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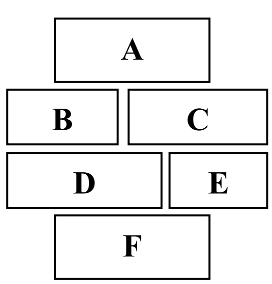

A: *Hemibagrus baramensis;* **B:** *Eugnathogobius kabilia;* **C:** *Theriodes sandakanensis*; **D:** *Redigobius chryososoma*; **E:** *Dichotomyctere kretamensis*; **F:** *Betta ocellata*

Front cover: Inland freshwater fish diversity in Kabili-Sepilok Forest Reserve. (Photos: Dr. Tan Heok Hui)

Avifaunal survey of Mensalong Forest Reserve, Ranau, Sabah, Malaysia

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Abstract. The main objective of the Mensalong Forest Reserve (MFR) survey was to provide a brief description of the avian community in the area, which could be used to support future forest management initiatives. The MacKinnon List method was used. The four-day survey at MFR recorded a total of 23 lists with 566 individuals detected. A total of 101 species from 37 families were recorded, with H=4.18 and E_H=0.75. True species richness was estimated (using SuperDuplicates® online calculator) to be 118 species, with approximately 17 species not detected. There were 10 species of Bornean endemics detected, namely the Bornean Banded Pitta, Bornean Ibon, Bornean Spiderhunter, Bornean Treepie, Chestnut-crested Yuhina, Dusky Munia, White-crowned Shama, Black-throated Wren-babbler, Blue-banded Pitta and the Kinabalu Serpent Eagle. The latter two species were categorised as Vulnerable in the IUCN Redlist. The top 4 most speciose families were Pycnonotidae (with 13 species), Nectariniidae/Pellorneidae (8 species each), Picidae (6 species) and Columbidae (5 species). The Pycnonotidae and Megalaimidae also had the highest percentage of individuals detected with 15.2% and 11.3% respectively. The top 5 species with the highest Relative Abundance Index were the Gold-whiskered Barbet (0.058), the Orange-bellied Flowerpecker (0.048), the Brown Fulvetta (0.041), the Chestnut-crested Yuhina (0.035) and the Blue-crowned Hanging Parrot (0.034). The majority of the species (93 species) detected were forest and forest-dependent species. Of these, 74 species were strictly forest birds. Insectivores made up the most dominant dietary guild, i.e. a total of 57 species (from 28 families) with 48 species (from 23 families) being strict insectivores and the rest were mixed-diet insectivores.

Keywords: avifaunal survey, MacKinnon List method, scientific expedition, Mensalong Forest Reserve, Ranau, Kinabalu Park, feeding guilds

INTRODUCTION

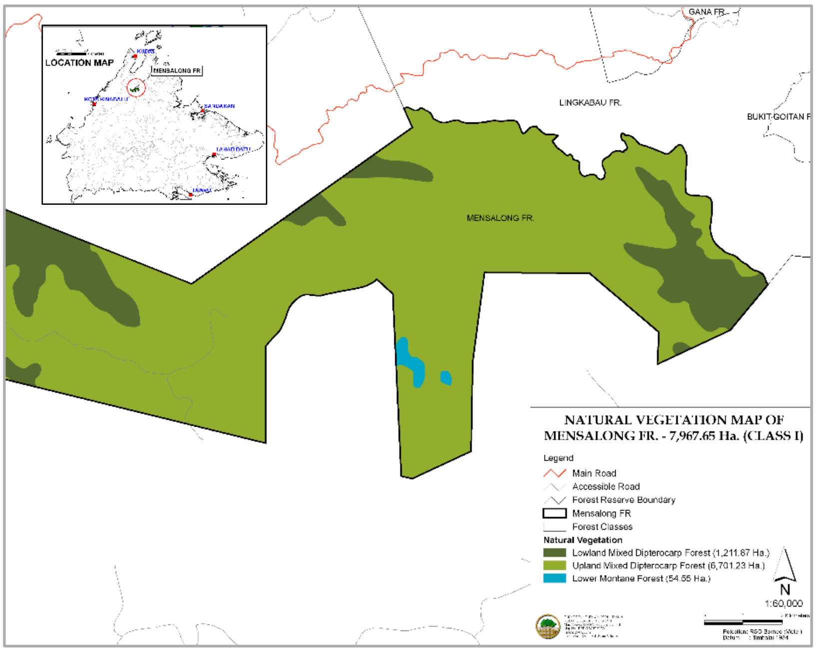
Birds are important indicators of the health of forest ecosystems and only recently have been included when surveying biodiversity in the forest reserves of Sabah, Malaysia. Hence, rapid assessments of avifaunal communities with respect to forest ecosystem health have only recently been conducted by the Sabah Forestry Department. This paper documents the outcomes of a brief bird survey conducted during the Mensalong Forest Reserve (MFR) Scientific Expedition on the 29th July-4th August 2019. The expedition was co-organised by the Forest Research Centre, Sabah Forestry Department, under the auspices of the Heart of Borneo Initiative. The main objective of this survey is to provide a brief description of the bird species and ecology present in the forest reserve to support future forest management initiatives. Surveys using the MacKinnon List (ML) method (MacKinnon & Phillips 1993) were conducted at 4 sites close to the expedition base camp at Kg. Turuntungon.

