L. arboreum*. Their structures were elucidated based on spectroscopic data analysis and the antifungal activities of compounds 1 and 2 were determined. In addition, the compound 2 showed highest antifungal activity against *Haliphthoros milfordensis*. As a result of comparison with previous literature, significant variations were observed in relation to structure types of secondary metabolites and bioactivities. Information from this study gives additional evidence of chemotaxonomic significance and baseline data for effective selection of suitable lead pharmaceuticals.

**Keywords:** Soft coral, *Litophyton arboreum*, Sesquiterpene, Chemotaxonomy, Antifungal activity.

## Introduction

Recently, natural products from marine resources have increasingly been recognized to have an important role for human health and a vital component of healing practices (Carroll et al., 2022). The soft corals produce an unprecedented diversity of secondary metabolites with a wide range of biological activities and are considered the largest remaining reservoirs of undiscovered natural molecules (Nurrachma et al., 2021). However, despite increasing attention, the soft coral species are particularly difficult to identify because there are still few tools specific to the classification group required to evaluate gene flow at the population level (Fujita et al., 2012, Metwally et al., 2020). Correctly identifying soft coral species that have significant potential as

drugs for human use is the first basic step in any development of marine natural products. Among them, the genus of *Litophyton* (phylum Cnidaria, class Octocorallia, order Alcyonacea, family Nephtheidae), which produces many secondary metabolites such as diterpenoids, sesquiterpenoids and steroids, is attracting attention due to their wide diversity of biological activities such as anti-cancer, anti-HIV and antiproliferative (Ellithey et al., 2013; Ghandourah et al., 2015; Yang et al., 2020) properties.

However, despite the development of chemistry research, taxonomical knowledge is still lacking and often confusing. It has come to a stage where many researchers have isolated interesting compounds but could not reproduce them since identification problems are still not resolved. Generally, their identification is characterized by morphological features such as polyps arranged on the terminal branches only, colour and external form, by the arrangement of sclerites and their DNA barcoding (McFadden et al., 2009; Santhanam, 2020). However, soft corals identification using these methods is quite difficult and the classification is often re-described (McFadden et al., 2014; Imahara et al., 2017). Actually, in 2007, it was reported that the genus *Litophyton* should be morphologically synonymous with the genus *Nephthea* (van Ofwegen, 2016). Hence, a simple and highly reliable new identification method is required and the soft corals secondary metabolites can be suitable as a taxonomic marker since they produce secondary metabolites that show variations by species (Aratake et al., 2012).

The existence of many genus *Litophyton* (synonymous with the genus *Nephthea*) has been found on Borneo Island, Sabah, Malaysia (Ishii et al., 2010a; Ishii et al., 2010b; Ishii et al., 2016; Ishii et al., 2018; Tani et al., 2019), but their identification and detailed information are still dearth. During this investigation, efforts were made to incorporate chemotaxonomic identification features for *Litophyton* species. As an initial step, comparative profiling of structure types of their secondary metabolites in *L. arboreum* were carried out for soft coral populations from Sepanggar Bay. In addition, to their importance as a tool for phylogenetic relation, bioactivity potentials of these secondary metabolites were also investigated and reported in this paper.

## Materials and Methods

### *General Experimental Procedures*

<sup>1</sup>H-NMR (600 MHz) and <sup>13</sup>C-NMR (150 MHz) spectra were recorded on a JEOL ECA 600 NMR spectrometer using CDCl<sub>3</sub> with tetramethylsilane as an internal

standard. The AUTOPOL IV automatic polarimeter (Rudolph Research Analytic) was used to acquire their physical data. Preparative thin layer chromatography (TLC) was performed with silica gel glass plates (Merck, Kieselgel 60 F<sub>254</sub>), and column chromatography (CC) with silica gel (Merck, Kieselgel 60, 70-230 mesh).

### **Sample Collection**

The specimen of *L. arboreum* was collected from Sepanggar Bay, Sabah, North Borneo (6°4.683'N, 116°4.710'E), in July 2016. The voucher specimen (BORMI0055) was deposited in the BORNEENSIS Collection of the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Malaysia.

### **Extraction and Isolation**

The fresh soft coral (2.5 kg wet wt) was extracted in methanol (MeOH) at room temperature (24 °C, 1.0 L × 3 each for five days), subsequently filtered, concentrated *in vacuo* and partitioned between ethyl acetate (EtOAc) / distilled water (H<sub>2</sub>O) followed by partitioned with *n*-hexane / 90% MeOH from EtOAc fraction. The resulting crude extracts were subjected to CC eluting with a gradient of *n*-hexane and EtOAc with increasing polarity. The MeOH fraction 1 gave **1** (3.1 mg; 0.6%) after purification by preparative TLC using *n*-hexane. In addition, the MeOH fraction 3 was subjected to preparative TLC in *n*-hexane-EtOAc (3:1 (v/v)) and toluene-EtOAc (3:1 (v/v)) to yield **2** (2.9 mg; 0.6%). Percentages of compounds were the average of the respective compounds in 90% MeOH (2.1 g) crude extracts.

**Alismol (1):** colourless oil; C<sub>15</sub>H<sub>25</sub>O:  $[\alpha]_D^{25} + 3.3$  (*c* 0.2, CHCl<sub>3</sub>): <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta_H$ : 5.56 (1H, s, H-6), 4.79 (2H, s, H-15), 4.70 (2H, s, H-15), 2.50 (2H, m, H-9), 2.27 (1H, s, H-5), 2.25 (1H, m, H-1), 2.22 (1H, m, H-11), 2.20 (2H, m, H-8), 2.05 (2H, m, H-9), 2.03 (2H, m, H-8), 1.90 (2H, m, H-2), 1.75 (2H, m, H-3), 1.75 (2H, m, H-3), 1.71 (2H, m, H-2), 1.23 (3H, s, H-14), 0.99 (3H, d, H-12, *J* = 6.9 Hz), 0.98 (3H, d, H-13, *J* = 6.9 Hz); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta_C$ : 48.0 (C-1), 25.4 (C-2), 41.0 (C-3), 81.0 (C-4), 55.7 (C-5), 122.0 (C-6), 150.5 (C-7), 30.7 (C-8), 37.8 (C-9), 154.6 (C-10), 38.1 (C-11), 22.2 (C-12), 22.0 (C-13), 24.8 (C-14), 107.2 (C-15).

**10 $\alpha$ -Methoxy-48-hydroxy guaian-6-ene (2):** colourless oil; C<sub>16</sub>H<sub>28</sub>O<sub>2</sub>:  $[\alpha]_D^{25} - 0.5$  (*c* 0.2, CHCl<sub>3</sub>): <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta_H$ : 5.43 (1H, s, H-6), 3.14 (3H, s, 4-OMe), 2.22 (1H, m, H-1), 2.21 (1H, m, H-11), 2.19 (2H, m, H-8), 2.03 (1H, m, H-5), 1.85 (2H, m, H-8), 1.73 (2H, m, H-3), 1.70 (2H, m, H-2), 1.65 (2H, m, H-9), 1.60 (2H, m, H-3), 1.59 (2H, m, H-9), 1.55 (2H, m, H-2), 1.19 (3H, s, H-14), 1.18 (3H, s, H-15), 0.97 (3H, s, H-12), 0.95 (3H, s, H-13); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta_C$ :



48.7 (C-1), 22.3 (C-2), 41.2 (C-3), 79.9 (C-4), 50.8 (C-5), 121.9 (C-6), 150.4 (C-7), 25.3 (C-8), 36.2 (C-9), 79.9 (C-10), 37.9 (C-11), 22.4 (C-12), 21.9 (C-13), 23.2 (C-14), 18.6 (C-15), 49.4 (10-OMe).

### Antifungal Assay

The *in vitro* screening used compounds **1** and **2** to evaluate the antifungal activity (minimum inhibitory concentration (MIC)) against eight marine fungal strains. The eight strains of marine fungi (*Exophiala* sp. NJM 1551, *Fusarium moniliforme* NJM 8995, *F. oxysporum* NJM 0179, *F. solani* NJM 8996, *Haliphthoros milfordensis* IPMB 1603, *H. sabahensis* IPMB 1402, *Lagenidium thermophilum* IPMB 1401, and *Ochroconis humicola* NJM 1503) used for screening were kindly provided by the Borneo Marine Research Institute (BMRI), Universiti Malaysia Sabah. The MIC was determined visually as the lowest concentration showing no hyphal growth when this was incubated at 25 °C for 7 days. Besides this, the assay was performed three times, and sterile seawater with same values of MeOH containing no compound was prepared as a control for this assay (Tani et al., 2019).

### Results and Discussion

The MeOH extract was chromatographed repeatedly over preparative TLC to obtain pure secondary metabolites **1** and **2**. Compounds **1** and **2** showed similar NMR spectroscopic data as the previous literature data (Peng et al., 2003; Rao et al., 2000). Thus, **1** and **2** were identified as alismol (**1**) and 10 $\alpha$ -methoxy-4B-hydroxy guaian-6-ene (**2**), respectively (Figure 1).

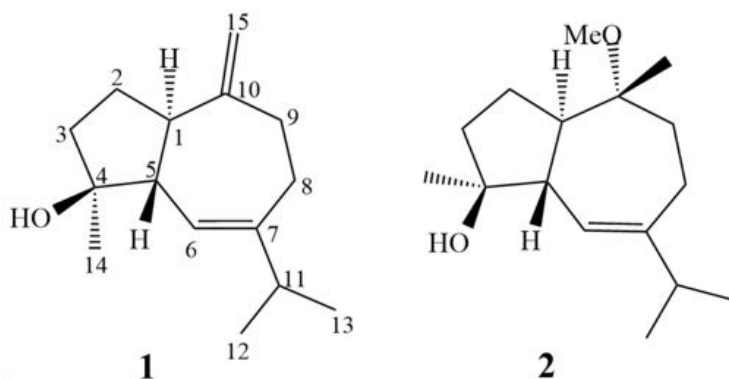


Figure 1. Structures of compounds **1** and **2** from *L. arboreum*.

Compound **1** was isolated as a colourless oil, with  $[\alpha]_D^{25} + 3.3$  (c 0.2, CHCl<sub>3</sub>). The <sup>13</sup>C-NMR spectroscopic data showed the presence of 15 carbon signals and four signals attributable to olefinic carbons ( $\delta_C$  154.6 (C-10), 150.5 (C-7), 122.0 (C-6), and 107.2 (C-15)). In addition, their multiplicities were confirmed by <sup>1</sup>H-NMR signal, DEPT, and HSQC measurements as three methyls at  $\delta_C$  24.8 (C-14), 22.2 (C-12), and 22.0 (C-13);  $\delta_H$  1.23 (H<sub>3</sub>-14), 0.99 (H<sub>3</sub>-12), and 0.98 (H<sub>3</sub>-13), four sp<sup>3</sup> methylenes at  $\delta_C$  41.0 (C-3), 37.8 (C-9), 30.7 (C-8), and 25.4 (C-2);  $\delta_H$  2.50 (H<sub>2</sub>-9), 2.20 (H<sub>2</sub>-8), 2.05 (H<sub>2</sub>-9), 2.03 (H<sub>2</sub>-8), 1.90 (H<sub>2</sub>-2), 1.75 (H<sub>2</sub>-3), 1.75 (H<sub>2</sub>-3), and 1.71 (H<sub>2</sub>-2), three sp<sup>3</sup> methines at  $\delta_C$  55.7 (C-5), 48.0 (C-1), and 38.1 (C-11);  $\delta_H$  2.27 (H-5), 2.25 (H-1), and 2.22 (H-11), one sp<sup>2</sup> methylene at  $\delta_C$  107.2 (C-15);  $\delta_H$  4.79 (H<sub>2</sub>-15) and 4.70 (H<sub>2</sub>-15), one sp<sup>2</sup> methine at  $\delta_C$  122.0 (C-6);  $\delta_H$  5.56 (H-6), and three quaternary carbons at 154.6 (C-10), 150.5 (C-7), and 81.0 (C-4) (Table 1).

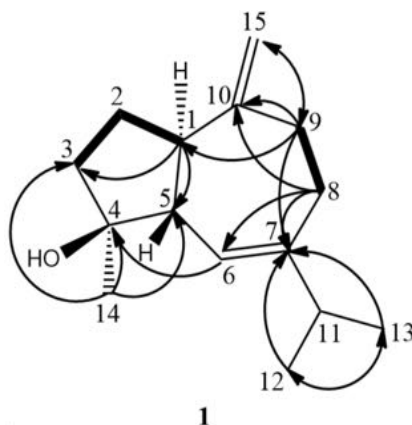


Figure 2. <sup>1</sup>H-<sup>1</sup>H COSY and key HMBC correlation of compound **1** and **2**.

Construction of compound **1** based on the <sup>1</sup>H-<sup>1</sup>H COSY and key HMBC correlations are shown in Figure 2. The cross peaks of <sup>1</sup>H-<sup>1</sup>H COSY determined the partial spin systems H-1/H<sub>2</sub>-2/H<sub>2</sub>-3. In addition, the HMBC correlations between H-1 to C-3/C-5, H<sub>2</sub>-9 to C-1/C-10, H<sub>3</sub>-14 to C-3/C-4/C-5 and H<sub>3</sub>-15 to C-9 revealed the connectivity for the two structures (5-membered ring and 7-membered ring). Thus, our results also support that the <sup>13</sup>C-NMR data for alismol in reference (Peng et al., 2003) at C-1 and C-5 are incorrect, and that a previously reported reference (Thin et al., 2019) is correct. In addition, the relative configuration of **1** was determined to be identical to previously reported compounds based on

comparison of chemical shifts, *J*-based configurations, and HMBC correlations (Peng et al., 2003).

On the other hand, compound **2** was isolated as colourless oil, with  $[\alpha]_D^{25}$  - 0.5 (*c* 0.2, CHCl<sub>3</sub>). The <sup>13</sup>C- NMR spectroscopic data of **2** indicated the presence of 16 carbon signals and two signals attributable to olefinic carbons ( $\delta_C$  150.4 (C-7) and 121.9 (C-6)), where their multiplicities were confirmed by <sup>1</sup>H-NMR spectroscopic data, DEPT and HSQC measurements as five methyls (including one methoxy) at  $\delta_C$  49.4 (10-OMe), 23.2 (C-14), 22.4 (C-12), 21.9 (C-13), and 18.6 (C-15);  $\delta_H$  3.14 (10-OMe), 1.19 (H<sub>3</sub>-15), 1.18 (H<sub>3</sub>-14), 0.97 (H<sub>3</sub>-12), and 0.95 (H<sub>3</sub>-13), four sp<sup>3</sup> methylenes at  $\delta_C$  41.2 (C-3), 36.2 (C-9), 25.3 (C-8), and 22.3 (C-2);  $\delta_H$  2.19 (H<sub>2</sub>-8), 1.85 (H<sub>2</sub>-8), 1.73 (H<sub>2</sub>-9), 1.70 (H<sub>2</sub>-2), 1.65 (H<sub>2</sub>-3), 1.60 (H<sub>2</sub>-9), 1.59 (H<sub>2</sub>-3), and 1.55 (H<sub>2</sub>-2), three sp<sup>3</sup> methines at  $\delta_C$  50.8 (C-5), 48.7 (C-1), and 37.9 (C-11);  $\delta_H$  2.22 (H-5), 2.21 (H-11), and 2.03 (H-1), one sp<sup>2</sup> methine at  $\delta_C$  121.9 (C-6);  $\delta_H$  5.43 (H-6), and three quaternary carbons at 150.4 (C-7), 80.9 (C-4), and 79.9 (C-10) (Table 1). Based on the above NMR spectroscopic data and comparison with literature, compound **2** was determined to be 10 $\alpha$ -methoxy-4  $\beta$ -hydroxy guaian-6-ene.

**Table 1.** <sup>1</sup>H (600 MHz) and <sup>13</sup>C NMR (150 MHz) spectra of **1** and **2** in CDCl<sub>3</sub>,  $\delta$  in ppm and *J* in Hz.

Position	1		2	
	$\delta_H$ (Mult. <i>J</i> )	$\delta_C$	$\delta_H$ (Mult. <i>J</i> )	$\delta_C$
1	2.25 (m)	48.0	2.03 (m)	48.7
2	1.90 (m)	25.4	1.70 (m)	22.3
	1.71 (m)		1.55 (m)	
3	1.75 (m)	41.0	1.65 (m)	41.2
	1.75 (m)		1.59 (m)	
4	-	81.0	-	80.9
5	2.27 (s)	55.7	2.22 (m)	50.8
6	5.56 (s)	122.0	5.43 (s)	121.9
7	-	150.5	-	150.4
8	2.20 (m)	30.7	2.19 (m)	25.3
	2.03 (m)		1.85 (m)	
9	2.50 (m)	37.8	1.73 (m)	36.2
	2.05 (m)		1.60 (m)	
10	-	154.6	-	79.9
11	2.22 (m)	38.1	2.21 (m)	37.9
12	0.99 (d, 6.9)	22.2	0.97 (d, 8.3)	22.4
13	0.98 (d, 6.9)	22.0	0.96 (d, 8.3)	21.9
14	1.23 (s)	24.8	1.18 (s)	23.2
15	4.79 (s)	107.2	1.19 (s)	18.6
	4.70 (s)			
4-OMe	-	-	3.14 (s)	49.4

In addition, the antifungal potential of compounds **1** and **2** were tested against eight fungal strains (*Exophiala* sp., *F. moniliforme*, *F. oxysporum*, *F. solani*, *H. milfordensis*, *H. sabahensis*, *L. thermophilum*, and *O. humicola*). The result of antifungal screening analysis showed that strongest activity was **2** with MIC 12.5 µg/mL against *H. milfordensis* (Table 2).

Table 2. Antifungal activities of compounds **1** and **2**.

Strains	MIC (µg/mL)	
	1	2
<i>Exophiala</i> sp.	50	50
<i>F. moniliforme</i>	50	50
<i>F. oxysporum</i>	100	100
<i>F. solani</i>	100	100
<i>H. milfordensis</i>	50	12.5
<i>H. sabahensis</i>	100	50
<i>L. thermophilum</i>	100	100
<i>O. humicola</i>	50	50

Note: Positive control; Clotrimazole with MIC 3.1 µg/mL.

The structures (guaiane types) similar to compound **1** and **2** have been reported to isolate from various terrestrial plants (Ma et al., 2019; Pan et al., 2021). Besides that, the guaiane type sesquiterpenes were isolated and reported from *L. arboretum*, among others in the genus *Litophyton* (Ellithey et al., 2013; El-Kassem et al., 2018). In addition, the results of antifungal activity provide important information on the functional quality of secondary metabolites from the genus *Litophyton* and can be used to better characterize the genus soft corals. Hence, secondary metabolites and their bioactivities can be considered a chemical taxonomic marker of the genus level.

## Conclusion

In this study, the variety in structure types and bioactivities of *Litophyton arboreum* in Malawali Island, Sabah, Northern Borneo, not fully studied previously was used as an additional tool for their identification. As a result, a total of two secondary metabolites (alismol (**1**) and 10 $\alpha$ -methoxy-4 $\beta$ -hydroxy guaian-6-ene (**2**)) were isolated from Bornean soft corals *L. arboretum*. Moreover, **2** showed relatively better inhibition against *H. milfordensis* at MIC value of 12.5 µg/mL. As far as we know, these results are the first record of sesquiterpenes isolated from the soft coral *L. arboreum* in Northern Borneo and

is a step towards enriching our knowledge of Bornean soft corals. These sesquiterpenes derivatives might be considered as a chemical taxonomic marker for *L. arboreum*.

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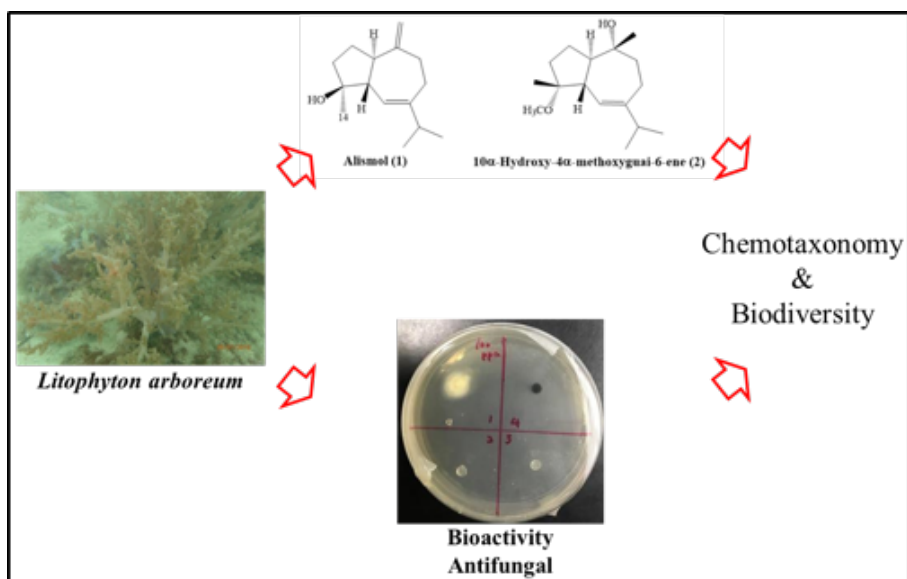
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### Graphical Abstract



## Research Article

# Phenolic Content and Antioxidant Activity of ethanol extracts of *Etlingera pubimarginata* (Zingiberaceae), including notes on its Morphology

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## ABSTRACT

Ethanol extracts of the dry weight leaves and rhizomes of *Etlingera pubimarginata* (Elmer) A.D.Poulsen were used in this study to determine its phenolic content and antioxidant activity. Total phenolic content was determined using the Folin-Ciocalteu method, while phosphomolybdenum method was used for the total antioxidant activity. Data revealed that the total phenolic content in *E. pubimarginata* leaves ( $27.25 \pm 0.72$  mg GAE/g dried sample) have greater amount of phenolics than its rhizomes ( $0.76 \pm 0.11$  mg GAE/g dried sample). Further, total antioxidant activity of *E. pubimarginata* was observed higher in leaves ( $34.83 \pm 0.49$  mg AAE/g dried sample) than rhizomes ( $1.82 \pm 0.09$  mg AAE/g dried sample), as well as the reducing power revealed to have higher amounts in the leaves ( $24.83 \pm 2.99$  mg GRPE/g dried sample) than the rhizomes ( $0.33 \pm 0.10$  mg GRPE/g dried sample). The high contents of phenolic compounds contribute to the antioxidant activity of extracts of *E. pubimarginata*. A perfect positive linear relationship was observed among the total phenolic content, total antioxidant activity, and reducing power ( $r=1$ ,  $p<0.001$ ) based on the correlation analysis. These imply that *E. pubimarginata* could be potentially used as a new source of natural antioxidant. Furthermore, a description of the species, including its updated distribution, phenology, and habitat and ecology are provided in this paper.

**Keywords:** ginger, total antioxidant activity, total phenolic content, Philippine endemic, reducing power



## Introduction

Phenolics, the most investigated group of secondary metabolites (Maulana et al., 2019), are known to have antioxidant activity (Lestari et al., 2015). Phenolic compounds exhibit peroxide decomposition, free radical inhibition, metal inactivation or oxygen scavenging in biological systems (Babbar et al., 2015). Antioxidants, on the other hand, are compounds that have the ability to either delay or inhibit the oxidation processes (Pisochi & Negulescu, 2011).

Zingiberaceae species are used in traditional medicine as well as spice agents, and food flavourings (Boonmee et al., 2011; Larsen et al., 1999). Many of these species are also used in traditional cures and are apparently associated with women-related illnesses, such as postpartum medicine for women after confinement (Larsen et al., 1999). In the Philippines, determination of total phenolic content and antioxidant activity of some ginger species have been carried out (Mabini & Barbosa, 2018; Redondo & Barbosa, 2018; Barbosa & Nueva, 2019). Philippine ginger species were also studied for their pollen morphology (Mendez et al., 2017; Acma & Mendez, 2018; Mendez & Acma, 2019) and phytochemical screening and antioxidant activity (Barbosa et al., 2016), and discovery of some new species in the Philippines (Docot et al., 2019, 2021, 2022; Mazo, 2022). However, with more than 100 species of Zingiberaceae in the Philippines, studies conducted are scanty, hence more studies are wanting.

*Etlingera pubimarginata* (Elmer) A.D.Poulsen is a ginger species endemic to the Philippines. This species was formerly known as *Amomum pubimarginatum* Elmer until this species was transferred to *Etlingera* by Poulsen & Docot (2018) on the bases of morphological characters that resemble some *Etlingera* species found in Sulawesi (Poulsen, 2012). This species is distinct from other Philippine *Etlingera* species by having coriaceous, sharply pointed, stiff bracts and its fruits are subglobose to ovoid, densely covered with golden brown hairs (Naive et al., 2019). Aside from its type locality in Mt. Apo (Davao del Sur) (Elmer, 1915), some populations of *E. pubimarginata* were also reported in Claveria (Misamis Oriental) (Naive et al., 2019), Marilog District in Davao (Acma et al., 2020), and Cinchona Forest Reserve in Bukidnon (Jayme et al., 2020). For over a century since its first collection, no laboratory studies on this species were conducted. Thus, this study was conducted to determine the total phenolic content and antioxidant activity of the leaf and rhizome extracts of *E. pubimarginata* collected from Mt. Malambo, Marilog District, Davao City. A description of the species updated distribution, phenology, habitat and ecology, and notes are also discussed in this paper.

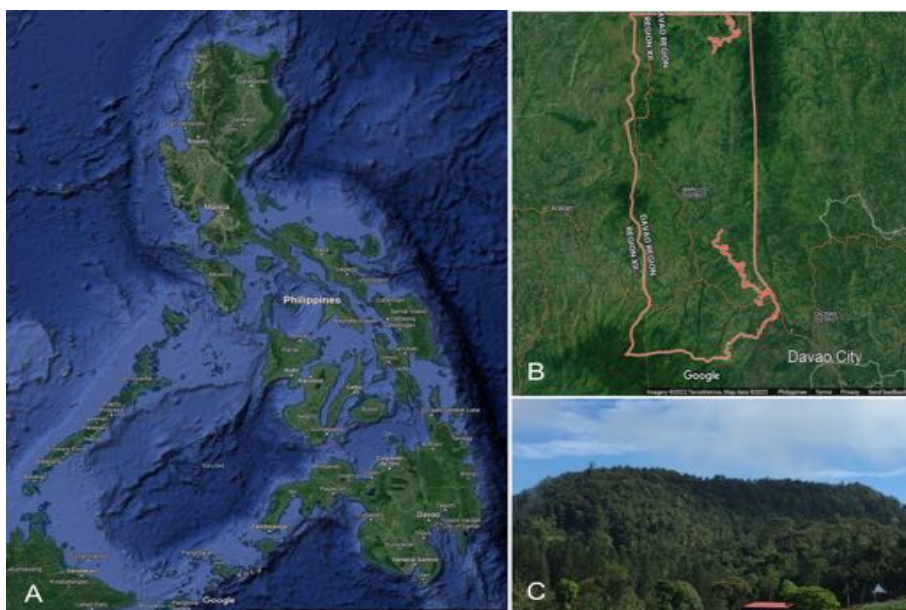
## Material and Methods

### *Entry Protocol*

A prior informed consent and a letter were personally submitted by NPM at the Barangay Captain's office of Barangay Datu Salumay to collect samples at Mt. Malambo. The gratuitous permit of Dr. Victor B. Amoroso issued by the Department of Environment and Natural Resources (DENR) - Region XI was used as the collection permit.

### *Place and Duration of the Study*

The leaf and rhizome samples and voucher specimens were collected from Mt. Malambo in Marilog District, Davao City on September 25, 2021 (**Figure 1**). These samples were transported to Central Mindanao University in Bukidnon province for processing. The determination of total phenolic content (TPC), total antioxidant activity (TAA), and reducing power (RP) of dry weight leaves and rhizomes of *E. pubimarginata* were conducted at the Natural Science Laboratory of Natural Science Research Center (NSRC) in Central Mindanao University from September 2021 – January 2022.



**Figure 1.** Site of collection of *E. pubimarginata* leaf and rhizome samples and voucher specimens. **A)** Map of the Philippines, **B)** Map of the Marilog District (red colour), **C)** Panoramic view of Mt. Malambo (A & B - ©2021 Google image).