The Forest Research Centre of the Sabah Forestry Department aims to develop a rapid assessment methodology based on a modified ML method specifically for the department's researchers and field staff with limited time (3-4 days) for field work. This survey was part of a series of on-going field trials.

MATERIALS & METHODS

Site description

The Mensalong Forest Reserve (MFR) approximately lies within latitudes 6º 17' 0" - 6º 12' 0" N and longitudes 116º 45' 0" - 116º 56' 0" E, or about 48 km from Ranau Town along the Ranau-Kota Marudu Road, adjacent to the northeast boundary of Kinabalu Park. With an area of 7,967.65 ha, MFR is located in the Kota Marudu district and is administered by the Kota Marudu District Forestry Office. It was formerly part of the Lingkabau Forest Reserve and was later gazetted as a Class I Protection Forest Reserve on 8th December 2016. Approximately 5 km of its western boundary is shared with Kinabalu Park. Like most of the surrounding area, the soils of MFR are of the Crocker Association, the most extensive soil association in Sabah. Typical of areas with this soil association, the slopes were normally greater than 25º, ridge crests and valley bottoms were narrow and landslips were said to be a common occurrence. The hills were formed of interbedded sandstone and mudstone. The elevation ranged 250–1,100 m a.s.l.



Map 1. Natural vegetation map of Mensalong Forest Reserve.

The natural vegetation of MFR comprised mainly of lowland and upland mixed dipterocarp forests, and small patches of lower montane forest located in the south-central part of the reserve (see Map 1). The forest reserve was logged-over while it was still part of Lingkabau FR, a Class II Commercial Forest. There were at least three villages located close to the reserve boundaries, i.e. Kg. Monggis, Kg. Turuntungon and Kg. Surun-Surun.

The bird surveys were conducted along old logging roads accessible from the Ranau-Kota Marudu main road that intersects the reserve in the west, and at the lower montane forest patch, which was accessible from the network of oil palm estate unsealed, earth roads from the expedition base camp at Kg Turuntungon.

Survey methods

The ML method, as a rapid assessment method, has been gaining popularity in the past 10 years as a cost-effective method of conducting bird surveys in the tropics. It was designed for those who have limited time, resources and personnel for surveys (MacKinnon & Phillips 1993), 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 comparison of data obtained by different observers or under varying field conditions (Herzog *et al.* 2002).

In the ML method, both aural and visual observations were grouped into consecutive lists of 15 species, and 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 is then plotted as a function of the list number. In addition, the number of individuals of the species observed within each list was recorded.

Observation methods

The survey team comprised the authors and was assisted by a volunteer from Universiti Putra Malaysia Kampus Bintulu, who had worked with the survey team on two previous surveys. Every observer had a pair of NIKON binoculars (8 x 42s). The reference field guide of choice was the 'Phillipps' Field Guide to the Birds of Borneo', 3rd edition (Phillipps & Phillipps 2014). Audio playback equipment consisting of an mp3 player connected to a small, battery-powered Bluetooth® loudspeaker was used to verify the species of the birds heard.

Surveys were conducted for 4-5 hours beginning at about 7 am, and again for 2-3 hours in the evenings from 5 pm onwards to detect nocturnal birds. Surveys were conducted along old, accessible logging roads and/or skid trails. All observations were recorded by a designated person. Care was taken to prevent intra-list and inter-list double-counts of individuals. As most species were detected by their calls/vocalizations, individuals were recorded only if the observers were certain that they were different individuals, for *e.g.*, when the calls originate from a different direction and/or there were more than one call heard from a similar direction of the previously recorded species. As the trails were not looped, only bird species not recorded earlier were recorded on the return leg of the trails. Only the first evening survey was conducted along the trail used in the morning; the two other evening surveys were conducted at the side of the main Ranau-Kota Marudu road, close to the trails surveyed earlier in the respective mornings.

Table 1. Timetable of bird surveys during the expedition.

Date	Survey site	Observation times	Observation hours (hrs)
30th July 2019	Trail 1, upland MDF	7:40 am – 11:40 am, 5:00 – 7:00 pm	6
1st August 2019	Trail 2, upland MDF	7:15 am – 11.15 pm, 5:30 – 7:30 pm	6
2nd August 2019	Trail 3, upland MDF	7:30 am – 11:30 am, 7:30 – 9:00 pm	5.5
3rd August 2019*	Trail 4, lower montane forest	9:30 am – 12:30 pm	3

*The survey started late due to the distance and accessibility to the site. Poor weather hampered the evening survey.

Analyses

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. To estimate true species richness of the area, the authors 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 Bornean endemics were also examined.

Analyses of feeding guilds provides information on how communities of species utilize certain forest resources (fruits, insects, arthropods, seeds, etc.) within MFR 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 are considered as omnivores if they are known to consume roughly similar amounts of animal- and plant-based food resources, such as Ins/Gra, Fru/Ins, Nec/Fru/Ins, etc. Guild information was mainly from Phillipps (2014) and Wells (1999 & 2007). The feeding guilds were then described according to habitat types (for e.g. forest, forest edge and open area) to examine the importance of habitats to different guilds.

RESULTS AND DISCUSSION

Avifaunal composition and species richness

The four-day survey yielded 23 lists and 566 detected individuals. Of these, about 297 (52.5%) were detected by their calls/vocalisations. A total of 101 species belonging to 37 families were recorded. The Shannon Diversity Index (H) value was 4.18. Compared to the lowland mixed dipterocarp forest of the Kabili-Sepilok Virgin Jungle Reserve (Class 6) with 308 species and 59 families (Petol & Ong 2013), the lower value for the total number of species may be, amongst others, due to the MFR having been logged in the past. It has to be stated that the avifaunal data of Sepilok had been collected since the 1970s and thus, provided a more accurate checklist compared to the rapid 4-day survey of MFR.

The survey also yielded ten species that were endemic to Borneo (Table 2). Besides the Black-throated Wren-babbler, Kinabalu Serpent Eagle and the Bornean Necklaced Partridge, all the other seven species were categorised as Least Concern (LC) in the IUCN Red List of Threatened Species. The Black-throated Wren-babbler was the sole species listed as Near Threatened (NT).

Table 2. Species endemic to Borneo and their respective categories in the IUCN Red List of Threatened Species.

No.	Species	Category
1	Black-throated Wren-babbler	NT
2	Bornean Banded Pitta	LC
3	Bornean Ibon	LC
4	Bornean Spiderhunter	LC
5	Bornean Treepie	LC
6	Kinabalu Serpent Eagle	VU
7	Bornean Necklaced Partridge	VU
8	Chestnut-crested Yuhina	LC
9	Dusky Munia	LC
10	White-crowned Shama	LC

Table 3 lists 28 species that were listed as NT and Vulnerable (VU) in the IUCN Red List of Threatened Species. Of those listed as NT, only the Black-throated Wren-babbler was endemic to Borneo. A majority of those in the said category were common lowland MDF species, with the exception of the Great Argus, Green Broadbill, Jambu Fruit Dove and the Rufous-tailed Shama. Two species in the VU category, namely the Kinabalu Serpent Eagle and the Bornean Necklaced Partridge, were Bornean endemics.

Table 3. Species listed as Near Threatened (NT) and Vulnerable (VU) in the IUCN Red List of Threatened Species.

No.	Species	Category	No.	Species	Category
1	Black-and-yellow Broadbill	NT	**15**	Maroon-breasted Philentoma	NT
2	Black-throated Wren-babbler	NT	**16**	Puff-backed Bulbul	NT
3	Brown Fulvetta	NT	**17**	Rufous-crowned Babbler	NT
4	Buff-vented Bulbul	NT	**18**	Rufous-tailed Shama	NT
5	Crested Jay	NT	**19**	Scaly-breasted Bulbul	NT
6	Dark-throated Oriole	NT	**20**	Scarlet-rumped Trogon	NT
7	Fiery Minivet	NT	**21**	Short-tailed Babbler	NT
8	Great Argus	NT	**22**	Sooty-capped Babbler	NT
9	Green Broadbill	NT	**23**	Streaked Bulbul	NT
10	Green Iora	NT	**24**	Yellow-crowned Barbet	NT
11	Grey-bellied Bulbul	NT	**25**	Blue-banded Pitta	VU
12	Grey-chested Jungle Flycatcher	NT	**26**	Bornean Necklaced Partridge	VU
13	Jambu Fruit Dove	NT	**27**	Kinabalu Serpent Eagle	VU
14	Lesser Green Leafbird	NT	**28**	Wreathed Hornbill	VU

Table 4 shows that the bulbuls (Pycnonotidae) had the highest number of species (13), followed by both the sunbirds/spiderhunters (Nectariniidae) and the babblers (Pellorneidae), each represented by 8 species respectively.