### ***Collection, Measurement, and Description of Plant Materials***

The vegetative and reproductive parts of *E. pubimarginata* were collected for voucher purposes. Reproductive materials were preserved in 80% ethanol as pickled collection, and the herbarium specimens were dried. The herbarium specimens were then deposited at the Central Mindanao University Herbarium (CMUH). Five fresh individuals of *E. pubimarginata* were measured and described in the field (Mendez et al., 2017; Acma & Mendez, 2018). For the small and detailed floral parts, a stereo microscope at the NSRC Plant Tissue and Spore Culture Laboratory was used to view the dissected parts for description. After the microscopic examination of the floral parts, these were then placed inside the prepared pickled collection.

### ***Sample Preparation and Extraction***

Leaves and rhizomes of *E. pubimarginata* were collected and placed separately inside labelled plastic bags with wet tissue paper to prevent dehydration. These samples were brought to Central Mindanao University for further processing. Leaf and rhizome samples were washed, and the earthy matters were removed prior to air-drying. Dried samples were powdered and stored until used.

Extracts were prepared following the method of Padda & Picha (2008) with some modifications. The dried leaf and rhizome powder were extracted with absolute ethanol with a ratio 1 g: 25.0 mL at room temperature ( $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ). The mixtures were shaken for 1 hour in an orbital shaker at 300 rpm and centrifuged for 5 minutes at 5,000 rpm. The supernatants herein referred as extracts were collected in separate 15 mL conical tubes. The concentration of the extracts was determined as 40,000 ppm (mg dried sample per liter test solution) based on the plant to solvent ratio used in extraction. These were then stored at  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and were used in the succeeding analyses. The ethanolic leaf and rhizome extracts of *E. pubimarginata* were subjected for the TPC, TAA, and RP determination in 96-well plate format colorimetric assays. Sample extraction was done in triplicates and analyzed in three trials per replicate.

### ***Total Phenolic Content***

The TPC of the extracts was determined using the method described by Ainsworth & Gillespie (2007) with some modifications. Briefly, 200  $\mu\text{L}$  of the extracts and 200  $\mu\text{L}$  of 10% Folin-Ciocalteu reagent were transferred in a 2-mL centrifuge tube. The reaction mixture was set aside for 5 minutes and added with 800  $\mu\text{L}$  of 10% sodium carbonate. The mixture was set aside at room temperature ( $25^{\circ}\text{C}$ ) for 30 minutes and centrifuged at 11,000 rpm for three minutes. A 200  $\mu\text{L}$  of the resulting solution was then transferred to the assigned

microplate wells. The absorbance was determined at 750 nm using a microplate reader (Molecular Devices Spectramax® 250). Likewise, the same method was done to prepare a standard calibration curve by using 200 µL of standard solutions with a concentration range of 0-100 ppm gallic acid (GA) in 10 ppm increment from a stock solution of 100 ppm GA in absolute ethanol. The TPC was determined and expressed as milligram gallic acid equivalent per gram dried sample (mg GAE/g dried sample) by interpolating sample absorbance against the standard calibration curve using the formula below:

$$\text{Total Phenolic Content} \left( \frac{\text{mg GAE}}{\text{g dried sample}} \right) = \frac{A}{B}$$

where: A = gallic acid concentration of the sample solution determined from the calibration curve (mg GAE/L)  
B = the concentration of test solution (g/L, gram dried sample per L solution)

#### **Total Antioxidant Activity**

The TAA was determined using phosphomolybdenum method of Prieto et al., (1999) with slight modifications. Briefly, 50 µL of extracts were placed in centrifuge tubes and diluted with 200 µL (1:1 ethanol: water). The solution was added with 600 µL of reagent solution (prepared by mixing equal amounts of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 95°C for 90 minutes. The samples were allowed to cool at room temperature (25°C) and centrifuged for three minutes at 11,000 rpm. The absorbance of the supernatant was measured at 695 nm against a blank using a microplate reader (Molecular Devices Spectramax® 250). Also, the same method was done to prepare a standard calibration curve by using 200 µL of standard solutions with a concentration range of 0-150 ppm ascorbic acid (AA) in 15 ppm increment from a stock solution of 300 ppm AA in absolute ethanol. TAA was determined by interpolating sample absorbance against the standard curve. The TAA was calculated using the equation as follows:

$$\text{Total Antioxidant Activity} \left( \frac{\text{mg AAE}}{\text{g dried sample}} \right) = \frac{A}{B}$$

where: A = ascorbic acid concentration of the solution determined from the calibration curve (mg AAE/L)  
B = concentration of the test solution (g/L, gram dried sample per L solution)

#### **Reducing Power**

The RP was determined by adapting the method described by Murugan & Iyer (2011) with some modifications. In a centrifuge tube containing 1 mL of

extracts, 200  $\mu\text{L}$  of 0.2 M phosphate buffer (pH 6.6) and 200  $\mu\text{L}$  of 1% (w/v) solution of potassium ferricyanide were added. After, the mixture was incubated at 50°C for 30 minutes. After cooling to room temperature (25°C), 200  $\mu\text{L}$  of 1% (w/v) trichloroacetic acid was added. The mixture was centrifuged for three minutes at 11,000 rpm. An aliquot of 200  $\mu\text{L}$  of the supernatant was transferred to a 96-well plate, and 20  $\mu\text{L}$  of 1% (w/v) solution of ferric chloride was added. The absorbance was measured at 620 nm using a microplate spectrophotometer (Molecular Devices Spectramax® 250). Likewise, the same method was done to prepare a standard calibration curve by using 1000  $\mu\text{L}$  of standard solutions with a concentration range of 0-100 ppm gallic acid (GA) in 10 ppm increment from a stock solution of 100 ppm GA in absolute ethanol. Sample concentration was determined by interpolating sample absorbance against the standard curve. The RP was expressed as milligram gallic acid reducing power equivalent per gram sample (mg GRPE/g sample) and calculated as follows:

$$\text{Reducing Power} \left( \frac{\text{mg GRPE}}{\text{g dried sample}} \right) = \frac{A}{B}$$

where: A = gallic acid concentration of the test solution determined from the calibration curve (mg GRPE/L)  
 B = concentration of the test solution (g/L, gram dried sample per L solution)

### ***Statistical Analysis***

The sample extraction was done in triplicates and TPC, TAA, and RP were analyzed in three trials. The data gathered among the TPC, TAA, and RP of *E. pubimarginata* were correlated using Pearson's correlation at 0.001 level of significance.

## **Results and Discussion**

***Gross Morphology of Etlingera pubimarginata (Elmer) A.D.Poulsen (Figure 2).***

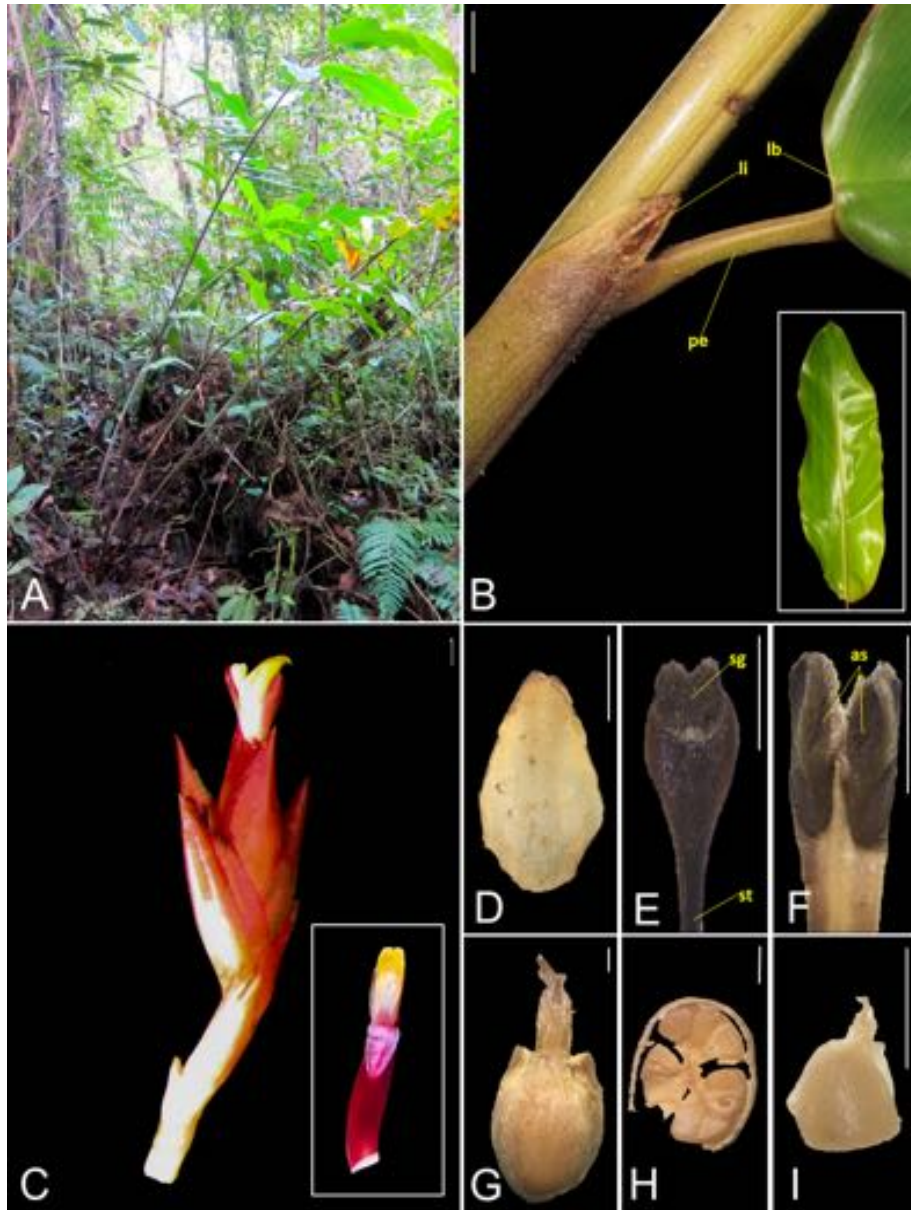
***Plant Description:*** Terrestrial clumping perennial herb and reaches a height of about 2–2.5 m tall. *Rhizome* below the ground, greenish, sometimes pinkish, 1.2–1.5 cm in diam. *Leaves* distichous, ascending, young leaves purple at lower surface, lower leaves reduced; *lamina* oblong to broad lanceolate, 35–43 cm long  $\times$  12–14 cm wide, adaxially paler green, glabrous, abaxially green, glabrous; *midrib* adaxially yellowish to brownish, ridged, glabrous, abaxially reddish to brown, pubescent; *margin* entire, wavy, brown, hairy on the edges, *base*

rounded; *apex* shortly acuminate, recurved; Leaf sheath green with violet marks, grooved. *Petiole* short, grooved, greenish to brown, 1.2–1.4 cm long by 0.4–0.7 cm wide. *Ligule* entire, oblong to truncate, brown, hairy velvety, 5–6 mm long × 2–4 mm wide. *Inflorescence* short, lateral, elongated spike, bearing 1–3 flowers, 7.5 cm long × 3 cm wide. *Peduncle* short, submerged in the ground, pinkish when young, reddish brown when adult, 1.2–1.8 cm long × 0.4–0.6 cm wide. *Bracts* narrowly ovate with sharp, sturdy apex, red and ribbed, glabrous, margin entire, apex acute, 2.2 cm long by 0.8 cm wide. *Bracteoles* tubular, red, 2-tipped with sharp acute apex, glabrous towards the top, pubescent towards the bottom, 1.7 cm long × 0.4 cm wide. *Calyx* lanceolate to oblanceolate, red, tubular with sharp apex, glabrous towards the top, pubescent towards the bottom, 1.7 cm long × 0.3 cm wide. *Flower* 3–4 cm long × 1–2 cm wide; *corolla tube* white. *Corolla lobes* lanceolate to narrow oblong, glabrous, trilobed, pinkish to red, with numerous distinct veins, margin entire, apex rounded; *dorsal corolla lobe*, bright yellow, apex inward infolded and rounded, 10 mm long by 3 mm wide; *lateral corolla lobes* 11 mm long × 2 mm wide; *staminal tube* 3–4 cm, creamy white, glabrous. *Labellum* ovate, lower lobes folded over the stamen, longer than corolla lobes, glabrous, bright yellow, margin entire, apex rounded, 11 mm long × 6 mm wide. *Stamen* 3.5–4 cm long × 1–2 cm wide; *anther* creamy yellow, hairy in the dehiscent part, 6–8 cm long × 2–3.5 cm wide; *filament* 3.9–4.1 × 1 cm, glabrous. *Pistil* 3.4–3.7 × 1 cm; *stigma* creamy white, apex bilobed, white, hairy, 0.8–1.2 cm long; *ostiole* 1 mm, pubescent; *style* white, 2.9–3.1 cm long; *ovary* 1–2 mm × 0.3–0.4 mm. *Infructescence* lax, 7.5 cm long × 4–5 cm wide; *fruits* subglobose and beaked, calyx persistent, pubescent, reddish, 1.5–1.9 cm; *seeds* creamy yellow, 0.6–0.9 mm.

**Phenology:** Flowering and fruiting from January to April.

**Local name and Use:** The species is known as “tagbak” and the fruits are edible according to the local people of Brgy. Datu Salumay (Jason Batawan, pers. comm.).

**Distribution:** *Etlingera pubimarginata* is endemic to the Philippines. This species has been recorded only in some provinces of Mindanao Island, viz., Cinchona Forest Reserve, Bukidnon (Jayme et al., 2020); Mt. Apo, Davao del Sur (Elmer, 1915); Claveria, Misamis Oriental (Naive et al., 2019); and Marilog District, Davao City (Acma et al., 2020).



**Figure 2.** *Etlingera pubimarginata* (Elmer) A.D.Poulsen. **A)** Habit, **B)** Ligule and petiole (inset: leaf blade), **C)** Inflorescence (inset: flower, anterior view), **D)** Labellum (flattened), **E)** Pistil (sg - stigma; st - style, **F)** Stamen (as - anther sacs), **G)** Fruit, **H)** Cross section of the fruit, **I)** Seed. Photographs: N.P. Mendez (A-I). Scale bars: 1 cm - B; 0.8 cm - C; 0.5 cm - D, E, F, G, H, I.

**Habitat and Ecology.** *Etlingera pubimarginata* was found growing along the edge of a relatively disturbed and partially open forest at 1,200masl. The populations were found growing with *Paspalum conjugatum* P.J.Berguis (Poaceae) and associated with *Psychotria cuernosensis* Elmer (Rubiaceae), *Elatostema* sp. (Urticaceae), *Medinilla clementis* Merr. (Melastomataceae), *Aeschynanthus cardinalis* (Copel. ex Merr.) Schltr. (Gesneriaceae), *Pinanga* spp. (Arecaceae), *Freycinetia* spp. (Pandanaeae), and other ginger species, such as *Adelmeria alpina* Elmer, *Hornstedtia conoidea* Ridl., and *Plagiostachys albiflora* Ridl. (Zingiberaceae).

**Specimens Examined:** PHILIPPINES. Mindanao: Davao City, Marilog District, Brgy. Datu Salumay, Mt. Malambo, 1,220masl, 17 December 2021, NPM010, N.P. Mendez with R.M. Tubongbanua.

**Other Specimens Examined:** PHILIPPINES. Mindanao: Davao City, Marilog District, Brgy. Datu Salumay, Mt. Malambo, 1,200masl, flowering and fruiting 7 May 2018, VBA10862, F.M. Acma with N.P. Mendez & V.B. Amoroso (CMUH 00012089).

**Notes:** *Etlingera pubimarginata* closely resembles to the Philippine endemic *E. pilosa* by having sessile to short leaves, spiny apex bracts, and yellow labellum. However, the species differ in leaf apex (shortly acuminate vs. obtuse), ligule (oblong to truncate vs. ovate), lamina (oblong to broad lanceolate vs. narrowly obovate), bracts (narrowly ovate with sharp sturdy apex vs. boat-shaped to ovate and mucronate to acute apex), and labellum (11 × 2 mm vs. 10-12 × 5-6 mm).

*Etlingera pubimarginata* is unique by having deep red, spiny apiculate and sturdy bracts, bracteoles and calyx. The dorsal and lateral lobes are oblong and reddish and its labellum is bright yellow with lower lobes folded over the stamen. The lax inflorescence of *E. pubimarginata* also differs from the other Philippine *Etlingera* species and is unusual to the group.

#### **Total Phenolic Content**

The results of the TPC were derived from a calibration curve ( $y = 0.0546x + 0.0658$ ,  $R^2 = 0.9946$ ) of gallic acid (0-200 mg/mL). The ethanolic extracts of *E. pubimarginata* revealed that the leaves ( $27.25 \pm 0.72$  mg GAE/g dried sample) exhibited higher phenolic content than the rhizomes ( $0.76 \pm 0.11$  mg GAE/g dried sample) (Table 1).



Table 1. Mean TPC extraction yield of leaves and rhizomes of *E. pubimarginata*.

Plant parts	mg GAE/g dried sample
Leaves	27.25 ± 0.72
Rhizomes	0.76 ± 0.11

The mean value of the TPC in leaves ( $27.25 \pm 0.72$  mg GAE/g sample) in this study is relatively higher than the studies of Mabini & Barbosa (2018) with 0.55 mg GAE/g sample on methanolic extracts of *Etlingera philippinensis* (Ridl.) R.M.Sm., 1.95 mg GAE/g sample on ethanolic extracts of *Hedychium coronarium* Koenig, and 1.67 mg GAE/g sample on methanolic extracts of *Hornstedtia conoidea* Ridl. The mean value of TPC in rhizomes ( $0.72 \pm 0.11$  mg GAE/g sample) of *E. pubimarginata* is higher than the study of Mabini & Barbosa (2018) with 0.35 mg GAE/g sample on *E. philippinensis*, but lower in the studies of Barbosa & Nueva (2019) with 1.28 mg GAE/g sample on *H. conoidea* and Redondo & Barbosa (2018) with 1.48 mg GAE/g sample on *H. coronarium*. These slight variations in the mean values of TPC might be due to the different amounts of sugars, ascorbic acid, duration, or methods of extraction that may change the amount of phenolics (Burri et al., 2017).

On TPC of other *Etlingera* species, the work of Chan et al. (2007) revealed to have high amounts of phenolics on the species of *E. maingayi* (Baker)R.M.Sm. ( $1110 \pm 93$  mg GAE/100 g of leaves and  $160 \pm 52$  mg GAE/100 g of rhizomes) and *E. elatior* (Jack.)R.M.Sm. ( $2390 \pm 329$  mg GAE/100 g of leaves and  $326 \pm 76$  mg GAE/100 g of rhizomes). The values of the TPC on the work Sabli et al. (2012) closely resemble in the present study, viz., *E. belalongensis* A.D.Poulsen ( $17.07 \pm 0.32$  mg GAE/1 g dried sample of rhizomes and  $10.07 \pm 0.25$  mg GAE/1 g dried sample of stems) and *E. velutina* (Ridl.)R.M.Sm. ( $25.03 \pm 0.46$  mg GAE/1 g dried sample of rhizomes and  $5.30 \pm 0.1$  mg GAE/1 g dried sample of stems), which also revealed to have high amounts of phenolics.

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity (Soobrattee et al., 2005). The total phenolic compounds also play an effective role in stabilizing lipid peroxidation (Yen et al., 1993). These phenolic compounds contribute to antioxidant activity due to the arrangement of functional groups (hydroxyl) about its nuclear structure for hydrogen donation in order to stabilize radical molecules (Soobarattee et al., 2008; Alam et al., 2018).

The quantification of TPC in ethanolic leaf and rhizome extracts of *E. pubimarginata* employing Folin-Ciocalteu method, is convenient, simple, and reproducible (Cirillo & Lemma, 2012; Danciu et al., 2015). This method involves electron transfer in alkaline medium from phenolic compound to phosphomolybdic/phosphotungstic acid complexes to form blue complexes that are determined spectroscopically (Singleton et al., 1999).

#### ***Total Antioxidant Activity and Reducing Power***

The results for the TAA of the ethanolic extracts of leaves and rhizomes of *E. pubimarginata* were calculated from a calibration graph which were linear over the calibration range with  $R^2$  value of 0.9963 ( $y = 0.0149 \times 0.0465$ ) of L-ascorbic acid (0-100 mg/mL). The ethanolic extracts of *E. pubimarginata* revealed that the leaves ( $34.83 \pm 0.49$  mg AAE/g sample) have higher antioxidants compared to the rhizomes ( $1.82 \pm 0.09$  mg AAE/g sample). On the other hand, the data for the RP were derived from a calibration curve ( $y = 0.0505 \times 0.5037$ ,  $R^2 = 0.9975$ ) of gallic acid (0-1000 mg/mL). The ethanolic extracts of *E. pubimarginata* revealed that the leaves ( $24.83 \pm 2.99$  mg GAE/g sample) is higher compared to the rhizomes ( $0.33 \pm 0.10$  mg GAE/g sample) (Table 2).

The mean value of TAA of the leaves ( $34.83 \pm 0.49$  mg TAA/g sample) of *E. pubimarginata* is higher compared to the studies of Mabini & Barbosa (2018) on *E. philippinensis* (0.79 mg AAE/g sample), Redondo & Barbosa (2018) on *H. coronarium* (2.94 mg AAE/g sample), and Barbosa & Nueva (2019) on *H. conoidea* (4.67 mg AAE/g sample). For the mean value of TAA of the rhizomes ( $1.82 \pm 0.09$  mg TAA/g sample), it is also higher than the studies of Mabini & Barbosa (2018) on *E. philippinensis* (0.55 mg AAE/g sample) and Redondo & Barbosa (2018) on *H. coronarium* (1.02 mg AAE/g sample), but lower than the study of Barbosa & Nueva (2019) on *H. conoidea* (with 2.03 mg AAE/g sample).

**Table 2.** Mean TAA and RP extraction yield of leaves and rhizomes of *E. pubimarginata*.

Plant parts	TAA (mg AAE/g dried sample)	RP (mg GRPE/g dried sample)
Leaves	$34.83 \pm 0.49$	$24.83 \pm 2.99$
Rhizomes	$1.82 \pm 0.09$	$0.33 \pm 0.10$

Recently, there has been an increase in research of the potential phytochemicals from plants for therapeutic uses because many phytochemicals have been demonstrated to have antioxidant activities (Kairupan et al., 2019). The TAA of plant extracts may be due to the presence of polyphenols, which act as reductants by donating electrons and reacting with free radicals converting them to a more stable product and subsequently terminating free radical chain

reaction (Gordon, 1990). Populations of *E. pubimarginata* were found at the montane forests of Mt. Malambo in partially open forest, which supported Frankel & Berenbaum (1999) that foliage of tropical forest plants produced more antioxidants when exposed to elevated light conditions.

### ***Correlation among TPC, TAA, and RP***

The contribution of the phenolic compounds in the ethanolic extracts of *E. pubimarginata* to the antioxidant activity was determined by Pearson's correlation coefficient. The results of the correlation analysis are summarized below (Table 3).

**Table 3.** Pearson's correlation coefficients among TPC, TAA, and RP.

Plant parts	TPC	TAA	RP
TPC	1	1**	1**
TAA	1**	1	1**
RP	1**	1**	1

Remarks: \*\*Correlation is significant at 0.001 level

A perfect positive linear relationship was observed among the TPC, TAA, and RP ( $r=1$ ,  $p<0.001$ ). Results indicate that phenolic compounds significantly contribute to the antioxidant activities of *E. pubimarginata*.

### **Conclusions and Recommendations**

The highest phenolic content was recorded higher in leaves ( $27.25 \pm 0.72$  mg GAE/g dried sample) than the rhizomes ( $0.76 \pm 0.11$  mg GAE/g dried sample). The TAA was also significantly higher in leaves ( $34.83 \pm 0.49$  mg AAE/g dried sample) than rhizomes ( $1.82 \pm 0.09$  mg AAE/g dried sample). In terms of the RP, the leaves ( $24.83 \pm 2.99$  mg GRPE/g dried sample) also obtained higher value than rhizomes ( $0.33 \pm 0.10$  mg GRPE/g dried sample). Overall, the leaves contributed the higher phenolic content and antioxidant activity than the rhizomes. A perfect positive linear relationship was observed among the TPC, TAA, and RP ( $r=1$ ,  $p<0.001$ ). These imply that the high contents of phenolic compounds contribute to the antioxidant activity of extracts of *E. pubimarginata*. Thus, the analyzed *E. pubimarginata* in this study contained phenolic compounds in good quality.

This calls for thorough phytochemical analyses to be done to identify the active phenolic and antioxidant components of this Philippine endemic ginger species, since this is the first report of the TPC, TAA, and RP of *E. pubimarginata*.

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## Research Article

# Which Protein Source is Best for Mass-Rearing of Asian Weaver Ants?

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## ABSTRACT

The Asian weaver ant (*Oecophylla smaragdina*) is sometimes used as a biocontrol agent against pests in tropical agriculture as part of integrated pest management programmes. However, the effectiveness of weaver ants as a predator depends on the abundance and activity of colonies in naturally occurring populations. Mass-rearing is a method that could be useful both for getting lots of colonies and for maximising colony growth and aggressiveness. The diet of mass reared weaver ants potentially impacts their growth rate and behaviour. In this study, we investigate the effect of four common commercial protein diets (mackerel, tuna, cricket and mealworms) on *O. smaragdina* colony growth and aggressiveness during mass-rearing over a two-month experimental study at the Smart Research Institute, Riau, Indonesia. Colonies fed on mackerel, the cheapest protein source, and mealworm were able to grow significantly larger than those fed on tuna, even though colonies fed with mackerel showed the lowest amount of mass intake of protein food. In contrast, colonies fed with crickets (the diet with the greatest proportion of protein) had the greatest aggression index against experimentally placed bagworms (a common pest in oil palm plantations), with their aggression being significantly greater than that for mackerel-fed colonies. Taken together, our results show that there are trade-offs between different protein diets for weaver ant colonies in mass rearing facilities. Protein diet can be chosen depending on whether colony growth rate, colony aggressiveness, or price is the main factor driving decisions.

## Keywords:

*Oecophylla smaragdina*, ant farming, biological control, aggression index, colony size, IPM



## Introduction

In many countries in Southeast Asia, harvesting Asian weaver ants (*Oecophylla smaragdina*, Fabricius 1775) from nature is a common activity. People harvest ant brood (larvae and pupae) and use them for bird feed (Césard, 2004), or fishing bait. The adults are also sometimes eaten by humans (Itterbeeck, 2016; Offenberger & Wiwatwitaya, 2010). This wild harvesting of colonies in Java, Indonesia due to high demand for brood for use as birdsong food is potentially reducing the abundance of colonies in natural habitats, something not yet seen in other countries (Itterbeeck, 2016). Perhaps because of limitations on the numbers of wild colonies available, farming of weaver ants is popular in Indonesia, Thailand, and Laos, either on a household scale, or in larger commercial operations (Offenberger & Wiwatwitaya, 2010; Césard, 2004). Using such mass-rearing methods, the ant brood supply is relatively stable compared to the harvesting of brood from nature (Prayoga, 2015). Because weaver ants are also a biological control agent, we suggest that the methods used in weaver ant farming could be deployed to increase availability of colonies as part of integrated pest management programmes.

As a generalist predator, *Oecophylla* spp. are commonly used as biocontrol agents in several agricultural systems such as cacao, cashew, mango, coconut and citrus (Thurman et al., 2019). This ant species has been reported to control major pests such as true bugs (Coreidae and Miridae), beetles (Chrysomelidae), aphids (Aphididae), caterpillars (Lepidoptera), leaf miners (Coleoptera), leafrollers (Lepidoptera), fruit flies (Drosophilidae), leafhoppers (Cicadellidae) and shoot borers (Lepidoptera) (van Mele, 2008). Weaver ant populations fluctuate due to factors such as temperature sensitivity of the pupae development, mortality caused by disease, intraspecific and interspecific competition (Crozier et al., 2010), and abiotic environmental factors. Because of this, mass-rearing methods are potentially useful for increasing the availability of colonies. Such mass-reared colonies could be deployed in mass-release in response to pest outbreaks as part of an integrated pest management programme (IPM), potentially reducing the need to use other less sustainable control methods such as pesticides.

An important consideration besides nectary-food availability and prey abundance that determines colony development (Crozier et al., 2010) is the diet composition used in the mass-rearing system. This is because diet is likely to impact colony growth rate and colony aggressiveness, both of which are important for the effectiveness of ants as biological control agents. Ant colony growth and survival is likely to be determined by the balance of protein diet and

carbohydrates (Dussutour & Simpson, 2012; Kay et al., 2012), while aggressiveness is likely to be determined by chemical cues and recognition systems (Newey et al., 2009; Newey et al., 2010). Moreover, synergistic effects between temperature and diet and its interaction can drive variation in aggressiveness within and between ant species (Barbieri et al., 2015). This study will investigate the effect of four common commercial protein diets on *O. smaragdina* colony growth and aggressiveness in a mass-rearing system.