Table 4. Top five most speciose families (with ranks 2 and 5 shared between 2 families respectively).

Rank	Family	No. of species
1	Pycnonotidae	13
2	Nectariniidae	8
2	Pellorneidae	8
3	Picidae	6
4	Columbidae	5
5	Cisticolidae	4
5	Megalaimidae	4
5	Muscicapidae	4
5	Timaliidae	4

As shown in Table 5, individuals from the bulbul and barbet families were the most commonly detected with 86 (15.2%) and 64 (11.3%) individuals respectively. The top 10 families with the highest percentage of individuals detected were species that were detected by their calls and/or are very conspicuous in nature. However, some species within a family were more conspicuous than others. For e.g., 68.6 % of the bulbuls detected were from 5 (out of 13) species, i.e. the Red-eyed Bulbul, Buff-vented Bulbul, Yellow-vented Bulbul, Hairy-backed Bulbul and the Cream-vented Bulbul. Similarly, about 75% of babblers detected were from just

2 (out of 4) species; the Gold-whiskered Barbet and the Blue-eared Barbet. Although the babblers (Pellorneidae) were well represented by 8 species, the most commonly detected was the Brown Fulvetta, as this species is normally detected on ridges far from valley streams, where other common babblers are normally detected.

Table 5. Top 10 families with the highest percentage of individuals detected (note similar rankings).

Rank	Family	Number of individuals	Percentage of individuals detected (%)
1	Pycnonotidae	86	15.2
2	Megalaimidae	64	11.3
3	Pellorneidae	40	7.1
4	Timaliidae	35	6.2
5	Dicaeidae	31	5.5
5	Nectariniidae	31	5.5
5	Zosteropidae	31	5.5
6	Cisticolidae	20	3.5
7	Psittaculidae	19	3.4
8	Columbidae	18	3.2
8	Picidae	18	3.2
9	Apodidae	16	2.8
10	Estrildidae	15	2.7
10	Muscicapidae	15	2.7

Being in an upland MDF, the avian community of Mensalong FR share many similar species with lowland MDF and, to a lesser degree, to the lower montane forest of the nearby Kinabalu Park to the east. Examples of species representing the latter were the Bornean Treepie, Bornean Ibon, Everett's White-eye, Spotted Fantail, Chestnut-crested Yuhina, Bornean Spiderhunter and the Kinabalu Serpent Eagle.

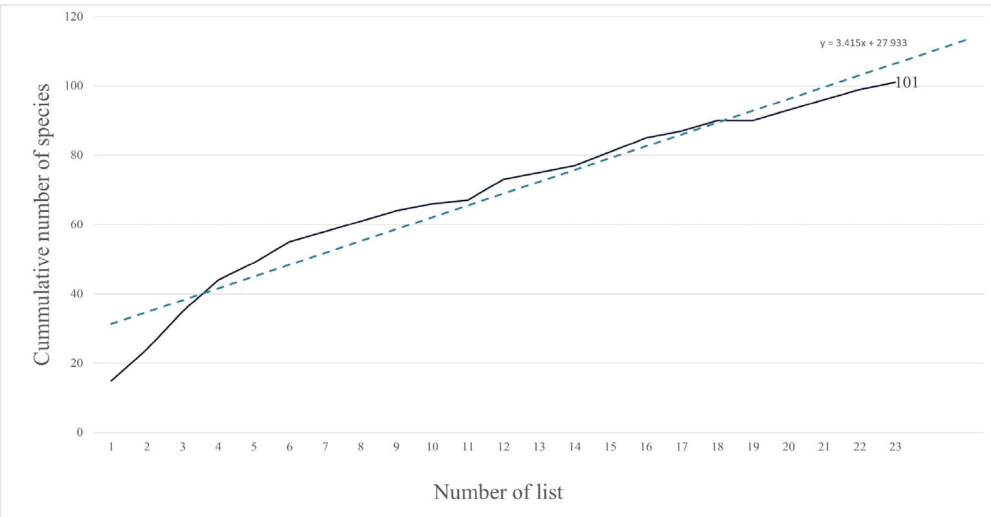


Figure 1. Species accumulation curve and linear regression line of bird species in MFR.

As expected for the ML rapid assessment method, and with a 4-day duration of the survey, the species accumulation curve (Figure 1) had not achieved asymptote. To estimate the true species richess, the SuperDuplicates® online calculator was used. It estimated Chao1 (species richness using abundance data) to be approximately 118 species, with an upper and lower threshold of approximately 133 and 110 species respectively, at 95% confidence interval. It also estimated that approximately 17 species were undetected, i.e. the survey managed to detect approximately 85.6% of the total species in the area (Table 6). Based on the linear regression line in Figure 1, about three more lists, or another survey day, were needed to detect the estimated 118 species of birds.

Table 6. Results from SuperDuplicates®.

Estimated number of doubletons	Estimate d species richness	Standar d error	95% C.I. lower	95% C.I. upper	Number of undetected species	Undetecte d percentage (%)
11.01	117.96	5.73	109.91	133.29	16.96	14.38

Relative abundance index

A total of 566 individuals from 101 species, with an Evenness (E_H) value of 0.75, were detected. Table 7 shows the top 5 species with the highest relative abundance index (RAI) with the Gold-whiskered Barbet ranked 1st. Of the five, only the Chestnut-crested Yuhina was observed visually (about 20 individuals in a flock); the individuals of the other 4 species were mostly detected by their calls. All species ranked 1st to 19th (292 individuals) were mainly detected by their calls and other vocalisations.

Table 7. Top 5 relative abundances of bird species in MFR.

Rank	Species	Number of individuals detected	Relative Abundance Index (RAI)
1	Gold-whiskered Barbet	33	0.058
2	Orange-bellied Flowerpecker	27	0.048
3	Brown Fulvetta	23	0.041
4	Chestnut-crested Yuhina	20	0.035
5	Blue-crowned Hanging Parrot	19	0.034

Habitat types and feeding guilds

The species were categorised according to their preferred habitats (e.g. forest, forest edges, open area) and respective feeding guilds (Figure 2). The majority of the species (88 species) detected were forest and forest-dependent species. Of these, 75 species were strictly forest birds. The high number of forest and forest-dependent species-and the low number of open area specialists (e.g. munias, Eurasian Tree Sparrows, Asian Glossy Starlings)- reflected the relatively intact forest ecological functions. It also showed that the road built through the forest reserve had very little impact on the forest ecosystem in general. Unusually, both the ubiquitous Indian and Plaintive cuckoos, very often heard in forest edges and open areas, were not detected during the survey.

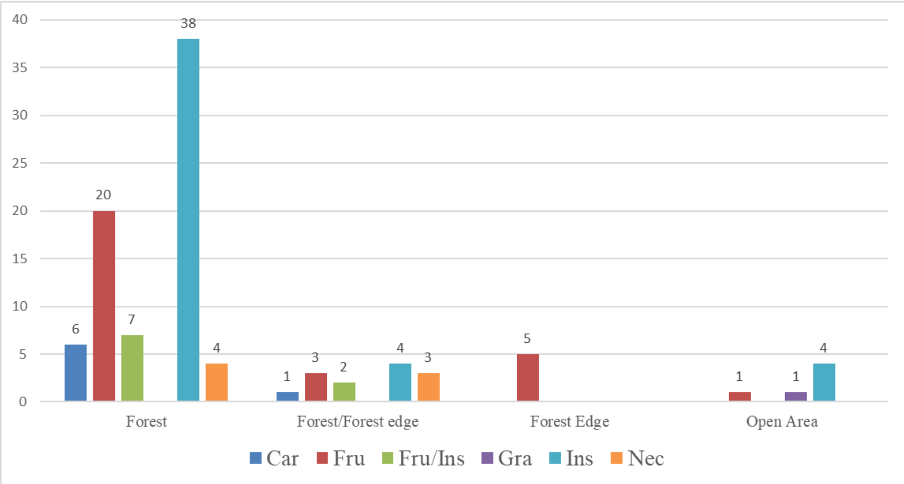


Figure 2. Number of species according to habitat types and feeding guilds in MFR.

As expected, insectivores made up the most dominant dietary guild, i.e. a total of 57 species (from 28 families) with 48 species (from 23 families) being strict insectivores and the rest were mixed-diet insectivores. The dominance of insectivorous (strict and otherwise) bird species in Mensalong FR indicated the presence of plentiful food resources. The second most dominant guild was the frugivores with 38 species, 9 of which were mixed-diet frugivorous species. All 7 species of nectarivores were from the spiderhunter/sunbird family, Nectariniidae. The sole granivore was the Bornean endemic and very common Dusky Munia.