## Materials and methods

### *Weaver ant collection and husbandry*

Weaver ant colonies were collected from oil palm plantations close to the mass-rearing facility. Only nests in which ants were present (confirmed using binoculars) and constructed from live green palm leaflets, were sampled. Nests were wrapped in a plastic bag, and then all connecting leaflets and fronds were cut using machetes and pruning shears. Ant nests from the field were transferred to racks in the mass-rearing facility. The mass-rearing facility was located at the Smart Research Institute (Smartri), (0° 55' 34.78" N, 101° 11' 38.57" E). The building was a simple 4 x 6 m square, wooden framed building with 60% black sun-shade nets covering the sides and a corrugated metal sheet roof (Figure 1). The interior ceiling was made with plywood with oil palm fibres used as insulation between the metal sheet and plywood. Colonies of *O. smaragdina* ants were reared in racks with plastic trays as shelves.



**Figure 1.** The mass-rearing facility used to propagate Asian weaver ant colonies.

Each colony was housed in a recycled 1.5 L plastic mineral water bottle (8 cm in diameter and 16 cm heights; **Figure 2**). The plastic bottles were thoroughly cleaned to remove any substance that may have negative effects on ants, including insecticide. The original leaf ball nests were broken open to facilitate migration into the empty bottles. At this time, water mist was sprayed into the nest with a hand-manual sprayer to protect the operator from the formic acid sprayed by ant workers into the air. We wore hand gloves and goggles during this work. Grease and used machine oil were used as barriers to avoid worker ants from attacking other colonies or from escaping from the facility. A Maxim iButton® datalogger that automatically recorded every three hours was installed in the centre of the facility to monitor ambient temperature and humidity. Overall mean temperature during the experiment was 27.2° C, with the mean daily maximum being 36.1° C and mean daily minimum being 22.6° C. The mean relative humidity was recorded at 85.7%, with mean daily maximum reaching 99% and daily minimum at 52.8%. After being transferred to mass-rearing racks, all the colonies were acclimated for three days. In the acclimation period, all colonies were fed only using sugar solution and fresh water.



**Figure 2.** A weaver ant colony growing in a recycled plastic bottle in the mass-rearing facility.

### ***Experimental design***

We conducted an experiment to see which protein food would be best for weaver ants. The commercial protein diets used in this experiment were: 1. canned tuna in vegetable oil (Kingfisher brand, claims to use *Katsuwonus*

*pelamis* tuna). 2. fresh mackerel- *Euthynnus affinis* (local name ikan tongkol). 3. fresh yellow mealworm (*Tenebrio molitor*), and 4. fresh cricket (*Gryllus bimaculatus*). The canned tuna was supplied from the retail market, while mackerel was supplied from the traditional market. Mealworms and cricket were bought from a bird pet shop.

Diet treatments were assigned randomly to avoid biases due to gradients in the microclimate or other conditions in the rearing facility. All ant colonies were also given access to 30% sugar solution and freshwater administered *ad libitum*. Each diet treatment consisted of five replicates (n = 20 colonies in total). Experimental feeding and observations of colonies were conducted for two months.

The diet experiment started after the three-day acclimation period. Tuna, fresh mackerel, and fresh mealworms were fed at a rate of 4 g per day, while fresh crickets were fed at a rate of five individuals (average total of five individuals was 1.5 grams) every two days. Each two-day period in this experiment is referred to as a feeding period. Food was placed into a special feeding arena around the ant nest. The amount of leftover protein diet was weighed at the end of the feeding period to determine the amount consumed (mass of food remaining subtracted from mass of food placed). All the food in the feeding arenas were checked for fungal growth every day, especially the canned tuna and fresh mackerel. Any food with fungal growth or fungal contamination was replaced. Based on pre-trial observations, in 48 hours, there was decrease in the mass of tuna and mackerel when these were kept under control conditions in the mass-rearing facility without ant access, presumably due to evaporation. Hence, to analyse the actual amount eaten by ants and to avoid biased weight measurements, final tuna and mackerel weights were counted by reducing final mass with the average control mass.

#### ***Ant colony size observation***

Observation of colony growth focused on occupation and colony survival in the plastic bottle or in any place within the plastic tray. Colony size was recorded on a scale ranging from zero (0) to five (5) (Table 1). The rating was assessed at the same time as the mass of food consumed was measured at the end of each two-day feeding periods.

**Table 1.** Scale used to assess ant colony size.

Rating	Definition
0	No ants in the colony area, and the colony failed to survive
1	Only major workers with a number less than or equal to 20
2	Only major workers with a number more than 20 to 50
3	Major and minor workers are present, foraging actively
4	Major workers, minor workers, and the queen are present, workers foraging actively
5	Major workers, minor workers, and the queen are present and workers forage actively. The colony has egg clusters (note that because this study uses transparent recycle-plastic bottle, the egg clusters are observable from outside).

### ***Ant aggressiveness observations***

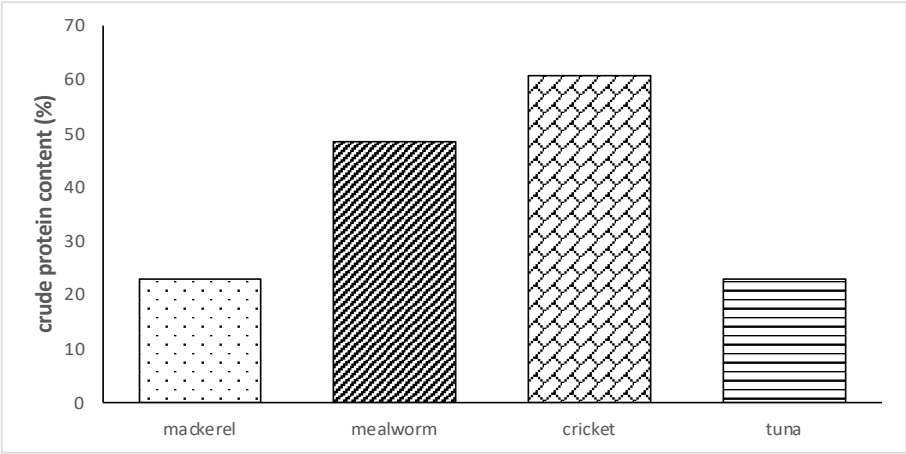
As predacious ants, Asian weaver ants show aggressive behaviour toward foreign objects and other species of animals. The aggressive behaviour of the weaver ant was examined using an "aggression index" (Newey et al., 2009), covering specific behaviours such as threatening, pursuit, biting, or grappling by worker weaver ants. The index aggression index was formulated as:

$$A = f_{\text{post}} * 1 + f_{\text{purs}} * 2 + f_{\text{bit}} * 3$$

Where A = aggression index,  $f_{\text{post}}$  = frequency of aggressive posturing,  $f_{\text{purs}}$  = frequency of pursuit, and  $f_{\text{bit}}$  = frequency of biting or grappling. The tests used a single fresh *Clania tertia* bagworm that was 2 cm long. This species is an important pest of oil palm. The bagworm was always placed in a testing area 5 cm away from the ant nest. Ant behaviour was recorded within a 3 cm diameter area centred on the bagworm. Frequencies of each kind of aggressive response were recorded for three minutes for each colony. The test was conducted twice for each colony, with a ten-second rest between the two tests. A replacement of bagworm was used for every trial. This test was carried out every two weeks on all ant colonies.

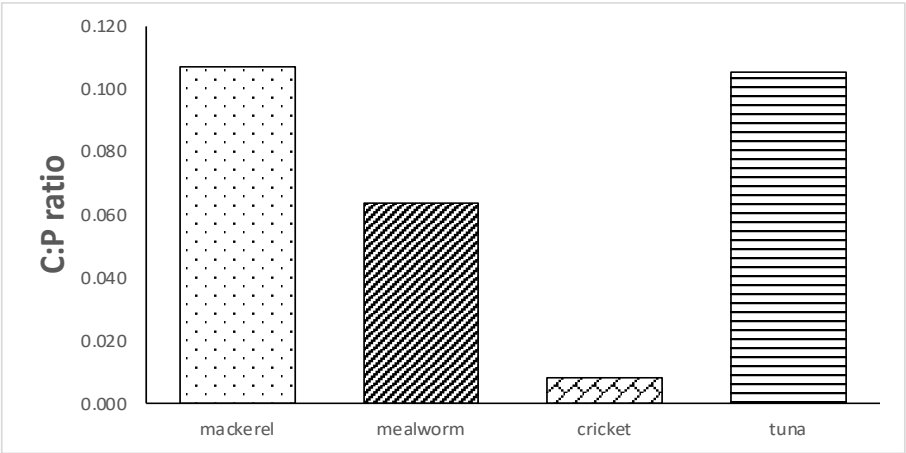
### ***Diet content and financial cost***

The amount of protein in the four different diets of the weaver ant colonies is an important consideration in this experiment. Although this study did not conduct a proximate analysis for protein content, we gathered data from existing sources for the protein content. The canned tuna was labelled as having 70% tuna contents, comprising 16% protein. Fresh mealworm (*Tenebrio molitor*) comprises 45% – 51% protein content (Costa et al., 2020; Zhao et al., 2016). According to proximate analysis (**Figure 3**), mackerel tuna (*Euthynnus affinis*) contains 23% protein (Hizbullah et al., 2019).



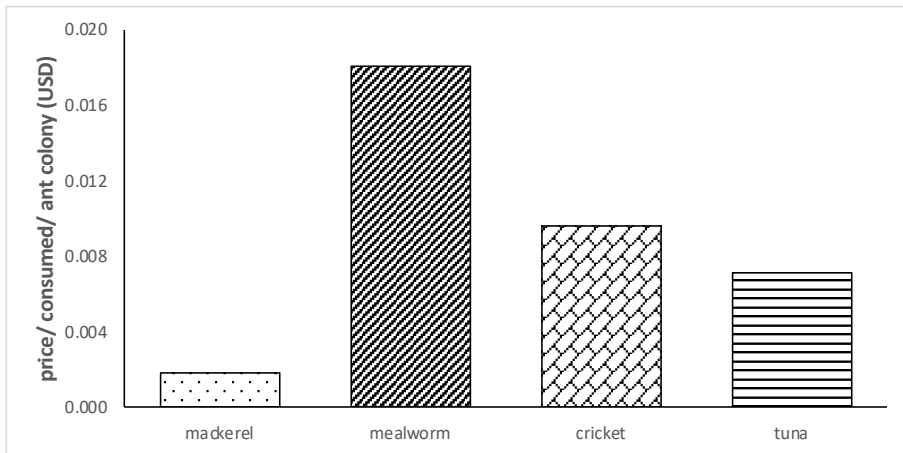
**Figure 3.** Proximate protein content of the different diets fed to the weaver ant colonies. These values are from previous laboratory analyses from Costa et al., (2020) and Zhao et al., (2016) for mealworm; Hizbullah et al., (2019) for mackerel; Jeong et al., (2021); Phesatcha et al., (2022); Udomsil et al., (2019) for cricket and nutritional data from Tuna King’s Fisher brand for canned tuna. All the values relate to percentage of dry matter.

However, the highest protein content is in cricket *Gryllus bimaculatus*, with 61% – 69% protein (this species was used in this study) (Jeong et al., 2021; Phesatcha et al., 2022; Udomsil et al., 2019). The carbohydrate-protein ratio from that report is also compared directly from the references (**Figure 4**).



**Figure 4.** The carbohydrate-protein ratio for the different diets fed to weaver ant colonies used in this experiment. High values on the y-axis indicate diets rich in carbohydrate relative to protein. See Figure 3 legend for data sources.

We conducted cost comparison of commercial protein used in this experiment for cost and benefit consideration in weaver ant mass-rearing. The protein diet cost was analyzed by comparing each feeding session's protein intake (i.e food that was actually consumed, ignoring any not eaten at the end of the experiment; **Figure 5**).



**Figure 5.** Comparison of the price of average protein diet intake per batch colony (in USD) per feeding session. Prices were recorded during the experiment, on the Indonesian market (July-September 2021).

### *Statistical analyses*

Differences in the amount of food consumed by weaver ant colonies across different diets was determined using a linear model, with amount of food consumed as the response, diet type, room temperature during the feeding session, and observation series as fixed predictors. Colony identity was included as a random factor since multiple measurements were made for each colony.

Differences in the size of weaver ant colonies between treatments were tested statistically using an ordinal regression. Colony size was used as a response variable, while diet type and mass of food consumed were set as predictor variables. Colony identity was set as a random effect. Tukey pairwise tests were used to determine which treatments differed if differences were found among diet treatments. The best fit model was selected based on the lowest Akaike Information Criterion (AIC) (Aho et al., 2017).

All statistical models were performed using the R statistical programme version 4.0.2 (R Core Team, 2020) in R Studio version 1.1.423 (RStudio Team, 2020). Graphs were created using "ggplot2" and "tidyverse" R packages (Wickham et al., 2019). The linear mixed model (lmer function) was performed using the "lme4" package (Bates et al., 2015) and the ordinal model (clmm function) was performed using the "ordinal" package (Christensen RHB, 2019).

## Results and discussion

All the protein diets were consumed to some degree by weaver ant colonies. Mackerel was consumed significantly less than other protein diets (t-value=-2.693, p=0.015, Table 2). Significantly more mealworm was eaten than cricket (t-value=2.657, p=0.016) but these did not differ significantly from tuna (t-value=0.522, p=0.606).

**Table 2.** The summary of the fitted model for the diet intake of weaver ant colonies.

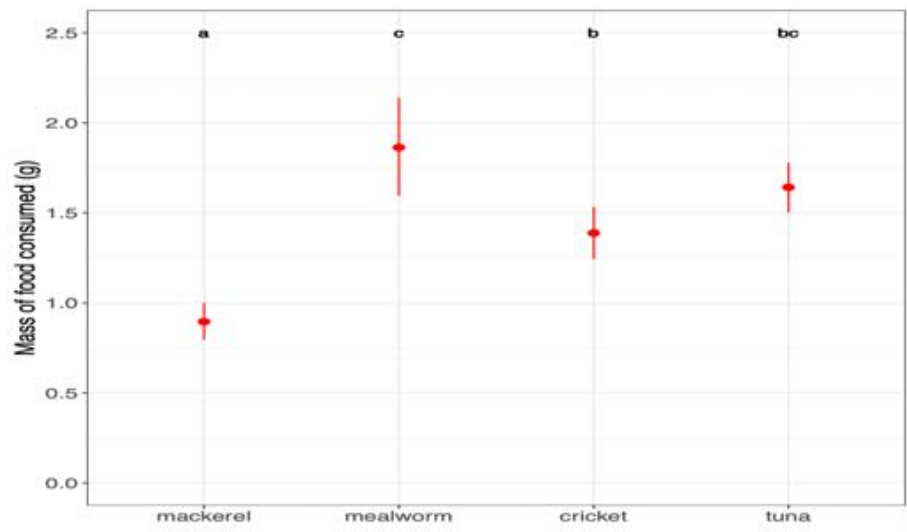
Variable	estimate	Std.err	t-value	p >  z
(Intercept)	1.6078	0.191	8.425	<0.001*
mackerel	-0.7265	0.270	-2.693	0.015*
mealworm	0.7183	0.270	2.657	0.016*
tuna	0.1551	0.297	0.522	0.606

Asterisks (\*) indicate statistically significant comparisons at p<0.05.

We predicted that the kind of protein fed to ant colonies would affect their growth. The tuna-fed colonies had significantly smaller colony sizes compared to colonies fed on mackerel and mealworm (**Figure 7**). This result is interesting because the amount of tuna consumed was the same as cricket and mealworm (p>0.05, **Figure 6**), but tuna has lower protein content (**Figure 3**).

In this experiment, protein content may have a role in colony development such as supporting egg production (Crozier et al., 2010; Way, 1954), especially during early colony establishment. However, high protein content in the diet can also decrease the number of workers in an ant colony. This case is observed in black garden ant workers (*Lasius niger*), where workers die at a young age, causing the colony to collapse when it was fed a high-protein diet (Dussutour & Simpson, 2012).





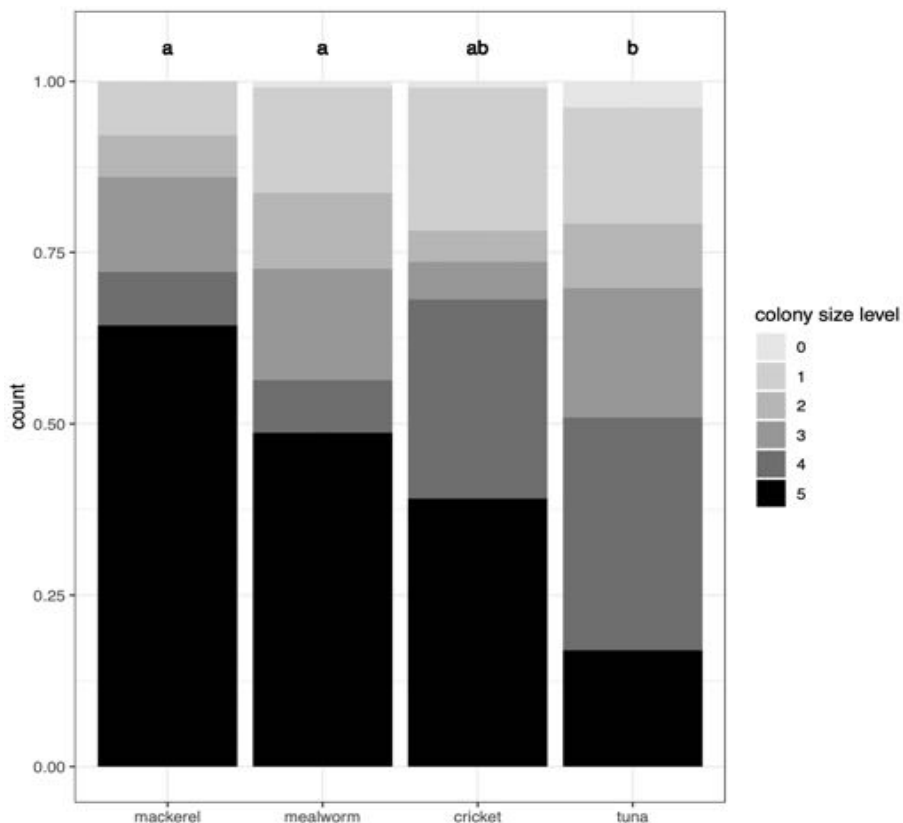
**Figure 6.** The mass of protein food consumed by weaver ant colonies during the mass-rearing experiment under different diet regimes. The dot and bar indicate means and standard error. The same letters at the top of the plot denote the diet that was consumed is not statistically significantly different (Tukey contrast,  $p>0.05$ ).

The freshness of the diet is also likely to determine nutrient components, such as protein, fat and carbohydrate. A decrease in nutrient content levels occurs in fresh tuna stored in the fridge, with protein content decreasing to 21% from an initial value of 23% protein 14 days after it was caught and stored (Hizbullah et al., 2019). However, the nutrient content in canned commercial food undergoes food processing including use of preservatives, likely minimizing this loss. Moreover, there is a possibility that a preservative may have negative effects on ant colonies. Thus, the actual protein content in tuna might be lower than reported in the label. Consequently, tuna fed-colonies may need more protein content to build a bigger colony.

**Table 3.** The summary of the fitted model predicting weaver ant colony size in relation to type and amount of food consumed and temperature during mass-rearing.

Variable	mean	Std.err	z value	p >  z
diet (cricket)	3.560	1.833	1.942	0.052
diet (mealworm)	4.318	1.872	2.306	0.021*
diet (mackerel)	5.620	1.890	2.974	0.003*
consume (gram)	0.103	0.145	0.709	0.479

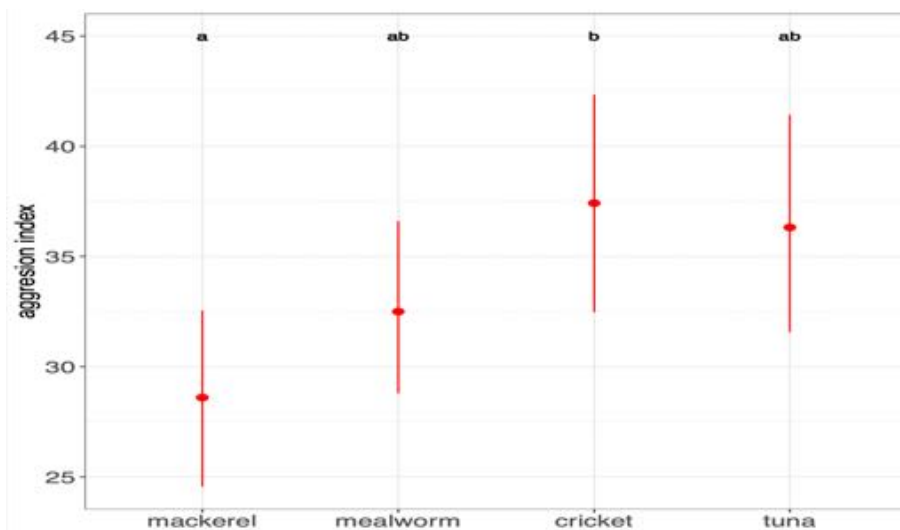
The star (\*) indicate significant at  $p<0.05$ .



**Figure 7.** Colony size level in the mass-rearing experiment in relation to diet. The rating observation was a cumulative score from 25 observation sessions. Colony size was not different statistically for treatments with the same letter label shown in the upper plot (Tukey contrast at  $p=0.05$ ).

In this study, the lowest ratio of carbohydrate: to protein was found in the cricket diet (**Figure 4**), which had the same effect on colony size as the tuna diet ( $z\text{-value}=1.884$ ,  $p=0.06$ , **Figure 7**). In contrast, mackerel-fed colonies that ate a diet with higher carbohydrate-protein ratio (**Figure 4**) could grow bigger than the cricket-fed colony during the experiment, even though this difference was not statistically significant ( $p>0.05$ , **Table 3**). Dietary carbohydrates can increase the total larva mass and hence influence colony growth in a longer-term experiment (Kay et al., 2012). Likewise, the combination of protein and carbohydrate in diet may play an important role in maintaining colony size.

The cricket-fed colony had the highest mean aggression index against experimentally placed bagworms, although it only differed significantly from the mackerel-fed colony (Tukey contrast,  $t\text{-value}=2.818$ ,  $p=0.034$ , **Figure 8**). The high aggression index in the cricket-fed colony is probably related to prey recognition (Newey et al., 2009), where ants are more likely to attack live intruders such as crickets and bagworms. The weaver ant colony use visual, auditory, tactile and olfactory cues to determine other organisms (Newey et al., 2009; Newey et al., 2010), which in this study is bagworm. The fact that the usual diet of these colonies was live insects might explain the high aggression index seen in the cricket-fed colony and the mealworm-fed colony (which were not significantly different: Tukey contrast,  $t\text{-value}=1.572$ ,  $p=0.474$ , **Figure 8**). Nevertheless, the effect of diet on ant aggression level depends on the ant species. For instance, the aggression and activity of Argentine ants (*Linepithema humile*) are higher on colonies reared on low protein, high carbohydrate diets (Kay et al., 2012).



**Figure 8.** Differences in aggression between weaver ant colonies fed on different diets as part of a mass-rearing colony experiment. The dot and bar indicate means and standard errors (Tukey contrast,  $p<0.05$ ).

Consideration of economic feasibility is important when conducting mass-rearing of biological control agents. In addition to labour costs, purchase of food is probably the highest cost for mass rearing facilities. Mackerel is the cheapest among the protein diets used in this experiment (**Figure 5**). Hence, when considering only cost of the food, mackerel can potentially support weaver ant

colonies to build larger colonies at the lowest expense. However, this is potentially offset by the lower amounts of mackerel eaten by weaver ant colonies (**Figure 6**) and the lower aggression exhibited by those colonies (compared to those fed on crickets; **Figure 8**).

Three parameters should be considered when selecting food for conducting mass-rearing of Asian weaver ants: colony size/growth, aggressiveness level, and price. The most crucial aspect for the goal of the mass-rearing to enhance the Asian weaver ant as a biological agent for controlling leaf-eating caterpillars is the capability to produce large ant colonies. For this, mackerel should be selected as food. Second, colonies reared in captivity should have similar aggression levels to wild colonies. For this, crickets should be selected as food. Hence, there is a trade off between these two goals and diet choice can be tailored as needed. If the financing factor for weaver ant protein food is limited, then mackerel should be chosen as the cheapest source. Our results show that it is possible to maintain and grow these natural enemies of leaf-eating caterpillars, with future potential to implement natural enemy population augmentation as part of IPM programmes.

## Conclusions

This study demonstrated that weaver ant colonies can be maintained in captivity with a sugar solution and various alternative protein sources and that they will grow larger while in captivity. There appear to be trade-offs between different protein regarding colony growth and aggression, with mackerel being best for the former and crickets for the latter. Furthermore, mackerel is the most economical protein food for weaver ants. A mixed-protein diet is probably best for ensuring growth of weaver ant colonies as well as to maintain their aggressiveness. The findings of this study could be useful in developing mass-rearing methods that uses the Asian weaver ant as a biocontrol agent in tropical agriculture systems.

## Author contribution

ADA, KMY, and TMF contributed to the conception and design of the study. ADA collected the data. ADA, KMY, and TMF wrote the first draft of the manuscript. All authors contributed to the manuscript revision and have read and approved the submitted version.

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## Conflict of Interest

The authors declare no conflict of interest.

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## Research Article

# Avifaunal Survey of Bukit Balingkadus, A Small Fragmented Forest in Ranau, Sabah, Malaysia

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## ABSTRACT

An avifaunal survey was carried out at Bukit Balingkadus Forest Reserve in Ranau, Sabah, Malaysia. The MacKinnon List method was used to assess species diversity. The four-day survey recorded a total of 16 MacKinnon lists with 388 individuals detected. A total of 86 species from 37 families were recorded, with  $H=3.89$  and  $E_H=0.65$ . True species richness was estimated (using SuperDuplicates® online calculator) to be approximately 114 species, with approximately 28 species not detected. There were 13 species of Bornean endemics, two of which were listed as Vulnerable in the IUCN Red List of Threatened Species. Pycnonotidae was the most speciose family with 10 species. Cuculidae and Megalaimidae were ranked second with 6 species each. Zosteropidae had the highest number of individuals at 47. The top ten most detected species made up 49.5 % of all species detected. The most detected species was the Chestnut-crested Yuhina (Zosteropidae). The majority of the species detected (81) were forest-dependent, of which 60 were strictly forest birds. Insectivores made up the most dominant dietary guild, i.e., a total of 41 species (from 22 families). Frugivores ranked second with 29 species from 10 families.

**Keywords:** avifaunal survey, MacKinnon List method Bukit Balingkadus Forest Reserve, Ranau district, feeding guilds.

## Introduction

Birds have evolved and diversified to a wide variety of habitats and foraging strategies (Naish 2014). They have intimate associations with their habitats, prey or food sources, and some have established strong symbiotic relationships, such as flower-pollinators. As such, they make effective bio-indicators of negative impacts on forest biodiversity due to natural and man-made disturbances (Karr et al. 1990). Due to their conspicuous nature and relative



ease of detection and study, they are among the best-researched animals in forest ecosystems.

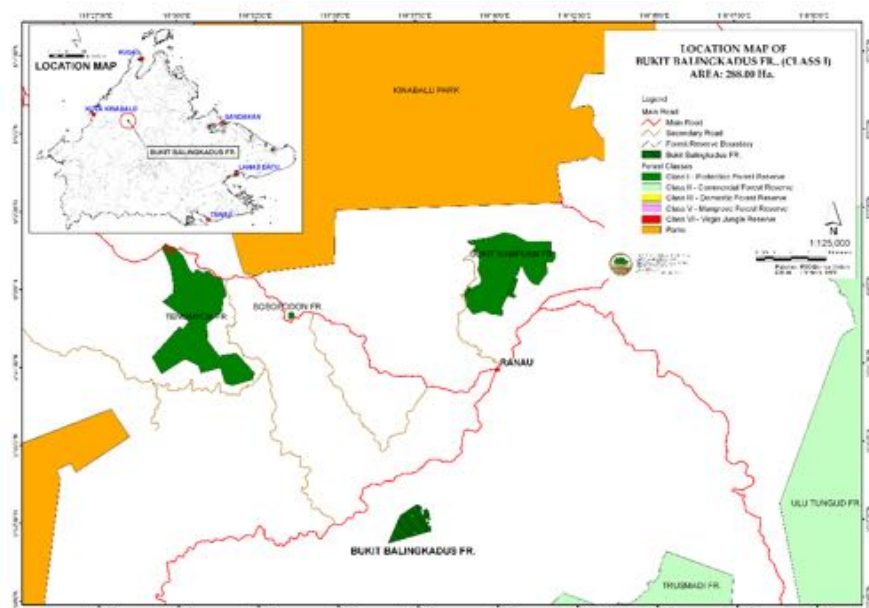
Recently, the Sabah Forestry Department began a rapid assessment of avifaunal communities to help it determine forest ecosystem health. This paper documents the outcome of a brief bird survey conducted in the Bukit Balingkadus Forest Reserve (BBFR) Scientific Expedition, 25-30<sup>th</sup> September 2021. The expedition was organised by the Forest Research Centre, Sabah Forestry Department, under the auspices of the Heart of Borneo Initiative. The main objective of this survey was to provide a brief description of the bird community and ecology in the forest reserve to provide information for future forest management initiatives. Surveys using the MacKinnon List (ML) method (MacKinnon & Phillipps, 1993) were conducted at three sites within the forest reserve.

The Forest Research Centre of the Sabah Forestry Department aims to develop a rapid assessment programme using a modified ML method, which will allow the department's researchers and field staff with limited time for fieldwork (three to four days) to collect data rapidly. The Balingkadus survey was part of a series of these ongoing field trials.

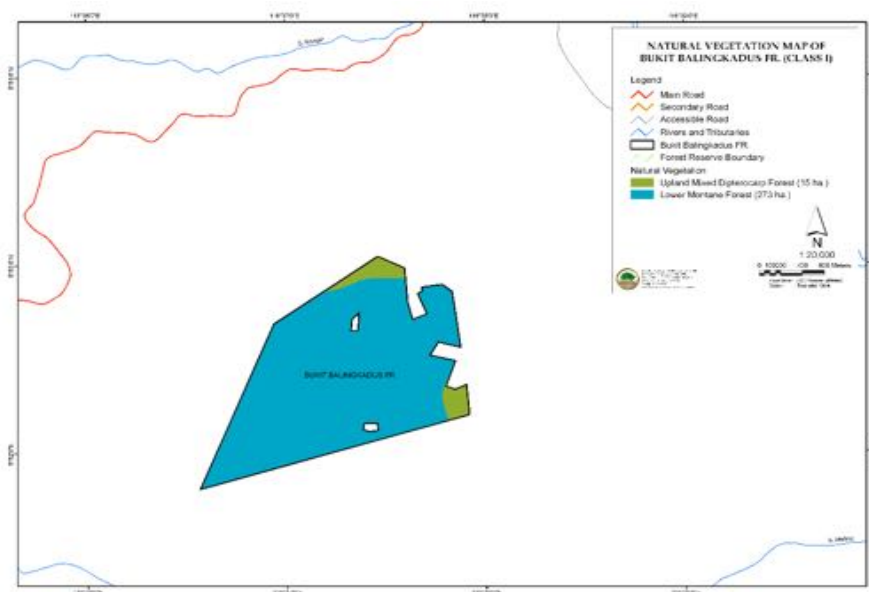
### *Site description*

Bukit Balingkadus Forest Reserve (BBFR), a Class I Protection Forest Reserve gazetted in 2012, lies approximately within latitudes 5.862-5.883 N and longitudes 116.612-116.635 E, or about 20 km southwest of Ranau town along the Ranau-Tambunan main road. The reserve is very small (approximately 288 ha) and is administered by the Ranau district forestry office. It is also situated within Kampung Kinarasan, about 7 km from Ranau town at its closest point. Access to the forest reserve is mainly through a network of earth roads that encircle the reserve. It is about a 50minute drive uphill to get to the reserve from Kampung Kinarasan.

The natural vegetation consisted of lower montane (273 ha) and upland mixed dipterocarp (15 ha) forest types over the Crocker soil association consisting of predominantly sandstone and mudstone-based soils. The hill itself has an amplitude in excess of 300 m and slope normally greater than 25°. Ridge crests and valley bottoms are narrow, and landslips are common (Bower et al., 1975).



**Map 1:** Location of Bukit Balingkadus Forest Reserve.



**Map 2:** Natural vegetation map of Bukit Balingkadus Forest Reserve.

Most of BBFR is relatively intact lower montane forest, although the eastern side is a mixture of agricultural land and degraded forest. This level of disturbance was likely in place when the reserve was gazetted in 2012 and not the result of recent encroachment activities. The forest edges at the boundaries have been negatively affected by the clearing of surrounding lands for agriculture. These surrounding areas are dominated by the highly invasive bracken fern, *Pteridium esculentum*, with occasional pioneer trees (*Trema* spp., *Macaranga* spp., and *Vitex* spp.).

On a larger scale, BBFR is an isolated forest reserve, surrounded on all sides by alienated land, much of which is planted with oil palm and miscellaneous fruit trees and cash crops. About 20 km to the north, east, and west of BBFR lie the large forested areas of Kinabalu Park, Tongod Forest Reserve, and the northern tip of the Crocker Range Park, respectively. The nearest forests are the northern tip of Trus Madi Forest Reserve to the south, and Tenompok Forest Reserve to the northwest, about 8 km and 12 km from BBFR, respectively.

### ***Survey methods***

The MacKinnon Lists (ML) method is a time-efficient and cost-effective sampling approach developed for studying avian tropical biodiversity, in which a series of lists of species recorded are collected from a single survey site (MacKinnon & Phillipps, 1993). It was designed for researchers who have limited time, resources, and personnel to carry out surveys, such as government agencies, non-governmental organisations, citizen scientists, and forest concessionaires. It also accounts for differences in effort, observer experience and knowledge, and weather (Poulsen et al., 1997). As the method relates species richness to the number of observations rather than to time, area, or walking speed, it allows for the comparison of data obtained by different observers or under varying field conditions (Herzog et al., 2002). The ML method has gained popularity since the 1990s, not just in avian surveys but also in biodiversity assessments of mammal and fish species (Bach et al., 2020).

To apply the ML method, we compiled lists of consecutive bird species recorded aurally and visually. Each list comprised 15 species. A species accumulation curve was generated from the addition of those species not recorded on any of the previous lists to the total species number, which was then plotted as a function of the list number. However, in contrast to the traditional ML method, the number of individuals in each species observed within each list was also recorded. This was to provide more accurate species abundance ranks and to decrease the chances of double-counting individuals.

### ***Observation methods***

Every observer had a pair of Nikon binoculars (8 x 42s). The reference field guide of choice was *Phillipps' Field Guide to the Birds of Borneo*, 3<sup>rd</sup> Ed., (Phillipps and Phillipps, 2014). The latest taxonomic changes were determined from online sources ([www.worldbirdnames.com](http://www.worldbirdnames.com) and <https://birdsoftheworld.org>) and published papers. For example, Ochraceous Bulbul (*Alophoixus ochraceus*) is now listed as Penan Bulbul (*Alophoixus ruficrissus*) as per del Hoyo et al. (2021). A Nikon P1000 mega-zoom camera (3000 mm equivalent) was used to photograph birds to confirm their identities.

Surveys were conducted over four days (26-29<sup>th</sup> September 2021), beginning at 7.30am and ending after 4 hours. Four sites in the western portion of the reserve were chosen for the surveys, as access to the eastern boundary was difficult due to rainy conditions. This point of entry allowed the team to conduct surveys close to the centre of the reserve, which is quite small. Night surveys to detect nocturnal birds were not conducted due to the rainy conditions during the evenings, which made the steep earth road extremely slippery.

All observations were recorded by a designated person. Care was taken to prevent intra-list and inter-list double-counts of individuals. As about half of the individuals were detected by their calls/vocalizations, individual birds were listed only when and if the observers were certain that they were different, especially when inputting abundance data within the same 15-species list. Criteria for determining distinct individuals of the same species were: a) their calls originated from different directions; b) there were two or more calls heard subsequently from a similar direction of a previously recorded individual; c) the distance from a previously recorded individual was deemed sufficient for a call to be considered as from a separate individual. For species in flocks, such as the Chestnut-crested Yuhina, photographs were taken and immediately viewed to estimate the number of individuals. Care was taken not to double-count the same flock. As the trails were not looped, only bird species not recorded earlier were recorded on the return leg of the trails.

### ***Analyses***

From survey observations, basic diversity information was extracted, including species richness, a diversity index (H), relative abundance ( $E_H$ ), species family, endemicity, etc. A species accumulation curve was generated from the addition of those species not recorded on any of the previous lists to the total species number, which was then plotted as a function of list number. To estimate species richness of the area, we used the SuperDuplicates® online calculator

developed by Chao et al. (2017), which requires only the total number of species observed and the number of species observed only once (uniques/singletons). The relative abundance indices of species observed were calculated. The most common families and species, and number of Bornean endemics, were also determined.

Analyses of feeding guilds provided information on how communities of species use certain forest resources (fruits, insects, arthropods, seeds, etc.) and may indicate the condition or health of the forest ecosystem. Thus, the species were categorised according to 6 feeding guilds based on their preferred diet; carnivores (Car), frugivores (Fru), insectivores (Ins), nectarivores (Nec), granivores (Gra) and omnivores (Omn). Species were considered as omnivores if they are known to consume roughly similar amounts of animal and plant-based food resources. Guild information was determined mainly from Phillipps (2014) and Wells (1999 & 2007). Feeding guilds were then described according to habitat type (e.g., forest, forest edge and open areas) to examine the importance of various habitats to different guilds.

## Results

### *Avifaunal Composition and Species Richness*

The four survey days yielded 16 lists and 388 individuals, of which 203 (52.3%) were detected by their calls/vocalisations. A total of 86 species belonging to 37 families were recorded (see **Appendix I** for the complete species list). The Shannon Diversity Index (H) value was 3.89, with an Evenness Index ( $E_H$ ) of 0.65. The survey also yielded 13 species that were endemic to Borneo (**Table 1**). Two species are listed as Vulnerable (VU) while the rest are considered of Least Concern (LC) by the IUCN (2022).

**Table 1.** Species endemic to Borneo and their respective categories in the IUCN Red List of Threatened Species.

No.	Species	Family	Category
1	Blue-banded Pitta	Pittidae	VU
2	Bornean Banded Pitta	Pittidae	LC
3	Kinabalu Serpent Eagle	Accipitridae	VU
4	Bornean Barbet	Megalaimidae	LC
5	Bornean Brown Barbet	Megalaimidae	LC
6	Pale-faced Bulbul	Pycnonotidae	LC
7	Penan Bulbul	Pycnonotidae	LC
8	White-crowned Shama	Muscicapidae	LC
9	Bornean Green Magpie	Corvidae	LC
10	Bornean Bulbul	Pycnonotidae	LC

11	Bornean Treepie	Corvidae	LC
12	Chestnut-crested Yuhina	Zosteropidae	LC
13	Dusky Munia	Estrildidae	LC

**Table 2** provides species that are listed as Near Threatened (NT) and Vulnerable (VU), respectively, by the IUCN (2022). All species categorized as NT are lowland mixed dipterocarp forest species. Of those listed as VU, the Blue-banded Pitta and the Kinabalu Serpent Eagle are Bornean endemics.

**Table 2.** Species listed as Near Threatened (NT) and Vulnerable (VU) by the IUCN (2022).

No.	Species	Category	No.	Species	Category
1	Giant Pitta	NT	11	Brown Fulvetta	NT
2	Crested Jay	NT	12	Buff-necked Woodpecker	NT
3	Dark-throated Oriole	NT	13	Diard's Trogon	NT
4	Green Broadbill	NT	14	Rufous-crowned Babbler	NT
5	Lesser Green Leafbird	NT	15	Green Iora	NT
6	Red-throated Barbet	NT	16	Blue-banded Pitta	VU
7	Scaly-breasted Bulbul	NT	17	Kinabalu Serpent Eagle	VU
8	Scarlet-breasted Flowerpecker	NT	18	Greater Green Leafbird	VU
9	Yellow-crowned Barbet	NT	19	Wreathed Hornbill	VU
10	Black-and-yellow Broadbill	NT			

Table 3 shows that Pycnonotidae (bulbuls) was the most speciose family (10 species). The six families in the table contributed 35 species or approximately 41% of the total number of species observed.

**Table 3.** Top four most speciose families (with shared rankings).

Rank	Family	No. of species
1	Pycnonotidae	10
2	Cuculidae	6
2	Megalaimidae	6
3	Columbidae	5
4	Eurylaimidae	4
4	Nectariniidae	4

As shown in **Table 4**, the Chestnut-crested Yuhina caused the Zosteropidae to be the most commonly detected family with 47 individuals, i.e., 12.1% of all individuals detected. Unlike most other species, yuhinas were detected visually as they normally flew in flocks of more than 15 individuals. Care was taken not to double-count the same flocks. Both second-ranked Megalaimidae (barbets)

and Pycnonotidae (bulbuls) had 38 individuals respectively. Of the former, the Gold-whiskered and Bornean barbets were the most commonly detected (all aurally), with 31 individuals compared to a total of 38 for the family. Among the bulbuls, the Yellow-vented bulbul was the most detected species with 13 individuals. Other bulbuls of interest were the Black-and-white, Bornean, Penan, Pale-faced and Scaly-breasted. Nectariniidae (sunbirds/spiderhunters) were mostly represented by the Little Spiderhunter, with 24 out of 35 individuals sighted for the family.

**Table 4.** Top ten families with the highest percentage of individuals detected (note shared rankings).

Rank	Family	No. of individuals	% of individuals detected
1	Zosteropidae	47	12.11
2	Megalaimidae	38	9.79
2	Pycnonotidae	38	9.79
3	Nectariniidae	35	9.02
4	Cisticolidae	31	7.99
5	Alcippeidae	20	5.15
6	Apodidae	18	4.64
7	Timaliidae	16	4.12
8	Columbidae	13	3.35
8	Eurylaimidae	13	3.35
9	Dicaeidae	11	2.84
9	Muscicapidae	11	2.84
10	Cuculidae	9	2.32

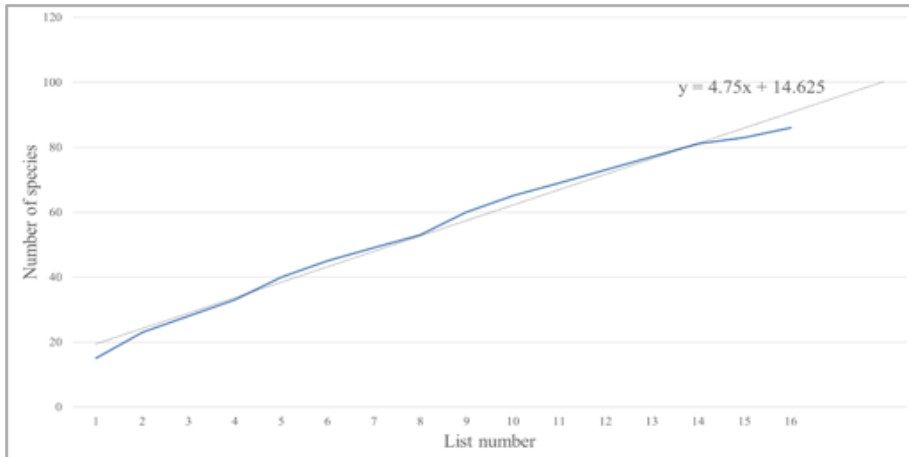
Amongst the Cisticolidae, which was ranked fourth, the Rufous-tailed Tailorbird and the Yellow-bellied Prinia contributed 13 and 11 individuals respectively out of 31. The Brown Fulvetta was the sole representative of the fifth-ranked Alcippeidae.

**Table 5.** Top ten most detected species (note shared rankings).

Rank	Species	Family	No. of individuals detected	% Detected visually	Relative abundance Index
1	Chestnut-crested Yuhina	Zosteropidae	44	88.6	0.1134
2	Little Spiderhunter	Nectariniidae	24	62.5	0.0619
3	Brown Fulvetta	Alcippeidae	20	65.0	0.0515
4	Gold-whiskered Barbet	Megalaimidae	19	0.0	0.0490
5	Plume-toed Swiftlet	Apodidae	17	100.0	0.0438
6	Rufous-tailed Tailorbird	Cisticolidae	13	23.1	0.0335
6	Yellow-vented Bulbul	Pycnonotidae	13	84.6	0.0335
7	Bornean Barbet	Megalaimidae	12	0.0	0.0309
8	Yellow-bellied Prinia	Cisticolidae	11	0.0	0.0284
9	White-crowned Shama	Muscicapidae	10	20.0	0.0258
10	Orange-bellied Flowerpecker	Estrildidae	9	33.3	0.0232

**Table 5** shows the top ten most detected species. These species made up approximately 49.5% of all species detected. The Chestnut-crested Yuhina was the most detected species with 44 individuals. It was easily detected as it usually travels in flocks and also vocalized while in flight. Similarly, all individuals of the fifth-ranked Plume-toed Swiftlet were seen while flying.

The Little Spiderhunter, Brown Fulvetta, and Yellow-vented Bulbul, being very vocal and conspicuous while feeding, were commonly detected visually. The Gold-whiskered Barbet, Bornean Barbet, and Yellow-bellied Prinia were all detected aurally and noted only when the individuals were approximately 10-20 m from the survey team. The Rufous-tailed Tailorbird and White-crowned Shama were more inclined to vocalize when the survey team was closer, i.e., less than 10 m away.



**Figure 1:** Species accumulation curve and linear regression line of birds in Bukit Balingkadus Forest Reserve.

As expected for the ML rapid assessment method, and with a 4-day duration of the survey, the species accumulation curve (**Figure 1**) did not achieve an asymptote. To estimate the probable species richness, the SuperDuplicates® online calculator was used (Chao et al., 2017). Only the total number of species detected and the number of singletons (species detected only once) were needed to input into the calculator. Table 6 displays the summary of results. It estimated Chao1 (species richness using abundance data) to be approximately 114 species, with an upper and lower threshold of approximately 138 and 101 species, respectively, in the 95% confidence interval. The number of doubletons was estimated to be 14, which was the actual number detected during the



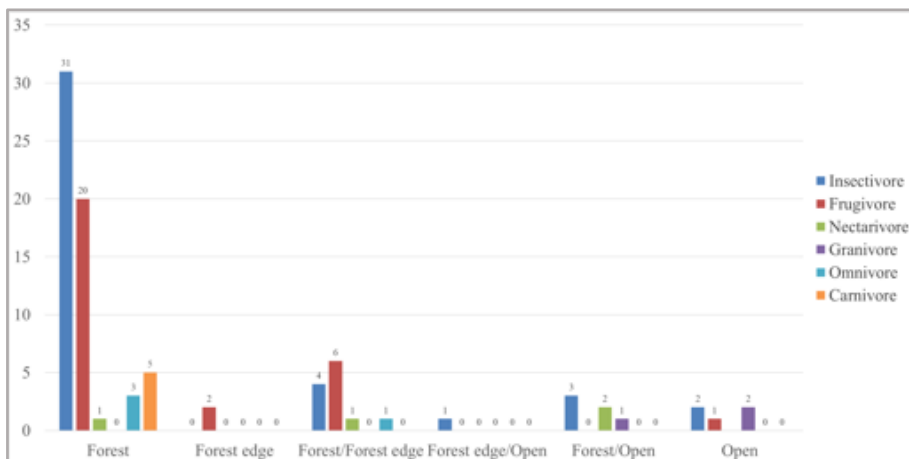
survey. The calculator also estimated that approximately 28 species were undetected, i.e., the survey detected approximately 75.4 % of the total species in the area. Based on the linear regression line in **Figure 1**, it estimated that another five lists, or an extra survey day, were needed to detect the estimated 114 species of birds in BBFR.

**Table 6.** Results from SuperDuplicates®.

Estimated number of doubletons	Estimated species richness	Standard error	95% C.I. lower	95% C.I. upper	Number of undetected species	Undetected percentage (%)
14.01	114.02	9.01	101.15	137.81	28.02	24.57

### *Habitat types and feeding guilds*

Species were categorised according to their preferred habitats (e.g., forest, forest edge, open areas) and feeding guilds (**Figure 2**). Of the 81 forest-dependent species, 60 were strictly forest birds. The high number of forest-dependent species—and the low number of open area specialists (5 species)—may possibly be a positive indication of the overall ecological health of BBFR. Most of the families common in Bornean forests were present. These included hornbills, leafbirds, tailorbirds, pigeons/doves, cuckoos, broadbills, barbets, woodpeckers, pittas, bulbuls and trogons. Furthermore, hill and lower montane species were well represented; these included the Kinabalu Serpent Eagle, Ruddy Cuckoo Dove, Bornean Green Magpie, Bornean Treepie, Ashy Drongo, Bornean Barbet, Bornean Bulbul, Pale-faced Bulbul, Penan Bulbul, Blue-banded Pitta and the Chestnut-crested Yuhina.



**Figure 2:** Number of species according to habitat types and feeding guilds in Bukit Balingkadus Forest Reserve.

In terms of feeding guilds, insectivores and frugivores comprised 82.5 % of the total individuals detected, with the former being the most abundant at 205 individuals. Of the 41 species (representing 22 families) of insectivores, 31 were strictly forest species. The Brown Fulvetta, Rufous-tailed Tailorbird, and the White-crowned Shama were the most commonly detected insectivores with 20, 13, and 10 individuals respectively. The second most dominant guild was frugivores, with 115 individuals (from 29 species, 10 families) detected. From their conspicuous calls, 38 individuals of barbets (6 species in total) were the most detected, with the Gold-whiskered and the Bornean barbets accounting for 19 and 12 individuals respectively. With 10 species, the bulbuls (Pycnonotidae) were the most speciose family amongst the frugivores. Three of the bulbul species were Bornean endemics (see **Table 1** above).

As in our previous surveys, the number of insectivorous and frugivorous species (31 & 20 respectively) were significantly higher in the 'Forest' habitat as compared to other habitats (Petol & Rudolf, 2019; Petol et al., 2021a; Petol et al., 2021b; Joeman et al., 2020a & Joeman et al., 2020b). Their dominance in BBFR may indicate the constant availability of their primary food sources, which may also reflect the overall good forest health.

## Discussion

Most of the families common in Bornean forests were present in BBFR. However, being surrounded by farmland for many years and located between 10-20 km away from large forest reserves, certain avifauna communities in the BBFR may have been negatively affected. For example, not a single species from Phasianidae (e.g., the Great Argus and the firebacks) was detected. Being surrounded by human settlements, it was assumed that the ground-dwelling species are regularly hunted or trapped. Curiously, the famous Whitehead trio (Whitehead's Broadbill, Spiderhunter, and Trogon)—although represented by other members of their respective families—were not detected.

Additionally, BBFR's high Bornean endemic count of 13 species was also the highest we have encountered in our avifaunal surveys of forest reserves. It is unclear whether the large swaths of forests between 8 to 20 km away on all sides may have contributed to its avian diversity over the years. It is possible that birds use BBFR to cross over to the other larger forest reserves/parks, hence explaining the reserve's relatively modest species count. This may be true for most species that are constantly airborne. However, the presence of 3 species of pittas, exclusively ground birds, may discount this simple explanation.

Another possibility might be that the forest—before it was gazetted as a forest reserve—served as a refugium when the surrounding areas were cleared for agriculture; thus, concentrating species into a small space. Further surveys in the coming years should be conducted to ascertain whether the composition of the bird community is stable, declining, or growing.

## Conclusions

The survey team obtained a preliminary insight into the avian diversity and ecology of BBFR. Although it is a small, fragmented forest surrounded by farmland, its avifaunal diversity (86 species from 37 families) was rather representative of lower montane forests in the surrounding areas. A further two or three surveys in the coming years are necessary to better understand the reserve's avian diversity.

## Acknowledgements

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## APPENDIX I

List of bird species detected at Bukit Balingkadus Forest Reserve. Common names in bold denotes Bornean endemics. Bird names are according to the classification in Gill, Donsker and Rasmussen (2022).

NO.	COMMON NAME	SPECIES	FAMILY
1	Golden-bellied Gerygone	<i>Gerygone sulphurea</i>	Acantizidae
2	<b>Kinabalu Serpent Eagle</b>	<i>Spilornis kinabaluensis</i>	Accipitridae
3	Changeable Hawk-eagle	<i>Spizaetus cirrhatus</i>	Accipitridae
4	Common Iora	<i>Aegithina tiphia</i>	Aegithinidae
5	Green Iora	<i>Aegithina viridissima</i>	Aegithinidae
6	Brown Fulvetta	<i>Alcippe brunneicauda</i>	Alcippeidae
7	Plume-toed Swiftlet	<i>Collocalia offinis</i>	Apodidae
8	Brown Needletail	<i>Hirundapus giganteus giganteus</i>	Apodidae
9	Wreathed Hornbill	<i>Rhyticeros undulatus</i>	Bucerotidae
10	Yellow-bellied warbler	<i>Abroscopus supercilialis</i>	Cettiidae
11	Lesser Green Leafbird	<i>Chloropsis cyanopogon</i>	Chloropseidae
12	Greater Green Leafbird	<i>Chloropsis sonnerati</i>	Chloropseidae
13	Red-headed Tailorbird	<i>Orthotomus ruficeps</i>	Cisticolidae
14	Rufous-tailed Tailorbird	<i>Orthotomus sericeus</i>	Cisticolidae
15	Yellow-bellied Prinia	<i>Prinia flaviventris</i>	Cisticolidae
16	Emerald Dove	<i>Chalcophaps indica</i>	Columbidae
17	Zebra Dove	<i>Geopelia striata</i>	Columbidae
18	Ruddy Cuckoo-dove	<i>Macropygia emiliana</i>	Columbidae
19	Little Cuckoo Dove	<i>Macropygia ruficeps</i>	Columbidae
20	Spotted Dove	<i>Streptopelia chinensis</i>	Columbidae
21	<b>Bornean Green Magpie</b>	<i>Cissa jeffreyi</i>	Corvidae
22	<b>Bornean Treepie</b>	<i>Dendrocitta cinerascens</i>	Corvidae
23	Crested Jay	<i>Platylophus galericulatus</i>	Corvidae
24	Plaintive Cuckoo	<i>Cacomantis merulinus</i>	Cuculidae
25	Greater Coucal	<i>Centropus rectunguis</i>	Cuculidae
26	Oriental Cuckoo	<i>Cuculus optatus</i>	Cuculidae
27	Raffles's Malkoha	<i>Phaenicophaeus chlorophaeus</i>	Cuculidae
28	Red-billed Malkoha	<i>Phaenicophaeus javanicus</i>	Cuculidae
29	Square-tailed Drongo-cuckoo	<i>Surniculus lugubris</i>	Cuculidae
30	Orange-bellied Flowerpecker	<i>Dicaeum trigonostigma</i>	Dicaeidae
31	Yellow-breasted Flowerpecker	<i>Prionochilus maculatus</i>	Dicaeidae
32	Scarlet-breasted Flowerpecker	<i>Prionochilus thoracicus</i>	Dicaeidae
33	Ashy Drongo	<i>Dicrurus leucophaeus</i>	Dicruridae
34	<b>Dusky Munia</b>	<i>Lonchura fuscans</i>	Estrildidae
35	Green Broadbill	<i>Calyptomena viridis</i>	Eurylaimidae
36	Dusky Broadbill	<i>Corydon sumatranus</i>	Eurylaimidae
37	Black-and-red Broadbill	<i>Cynbirhynchus macrorhynchos</i>	Eurylaimidae
38	Black-and-yellow Broadbill	<i>Eurylaimus ochromalus</i>	Eurylaimidae
39	Pacific Swallow	<i>Hirundo tahitica</i>	Hirundinidae
40	Asian Fairy-bluebird	<i>Irena puella</i>	Irenidae
41	<b>Bornean Brown Barbet</b>	<i>Calorhamphus fuliginosus</i>	Megalaimidae