CONCLUSIONS

Using the MacKinnon List method, the survey team managed to obtain a preliminary insight on the avian diversity and ecology in MFR. Its avian diversity (101 species from 37 families) can still be considered as high, taking into account the low observation hours of approximately 20.5 hours. The families were similar to those found in lowland MDF, albeit a few elements of montane avifauna, such as the Bornean Treepie, Bornean Ibon, Everett's White-eye, Spotted Fantail, Chestnut-crested Yuhina, Bornean Spiderhunter and the Kinabalu Serpent Eagle. The high number of forest and forest-dependent species-and the apparent lack of open area specialists (e.g. munias, Eurasian Tree Sparrows, Asian Glossy Starlings)-reflected the relatively intact forest ecological functions. The feeding guilds further supports this statement with insectivores (57 species from 28 families) being the most dominant guild followed by frugivores (38 species). From the results of the survey, it can be assumed that MFR had a very healthy avian diversity and community.

ACKNOWLEDGEMENTS

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Comparison of insect assemblages (butterfly, dragonfly and moth) in different lowland forest types in Sabah, Malaysia

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Abstract. A comparative study on butterfly, dragonfly and moth assemblages was conducted in different lowland forest types (mangrove, plantation and dipterocarp forests) in Sandakan, eastern Sabah. The family and species composition of the three insect orders in various sites are highlighted. Highest insect diversity was recorded in the dipterocarp forest, followed by plantation forest and mangrove forest. The variety and abundance of food sources for insects are higher in the dipterocarp forest compared to the other forests due to the higher plant diversity. However, the presence and abundance of host-specific insects depend on the availability of their host plant in the habitat. Of the three insect orders, moth was the most diverse, followed by butterfly and dragonfly in all the study sites. The similarity among species for the three insect orders among the forests was relatively low although they were located within the same district. This could have been affected by the adjacent land-use changes as well as forest fragmentation. Inventories from this study on insect assemblages have identified some species with conservation interest, nature tourism potential and insects that can be detrimental to the habitat. Such information would contribute towards best practices in sustainable forest management in Sabah.

Keywords: insects, assemblages, diversity, butterflies, moths, dragonflies, damselflies, forest types, mangrove forest, lowland forest, plantation forest

INTRODUCTION

The tropics are truly diverse with various habitat types inhabited by a diversity of life forms, including insects (Eggleton 2020 & Kitching *et al*. 2020). Understanding the extent of insect assemblages in the tropics has always been a major challenge because of its high diversity and abundance compared to those in the temperate region (Godfray *et al*. 1999). Insects are ecologically crucial in structuring and maintaining communities, forming intricate networks that can influence species' coevolution, coexistence and community stability (Cardoso *et al*. 2020). Lately, there has been much discussion on the decline of global insect population, e.g. Hallmann *et al*. (2017), Lister & Garcia (2018) and Sanchez-Bayo & Wyckhuys (2019) although it may not affect all species equally (Didham *et al*. 2020). In view of that, scientific research on wildlife diversity is systematically biased towards vertebrates and temperate regions (Titley *et al*. 2017). Hence, more research should be conducted on invertebrate assemblages and diversity in the tropics. In Sabah, much is still not known about many of the insect group assemblages in different habitat types although some research has been conducted on selected insect groups in the past, e.g. on moths (Chey *et al*. 1997) and beetles (Chung *et al*. 2000).

In studying insect assemblages, inventories are important as they provide the baseline information with scientifically named species for further research and implementation, such as legislation and determining levels of endemism, as well as a base for biodiversity informatics, ecology, and ultimately, for assessment and conservation (Samways *et al*. 2020). With such data in place for a study area, monitoring the status of insect diversity would be more effective and accurate. In long term, this can be a continuous reassessment of an assemblage, population or community in a habitat over time in terms of improvement or deterioration.

The focus of this insect assemblage study is on three major insect orders, namely butterfly, moth and dragonfly (including damselfly). Butterfly is easy to study due to its high sensitivity towards the environment. The butterfly species composition will change according to food availability or vegetation type (Curtis *et al.* 2015) due to the preference in food selection. Thus, butterfly can be used to indicate the presence of certain plant species as food source, making this insect as a bioindicator of the environment and habitat (Miller *et al.* 2011).

Dragonfly is beneficial to human beings. It is an indicator for aquatic ecosystem health and also for diversity of other smaller insects as it preys on them (Hykel *et al.* 2016). Vegetation plays a role in the regulation of dragonfly species richness. Dragonfly assemblage is strongly dependent on the composition and structure of vegetation (Korkeamaki & Suhonen 2002). Similarly, moth is an effective ecological indicator that can reflect the diversity of flora communities, health and quality of forest (Kitching *et al.* 2000). Hence, the environmental status of a forest can be looked upon through the assessment of moth diversity. Some are pollinators for the timber trees while some of their larvae are pests in forestry.