42	Blue-eared Barbet	<i>Megalaima australis</i>	Megalaimidae
43	Gold-whiskered Barbet	<i>Megalaima chrysopogon</i>	Megalaimidae
44	<b>Bornean Barbet</b>	<i>Megalaima eximia</i>	Megalaimidae
45	Yellow-crowned Barbet	<i>Megalaima henricii</i>	Megalaimidae
46	Red-throated Barbet	<i>Megalaima mystacophanos</i>	Megalaimidae
47	Red-bearded Bee-eater	<i>Nyctyornis amictus</i>	Meropidae
48	Black-naped Monarch	<i>Hypothymis azurea</i>	Monarchidae
49	<b>White-crowned Shama</b>	<i>Copsychus stricklandi</i>	Muscicapidae
50	Hill Blue Flycatcher	<i>Cyornis whitei</i>	Muscicapidae
51	Temminck's Sunbird	<i>Aethopyga temminckii</i>	Nectariniidae
52	Little Spiderhunter	<i>Arachnothera longirostra</i>	Nectariniidae
53	Long-billed Spiderhunter	<i>Arachnothera robusta</i>	Nectariniidae
54	Rubycheek	<i>Chalcoparia singalensis</i>	Nectariniidae
55	Dark-throated Oriole	<i>Oriolus xanthonotus</i>	Oriolidae
56	Rufous-crowned Babbler	<i>Malacopteron magnum</i>	Pellorneidae
57	Black-capped Babbler	<i>Pellorneum capistratum</i>	Pellorneidae
58	Ferruginous Babbler	<i>Trichastoma bicolor</i>	Pellorneidae
59	Maroon Woodpecker	<i>Blythipicus rubiginosus</i>	Picidae
60	Grey-and-buff Woodpecker	<i>Hemicircus concretus</i>	Picidae
61	Buff-necked Woodpecker	<i>Meiglyptes tukki</i>	Picidae
62	<b>Blue-banded Pitta</b>	<i>Pitta arquata</i>	Pittidae
63	Giant Pitta	<i>Pitta caerulea</i>	Pittidae
64	<b>Bornean Banded Pitta</b>	<i>Pitta schwaneri</i>	Pittidae
65	<b>Penan Bulbul</b>	<i>Alophoixus ruficrissus</i>	Pycnonotidae
66	Spectacled Bulbul	<i>Ixodia erythrophthalmos</i>	Pycnonotidae
67	Scaly-breasted Bulbul	<i>Ixodia squamata</i>	Pycnonotidae
68	Black-and-white bulbul	<i>Microtarsus melanoleucos</i>	Pycnonotidae
69	Red-eyed Bulbul	<i>Pycnonotus brunneus</i>	Pycnonotidae
70	Yellow-vented Bulbul	<i>Pycnonotus goiavier</i>	Pycnonotidae
71	<b>Pale-faced bulbul</b>	<i>Pycnonotus leucops</i>	Pycnonotidae
72	Olive-winged Bulbul	<i>Pycnonotus plumosus</i>	Pycnonotidae
73	Cream-vented Bulbul	<i>Pycnonotus simplex</i>	Pycnonotidae
74	<b>Bornean Bulbul</b>	<i>Rubigula montis</i>	Pycnonotidae
75	Spotted Fantail	<i>Rhipidura perlata</i>	Rhipiduridae
76	Grey-headed Canary-flycatcher	<i>Culicicapa ceylonensis</i>	Stenostiridae
77	Hill Myna	<i>Gracula religiosa</i>	Sturnidae
78	Bold-striped Tit-babbler	<i>Mixornis bornensis</i>	Timaliidae
79	Chestnut-backed Scimitar Babbler	<i>Pomatorhinus mantanus</i>	Timaliidae
80	Chestnut-winged Babbler	<i>Stachyris erythroptera</i>	Timaliidae
81	Diard's Trogon	<i>Harpactes diardii</i>	Trogonidae
82	Black-winged Flycatcher Shrike	<i>Hemipus hirundinaceus</i>	Vangidae
83	Bar-winged Flycatcher-shrike	<i>Hemipus picatus</i>	Vangidae
84	Erpornis	<i>Erpornis zantholeuca</i>	Vireonidae
85	<b>Chestnut-crested Yuhina</b>	<i>Yuhina everetti</i>	Zosteropidae
86	Everett's White-eye	<i>Zosterops everetti</i>	Zosteropidae



## Research Article

# Preliminary Survey on Clam Based Tourism and Conservation in Sabah

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## ABSTRACT

One nature-based attraction for nature lovers and ecotourists in Sabah is the variety of clam resources where exotic species such as Giant clam are protected under CITES and the Sabah Department of Fisheries. Clams are highly distributed in Sabah, attracting tourists and providing food sources for local people. Despite the popularity of clams, the relationship between clam consumption by the local community and tourists are least documented and studied in Sabah, implicating the management and conservation of clam resources through the perspective of local people and tourists. This research is significant in improving policies and decision-making for the long-term ecological and socio-economic sustainability of clam-based tourism and conservation in Sabah. Online surveys and in-person interviews on local people and tourists were conducted in Sandakan, Kota Kinabalu, Tawau and Kudat. Descriptive statistical methods, Chi-squared analysis, and factor analysis were used for analysing data. Findings suggest a relationship between clam consumption by the respondents with clam as traditional food of the respondents and the popularity of clam in the visited area. Factor analysis yielded two factors on the involvement of the local community in clam conservation and utilization in the visited area, and another two factors on the respondent's expectation based on their trip experiences in Sabah. The results also suggest that tourists commonly viewed the involvement of the local community in their visited area as more traditional-like. These views contradict clam conservation efforts in Sabah that adopt modern approaches while excluding the role of local people. This information is crucial in understanding the consumption of clam resources in Sabah's tourism industry, integrating with local cultures and adding value to clam conservation in Sabah.

**Keywords:** Consumption, managements, conservation, nature-based tourism, clam resources

## Introduction

Clam populations are subject to natural demographic fluctuation. Still, the benefit of clam resources to the local community may alter their population dynamic because it is the source of food that benefits local communities.



However, reviving the clam population is not feasible due to miscommunication, conflict between enforcers and local people, and rapid development for tourism activities, especially in coastal areas. The current fields, consumption in economic views and conservation of resources, would present difficulties to understand more precisely the relationship between human consumption and conservation of clams (Anderson, Pearsall, Hunn, 2011). These difficulties seem to be limitations of both fields: while conservation focuses more on the current modern efforts to conserve and increase the population of clam resources and exclusively ignore the rights of the local community or indigenous people in utilizing resources, consumption in economic views focus on the method to consume and generate financial benefits from clam resources (Anderson, Pearsall, Hunn, 2011).

The recurrent contact with local communities, or even tourists and researchers can cause disturbance to the population and abundance, especially in threatened and protected clams, such as giant clams (Copland & Lucas, 1988). Specifically related to tourism, this practice can benefit from the presence of clams, like in several countries such as Fiji, the Philippines and Southeast Asia, which promote clam consumption as traditional cuisines to attract tourists (Copland & Lucas, 1988). Practices like these can help people better understand clams and add value to their conservation through consumption and utilization. Despite the popularity of clams as seafood products among international and local tourists (Copland & Lucas, 1988; Fredericks, 2018), clam consumption by the local community and tourists is least documented and studied in Sabah. Therefore, it is hard to perceive the importance of clams through the perspective of local people and tourists for management and conservation efforts. The food systems of local people offer essential information in understanding the functional aspects of the culture, environment, and health of the people consuming the resources (Albuquerque & Alves, 2016). Data on this research could be an essential tool to understand the knowledge and conceptualization of tourists, researchers, and local communities regarding clams (Ladio & Molares, 2014). Therefore, this research is crucial in determining the level of clam resources that could be harvested and consumed in Sabah's tourism industry, infused under the culture of locals to understand better and add value to their conservation in Sabah.

## Methods

### *Study Site*

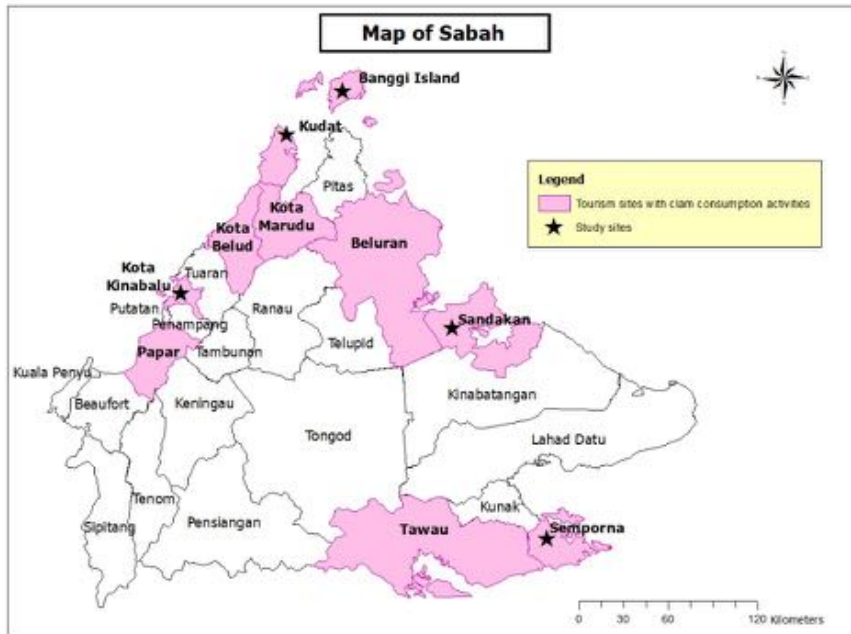


Figure 1. Map shows the location of data sampling.

Semi-structured interviews and open-ended questionnaires (Teh & Sumaila, 2007) were conducted in four main sites involved in giant clam conservation and clams trading. Giant clam conservation at the Marine Ecology Research Centre (MERC) in Malohom Bay of Gaya Island, Banggi Island of Tun Mustapha Park (TMP), Sandakan wet market and Fisheries Department of Kota Kinabalu. These sites were chosen because the conservation area emphasized the giant clam propagation programme and coral reef restoration. Activities such as nursery and re-stocking giant clams are currently active in these areas; individuals involved directly in the effort of giant clam conservation were interviewed (Ladio & Molares, 2014). Field interviews targeted local communities (Teh et al., 2005; Teh & Sumaila, 2007), tourists and individuals involved in giant clam professions, as these were the three stakeholder groups with the highest potential for interaction with giant clams. Government officers were interviewed to gain their perspective on their professions in managing marine resources. Giant clams also are food resources for local people (Teh & Sumaila, 2007), such as the Bajau people (Stacey et al., 2018).

### ***Sampling Method***

The survey instrument was self-developed, and pilot tested among randomly selected participants (Creswell, 2014). The core survey items formed 5-point Likert-type scales related to the importance of clam consumption and conservation to the tourism industry in Sabah. Based on the pilot testing, some survey items were revised slightly. The survey was administered face-to-face with the respondents (Creswell, 2014), distributed manually to the department, and lastly, administered online due to pandemic outbreaks. The data collection took place from September to November of 2019 because the location was distributed far from each other and aimed to be sufficient with online surveys. The procedure was complicated by having a small sample due to lack of tourists, thus, changed to local tourists, represented by the local community and only some international tourists. Therefore, the data was administered online as a method to collect a larger sample. Respondents were recruited from Sandakan, Kota Kinabalu, Papar, Penampang, Semporna and Tuaran for the two months operation. The survey assessed the perspectives of local people and tourists on clam conservation and consumption through their experiences (Albuquerque & Alves, 2016). The outcome measures were: (1) Demographic background (Creswell, 2014), (2) Ordinal form of questions on the importance of clam consumption and conservation and clam consumption in the visited area; (3) Subjective form of questions for clam consumption; 4-point Likert scale on the trip experiences based on the respondent expectations (Creswell, 2014) and another 6-point Likert scale of the respondent perspectives on clam consumption and conservation in Sabah (Creswell, 2014).

### ***Data Analysis***

This research applies descriptive and inferential analysis with Statistical Packages for Social Sciences version 27 (SPSS 27) (Field, 2009). The descriptive findings help explain the survey results and provide insights into the results (Fetterman, 2010). This research would use descriptive statistics to present the demographic background of the respondents, respondent experiences on clam consumption, and conservation and expectation of the respondents based on their trip experiences through frequency and percentages to describe and display the results.

The main reason that Chi-square tests were selected to analyze data in this research was to test hypotheses on nominal types of data (Field, 2009; Mchugh, 2013). This test is suitable for studying the situation of clam consumption and conservation and stipulating the extent of the relationship between each variable, reflecting the magnitude. This research also applied factor analysis to

reduce measurable and observable variables with similar variance by reducing the dimensionality (Yong and Pearce, 2013). Thus, 5-point Likert scale questions and 4-point Likert scale were analyzed using factor analysis and reduced into comprises factors (Yong and Pearce, 2013). Factor analysis helps reduce the questionnaires to a smaller set and acquire an underlying concept to facilitate the interpretation of the survey (Yong and Pearce, 2013). Therefore, descriptive analysis on respondents' expectations matches their trip experiences and local community involvement in clam conservation, and utilization in the visited area applied factor analysis to reduce the data into several categories.

## Results

### *Demographic of the Respondent Background*

As shown in the table below, this study yielded 48 % male and 52 % female respondents. Most of the respondents were dominated by local tourists (86%) and the remaining 14 % were international tourists at the study sites. In terms of age composition, most of the respondents were more than 40 years old (37%), followed by 25 % of respondents aged 31 to 35 years old. Another 22 % were 26 to 30 years old, which is less than 10 % of 25 years old and 7 % of 36 to 40 years old. In terms of occupation, three sectors were highlighted: 44 % government officers, 28 % tourists, and 13 % traders. The response rate from Kota Kinabalu was 62 % and Sandakan (27 %), with locals making up the majority of respondents because most of the study sites lacked tourists. This uneven distribution was due to a lack of tourists during the school season and a dry period with lack of clam supplies from fishermen.

Table 1. Demographic variable of the respondents.

Item	Frequency (%)	Value	Frequency (%)
<b>Gender</b>		<b>Occupation</b>	
Male	48	Administrator	3
Female	52	Blogger	3
<b>Age</b>		Chef	0
<25 years old	10	Government	44
26-30 years old	22	Officer	
31-35 years old	25	Restaurant	1
36-40 years old	7	Manager	
>40 years old	37	Security	0
<b>Type of Tourists</b>		Traders	13
		Teacher	0
		Tourist	28
Local Tourist	86	Waitress	0

International Tourist	14	Web Developer	1
		No Answer	6
		<b>Location</b>	
		Sandakan	27
		Kota Kinabalu	62
		Papar	1
		Penampang	1
		Semporna	5
		Tuaran	4

### ***Respondent Experiences on Clam Consumption in the Visited Site***

Based on Table 2 below, most respondents consumed clam meat with a response rate of 87 %, while a minority did not consume the meat mainly due to lack of preferences and allergic reasons. In terms of clam products in the visited area, the table lists eight types of clam products comprising 10 % canned food, 1 % of clam collection, 41 % of clam cuisine, 4 % of dried clams, 4 % of exotic pet, another 34 % of clam handicraft, 2 % of skincare cosmetic, and 1 % of snacks from clam—only 3 % of the respondents did not provide answers. The respondents also highlighted several locations that commercialized clam products. 11 % of the respondents listed beaches due to abundance and easy access to calm supplies by traders. As shown in the table below, local consumers and tourists listed fresh clam supplies for cooking and cuisine methods often found in fish, local and wet markets. Readily cooked clam cuisines for consumption are often found in restaurants, local stalls and night markets that are concentrated with local and international tourists.

**Table 2.** Information on clam products and consumption location.

Information	%	Information	%
<b>Consume Clam</b>		<b>Consumption Location</b>	
Yes	87%	Beaches	11%
No	13%	Coral Reef Area	0%
Total	100%	Fish Market	8%
<b>Products from clam</b>		Houses	2%
Canned Food	10%	Local Market	12%
Collection	1%	Night Market	3%
Cuisine	41%	Restaurant	23%
Dried	4%	Sea	1%
Exotic Pet	4%	Seaside	1%
Handicraft	34%	Souvenir store	5%
Skincare Cosmetic	2%	Stall	16%
		Supermarket	4%

Snacks	1%	Local Supplier	1%
No Answer	3%	Wet Market	10%
Total	100%	Not Sure	1%
		No Answer	1%
		Total	100%

**Table 3.** Relationship between clam consumption by the respondents and clam conservation in the visited area

a. Relationship between clam consumption and awareness of clam conservation in the tourism area

Clam consumption area	Awareness of clam conservation				
	Are you aware of clam conservation in the tourism site?				
	Yes	No	$\chi^2$	df	P
Clam Consumption					
Do you consume clam meats?					
Yes	107	130	0.006	1	0.937
No	16	20			

The relationship between clam consumption among respondents and awareness of clam conservation in the tourism area had a p-value greater than the chosen significance level of  $\alpha=0.05$  ( $\chi^2(2) > .006$ ,  $p = 0.937$ ). The null hypothesis is not rejected because there is not enough evidence to suggest an association between clam consumption and awareness of clam conservation in the visited area.

b. Relationship between clam consumption and clam as traditional food for the respondent.

		Clams as traditional food for respondents		$\chi^2$	Df	P
		Is clam part of your traditional food?				
Clam Consumption		Yes	No			
Do you consume clam meats?						
	Yes	138	99	16.285	1	0.000
	No	8	28			

The relationship between clam consumption and clam as traditional food of the respondent in the tourism area had a p-value less than the chosen significant level of  $\alpha=0.05$  ( $\chi^2(2) > 16.285$ ,  $p = 0.00$ ). Thus, the null hypothesis is rejected

and concluded that there is an association between clam consumption and clam as traditional food of the respondents.

c. Relationship between clam consumption and increased trend of clam consumption at the respondent and visited area.

		Trends of clam consumption				
		Has clam consumption or utilization increased in your community or visited area?				
Clam Consumption		Yes	No	$\chi^2$	df	P
Do you consume clam meats?						
	Yes	136	102	2.120	1	0.145
	No	16	20			

The relationship between clam consumption of the respondent and the increase of clam consumption in the visited area had a p-value greater than the chosen significance level of  $\alpha=0.05$  ( $\chi^2(2) = 2.120$ ,  $p = 0.145$ ). The null hypothesis is not rejected because there is not enough evidence to suggest an association between clam consumption and increased clam consumption in the visited area. (The increase of clam consumption could not be investigated due to seasonal factors limiting clam resources in the studied area, limited time, Covid 19 pandemic related restrictions, and lack of documentation; therefore, it is based on respondent experiences only).

**Table 4.** Expectation of the Respondents on their Trip Experiences

Factor analysis on expectation of the respondent on their trip experiences

Items	Factor Loading	Mean <sup>a</sup>	Eigen-Value	Total Rotated SS <sup>b</sup>	Variance Explained (%)	Cronbach's Alpha
<b>Factor 1:</b>						
<b>Tourism activities in the visited area</b>			<b>3.303</b>	<b>2.718</b>	<b>55.048</b>	<b>0.850</b>
How the area (visited area) looked	0.830	2.58				
How are the people	0.701	2.88				
Authenticity of arts and crafts	0.858	3.09				
Tradition and cultural of local people	0.834	2.96				

<b>Factor 2:</b>				
<b>Conservation activities in the visited area</b>			<b>1.059</b>	<b>1.644</b>
Development of technology	0.801	2.90		<b>17.643</b>
Conservation activities in the area	0.893	1.96		<b>0.689</b>
<b>Overall Scale</b>				<b>72.691</b>
				<b>0.830</b>

Varimax rotation was used; Kaiser-Meyer-Olkin = 0.623; Barlett's test of sphericity-significance = 0.000. Factor loading smaller than 0.3 are not included. <sup>a</sup>Items measured in a 4-point scale (1, Not at all as anticipated;4, More traditional than anticipated). <sup>b</sup>Sum of squares.

The table above contains an analysis on expectations of respondents based on their trip experiences via exploratory factor analyses. The suitability of the items and the strength of the inter-correlations were checked based on statistic indicators. Barlett's test sphericity results show that the analyses were highly significant ( $p < 0.001$ ). The Kaiser-Meyer-Olkin measure of sampling adequacy was above 0.623, exceeding the recommended value of 0.5. Varimax rotation produces factor structure and meaningful interpretation by maximizing the variances of loadings factors. As shown in the table above, the exploratory factor analysis identified two factors with eigenvalues greater than one: tourism activities in the visited area and clam conservation activities in the visited area. These factors explained over 72.30% of the variation. Factor 1 on tourism activities in the visited area yielded 4-factors, which is how the visited area looked like or the infrastructure in the visited area, what the local people are like or attitudes of local people towards tourists, authenticity of arts and crafts, including tradition and culture of local people in the visited area. These factors had variance explained over 55.048 % with eigenvalues greater than one. Factor 2 on clam conservation activities in the visited area yielded 2-factors, which is the development of technology and conservation activities in the conservation area with variance explained 17.643 %. All of these factors have eigenvalues more significant than one and explained over 68.69 % of the variation.

#### ***Correlation between respondents' expectation on willingness to return to Sabah to experience clam consumption***

Chi-squared analyses were applied to test the influence on the willingness to return to Sabah to experience clam consumption and the respondent expectation. The analysis of the relationship between the two variables do not show enough evidence to suggest an association between respondent expectation and their willingness to return and consume clam ( $p = .008$ ). On the other hand, descriptive analysis shows that 65 % of the respondents positively



agree on the willingness to return to Sabah to experience clam consumption. The promotion of Sabah as a 'seafood paradise' mainly influenced these results in several tourism sites due to concentrated seafood restaurants in areas such as Kota Kinabalu, Sandakan and Semporna. A small percentage of respondents disagree on the willingness to return to Sabah to experience clam consumption mainly due to lack of seasonal seafood supplies, or lack of preferences or allergic and health issues concerning clam consumption. Issues on the price or cost for clam consumption may not be the main issues due to low prices in competing with other traders. Some of the respondents explained this during face-to-face surveys and interviews. However, this study does not include the situation post-Covid 19 restrictions, where the prices may change and affect the overall results.

**Table 5.** Involvement of local community into clam conservation and utilization in the visited area

Exploratory factor Analysis: Involvement of local community into clam conservation and utilization in visited area

Items	Factor Loading	Mean <sup>a</sup>	Eigen-Value	Total Rotated SS <sup>b</sup>	Variance Explained (%)	Cronbach's Alpha
<b>Factor 1: Involvement of local community</b>			<b>3.192</b>	<b>2.097</b>	<b>53.192</b>	<b>0.747</b>
Do you agree that it is important for the community to be involved in clam conservation based on nature tourism?	0.842	4.30				
Do you agree that utilization of clams in tourism can help the community?	0.829	4.22				
Do you agree that the opportunities in training local people for tourism is important to the clam conservation and community?	0.557	4.36				
			<b>0.946</b>	<b>2.041</b>	<b>15.767</b>	<b>0.731</b>

<b>Factor 2: Clam conservation in tourism area</b>		
Do you agree that clam conservation should be stricter?	0.849	4.00
How important do you think it is that tourism in this area promote your involvement in conservation practices?	0.749	4.24
How important do you think it is that tourism provide educational material on the conservation of clam in tourism site?	0.641	4.18
<b>Overall Scale</b>	<b>68.959</b>	<b>0.815</b>
Varimax rotation was used; Kaiser-Meyer-Olkin = 0.820; Barlett's test of sphericity-significance = 0.000. Factor loading smaller than 0.3 are not included. <sup>a</sup> Items measured in a 5-point scale (1, Strongly disagree;5, Strongly agree). <sup>b</sup> Sum of squares.		

Involvement of the community and clam conservation in tourism efforts were examined via exploratory factor analyses to describe and understand the relationships. Barlett's test sphericity shows that the analyses were highly significant ( $p < 0.001$ ). The Kaiser-Meyer-Olkin measure of sampling adequacy was above 0.820, exceeding the recommended value of 0.5. Varimax rotation produced a simple factor structure and interpretation by maximizing the variances of loadings factors. The exploratory factor analysis in the table identified two factors with eigenvalues greater than one, which is Factor 1 on local community involvement in clam conservation and utilization in the visited area, and Factor 2 on clam conservation for tourism in the visited area. These factors all had eigenvalues more significant than one and explained over 68.69% of the variation. Factor 1 yielded 3-subfactors with over 53.192 % of variance explained whether respondents agree with the involvement of the community in clam conservation based on nature tourism, benefits of utilization of clams to the community in tourism activities, and the importance of the opportunities in training local people or communities for clam conservation in the visited area. Factor 2 yielded 3-subfactors with variance explained of 15.767 % on whether the respondents agree about strict conservation, promoting the involvement of

tourists and local people in conservation activities, and the importance of educational material on clam conservation in tourism activities of the visited area. This information is crucial in understanding the involvement of local people or communities in clam conservation and utilization in tourism activities of the visited area, which is based on respondent experiences.

## Discussion

Sabah has unique natural attractions, such as tropical marine environment habituated by clam species varieties. The enormous diversity of coastal marine habitats, ranging from deep fjords to atolls and coastal shelves to immense lagoon systems, contributes to a greater diversity of clams (Copland and Lucas, 1988). In the same context, environmental factors in these habitats provide optimum requirements needed by the population for growth and distribution around the coastal area of Sabah. Therefore, an increase in population and distribution provides food supplies for the local communities and is exploited for monetary benefits through seafood consumption, Tisdell (1990) stated. This led to concentrated seafood consumption around city and coastal areas such as the Kota Kinabalu waterfront and Sandakan's Sim-Sim restaurants (The Star, 2018). Meanwhile, seascapes and landscapes, including cultural and traditional richness, attract local and international tourists.

### *Consumption and utilizing clam products by visitors as tourism products in Sabah's tourism*

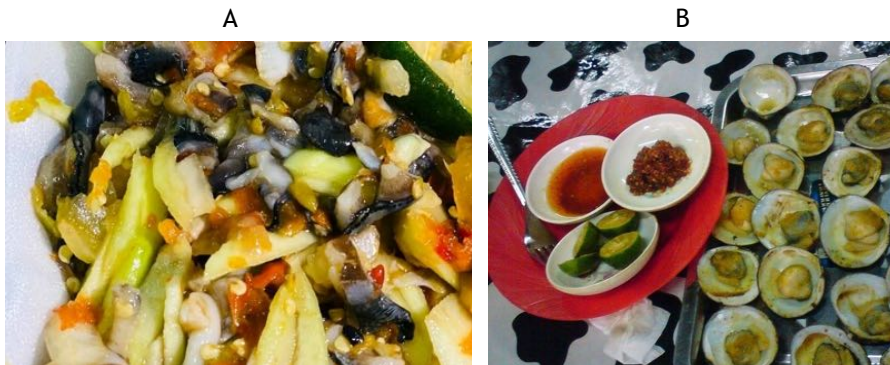


Figure A and B shows clam cuisines as clam products in the visited area (Study Site)

Clam products such as handicrafts and cuisines in Sabah are likely influenced and affected by local cultures in the visited area as tourism products. Copland and Lucas (1988) highlight the fusion of local tradition and cultures into the

production of clam products such as handicrafts and cuisines in tourism sites such as Fiji, the Philippines, and Palau, to attract tourists. This explains the influence of local tradition on clam products in Sabah, which is shown in the figure above and below. Such approaches have caused seafood cuisines in Sabah to be promoted massively as tourist attractions at coastal areas such as Sandakan, Kota Kinabalu and Semporna. However, this research's distribution of tourist data shows uneven distribution because of lack of interest in clam consumption and transportation factors, and current supplies from local fishers. The fusion of cultures into the production of clam products helps people better understand and add value to their conservation through consumption and utilization of the clam resources. Clam consumption of local people within coastal areas can provide significant economic benefits and social function, even in protected or conserved areas.

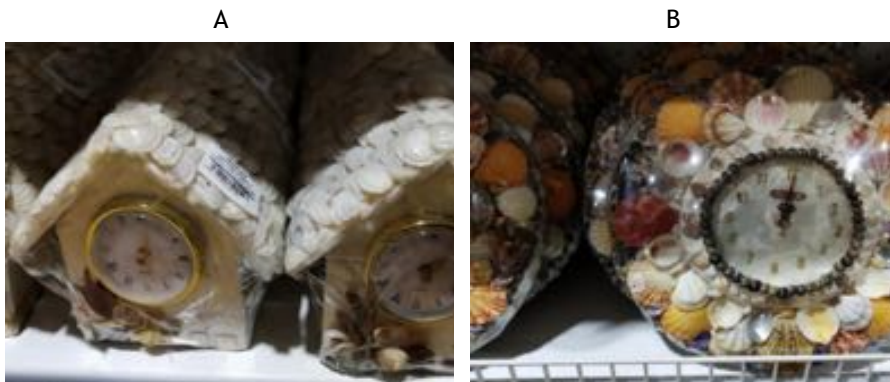


Figure A and B above shows the clam handicraft product that are sold in the local handicraft shop on the visited area (study site)

Promotion of seafood industry in Sabah through media such as travel advisories, magazines, blogs and by recommendation from tourists, and websites such as Portview, Ocean, Welcome, Sri Mutiara, Gayang, Salut, Windbell, and Kampung Nelayan often point at Sabah's seafood restaurant as an exclusive destination for potential tourists (New Straits Times, 2014). The news often reported that the traders provide two choices in selling fresh seafood: either selling fresh supplies to be cooked by customers, or served at the restaurants. This was concurrent with the finding in this study on types of clam products provided by traders in the result section. New Straits Times (2014) reports that seafood consumption in Sabah is affordable and reasonable, and fresh supplies consistently attract tourists and consumers for seafood consumption. Prices of seafood consumption generally ranged between RM30 to RM200, depending on the order (New Straits Times, 2014). Where mollusks are known as exotic

seafood in the seafood market, it is revealed that the bigger branches of restaurants profited approximately RM50,000 per day and even more on exceptional days (New Straits Times, 2014). Local and foreign tourists highly anticipate seafood products with lower prices. This generates income for fishermen, seafood farm owners, boatmen, suppliers, transporters and retailers. Previous research proved that understanding the expectations and experiences of visitors helps in planning, marketing and promoting Sabah's tourism industry. Moreover, the perspectives of visitors and tourists provide opportunities to improve and enhance tourism sites while preserving biodiversity. Improved tourism areas help create experiences that match the tourist's expectation, thus influencing their decision to return and recommend the tourism area. Expectations of tourists are crucial for efficient marketing destination based on tourist's destination selections, consuming goods and services, and deciding to revisit the tourism areas (Aksu, 2010). Following the respondents' expectation, it is likely that they expected the visited area to be traditional and infused with cultural aspects as promoted in many tourism magazines and sites. These expectations were met in several sites that prioritize local cultures, such as Semporna. However, clam conservation activities in the visited area are not likely to be expected by the local tourists due to low promotion of environmental awareness. The respondent responses that conservation activities in the visited site are more likely to be a modern approach in the development of technology and conservation activities. Such approaches may be necessary to utilize developed technology to conserve the clam population, such as advanced aquaculture or mariculture techniques. Modern and recent approaches to conservation activities mainly promote awareness and environmental education to the younger generation, local people and tourists. This study also assessed the condition of the visited area determined by tourist expectations and willingness to visit Sabah for clam consumption purposes and whether it was a more traditional or modern approach (Nkwanyana & Bekebu, 2018). In other words, tourist satisfaction was directly influenced by perceived, experienced value, either modern or traditional (Nkwanyana & Bekebu, 2018). It is questioned in this research whether modern or traditional approaches in the tourism site affects the willingness of visitors to return and recommend clam consumption in the visited area. As discussed above, the majority of visitors expected the visited area to encompass natural attractions with traditional and cultural approaches. Artistic approaches are also included in producing tourism products such as clam cuisines and handicraft products seen as rare and different from other locations or tourism locations. However, they also expected modern approaches to ease their visitation through developed facilities, transportation and land connection. This information helps create

supporting images on clam conservation and products in the tourism industry by promoting plans and triggering expectations of tourists. Thus, the most crucial factors are comparing tourism image with the experience during visits, which eventually influences the satisfaction for willingness to return (Pinto and Guerreiro, 2010).

***The importance of clam consumption and utilization in the clam management and conservation activities in Sabah***

Tourism promotion has increased tourist arrival beyond the carrying capacities of the environment, eventually leading to degradation of the environment and exhausting clam supplies for consumption activities. Therefore, to minimize the effect and pressure of mass tourism, nature-based tourism is seen as an alternative (TTR, 2020) to promote natural attractions (Chok Sim Yee, 2018), awareness (Daily Express, 2019), and conservation (Daily Express, 2014) effort in the tourism industry. It helps highlight and prioritize natural attractions and culture in Sabah (Chok Sim Yee, 2018). Concurrent with the results in this research, the respondents emphasized the community's involvement in clam conservation on nature tourism in the visited area. Mereniuc (2015) described nature-based tourism generates economic advantages through financial benefits and employment opportunities to the local community. It provides additional jobs, ranging from low-wage entry-level to high-paying professional positions in management and technical fields, generating income and raising living standards for the local people (Mereniuc, 2015). Professional positions and skills in managing and planning, including decision-making in both clam conservation and tourism industry, help train the community, especially in managing clam resources and in increasing environment consciousness among locals. As tourism is developed and promoted, additional opportunities are created for investment, development and to alleviate poverty in the rural community (Mereniuc, 2015) while increasing environmental awareness (Chheang, 2013); environmental preservation; employment and educational opportunities for local communities; empowerment of the communities; economic development as a result of tourist expenditures (SAN Parks Management, 2012), tax revenues (Vianna et al., 2018) and governmental investments; cultural viability and heritage maintenance, which could only be maintained along with efficient management and monitoring plans. Therefore, this research is crucial in determining the importance of consumption and utilization of clam resources in Sabah's tourism industry, under the cultures of locals to better understand clams and add value to their conservation in Sabah.

## **Conclusion**

This research is crucial in determining the level of clam resources that could be harvested and consumed in Sabah's tourism industry, infused under the cultures of locals to understand clams better and add value to their conservation in Sabah. Data on this research could be an essential tool to understand the knowledge and conceptualization of tourists, researchers and local communities regarding clams (Ladio & Molares, 2014). However, reviving the clam population is not feasible due to miscommunication, conflicts between enforcers and local people, and rapid development for tourism activities, especially in the coastal area. In conclusion, this study suggests that local cultures influence clam products such as handicrafts and cuisines in Sabah in the visited area. This fusion of cultures into the production of clam products helps understand and add value to their conservation activities through consumption and utilization of clam resources. Following the respondents' expectations, the visited area was expected to be infused with traditional and cultural aspects promoted in tourism magazines and websites. However, clam conservation activities in the visited area are more likely to be a modern approach in developing technology and conservation activities due to necessities in utilizing developed technology to conserve the clam population. This also includes modern approaches to conservation activities to promote awareness and environmental education to the visitors. This study also questioned whether these tourism site approaches affect the visitors' willingness to return and recommend clam consumption in the visited area. Concurrent with the results, the respondents emphasized that the community's involvement in clam conservation for tourism is mainly because of advantages through financial benefits and employment opportunities to the local community. On the other hand, they cultivated professional skills in managing and planning in clam conservation and tourism industry to help the community, including increasing environmental consciousness, environmental awareness and preservation for local communities.

## **Acknowledgments**

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**Research Article**

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## Tree Species Diversity and Stand Structure in Selected Forest Patches of Marilog Forest Reserve, Southern Philippines

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### ABSTRACT

Tree species diversity and stand structure investigations were conducted in the forest patches of Barangay Baganihan (site 1) and Mt. Malambo in Barangay Datu Salumay (site 2), Marilog District, Southern Philippines. Thirty-three 20 × 20 m plots were established at 1,197–1,345 masl in the sites. A total of 900 individuals belonging to 33 families, 43 genera, and 65 species of trees were recorded. Analyses of data revealed that the two sites could be categorised as montane forests based on the forest structure and species composition. *Palaquium philippense* (Perr.) C.B. Rob., *Syzygium tula* (Merr.) Merr., and *Astrocalyx calycina* (S.Vidal) Merr. obtained the highest species importance value index in site 1, while *Palaquium* sp., *P. philippense*, and *Lithocarpus caudatifolius* (Merr.) Rehder in site 2. Shannon-Weiner indices ( $H'$ ) in sites 1 and 2 are relatively low with  $H' = 1.22$  and  $H' = 1.38$ , respectively. A total of eight threatened species were recorded, viz., *A. calycina* as endangered; *Agathis philippinensis* Warb., *Becarianthus pulcherrimus* (Merr.) Maxw., *Camellia lanceolata* (Blume) Seem., *Dillenia megalantha* Merr., *P. philippense* and *Shorea contorta* S.Vidal as vulnerable; and *Cinnamomum mercadoi* S.Vidal as other threatened species. A total of 14 endemic species of trees were also recorded in the study sites. The biodiversity in the two sites is under severe threat due to the different anthropogenic disturbances. A new population of *A. calycina*, a monotypic genus in the Philippines, was collected and the flowers were documented for the first time after a century. The present study is significant since it would help elucidate the patterns of tree species composition and diversity in the forest patches of Barangay Baganihan and Mt. Malambo in Barangay Datu Salumay. Further, this paper adds to the rich biological diversity in Mt. Malambo by supporting its great potential as an ecotourism site.

**Keywords:** angiosperms, Philippine endemics, species richness, threatened species, tree profile, Mindanao

## Introduction

Tropical forests have been recognized to harbour a significant proportion of global biodiversity (Myers et al., 2000). It is also the most species-rich biome on Earth harbouring over 50% of species on just 7% of the land area (Wilson, 1988). The Philippines is one of the most important biodiversity hotspots (Lagenberger, 2004) and one of the world's megadiverse countries with high degrees of species richness and endemism (Ashton, 1993; Heaney & Regalado, 1998; Myers et al., 2000). Plant diversity is threatened by different factors that include rapidly changing land use patterns in tropical Asia (Sodhi et al., 2010), and more efforts should be made to document its biodiversity (Webb et al., 2010).

Marilog Forest Reserve in Davao City, Philippines has rich floral diversity which makes it a priority area for biodiversity studies by Conservation International. The area has a very pleasant, cool and breezy climate due to its elevation and presence of forest patches. Many have settled in the area to establish rest houses and mountain resorts. However, several disturbances have been documented, such as rampant small-scale logging activities, conversion of the forest lands into agricultural use, the proliferation of residential and mountain resorts, over-harvesting, trading of ornamental plants and spread of invasive alien species, such as *Piper aduncum* L. Due to these disturbances, the forest cover in the area has drastically changed and is now limited to a few remnants forest patches.

The present study is significant in generating valuable baseline data to conserve and manage the native tree flora in this tropical forest ecosystem. The results of this research would provide valuable data for forest assessment and identification of ecologically-useful species. This study was conducted to determine the community composition, species diversity, and tree population structure in the established plots in the forest patches of Barangay Baganihan and Mt. Malambo in Barangay Datu Salumay, Marilog District, Southern, Philippines.

## Materials and Methods

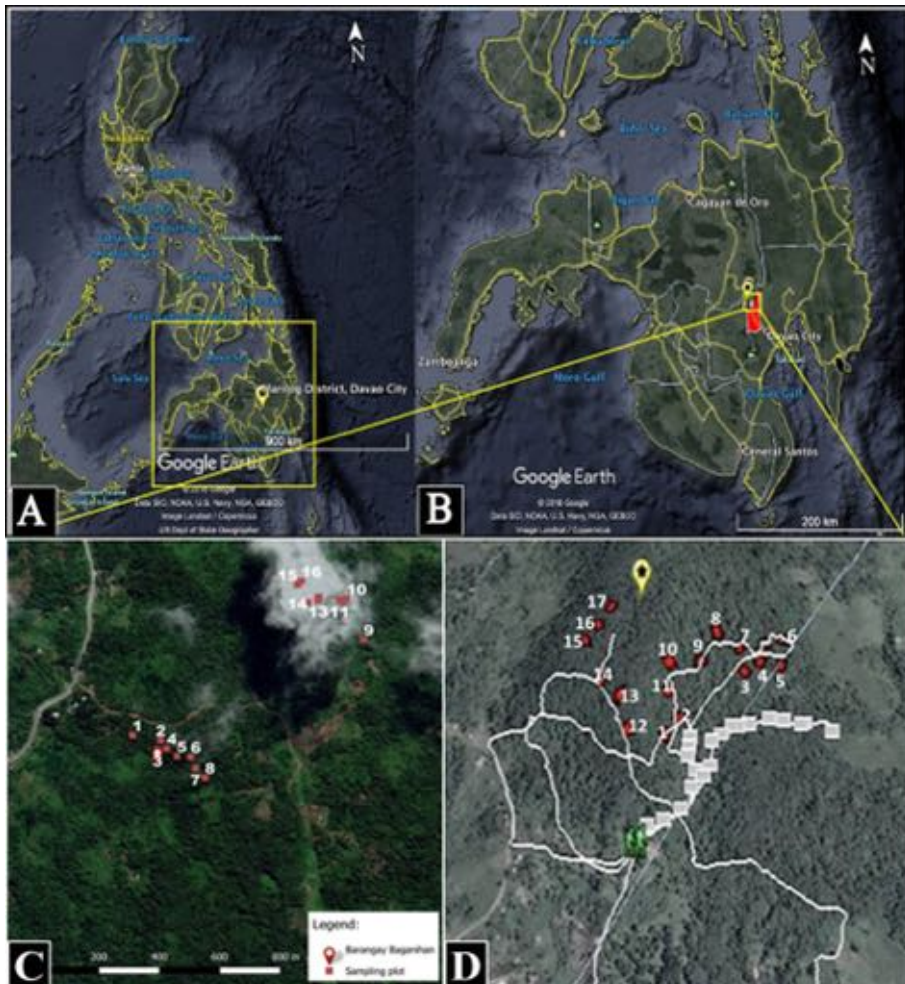
### *Permit Statement*

Necessary permits were obtained, such as Prior Informed Consent from the local people of Marilog District and gratuitous permit from the Department of Environment and Natural Resources (DENR). A memorandum of agreement (MOA) between Central Mindanao University (CMU) and the Manobo-Matigsalug Tribal

People Council for Elders of Davao, Inc. (MAMATRIPCEDI) was also done before the conduct of the study.

### *Study Sites*

This study was carried out in Barangay Baganihan (N 07° 27'13.74"- E 125° 15'1.12") and in Mt. Malambo, Barangay Datu Salumay (07°29'87" N, 125° 15'22.23" E) (**Figure 1**), both at the northern part of Marilog District, Davao City in March and May 2018, respectively. The two sites are found at the montane forest at elevations ranging from 1,197–1,345 masl.



**Figure 1.** Study sites. A) Philippine map, B) Map of Mindanao Island, C) sampling plots in the forest patches of Barangay Baganihan, Marilog District, D) sampling plots in Mt. Malambo, Brgy. Datu Salumay, Marilog District.

### ***Establishment of Sampling Plots and Collection of the Specimens***

A total of 33 20 × 20 m plots were established, of which 16 plots were in the forest patches of Brgy. Baganihan and 17 plots in Mt. Malambo, Brgy. Datu Salumay. The established plots represent the community characteristics of a tropical montane forest in the Philippines. All trees with at least 10 cm in diameter at breast height (DBH) in each plot were measured and by taking notes of the x and y coordinates and crown cover. A minimum of four voucher specimens for each species were collected using clipper/shear and trimming cutters. Each specimen was placed inside plastic bags and labeled with necessary information. Representative voucher specimens were deposited at the Central Mindanao University Herbarium (CMUH).

### ***Identification of the Specimens***

Species of the voucher specimens were identified by cross-referencing, monographs, floras, other scientific articles, online websites (e.g., Co's Digital Flora of the Philippines (Pelser et al., 2011 onwards), and compared using the digitized plant specimens available in Global Plants on JSTOR. In addition, the identification of some species collected was validated by the experts. Classification followed the Angiosperms Phylogeny Group (APG), 2016.

### ***Assessment of Conservation Status and Endemism***

Information of the conservation status of trees was referred to Fernando et al. (2022). In contrast, the endemism to the IUCN Red List of Threatened Species (2019) and Co's Digital Flora of the Philippines (Pelser et al., 2011 onwards).

### ***Data Treatment and Analysis***

Tree diversity was calculated using the formula of Shannon-Weiner diversity index (Shannon and Weiner, 1963). All data to represent tree density and basal area were pooled by each site. Calculation for frequency, relative frequency, density, relative density and Importance Value Index (IVI) were derived from Curtis & McIntosh (1951). The following formulae were used:

$$\text{Density} = \frac{\text{number of species}}{\text{Total area sampled}}$$

$$\text{Relative Density} = \frac{\text{density for a species}}{\text{total density for all species}} \times 100$$

$$\text{Frequency} = \frac{\text{number of plots in which species occur}}{\text{total number of plots sampled}}$$

$$\text{Relative Frequency} = \frac{\text{frequency value for a species}}{\text{total frequency for all species}} \times 100$$

$$\text{Dominance} = \frac{\text{basal area or volume for a species}}{\text{area sampled}}$$

$$\text{Relative Dominance} = \frac{\text{dominance for a species}}{\text{total dominance for all species}} \times 100$$

$$\text{Importance Value Index} = \text{Relative Density} + \text{Relative Frequency} + \text{Relative Dominance}$$

Note: basal area equation is  $\pi r^2$  or  $\pi (\text{dbh}/2)^2$

## Results and Discussion

### *Species Richness and Diversity*

A total of 65 species belonging to 33 families and 43 genera of trees were recorded (Table 1). The species richness of trees in the present study is lower compared to Mt. Apo, North Cotabato with 68 species (Silverio, 2014) and Pasonanca Natural Park, Zamboanga City with 78 species (Andas, 2015). The families with the most number of species are Euphorbiaceae and Meliaceae with five species each. These families were also commonly observed in the different Mindanao Long Term Ecological Research (LTER) Sites viz., Mt. Kitanglad, Mt. Apo, Mt. Malindang and Mt. Hamiguitan (Amoroso et al., 2009; 2011; Zapanta et al., 2019) and also in Balinsasayao, Sibulan, Negros Oriental (Amoroso et al., 2017). The most dominant species found within the established plots in site 1 are *Astrocalyx calycina* (S.Vidal) Merr., *Lithocarpus caudatifolius* (Merr.) Rehder, *Lithocarpus submonticolus* (Elmer) Rehder, J. Arnold Arb. and *Syzygium tula* (Merr.) Merr., while in site 2 are *Palaquium* sp., *Palaquium philippense* (Perr.) C.B.Rob., *Syzygium* sp. 2, *Dendrocnide* sp. and *Syzygium tenuirame* (Miq.) Merr. This study supported that the pattern of the species richness may vary in different groups of plants (Kessler et al., 2011). Kromer et al., (2005) also reported different patterns of species diversity among different plant groups, such as epiphytic ferns, orchids and some tree species in tropical America.

The forest type observed in the two sites can be classified as a montane forest based on the classification of Fernando et al. (2008) and Amoroso et al. (2012). Usually, the lower montane forests in the Philippines are characterised by the presence of *Lithocarpus* spp., *Syzygium* spp., gymnosperms such as *Agathis*

*philippinensis* Warb., *Phyllocladus hypophyllus* Hook.f. and tree ferns of family Cyatheaceae and Dicksoniaceae (Whitford, 1911; Gruezo, 1997; Buot & Okitsu, 1998; Fernando et al., 2004; Amoroso et al., 2011). There is also an abundance of terrestrial and epiphytic ferns, lycophytes and bryophytes in the lower montane forests (Ashton, 2003).

One of the forest types in the Philippines is commonly known as mossy forest, with richness and abundance of mosses and liverworts which cover the tree trunks and branches and even the forest floor (Whitford, 1911). It occurs in mountains above 1,000 masl depending on the locality, size and height of the mountain (Fernando et al., 2008). The upper montane forest is the most common forest type in the Philippines together with the lower montane forest. It is also one of the four definable forest formations on the higher tropical mountains (Ashton, 2003). This montane forest is also similar to the different mountain ecosystems in the Philippines that have similar species composition and other structure and physiognomy characteristics, such as canopy height, emergent trees, and presence of buttresses, vascular epiphytes and non-vascular epiphytes. There is also a high abundance of terrestrial and epiphytic ferns, lycophytes, bryophytes and other understory plants observed in this vegetation.

**Table 1.** Checklist of trees in the forest patches of Barangay Baganihan and Mt. Malambo in Brgy. Datu Salumay, Marilog Forest Reserve, Southern Philippines.

Family	Scientific Name	Common Name	Site	
			1	2
Actinidiaceae	<i>Saurauia</i> sp.			/
Apocynaceae	<i>Alstonia parvifolia</i> Merr.	Dita-dita	/	/
	<i>Alstonia</i> sp.			/
Araucariaceae	<i>Agathis philippinensis</i> Warb.	Almaciga	/	
Burseraceae	<i>Canarium asperum</i> Benth.	Pili	/	
	<i>Canarium</i> sp.	Malabasag	/	/
Calophyllaceae	<i>Calophyllum</i> sp.	Palamanyal	/	/
Chloranthaceae	<i>Ascarina philippinensis</i> C.B.Rob.	Ascarina	/	/
	<i>L</i>			/
Clethraceae	<i>Clethra canescens</i> Reinw.			/
Combretaceae	<i>Terminalia catappa</i> L., Mant.	Talisay-talisay (Manili)	/	
Cunoniaceae	<i>Weinmannia hutchinsonii</i> Merr.			/
Dilleniaceae	<i>Dillenia megalantha</i> Merr.	Kalaambog	/	
	<i>Dillenia philippinensis</i> Rolfe.	Kalagtimon	/	
Dipterocarpaceae	<i>Shorea contorta</i> S.Vidal	Lawaan puti	/	
Ebenaceae	<i>Diospyros</i> sp.			/

<b>Elaeocarpaceae</b>	. <i>Elaeocarpus</i> sp.	Kalaw	/	
<b>Escalloniaceae</b>	. <i>Polyosma</i> sp.	Kanduli	/	
<b>Euphorbiaceae</b>	. <i>Macaranga hispida</i> (Blume) Müll.Arg.	Aha	/	
	. <i>Macaranga sinensis</i> Baill.ex.Mull.Arg.	Hindang	/	/
	. <i>Malutos</i> sp.	Malutos	/	
	. <i>Omalanthus populneus</i> Geiseler		/	
	. <i>Omalanthus fastuatus</i> (Linden) Fern.		/	
<b>Fabaceae</b>	. <i>Archidendron clypearia</i> (Jack) Nielsen	Sili-sili like	/	
<b>Fagaceae</b>	. <i>Lithocarpus submonticolus</i> (Elmer) Rehder, J. Arnold Arb.	Ulayan Red	/	
	. <i>Lithocarpus caudatifolius</i> (Merr.) Rehder	Ulayan white	/	/
	. <i>Lithocarpus</i> sp.		/	
<b>Gentianaceae</b>	. <i>Fagraea auriculata</i> Jack	Banati	/	
	. <i>Fagraea blumei</i> G.	Banati	/	
<b>Lauraceae</b>	. <i>Actinodaphne apoensis</i> (Elmer) ined.		/	
	. <i>Cinnamomum mercadoi</i> S.Vidal	Kalingag	/	
	. <i>Cinnamomum uteli</i> Kosterm	Kalingag	/	
	. <i>Litsea segregata</i> Elmer	Dila sa manok	/	
<b>Malvaceae</b>	. <i>Grewia</i> sp.	Alimoong	/	
<b>Melastomataceae</b>	. <i>Astrocalyx calycina</i> (S.Vidal) Merr.	Bahawbaw	/	/
	. <i>Astronia ferruginea</i> Elmer		/	
	. <i>Astronia</i> sp.	Tungaw	/	
	. <i>Beccarianthus pulcherrimus</i> (Merr.) Maxw.	Palamanyal	/	/
<b>Meliaceae</b>	. <i>Dysoxylum arborescens</i> (Blume) Miq.	Mahalimoko n	/	
	. <i>Dysoxylum parasitum</i> (Osbeck) Kosterm.	Kalaantos	/	
	. <i>Dysoxylum</i> sp. 1	Lumbia, Kalaantas Red	/	/
	. <i>Dysoxylum</i> sp. 2	Kalaantas White	/	
	. <i>Reinwardtia</i> sp.		/	
<b>Moraceae</b>	. <i>Ficus benguetensis</i> Merr.	Tubog	/	/
	. <i>Ficus benjamina</i> L.	Baliti	/	/
	. <i>Ficus nota</i> (Blanco) Merr.	Anonang	/	
	. <i>Ficus</i> sp.	Anonang	/	
<b>Myrtaceae</b>	. <i>Syzygium tenuirame</i> (Miq.) Merr.	Lupit	/	/
	. <i>Syzygium tula</i> (Merr.) Merr.	Sagimsim	/	
	. <i>Syzygium</i> sp. 1		/	



	. <i>Syzygium</i> sp. 2		/
Phyllanthaceae	. <i>Bischofia javanica</i> Blume	Ube	/
	. <i>Breynia cernua</i> (Poir.) Müll.Arg.	Ulingon/Kari	/
		is	
	. <i>Huberantha</i> sp.	Balyok	/
Piperaceae	. <i>Piper aduncum</i> L. Var	Buyo-buyo	/ /
Pittosporaceae	. <i>Pittosporum ramiflorum</i> Zoll.		
Rubiaceae	. <i>Nauclea orientalis</i> (L.) L.		/
	. <i>Psychotria</i> sp.		/
Rutaceae	. <i>Melicope triphylla</i> (Lam.) Merr.	Kalaw/Buga	/ /
	. <i>Melicope</i> sp.		/
Sapotaceae	. <i>Palaquium philippense</i> (Perr.) C.B.	Natu	/ /
	Rob.		
	. <i>Palaquium</i> sp.		/
Staphyleaceae	. <i>Turpinia ovalifolia</i> Elmer.		/
Theaceae	. <i>Camellia lanceolata</i> (Blume) Seem.		/
Urticaceae	. <i>Dendrocnide</i> sp.	Alingatong	/

It is noteworthy that the monotypic genus, *Astrocalyx calycina* (S. Vidal) Merr. (Figure 2 (a)) was first recorded in the area. Its flowers were also documented and collected for the first time since its first description in 1910. It is a tree measuring 8–25 m tall with bole measuring 27–65 cm in diameter and with elongated, star-like calyx lobes. This is an endemic and endangered species and can only be found in the primary rainforests in the Philippine Islands (Mancera et al., 2017). This species is previously known in different localities in the country from 300–1830 masl in Luzon; Laguna (Mount Makiling); Rizal, Quezon, Camarines Norte and Sorsogon, Catanduanes, Visayas; Samar and Leyte, Mindanao; Lanao del Norte, Bukidnon (Mount Kitanglad Range), and Davao del Sur (Mount Apo). However, because of the different threats due to deforestation, over-exploitation of forest resources and conversion of natural lowland forests to agricultural lands, this species has been listed under the endangered category. Fortunately, there are still 55 individuals of *A. calycina* found in the area. This is commonly known as “Bahawbaw” by the local people and is also a habitat for various species of epiphytic ferns *Asplenium apoense* Copel., *Davallia wagneriana* Copel., *D. repens* (Lf) Kuhn, *Elaphoglossum blumeum* J.Sm., *Hymenophyllum acanthoides* (Bosch) Rosenstock, *Nephrolepis cordifolia* (L.) C. Presl, *Oleandra neriiformis* Cav., *Aglaomorpha heraclea* (Kunze) Copel., *Selliguea albidosquamata* (Blume) Parris and *Haplopteris alternans* (Copel.) S.Linds. & C.W.Chen), and lycophytes: *Selaginella involvens* (Sw.) Spring and *Phlegmariurus salvinioides* (Herter) Ching), and many species of mosses and liverworts (*Plagiochila* spp., *Bazzania* spp.).

*Dillenia megalantha* Merr. (**Figure 2 (b)**) is also an interesting species found in the area. It is a medium-sized tree up to 25 m tall, about 50 cm in diameter, with bright yellow flowers. This species has edible fruits eaten by the local people. It has been listed recently as a vulnerable species based on the IUCN assessment of 2020. This species can be found in Quezon, Albay, Sorsogon (Mt Bulusan), Samar, Dianagat, Mindanao; Zamboanga peninsula, Agusan del Norte (Mt Urdaneta), Surigao del Norte and in Mt. Apo, Davao which is the type locality (Pelser et al., 2011).

*Piper aduncum* L. (**Figure 2 (c)**) was found growing rampantly within Marilog District. This species is considered as an invasive alien species (IAS) and may compete with the indigenous species present in the area. It is now widely distributed in different parts of the country especially in Mindanao Island, because the seeds of this species are easily dispersed by wind and birds (Bonaccorso et al., 2002). However, the trunks and branches of this species are known to be inhabited by unique ferns, such as *Lecanopteris deparioides* (Cesati) Baker (Ant fern) and many other species of ferns and mosses.

The montane species, such as *Lithocarpus submonticolus* (Elmer) Rehder, J. Arnold Arb. and *L. caudatifolius* (Merr.) Rehder are known as “Ulayan” in the area and commonly harvested for timber and charcoal making. Furthermore, *Cinnamomum mercadoi* S.Vidal “Kalingag” is also frequently extracted from the forest for its medicinal uses.



**Figure 2.** Some noteworthy species of trees in the areas. A) *Astrocalyx calycina* (S.Vidal) Merr., B) *Dillenia megalantha* Merr., and C) *Piper aduncum* L.