These insect orders were chosen for this study because references for identification and expertise are easily available compared to the lesser-known and more difficult insect orders. Moreover, these three insect orders are more conspicuous, and hence, sampling is straightforward.

MATERIALS & METHODS

Study sites

The survey was conducted in late June until mid-July 2019 in three forest types in Sandakan, Sabah, namely a mangrove forest, a lowland dipterocarp forest and a plantation forest. All sites are below 50 m a.s.l. and they are within the jurisdiction of the Sabah Forestry Department. Hence, future monitoring on the status of insect diversity can be conducted without much difficulty. The location of all sites is shown in Figure 1 and the details are as follows:

Mangrove Forest (MF) – the Sepilok Laut mangrove (N05˚49', E117˚56') is located within a Virgin Jungle Reserve (Class VI), comprising 1,235 ha and is connected to Kabili-Sepilok Forest Reserve (SFD 2017). The management of this forest reserve is under the jurisdiction of the Sandakan District Forestry Officer, with his staff based at the Sepilok Laut Reception Centre. This area also covers the Long Term Ecological Research (LTER) plots in Sepilok Laut (Tangah *et al*. 2018), in which insect sampling was carried out. The common mangrove species found here are *Ceriops tagal*, *Rhizophora apiculata*, *Syzygium leucoxylon*, *Bruguiera sexangular* and *Pandanus affinis*.

Plantation Forest (PF) – insect sampling was carried out at the Gum Gum Plantation Station (N05˚52', E117˚55'), which is an Amenity Forest Reserve (Class IV) of 48 ha., gazetted in 1984 (SFD 2017). The station is managed by the Forest Plantation Section of the Forest Research Cente, Sepilok. There are research plots covering spacing and tree improvement trials of indigenous and exotic tree species (SFD 2018a). Some of the trees are fast-growing species, such as *Neolamarckia cadamba*, *Octomeles sumatrana*, *Falcataria moluccana*, *Eucalyptus pellita* and *Terminalia copelandii*.

Dipterocarp Forest (DF) – this site (N05˚52', E117˚56') is located in the Kabili-Sepilok Forest Reserve of 4,294 ha. Insect sampling was conducted at the Rainforest Discovery Centre (RDC) within the reserve. The RDC is opened to public and it promotes environmental education and nature tourism with a network of trails within this area. Among the prominent trees found here are *Parashorea malaanonan*, *Parashorea tomentella*, *Shorea multiflora*, *Dipterocarpus acutangulus*, *Ixonanthes reticulata* and *Eusideroxylon zwageri* (Fox 1973).