The Shannon diversity index in site 1 was  $H' = 1.22$  and site 2 was  $H' = 1.22$ . These diversity indices are higher compared to the other mountain ecosystems in Mindanao, viz., Leaño (2004) in Mt. Malindang with  $H' = 0.51$ , Silverio (2014) with  $H' = 0.59$  in Mt Apo, Demetillo (2003) in Mt. Lumot with  $H' = 0.90$ , and closely resembles with Polizon (2006) in Mt. Hamiguitan with  $H' = 1.24$  and to the protected forest of Mount Masinggi, Indonesia with  $H' = 1.19$  (Mokoginta, 2016). However, the result was relatively lower compared to Causaren et al. (2017) in the Remaining Forest Fragments in Cavite, Luzon Island with values ranging from range of  $H' = 1.5$  to  $H' = 3.5$ ; Malabrigo et al. (2018) in Mt. Calavite Wildlife Sanctuary, Mindoro Island with  $H' = 4.41$ , and in Pasonanca Natural Park with  $H' = 1.68$  (Andas, 2015). It is also lower compared to the tropical forest of Congo (Ifo et al., 2016; Ekoungoulou et al., 2018), Pahang National Park, Malaysia (Suratman, 2012) and in Northern and Eastern Thailand (Podong & Poolsiri, 2013; Glumphabutr et al., 2006).

The low species diversity in the forest patches of the two sites is due to the anthropogenic activities present in the area as observed by the authors during repeated botanical expeditions. The area is presently subjected to small scale logging, conversion of the forests to agricultural lands, residential areas and mountain resorts, overharvesting and trading of ornamental plants from the wild, and proliferation of invasive species. Tree species diversity in tropical forests differs significantly from one location to another mainly because of the variation in ecology, habitat, and disturbance (Neumann & Starlinger, 2001; Padalia et al., 2004). Forest degradation due to anthropogenic activities or human impacts was also cited as the main driver of the decreasing diversity of plants (Malabrigo et al. 2018; Naidu & Kumar, 2016). Woody plant species are critical components of the forest ecosystem and documenting the patterns of tree diversity and distribution provides a good database, useful for management measures in the forest ecosystem (Naidu & Kumar, 2016).

### ***Species Composition***

The current result revealed that the species with the highest importance value in the areas are *P. philippense*, *S. tula*, *A. calycina*, *L. submonticolus*, and *L. caudatifolius* in site 1 and *Palaquium* sp., *P. philippense*, *L. caudatifolius*, *Diospyros* sp., and *S. tenuirame* in site 2 (Table 2). This conforms to the vegetation type described in Mt. Apo, North Cotabato (Silverio, 2014), Mt. Halcon Range in Mindoro (Merrill, 1907), Mt. Kiamo, Bukidnon (Coritico & Amoroso, 2017) that the montane forests are typically dominated by the *Palaquium* spp., *Lithocarpus* spp. and *Syzygium* spp. Some of the species with high species IV (e.g., *Canarium asperum* Benth., *Melicope triphylla* (Lam.)

*Merr.* and *P. philippense*) were also observed in the permanent plots in Mindanao LTER Sites (Amoroso et al., 2014). The seeds and fruits of these tree species might be easily carried by animals or other seed dispersal agents and would eventually grow provided there is enough moisture. These tree species may also have an essential role in regulating ecosystem stability as cited by Tagupa (2006). The species with high IV determine the principal species present in the sampling area and ultimately dominate and provide overall estimates of the influence of the different plant species in a particular community. Thus, it plays an essential role in a particular community. Removal or loss of the said species of plants would significantly affect the biological and physical structure and function of the ecosystem (Amoroso et al., 2011).

**Table 2.** The five highest Importance Value Index (IVI) species derived from the pooled data of all plots in Site 1 in Brgy. Baganihan and Site 2 in Mt. Malambo, Brgy. Datu Salumay.

Species	Density/ba	Basal area/ba	Frequency	Relative Density (%)	Relative Frequency (%)	Relative Dominance (%)	IV	Rank
<b>SITE 1</b>								
<i>Palaquium philippense</i> (Perr.) C.B. Rob.	69	1769	16	14.44	7.58	15.63	37.64	1 <sup>st</sup>
<i>Syzygium tula</i> (Merr.) Merr.	62	1920	16	12.97	7.58	16.96	37.51	2 <sup>nd</sup>
<i>Astrocalyx calycina</i> (S.Vidal) Merr.	55	1380	14	11.51	6.64	12.19	30.33	3 <sup>rd</sup>
<i>Lithocarpus submonticolus</i> (Elmer) Rehder, J. Arnold Arb.	43	1054	15	9.00	7.11	9.31	25.41	4 <sup>th</sup>
<i>Lithocarpus caudatifolius</i> (Merr.) Rehder	34	818	12	7.11	5.69	7.23	20.03	5 <sup>th</sup>
<b>TOTAL</b>			<b>16</b>					
<b>SITE 2</b>								
<i>Palaquium</i> sp.	57	2837	13	6.02	6.02	17.18	29.22	1 <sup>st</sup>
<i>Palaquium philippense</i> (Perr.) C.B. Rob.	38	1672	11	5.09	5.09	10.13	20.31	2 <sup>nd</sup>
<i>Lithocarpus caudatifolius</i> (Merr.) Rehder	22	979	11	5.09	5.09	5.93	16.12	3 <sup>rd</sup>
<i>Diospyros</i> sp.	24	713	12	5.56	5.56	4.32	15.43	4 <sup>th</sup>
<i>Syzygium tenuirame</i> (Miq.) Merr.	25	986	2	3.24	3.24	5.97	12.45	5 <sup>th</sup>
<b>TOTAL</b>			<b>17</b>					

### Forest Structure

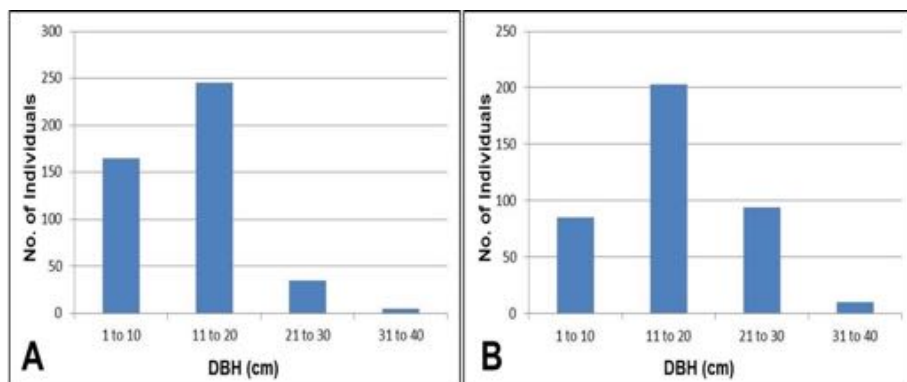
The mean stand density was 30 individuals/20 x 20 m<sup>2</sup>. The highest stand density in site 1 was observed in plot 16 (39 individuals/20 x 20 m<sup>2</sup>), while the lowest stand density was observed in plots 2 and 9 (21 individuals/20 x 20 m<sup>2</sup> each). The other plots showed moderate densities. For site 2, the highest stand density was observed in plot 15 (33 individuals/20 x 20 m<sup>2</sup>) and the lowest was observed in plot 3 (16 individuals/20 x 20 m<sup>2</sup>). The established plots comprised of tree species reaching up to 40 m high and with highest diameter of 90 cm DBH. In site 1, the highest mean diameter was at plot 1 with 26.0 cm, and the least in plot 14 with 17.5 cm, and the highest mean height was recorded at plot 2 with 20.8 m and the least in plot 6 with 11.5 m. In site 2, the highest mean diameter was obtained by plot 17 with 40.6 cm, and least in plot 1 with 18.8 cm, and the highest mean height was obtained by plot 17 with 21 m and the least in plot 14 with 13.0 m (Table 3). The total mean height of trees in the current study is comparably lower than the studies conducted in other mountain sites in the Philippines, viz., Andas (2015) with mean height of 18.82 m in the montane forest of Pasonanca Natural Park, Zamboanga City; Tagupa (2006) with 16.57 m in Mt. Hamiguitan, Davao Oriental; and Silverio (2014) with 23 m in Mt. Apo, North Cotabato. These differences may be consistent with the report of Amoroso et al. (2011) that as altitude increases, the mean number of individuals, mean height and dbh decreases.

**Table 3.** The total number of species and individuals, and mean diameter and height of trees in all the plots in Site 1 in Brgy. Baganihan and Site 2 in Mt. Malambo, Brgy. Datu Salumay.

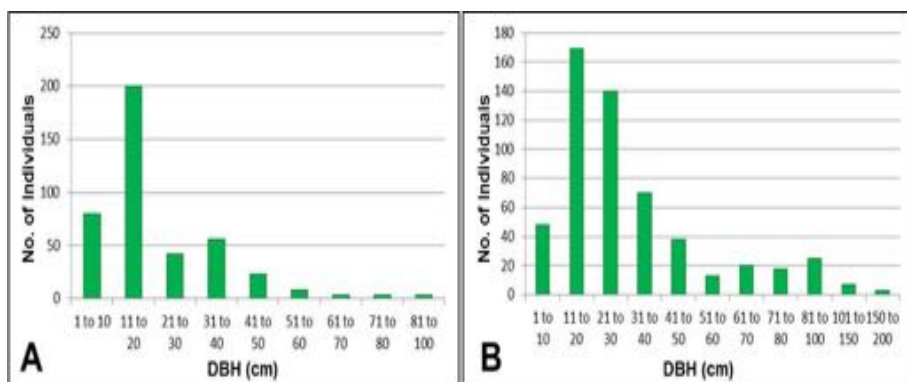
Plot Number	Total number of species	Total number of individuals	Mean ( $\pm$ SE) Diameter (cm)	Min Diameter (cm)	Max Diameter (cm)	Mean Height (m)	Min Height (m)	Max Height (m)
<b>Site 1</b>								
1	17	33	26.0	10	45	20.8	5	25
2	13	21	25.4	10	95	20.8	5	21
3	14	20	24.0	10	95	16.6	7	26
4	14	28	19.0	10	120	13.1	8	29
5	13	38	21.2	10	100	9.49	10	25
6	14	30	21.3	10	120	11.5	10	25
7	14	26	22.5	10	80	14.0	9	25
8	10	27	21.0	10	140	12.7	8	25
9	10	21	22.6	10	70	13.0	10	23
10	16	33	24.0	10	200	14.1	10	35
11	15	33	23.6	10	80	12.0	10	26
12	18	38	19.2	10	75	12.7	10	25
13	15	31	24.2	10	100	14.0	8	25
14	14	37	17.5	10	60	13.3	10	22
15	10	33	25.0	10	100	17.1	10	27
16	14	39	22.1	10	95	13.6	10	30
<b>Total</b>								
	221	488	22.4	10	98.4	14.3	8.75	25.9

Site 2								
1	14	28	18.8	10	45	14.4	5	25
2	11	27	31.5	10	95	14.5	5	21
3	11	16	34.5	10	95	16.3	7	26
4	14	23	36.0	10	120	17.1	8	29
5	9	28	34.4	10	100	16.1	10	25
6	9	18	33.5	10	120	17.9	10	25
7	11	20	26.6	10	80	16.6	9	25
8	12	25	35.8	10	140	16.6	8	25
9	8	26	23.0	10	70	17.1	10	23
10	8	24	33.8	10	200	15.5	10	35
11	11	20	22.1	10	80	15.2	10	26
12	11	18	28.4	10	75	18.5	10	25
13	9	27	28.3	10	100	18.4	8	25
14	7	32	23.4	10	60	13.0	10	22
15	7	33	34.8	10	100	18.8	10	27
16	11	27	34.8	10	95	18.8	10	30
17	11	30	40.6	12	90	21.0	18	29
Total		Total	Mean	Mean	Mean	Mean	Mean	Mean
174		422	28.6	10.1	97.9	16.8	9.3	26.1

For the height classes of trees in site 1, out of 478, a total of 255 individuals (53.4%) attained a height range of 11–20 m, followed by 171 individuals (35.8%) with height ranging from 1–10 m, 42 individuals (8.8%) with 21–30 m height range, and the least with only 10 individuals are the tallest trees with height ranging from 31–40 m. In terms of diameter, species with diameter range of 11–20 cm had the highest records with 203 individuals comprising 42.5% and the least are species with 51–102 cm with one to six numbers of individuals. The species with the widest DBH recorded in the area include *P. philippense*, *S. tula*, *M. triphylla* and *L. caudatifolius* ranging from 61–90 cm, while the tallest species include *P. philippense* with 40 m and *Syzygium* spp. with 30–35 m. For the height classes of trees in site 2, trees in the area reached about 31–40 m tall. About 67% of all individuals fall under 11–20 m height. The tallest trees in the area are *L. caudatifolius* and *Weinmannia* sp. with a mean height of 35 m. Moreover, DBH reaches about 150–200 cm in width. The species with the widest DBH is *Weinmannia* sp. at 200 cm. **Figures 3 and 4** represent the height and diameter classes of all tree species recorded in the established plots in sites 1 and 2.



**Figure 3.** Height classes of tree species in the established plots. **A)** site 1: Forest patches in Barangay Baganihan, **B)** site 2: Mt. Malambo, Barangay Datu Salumay.



**Figure 4.** Diameter classes of tree species in the established plots. **A)** site 1: Forest patches in Barangay Baganihan, **B)** site 2: Mt. Malambo, Barangay Datu Salumay.

### ***Conservation Status and Endemism***

There are eight threatened and 14 endemic species recorded in the forest patches of Brgy. Baganihan and Mt. Malambo, Brgy. Datu Salumay. The threatened species include *Astrocalyx calycina* as endangered; *Agathis philippinensis*, *Becarianthus pulcherrimus*, *Camellia lanceolata*, *Palaquium philippinense* and *Shorea contorta* as vulnerable, while *Cinnamomum mercadoi* as other threatened species based on Fernando et al. (2022). *Dillenia megalantha* was recently assessed by IUCN (2020) as a vulnerable species. The endemic species consists of viz., *Actinodaphne apoensis*, *Alstonia parvifolia*, *Astrocalyx calycina*, *B. pulcherrimus*, *Cinnamomum uteli*, *D. megalantha*, *D.*

*philippinensis*, *Lithocarpus submonticolus*, *Cinnamomum mercadoi*, *Litsea segregata*, *Syzygium tula*, *Aidia pulcherrima*, *P. philippense*, and *Weinmannia hutchinsonii*.

The threatened species of trees in the two sites are comparatively lower compared to the threatened species in the forest fragments in Cavite with 39 species (Causaren et al., 2018). The endemism is also low compared to the study of Causaren et al. (2018) with 19 endemic species and Lillo et al. (2019) in Mt. Lantoy, Agra, Cebu with 76 Philippine endemics and eight species being island endemics. The preservation of the forest patches in Brgy. Baganihan and Mt. Malambo is very crucial for conservation of the remaining threatened and endemic species of plants.



**Figure 5.** Some threatened and endemic species of trees in the forest patches of Brgy. Baganihan and Mt. Malambo, Brgy. Datu Salumay, Marilog District. **A)** *Astrocalyx calycina* (S.Vidal) Merr., **B)** *Cinnamomum mercadoi* S.Vidal, **C)** *Dillenia philippinensis* Rolfe., **D)** *Ficus benguetense* Merrill., **E)** *Palaquium philippense* (Perr.) C.B. Rob., **F)** *Piper aduncum*

### ***Management recommendation***

Assisted Natural Regeneration (ANR) activity was employed to support the remaining natural habitat of the disturbed forest patches in Barangay Bahanihan



and Mt. Malambo, Barangay Datu Salumay. The trees with the highest IVI in the two sites, such as *P. philippense*, *S. tula*, *A. calycina*, *L. submonticolus*, *L. caudatifolius*, *Palaquium* sp., *L. caudatifolius*, *Diospyros* sp., and *S. tenuirame* were prioritized for ANR, and the seedlings of these species were collected as base models. As it is a low-cost and straightforward method for forest restoration (Shono et al., 2007), ANR activity will help conserve the natural forest patches in the areas as it increases forest cover and attains the recovery of the native ecosystem or some of its functions. With some assistance, native species of trees that have adapted to the natural conditions achieve accelerated growth by natural progression, leading to the recovery of native ecosystems (FAO, 2019). Monitoring of high conservation value of the threatened and endemic tree species found in the study sites should be given specific attention.

## Conclusions

The forest patches of Brgy. Baganihan and Mt. Malambo, Brgy. Datu Salumay can be categorised as montane forests based on forest structure and species composition. The species index in this study is low compared to the other sites, mainly because of its ecological reasons. However, the sites have Philippine endemic and threatened species, such as *A. calycina*, *P. philippense*, and *D. philippinensis* to name a few. It is highly recommended that the protection and conservation of these areas should be enhanced. Conservation action to propagate the Philippine endemic and threatened species is crucial with *in situ* and *ex situ* conservation. Results of this study will be used to support the designation of the areas as local conservation sites by the officials of the local government units of Brgy. Baganihan and Brgy. Datu Salumay for long-term conservation and protection of biological diversity.

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Appendix 1. Summary table of the diversity indices of trees in the forest patches of Barangay Baganihan, Marilog District.

Scientific Name	No. of Individual	Occurrence	Total DBH	Density	Relative Density	Frequency	Relative Frequency	Dominance	Relative Dominance	IV	Diversity Values
<i>Alstonia parvifolia</i> Merr.	10	8	206	2.092	0.500	3.791	0.032	1.820	7.703	2.092	0.032
<i>Agathis philippinensis</i> Warb.	2	2	98	0.418	0.125	0.948	0.015	0.866	2.232	0.418	0.018
<i>Canarium asperum</i> Benth.	20	10	345	4.184	0.625	4.739	0.054	3.047	11.971	4.184	0.046
<i>Canarium</i> sp.	6	3	92	1.255	0.188	1.422	0.014	0.813	3.490	1.255	0.017
<i>Ascarina philippinensis</i> C.B. Rob.	5	3	93	1.046	0.188	1.422	0.015	0.821	3.289	1.046	0.017
<i>Terminalia catappa</i> L., Mant.	4	4	97	0.837	0.250	1.896	0.015	0.857	3.589	0.837	0.018
<i>Dillenia megalantha</i> Merr.	5	4	120	1.046	0.250	1.896	0.019	1.060	4.002	1.046	0.021
<i>Polyosma</i> sp.	16	9	331	3.347	0.563	4.265	0.052	2.924	10.536	3.347	0.045
<i>Macaranga hispida</i> (Blume) Müll.Arg.	1	1	15	0.209	0.063	0.474	0.002	0.132	0.816	0.209	0.004
<i>Macaranga sinensis</i> Baill.ex.Müll.Arg.	10	9	160	2.092	0.563	4.265	0.025	1.413	7.771	2.092	0.026
<i>Lithocarpus submonticolus</i> (Elmer) Rehder, J. Arnold Arb.	43	15	1054	8.996	0.938	7.109	0.165	9.310	25.415	8.996	0.096
<i>Lithocarpus caudatifolius</i> (Merr.) Rehder	34	12	818	7.113	0.750	5.687	0.128	7.226	20.026	7.113	0.082
<i>Fagraea auriculata</i> Jack	8	6	181	1.674	0.375	2.844	0.028	1.599	6.116	1.674	0.029
<i>Cinnamomum mercadoi</i> S.Vidal	10	6	176	2.092	0.375	2.844	0.028	1.555	6.490	2.092	0.028
<i>Litsea segregata</i> Elmer	13	8	177	2.720	0.500	3.791	0.028	1.563	8.075	2.720	0.028
<i>Archidendron clypearia</i> (Jack) Nielsen	2	2	36	0.418	0.125	0.948	0.006	0.318	1.684	0.418	0.008
<i>Grewia</i> sp.	3	1	56	0.628	0.063	0.474	0.009	0.495	1.596	0.628	0.011
<i>Astrocalyx calycina</i> (S.Vidal) Merr.	55	14	1380	11.506	0.875	6.635	0.216	12.190	30.331	11.506	0.111
<i>Astronia</i> sp.	3	2	25	0.628	0.125	0.948	0.004	0.221	1.796	0.628	0.006
<i>Beccarianthus pulcherrimus</i> (Merr.) Maxw.	7	3	97	1.464	0.188	1.422	0.015	0.857	3.743	1.464	0.018

(Continued on next page)

Appendix 1 (continued)

<i>Dysoxylum arborescens</i> (Blume) Miq.	11	4	162	2.301	0.250	1.896	0.025	1.431	5.628	2.301	0.026
<i>Dysoxylum</i> sp.	8	6	168	1.674	0.375	2.844	0.026	1.484	6.001	1.674	0.027
<i>Ficus benguetensis</i> Merr.	2	2	20	0.418	0.125	0.948	0.003	0.177	1.543	0.418	0.005
<i>Ficus benjamina</i> L. Mant.	5	3	323	1.046	0.188	1.422	0.050	2.853	5.321	1.046	0.044
<i>Ficus</i> sp.	10	5	205	2.092	0.313	2.370	0.032	1.811	6.273	2.092	0.032
<i>Syzygium tula</i> (Merr.) Merr.	62	16	1,920	12.971	1.000	7.583	0.300	16.960	37.513	12.971	0.131
<i>Syzygium tenuirame</i> (Miq.) Merr.	8	6	107	1.674	0.375	2.844	0.017	0.945	5.462	1.674	0.019
<i>Bischofia javanica</i> Blume	3	3	78	0.628	0.188	1.422	0.012	0.689	2.738	0.628	0.015
<i>Breynia cernua</i> (Poir.) Müll.Arg.	15	10	278	3.138	0.625	4.739	0.043	2.456	10.333	3.138	0.040
<i>Huberantha</i> sp.	5	3	125	1.046	0.188	1.422	0.020	1.104	3.572	1.046	0.022
<i>Piper aduncum</i> L. Var	3	2	36	0.628	0.125	0.948	0.006	0.318	1.893	0.628	0.008
<i>Melicope triphylla</i> (Lam.) Merr.	20	13	573	4.184	0.813	6.161	0.090	5.061	15.407	4.184	0.066
<i>Palaquium philippense</i> (Perr.) C.B. Rob.	69	16	1769	14.435	1.000	7.583	0.276	15.626	37.644	14.435	0.126
<b>TOTAL</b>	<b>478</b>		<b>11321</b>	<b>0.074</b>	<b>100</b>	<b>13.187</b>	<b>100</b>	<b>1.768</b>	<b>100</b>	<b>300</b>	<b>1.220</b>

Appendix 2. Summary table of the diversity indices of trees in Mt. Malambo, Barangay Datu Salumay, Marilog District.

Scientific Name	No. of Individuals	Occurrence	Total DBH	Density	Relative Density	Frequency	Relative Frequency	Dominance	Relative Dominance	IV	Diversity Values
<i>Actinodaphne</i> sp.	11	5	176	0.595	2.315	0.238	2.315	0.021	1.066	5.696	0.021
<i>Alstonia parvifolia</i> Merr.	2	1	29	0.119	0.463	0.048	0.463	0.003	0.176	1.102	0.005
<i>Alstonia</i> sp.	12	7	272	0.833	3.241	0.333	3.241	0.032	1.648	8.129	0.030
<i>Arthocarpus</i> sp.	1	1	23	0.119	0.463	0.048	0.463	0.003	0.139	1.065	0.004
<i>Ascarina philippinensis</i> C. B. Rob.	6	4	122	0.476	1.852	0.190	1.852	0.014	0.739	4.443	0.016
<i>Ascarina</i> sp.	18	8	406	0.952	3.704	0.381	3.704	0.048	2.459	9.867	0.040
<i>Astronia ferruginea</i> (Elmer)	3	1	100	0.119	0.463	0.048	0.463	0.012	0.606	1.532	0.013
<i>Beccarianthus</i> sp.	1	1	20	0.119	0.463	0.048	0.463	0.002	0.121	1.047	0.004
<i>Calophyllum</i> sp.	10	6	368	0.714	2.778	0.286	2.778	0.044	2.229	7.785	0.037
<i>Canarium</i> sp.	19	7	529	0.833	3.241	0.333	3.241	0.063	3.204	9.686	0.048
<i>Cinnamomum mercadai</i> S. Vidal.	18	9	372	1.071	4.167	0.429	4.167	0.044	2.253	10.587	0.037
<i>Clethra</i> sp.	6	1	72	0.119	0.463	0.048	0.463	0.009	0.436	1.362	0.010
<i>Dendrocnide</i> sp.	28	7	523	0.833	3.241	0.333	3.241	0.062	3.168	9.649	0.047
<i>Dillenia philippinensis</i> Rolfe.	7	3	253	0.357	1.389	0.143	1.389	0.030	1.532	4.310	0.028
<i>Diospyros</i> sp.	24	12	713	1.429	5.556	0.571	5.556	0.085	4.319	15.430	0.059
<i>Dysoxylum parasitum</i> (Osbeck) Kosterm.	5	3	136	0.357	1.389	0.143	1.389	0.016	0.824	3.602	0.017
<i>Dysoxylum</i> sp. 1	3	3	75	0.357	1.389	0.143	1.389	0.009	0.454	3.232	0.011
<i>Dysoxylum</i> sp. 2	3	2	153	0.238	0.926	0.095	0.926	0.018	0.927	2.779	0.019
<i>Elaeocarpus</i> sp.	8	6	246	0.714	2.778	0.286	2.778	0.030	1.490	7.046	0.027
<i>Fagraea</i> sp.	3	3	66	0.357	1.389	0.143	1.389	0.008	0.400	3.178	0.010
<i>Ficus benguetense</i> Merril.	24	10	474	1.190	4.630	0.476	4.630	0.056	2.871	12.130	0.044
<i>Ficus benjamina</i> L.	3	3	255	0.357	1.389	0.143	1.389	0.030	1.545	4.322	0.028
<i>Ficus</i> sp. 1	11	8	361	0.952	3.704	0.381	3.704	0.043	2.187	9.594	0.036
<i>Ficus</i> sp. 2	5	4	185	0.476	1.852	0.190	1.852	0.022	1.121	4.824	0.022

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## Appendix 2. (continued)

<i>Lithocarpus caudatifolius</i> (Merr.) Rehder	22	11	979	1.309	5.093	0.524	5.093	0.117	5.930	16.115	0.073
<i>Lithocarpus</i> sp.	21	8	673	0.952	3.704	0.381	3.704	0.080	4.077	11.484	0.057
<i>Macaranga sinensis</i> Baill. Muell. Arg.	33	7	605	0.833	3.241	0.333	3.241	0.072	3.665	10.146	0.053
<i>Malutos</i> sp.	23	8	530	0.952	3.704	0.381	3.704	0.063	3.210	10.618	0.048
<i>Melicope triphylla</i> (Lam.) Merr.	2	1	49	0.119	0.463	0.048	0.463	0.006	0.297	1.223	0.008
<i>Melicope</i> sp.	2	1	35	0.119	0.463	0.048	0.463	0.004	0.212	1.138	0.006
<i>Nauclea</i> sp.	1	1	10	0.119	0.463	0.048	0.463	0.001	0.061	0.986	0.002
<i>Omalanthus populneus</i> Geiseler	1	1	14	0.119	0.463	0.048	0.463	0.002	0.085	1.011	0.003
<i>Omalanthus</i> sp.	4	2	50	0.238	0.926	0.095	0.926	0.006	0.303	2.155	0.008
<i>Palaquium philippinense</i> (Perr.) C. B. Rob.	38	11	1672	1.309	5.093	0.523	5.093	0.199	10.128	20.313	0.101
<i>Palaquium</i> sp.	57	13	2837	1.548	6.019	0.619	6.019	0.338	17.185	29.222	0.131
<i>Piper aduncum</i> L.	6	4	85	0.476	1.852	0.190	1.852	0.010	0.515	4.219	0.012
<i>Pittosporum</i> sp.	3	2	65	0.238	0.926	0.095	0.926	0.008	0.394	2.246	0.009
<i>Psychotria</i> sp.	9	5	175	0.595	2.315	0.238	2.315	0.021	1.060	5.690	0.021
<i>Saurauia</i> sp.	1	1	28	0.119	0.463	0.048	0.463	0.003	0.170	1.096	0.005
<i>Shorea contorta</i> S. Vidal	1	1	40	0.119	0.463	0.048	0.463	0.005	0.242	1.168	0.006
<i>Syzygium tenuirame</i> (Miq.) Merr.	25	7	986	0.833	3.241	0.333	3.241	0.117	5.972	12.454	0.073
<i>Syzygium</i> sp. 1	3	2	95	0.238	0.926	0.095	0.926	0.011	0.575	2.427	0.013
<i>Syzygium</i> sp. 2	31	5	1100	0.595	2.315	0.238	2.315	0.131	6.663	11.293	0.079
<i>Thea</i> sp.	4	2	77	0.238	0.926	0.095	0.926	0.009	0.466	2.318	0.011
<i>Turpinia</i> sp.	2	2	100	0.238	0.926	0.095	0.926	0.012	0.606	2.458	0.013
<i>Weinmannia</i> sp.	9	6	375	0.714	2.778	0.286	2.778	0.045	2.271	7.827	0.037
<b>TOTAL</b>	<b>529</b>	<b>216</b>	<b>16509</b>	<b>0.026</b>	<b>100</b>	<b>10.286</b>	<b>100</b>	<b>1.965</b>	<b>100</b>	<b>300</b>	<b>1.378</b>

## Research Article

# The mosses of Mount Tambuyukon, Kinabalu Park, Malaysian Borneo

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## ABSTRACT

A total of 103 species, two subspecies and two varieties of mosses in 55 genera and 26 families were collected from Mount Tambuyukon in August 2008. This represents ca. 16.3% of the mosses reported from Sabah and ca. 13.9% of the mosses reported for Borneo. Amongst the 107 taxa, three species of mosses are new for Sabah, namely *Acroporium aciphyllum* Dixon, *Garovaglia baeuerlenii* (Geh.) Paris and *Macromitrium angustifolium* Dozy & Molk. The largest moss family collected here is Sematophyllaceae (13 taxa) followed by Leucobryaceae (11 taxa), and both Dicranaceae and Orthotrichaceae (10 taxa). In conclusion, Mount Tambuyukon sustains a rich and interesting moss flora, including several rare and endemic species.

**Keywords:** Borneo, moss flora, Mount Tambuyukon.

## Introduction

Mount Tambuyukon (2,580 m), despite being the third highest mountain in Malaysia, is left far behind in terms of botanical research as compared to Mount Kinabalu. Many bryologists have visited the latter mountain since the 19<sup>th</sup> century leading to numerous reports on bryophytes published since. However, there is no report on the bryophyte flora of Mount Tambuyukon although it is located very close to Mount Kinabalu. This could be due to poor accessibility to Mount Tambuyukon until recent decades.

Thus far, only a few species of bryophytes have been reported from Mount Tambuyukon, such as *Ectropothecium ptychofolium* Nishimura (Nishimura, 1984), *Dicranoloma assimile* (Hampe) Ren., *D. blumei* (Nees) Ren. and *D.*

*brevisetum* (Dozy & Molk.) Paris (Klazenga, 1999). These collections were made by Willem Meijer in the 1950s and are housed in Rijksherbarium Leiden, the Netherlands. Unfortunately, Meijer's collection from Mount Tambuyukon was not studied as a whole but only mentioned in several monographs and taxonomic publications on bryophytes (Frahm et al., 1990). Hence, this study is the first attempt to document the moss species that are found on Mount Tambuyukon.

## Methodology

Mosses were collected over a period of four days from 12–15 August 2008, on the eastern side of Mount Tambuyukon (Table 1). All habitats found along and beside the existing climbing trail from Monggis Substation (N6°12'4.4", E116°44'58.7") to the summit (6°11'56.49"N, 116°39'41.44"E) of the mountain were surveyed. Due to heavy rain during the fieldtrip, only 194 moss specimens were collected, which covered the elevation from the foothill (200 m a.s.l.) to the summit zone (2500 m a.s.l.).

The classification of moss families and genera is based on Goffinet and Buck (2020), while species names and authority are based on the Tropicos database. Specimens were deposited at the BORNEENSIS Herbarium (BORH) of Universiti Malaysia Sabah, with duplicates in Sabah Parks Herbarium (SNP) and in the University of Malaya Herbarium (KLU).

**Table 1.** Collection details of mosses collected at Mount Tambuyukon.

Collection No.	Collection detail
MS-DPM 3547-3571	Mt. Tambuyukon, Monggis Substation to Kopuakan Camp (Km 8), lowland forest, 12 August 2008.
MS-DPM 3572-3612	Mt. Tambuyukon, Kopuakan Camp (Km8) to Musang Camp (Km 10), montane forest, 13 August 2008.
MS-DPM 3613-3759	Mt. Tambuyukon, Musang Camp (Km10) to the summit, mossy forest, 14 August 2008.
MS-DPM 3760-3775	Mt. Tambuyukon, Musang Camp (Km 10) to Monggis Substation, 15 August 2008.

Note: MS-DPM = Monica Suleiman and Dunstan Polus Masundang.

## Results and Discussion

A total of 107 taxa consisting of 103 species, two subspecies and two varieties in 55 genera and 26 families of mosses were collected from Mount Tambuyukon (**Appendix 1**). This number is far less than what has been reported from Mount Kinabalu; only 27% of the 386 taxa of mosses that are reported for the latter mountain. This difference may be due to the limited collection time spent on the upper montane and summit region of the mountain. Bad weather during the field trip also prevented the team from collecting more specimens and hampered exploration to areas away from the main trail. Nonetheless, among the collection was a species new to science, *Bryobrothera tambuyukonensis* (Akiyama & Suleiman, 2015). This collection has also contributed three new records for Sabah, namely *Acroporium aciphyllum*, *Garovaglia baeuerlenii* and *Macromitrium angustifolium*. In addition, four taxa of mosses that were collected during this visit are new records for Kinabalu Park, namely *Braunfelsia dicranoides*, *Ectropothecium elegantipinnatum*, *Macromitrium salakanum* and *Macrohymenium muelleri*. This is indeed a fairly significant contribution to the bryoflora of the park.

It is worth pointing out that amongst the 107 taxa collected, four species are Bornean endemics, namely *Bryobrothera tambuyukonensis*, *Ectropothecium ptychofolium*, *Macromitrium ochraceoides* and *Schlotheimia rubiginosa*. Borneo holds only about 10 endemic species of mosses, which is ca. 1.30% of the 772 taxa of mosses recorded from Borneo. Hence, ca. 40% of the Bornean endemic species are found here, which indicates the importance of Mount Tambuyukon in the conservation of bryophytes flora.

The largest moss family collected here is Sematophyllaceae with 12 species and one variety, followed by Leucobryaceae with 14 species, Dicranaceae with 10 species and Orthotrichaceae with nine species and one variety. Sematophyllaceae is known to have a wide elevational range; it was collected from 500 m to 2500 m a.s.l. Meanwhile, members of the Dicranaceae and Orthotrichaceae were mostly collected at an elevation above 1,200 m. It is interesting to note that members of Dawsoniaceae and Sphagnaceae are not represented here, although these two families are abundant on other mountains in Sabah, such as Mount Kinabalu (Akiyama et al., 2001) and Mount Trus Madi (Suleiman & Edwards, 2002). More explorations, especially to other parts of the mountain and the summit region are needed to determine a more comprehensive understanding about the moss flora of this mountain.

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## Appendix 1. Species checklist of mosses from Mount Tambuyukon

Species marked with an asterisk (\*) are new to Kinabalu Park and double asterisks (\*\*) are new to Sabah. MS-DPM denotes M. Suleiman and D.P. Masundang.

### BARTRAMIACEAE

*Breutelia arundinifolia* (Duby) M. Fleisch.

On humus, 1550 m alt., MS-DPM 3682.

In Borneo, this species has only been reported from Mount Kinabalu and Mount Trus Madi.

*Breutelia kinabaluensis* Dixon.

On humus, 2250 m alt., MS-DPM 3750.

Mount Tambuyukon is a new locality for this species, beside Mount Kinabalu, in Borneo (Suleiman et al., 2006).

*Philonotis secunda* (Dozy & Molk.) Bosch & Sande Lac.

On gravel, 830 m alt., MS-DPM 3569.

### BRYACEAE

*Bryum russulum* Broth. & Geh.

On boulders and humus, 2150–2300 m alt., MS-DPM 3721, 3728, 3738b, 3747, 3754.

This is a large *Bryum* species and is known to be disjunctive in its distribution. The plant is thus far only reported from New Guinea, Sulawesi, Borneo and Thailand (Frahm et al., 2009). In Borneo, the species was formerly only known to Mount Kinabalu (Ochi, 1971; Akiyama et al., 2001).

### CALYMPERACEAE

*Calymperes fasciculatum* Dozy & Molk.

On tree trunk, 1200 m alt., MS-DPM 3595.

*Mitthyridium luteum* (Mitt.) H. Rob.

On tree trunk, 550 m alt., MS-DPM 3556.

*Syrrhopodon trachyphyllus* Mont.

On tree trunk, 1200 m alt., MS-DPM 3594.

*Syrrhopodon muelleri* (Dozy & Molk.) Sande Lac.

On tree buttress, 520 m alt., MS-DPM 3550.

*Syrrhopodon tristichus* Nees ex Schwägr.

On treelet trunk, 1550 m alt., MS-DPM 3688.

## DALTONIACEAE

*Benitotania elimbata* H. Akiyama, T. Tamag. & M. Suleiman

On shrub and tree trunks, 1400–1480 m alt., MS-DPM 3617, 3649, 3654, 3656, 3647, 3667.

Some of the specimens have filamentous gemmae on branch tips; the gemmae are papillose and branched.

*Calypstrochaeta remotifolia* (Müll. Hal.) Z. Iwats., B.C. Tan & Touw.

On rock and trunk of shrub, 1450–1600 m alt., MS-DPM 3653, 3699.

*Distichophyllum cuspidatum* (Dozy & Molk.) Dozy & Molk.

On trunk of shrub, 1630 m alt., MS-DPM 3697.

*Distichophyllum malayense* Damanhuri & Mohamed

On trunk of shrub, 1550 m alt., MS-DPM 3691.

*Distichophyllum subcuspidatum* Nog. & Z. Iwats.

On rotten log, 750 m alt., MS-DPM 3574.

*Bryobrothera tambuyukonensis* H. Akiyama & M. Suleiman

On treelet trunks and climber, 1450–1670 m alt., MS-DPM 3640, 3687, 3690, 3698, 3700, 3702, 3704.

This species was described as a new species based on the specimens collected in this study and from Sungai Imbak Forest Reserve (Akiyama & Suleiman, 2015). This is also the first record of the *Bryobrothera* genus in Borneo.

## DICRANACEAE

\**Braunfelsia dicranoides* (Dozy & Molk.) Broth.

On tree base and trunk, 1450–1550 m alt., MS-DPM 3631, 3679.

The only record of this species in Borneo was from Crocker Range Park (Suleiman et al., 2017).

*Braunfelsia* cf. *enervis* (Dozy & Molk.) Paris

On tree trunks and climber, 1450–1550 m alt., MS-DPM 3630, 3661, 3674.

These specimens have short and broadly ovate leaves, as in *B. enervis*, but some leaves are clearly costate. This is the second record of this species in Sabah.

*Braunfelsia edentula* (Mitt.) Wijk & Margad.

On tree trunks, humus and boulders, 1450–2450 m alt., MS-DPM 3645, 3666, 3671, 3681, 3724, 3729, 3759.

Specimens MS-DPM 3645, 3666 and 3671 are small for the species and green-brown in colour, as opposed to large and yellow-brown in typical ones.

*Dicranoloma assimile* (Hampe) Broth. ex Renauld

On humus and tree base, 1350–1450 m alt., MS-DPM 3612, 3629, 3766.

*Dicranoloma billardierii* (Brid.) Paris

On humus, 1400–1450, MS-DPM 3619, 3628.

*Dicranoloma blumei* (Nees) Broth. ex Renauld

On tree trunks, 1400–1450 m alt., MS-DPM 3615, 3660, 3626, 3627.

*Dicranoloma braunii* (Müll. Hal.) Paris

On tree trunks and rotten logs, 750–1550 m alt., MS-DPM 3573, 3596, 3620, 3762a, 3684.

*Dicranoloma brevisetum* (Dozy & Molk.) Paris

On tree trunks, 1350–1520 m alt., MS-DPM 3602, 3762b, 3685, 3764, 3765.

*Dicranoloma* aff. *daymannianum* E.B. Bartram

On tree trunk, 1150m alt., MS-DPM 3585.

The specimen is a soft-textured *Dicranoloma*, which looks like *D. braunii* in the field but the former has stem central strand and teeth of costa are scattered. It is close to *D. daymannianum* but its leaves are not brittle (Klazenga, 1999).

*Leucoloma molle* (Müll. Hal.) Mitt.

On tree trunk, 1150 m alt., MS-DPM 3584.

**FISSIDENTACEAE***Fissidens crassinervis* Sande Lac.

On soil, 1170 m alt., MS-DPM 3587.



*Fissidens crispulus* Brid.

On rock, 780 m alt., MS-DPM 3582a.

*Fissidens javanicus* Dozy & Molk.

On soil and tree root, 780 m alt., MS-DPM 3580, 3581.

*Fissidens nobilis* Griff.

On boulder, 750 m alt., MS-DPM 3565.

*Fissidens pallidus* Hook. F. & Wils.

On soil, 550 m alt., MS-DPM 3558.

*Fissidens pellucidus* Hornsch.

On rock, 780 m alt., MS-DPM 3582b.

*Fissidens* cf. *polypodioides* Hedw.

On soil, 1170 m alt., MS-DPM 3586.

#### **HYPNACEAE**

*Ctenidium* sp.

On tree trunk, 1450 m alt., MS-DPM 3652.

*\*Ectropothecium elegantipinnatum* (Müll. Hal.) A. Jaeger

On rotten log, 1450 m alt., MS-DPM 3625.

This is a common species in Borneo but has rarely been reported. In Sabah, it has been reported from Maliau Basin Conservation Area (Mohamed et al., 2010; Suleiman et al. 2017).

*Ectropothecium ptychofolium* Nishimura

On rotten log, 1300 m alt., MS-DPM 3598.

This distinctive species is endemic to Borneo. It was previously collected from Mount Tambuyukon by Willem Meijer (Nishimura, 1984).

*Ectropothecium* sp. 1

On rock and humus, 2150–2230 m alt., MS-DPM 3727, 3746.

*Ectropothecium* sp. 2

On boulder, 1450 m alt., MS-DPM 3659.

**HYPNODENDRACEAE**

*Hypnodendron dendroides* (Brid.) Touw

On humus and rotten log, 1200–1900 m alt., MS-DPM 3592, 3714.

*Hypnodendron diversifolium* Broth. & Geh.

On tree root, 2000 m alt., MS-DPM 3718.

*Dendro-hypnum milnei* subsp. *korthalsii* (Bosch & Sande Lac. ex Paris) N.E. Bell, A.E. Newton & D. Quandt

On boulder by a river, 750 m alt., MS-DPM 3575.

*Dendro-hypnum reinwardtii* subsp. *caducifolium* (Herzog) N.E. Bell, A.E. Newton & D. Quandt

On tree trunk and buttress, 1400–1800 m alt., MS-DPM 3618, 3693, 3713.

**HYPOPTERYGIACEAE**

*Lopidium struthiopteris* (Brid.) M. Fleisch.

On tree trunk, 750 m alt., MS-DPM 3566.

**LEUCOBRYACEAE**

*Campylopus comosus* (Schwägr.) Bosch & Sande Lac.

On rotten log, 1200 m alt., MS-DPM 3593.

*Campylopus exasperatus* (Nees & Blume) Brid

On boulders and humus, 2150–2250 m alt., MS-DPM 3725, 3732, 3736, 3738, 3745, 3749.

*Campylopus flagellifer* (Müll. Hal.) A. Jaeger

On rotten log and tree trunk, 1900–2450 m alt., MS-DPM 3715, 3755.

This species is only known from Borneo, South India, Sri Lanka and Thailand (Frahm et al., 2009). Thus far, this is the second record of this species for Borneo (Frahm & Mohamad, 1987).

*Cladopodanthus speciosus* (Dozy & Molk.) M. Fleisch.

On rotten log, 750 m alt., MS-DPM 3568.

*Dicranodontium uncinatum* (Harv.) J. Jaeger [syn. *D. fleischerianum* W. Schultze-Motel var. *fleischerianum*]

On humus, 2470 m alt., MS-DPM 3757.

*Leucobryum chlorophyllosum* Müll. Hal.

On tree buttress, 520 m alt., MS-DPM 3551.

*Leucobryum javense* (Brid.) Mitt.

On humus, 1350 m alt., MS-DPM 3767.

*Leucobryum sanctum* (Nees ex Schwägr.) Hampe.

On rotten logs, 520–850 m alt., MS-DPM 3549, 3771.

*Leucobryum sumatranum* Broth. ex M. Fleisch.

On humus, 1350–1400 m alt., MS-DPM 3609, 3760.

*Schistomitrium apiculatum* (Dozy & Molk.) Dozy & Molk.

On tree trunk, 1400 m alt., MS-DPM 3621.

*Schistomitrium mucronifolium* (A. Braun ex Müll. Hal.) M. Fleisch.

On tree trunk, 1350 m alt., MS-DPM 3643, 3763.

#### **METEORACEAE**

*Cryptopapillaria* sp.

On boulders in open areas, 2200 m alt., MS-DPM 3723, 3738a.

This species has the leaf characteristics of the genus but has different habits. The plant is much larger than the only *Cryptopapillaria* species (viz. *C. fuscescens* (Hook.) M. Menzel) known to Borneo. Unlike *C. fuscescens* which has flaring and auriculate leaf base, it is gradually narrow to insertion in this plant.

*Pseudotrachypus wallichii* (Brid.) W.R. Buck

On boulder, 600 m alt., MS-DPM 3560.

#### **MNIACEAE**

*Plagiomnium succulentum* (Mitt.) T.Kop.

On boulder by a river, 750 m alt., MS-DPM 3578.

#### **MYURACEAE**

*Oedicladium rufescens* (Reinw. & Hornsch.) Mitt.

On treelet trunk, 1350 m alt., MS-DPM 3761.

*Piloecium pseudorufescens* (Hampe) Müll. Hal. ex Broth.

On tree trunk, 550 m alt., MS-DPM 3553.

**NECKERACEAE**

*Circulifolium exiguum* (Bosch & Sande Lac.) S. Olsson, Enroth & D. Quandt  
On tree trunk, 650 m alt., MS-DPM 3561.

*Circulifolium microdendron* (Mont.) S. Olsson, Enroth & D. Quandt  
On boulder, 550 m alt., MS-DPM 3554.

*Homaliodendron flabellatum* (Sm.) M. Fleisch.  
On tree root, 750 m alt., MS-DPM 3577.

*Neckeropsis cyclophylla* (Müll. Hal.) S. Olsson, Enroth & D. Quandt  
On boulder, 750 m alt., MS-DPM 3576.

**OCTOBLEPHARACEAE**

*Octoblepharum albidum* Hedw.  
On tree trunk, 800 m alt., MS-DPM 3770.

**ORTHOTRICHACEAE**

**\*\****Macromitrium angustifolium* Dozy & Molk.

On tree and shrub trunks and boulder in open and partially shaded areas,  
1480–2200 m alt., MS-DPM 3664, 3668, 3677a, 3709, 3733, 3740.

Thus far, this species has only been reported from West Kalimantan in Borneo.  
It was previously reported from China, Papua New Guinea, Japan and the  
Philippines.

*Macromitrium blumei* Nees ex Schwägr.

On tree and shrub trunks and branch as well as boulders in open and partially  
shaded areas, 1480–2200 m alt., MS-DPM 3669, 3676, 3677b, 3680, 3730, 3735,  
3737.

*Macromitrium blumei* var. *zollingeri* (Mitt. ex Bosch & Sande Lac.) S.L. Guo,  
B.C. Tan & Virtanen [syn. *M. zollingeri* Mitt. ex Bosch & Sande Lac.]  
On treelet trunk, 1450 m alt., MS-DPM 3642.

*Macromitrium cuspidatum* Hampe

On shrub and tree trunk, 1400 m alt., MS-DPM 3614, 3646.

*Macromitrium longipilum* A.Braun ex Müll. Hal.

On tree trunks, 1450–1550 m alt., MS-DPM 3658, 3663, 3678.

This species is the largest member of the genus in Borneo, with distributional range restricted to the Malesian region. Although the species is highly polymorphic in its vegetative character, it can generally be recognised by the presence of its long piliferous costa and robust size.

*Macromitrium ochraceoides* Dixon

On boulders and shrub branch in open and partially shaded areas, 2230 m alt., MS-DPM 3741, 3742, 3743.

This Bornean endemic species is closely allied to *M. ochraceum* and has been considered as a form of the latter by Eddy (1996). However, *M. ochraceoides* can always be distinguished from *M. ochraceum* by the presence of long-piliferous costa and smooth laminal cells at the lower half of the leaf which are not found in the latter species. Although this species is common at the higher elevation of Mount Kinabalu, and now reported for Mount Tambuyokon, it has never been collected outside of Kinabalu Park.

*Macromitrium ochraceum* (Dozy & Molk.) Müll. Hal.

On tree trunk and rotten log in open and partially shaded areas, 1200–1450 m alt., MS-DPM 3590, 3641.

\**Macromitrium salakanum* Müll. Hal.

On fallen branch, 550 m alt., MS-DPM 3552.

A common species in Malesia region, with distributional range extended eastward to Solomon Islands and New Caledonia. This species is morphologically allied to *M. angustifolium*, which could only be separated from the latter species by its long-lingulate perichaetial leaves that end in a blunt or obtuse apex, whereas the leaf apex is acuminate in *M. angustifolium*.

*Schlotheimia rubiginosa* C.H. Wright

Fallen from tree canopy, 1720 m alt., MS-DPM 3708.

A Bornean endemic species with very narrow distributional range. Formerly, it was only collected from higher elevation of Mount Kinabalu, at upper montane to subalpine forest, and Mount Tambuyokon is now the second locality known for this species. The species is easily recognised by its robust plant size with its peculiar reddish-brown colour. In addition to these, the species is characterized by having long oblong leaves with obtuse to retuse apex, and prorate basal laminal cells.

*Schlotheimia wallisii* Müll. Hal.

On tree trunk and base of shrubs, 1480–1520m, MS-DPM 3665, 3672, 3675.

In Borneo, this species has only been reported from Mount Kinabalu 79 years ago (Suleiman et al., 2006).

### POLYTRICHACEAE

*Pogonatum cirratum* (Sw.) Brid. subsp. *cirratum*

On soil, 800 m alt., MS-DPM 3564.

*Pogonatum cirratum* subsp. *fuscatum* (Mitt.) Hyvönen

On rock, 1400 m alt., MS-DPM 3608.

*Pogonatum cirratum* subsp. *macrophyllum* (Dozy & Molk.) Hyvönen

On soil, 1300 m alt., MS-DPM 3599.

*Pogonatum piliferum* (Dozy & Molk.) Touw

On soil, 1170 m alt., MS-DPM 3588.

### POTTIACEAE

*Barbula consanguinea* (Thwaites & Mitt.) A. Jaeger

On boulder, 700 m alt., MS-DPM 3772.

*Hydrogonium orientale* (F. Weber) Jan Kučera

On soil, 830 m alt., MS-DPM 3570.

*Pseudosymblepharis bombayensis* (Müll. Hal.) P. Sollman

On boulders in open and partially shaded areas 1550–2200 m alt., MS-DPM, 3683, 3722, 3731, 3734, 3739.

### PTEROBRYACEAE

*Calyptothecium recurvulum* (Müll. Hal. ex Broth.) Broth.

On tree trunk, 750 m alt., MS-DPM 3579.

This large species is fairly common in lower montane forests in Sabah, especially along rivers.

*Cryptogonium phyllogonioides* (Sull.) Isov.

On tree trunk, 400–550 m alt., MS-DPM 3559, 3775.

*Neolindbergia rigida* (Bosch & Sande Lac.) M. Fleisch.

On tree trunk, 800 m alt., MS-DPM 3571.

**PTYCHOMNIACEAE**

**\*\**Garovaglia baeuerlenii* (Geh.) Paris**

On humus and tree trunk, 1670–1900 m alt., MS-DPM 3703, 3716.

This species has only been reported twice from Borneo; both records are from Sarawak (Suleiman et al., 2006).

*Garovaglia* sp.

On tree trunk, 700 m alt., MS-DPM 3567.

**PYLAISIADELPHACEAE**

*Clastobryum indicum* (Dozy & Molk.) Dozy & Molk.

On trunk of treelet, 1450 m alt., MS-DPM 3623.

*Isocladiella surcularis* (Dixon) B.C.Tan & Mohamed

On tree trunk, 750 m alt., MS-DPM 3774.

*Trismegistia calderensis* (Sull.) Broth.

On boulder and shrub trunk, 1600 m alt., MS-DPM 3692, 3706.

*Trismegistia lancifolia* (Harv.) Broth.

On rotten log, 760 m alt., MS-DPM 3562.

*Trismegistia panduriformis* (C.H.Wright) Broth.

On trunk of shrub, 1550 m alt., MS-DPM 3689.

**RACOPILACEAE**

*Racopilum spectabile* Reinw. & Hornsch.

On rotten rock, logs and tree root, 700– 1760 m alt., MS-DPM 3563, 3572, 3591, 3712.

**RHIZOGONIACEAE**

*Pyrrhobryum spiniforme* (Hedw.) Mitt.

On tree buttress, 500 m alt., MS-DPM 3548.

*Rhizogonium graeffeanum* (Müll. Hal.) A.Jaeger.

On tree base, 1300 m alt., MS-DPM 3601.

**SEMATOPHYLLACEAE**

*Acanthorrhynchium papillatum* (Harv.) M.Fleisch.

On rotten log, 500 m alt., MS-DPM 3547.

**\*\**Acroporium aciphyllum* Dixon**

On humus, 1750 m alt., MS-DPM 3711.

Dixon (1935) first reported this species for Borneo (Sarawak) but without citing any reference material. Thus, this finding represents the second record of this species in Borneo and confirms its presence on this island.

*Acroporium convolutum* (Sande Lac.) M. Fleisch.

On rotten log, 550–900 m alt., MS-DPM 3555, 3583.

*Acroporium diminutum* (Brid.) M. Fleisch.

On tree and shrub trunks, 1650–1750 m alt., MS-DPM 3701, 3710.

*Acroporium lamprophyllum* Mitt.

On shrub trunk, 1450 m alt., MS-DPM 3657.

*Acroporium macro-turgidum* Dixon

On humus and rotten log, 2100 m alt., MS-DPM 3719, 3720.

This species is the largest member of the genus. It is only known from the Malay Peninsula and Borneo, and thus far this is the second record for Sabah.

*Acroporium rigens* (Broth. ex Dixon) Dixon

On humus and rotten logs, 1200–1450 m alt., MS-DPM 3589, 3624.

*Acroporium rufum* (Reinw. & Hornsch.) M. Fleisch.

On tree trunk, 2150 m alt., MS-DPM 3726.

*Acroporium hyalinum* (Reinw. ex Schwägr.) Mitt. var. *hyalinum*

On tree trunks and humus, 1400–2470 m alt., MS-DPM 3616, 3694, 3758.

*Acroporium hyalinum* var. *hamulatum* (M. Fleisch.) M.S. Chua & B.C. Ho

On trunk of shrub, 1450 m alt., MS-DPM 3655.

*Acroporium strepsiphyllum* (Mont.) B.C. Tan

On shrub trunk, 1480 m alt., MS-DPM 3670.

*Clastobryophilum bogoricum* (Bosch & Sande Lac.) M. Fleisch.

On tree trunk, 1450 m alt., MS-DPM 3651.

**\**Macrohymenium muelleri* Dozy & Molk.**



On tree trunks of shrub and tree, 2300–2450 m alt., MS-DPM 3744, 3748, 3752, 3756.

The members of this genus are characterized by having inner peristome teeth that are much longer than those found at the outer peristome row, which is unique among the members of Sematophyllaceae.

#### **THUIDIACEAE**

*Thuidium pristocalyx* (Müll. Hal.) A. Jaeger

On rotten log, 720 m alt., MS-DPM 3773.

#### **TRACHYLOMATACEAE**

*Trachyloma indicum* Mitt.

On root of tree, 1350 m alt., MS-DPM 3613.

The apical leaves cells of this specimen are not pitted, in contrast to Hyvonen (1989). This species has the habits and size of *Hypnodendron reinwardtii*, and thus difficult to distinguish in the field, but the inconspicuous costa and asymmetric leaves will separate it from the latter.

## Instructions for Authors

**Managing Editors:** Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Sabah, MALAYSIA. Tel: +60-88-320000 ext. 103134; Fax: +6088-329246  
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