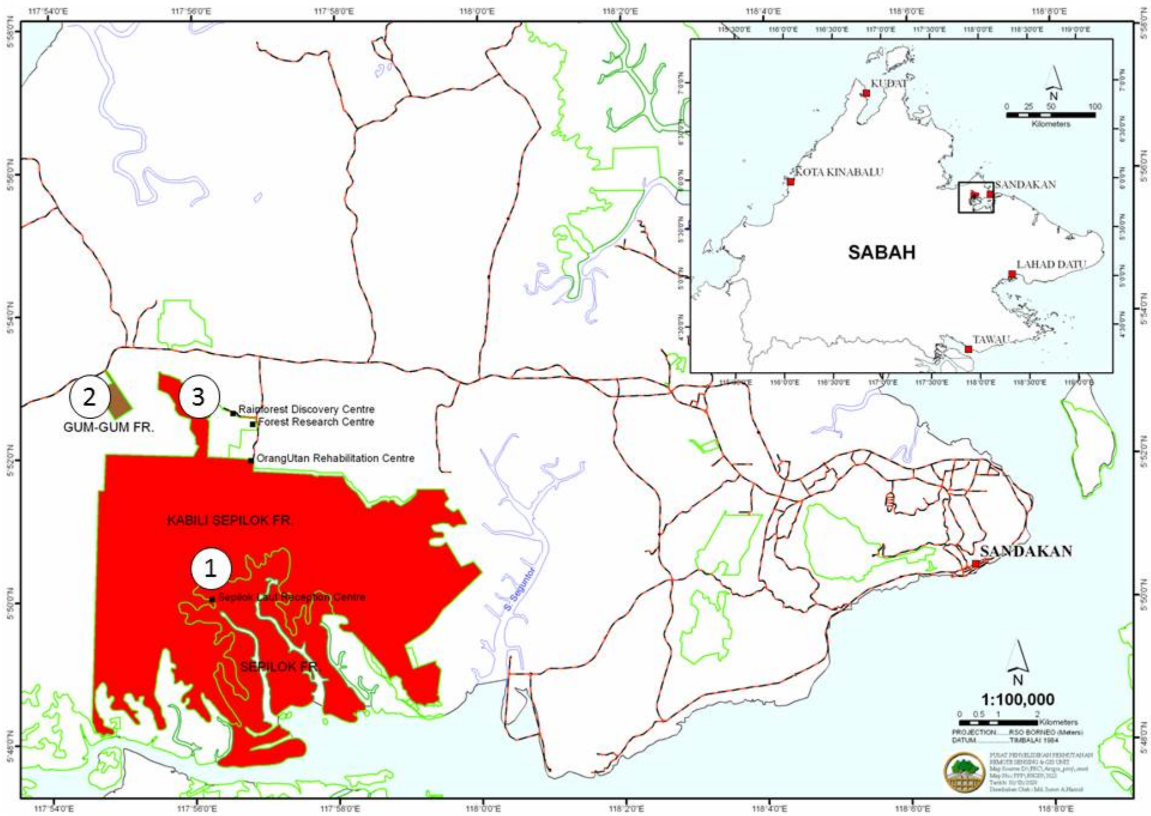

Figure 1. Location of the study sites (1=Mangrove Forest, 2=Plantation Forest & 3=Dipterocarp Forest) in Sandakan. Red area denotes Virgin Jungle Reserve while brown denotes Amenity Forest Reserve. The location of all sites in eastern Sabah is also shown (inset).

Sampling methods

Butterfly and dragonfly – these two diurnal insect orders were sampled using sweep net along three 500-m transects, based on a modified method adopted from Pollard (1977 & 1982) to fit the site condition. Sampling was conducted by two persons, twice a day, i.e., 8:30–10:30 am and 3:00–5:00 pm, for three days in each site. Those flying or resting at an unreachable visible distance within the sampling trail (e.g. above the understorey or within water body) were captured using camera with zoom lens. Whenever possible, only one individual from each species was captured in each site. Collected specimens were kept in triangle paper for curation and identification. A GPS (Model: Garmin GPSMAP 60CSx) was used to record the coordinates for each sampling site. Temperature and humidity were taken with a digital hygrometer (Extech Instruments: model no. 445702) (Table 1).

Moth - sampling was conducted for three consecutive nights at each site through light-trapping. The light trap consisted of a vertical white sheet (2 X 2 m) with a 250 W mercury-lithium bulb powered by a portable Yamaha generator. The light trap was set up in an open area, facing the vegetation, from 6:30 to 9:00 pm. The number of moth species and individuals resting on the white sheet were enumerated from 8:30 to 9:00 pm. Sampling was carried out at different locations in each study site. Mean temperature, humidity and weather condition are provided in Table 1.

Table 1. Details of sampling sites for the respective insect orders.

Butterfly & dragonfly (diurnal)				
Site	Coordinates	Mean ºC	Mean humidity	Weather*
MF	N05˚49', E117˚56'	29.2	74	Cloudy
PF	N05˚52', E117˚55'	31.0	66	Sunny & partial cloudy
DF	N05˚52', E117˚56'	28.8	76	Cloudy & partial sunny
Moth (nocturnal)				
Site	Coordinates	Mean ºC	Mean humidity	Weather*
MF	N05˚49', E117˚56'	25.8	80	Cloudy
PF	N05˚52', E117˚55'	24.1	83	Moon visible
DF	N05˚52', E117˚56'	25.5	82	Cloudy

*Overall weather condition throughout the sampling period.

Insect specimens and identification

Selected specimens were dry-mounted and sorted to species level. Unidentified specimens were morphotyped. The specimens are deposited at the Forest Research Centre, Sepilok, Sabah. Dry-mounted specimens were identified based on the FRC Entomology Collection and various reference materials, e.g. Otsuka (1988 & 2001) and Kirton (2014) for butterflies; Holloway (1983, 1985, 1986, 1988, 1989, 1993, 1996, 1997, 1998a & b, 1999, 2001, 2003, 2005, 2008, 2009 & 2011), Robinson *et al.* (1994) and Sutton *et al*. (2015) for moths; Orr (2003), Tang *et al*. (2010) and Choong *et al*. (2018) for dragonflies and damselflies.

Data analysis

Data obtained were analyzed to assess the diversity of the three insect orders in different forest types. The diversity indices, namely Shannon Wiener Index and Simpson Reciprocal Index, were calculated through a diversity analysis software by Seaby and Henderson (2007), based on Magurran (2004), and Southwood and Henderson (2000). Sorenson Similarity Index was used to compare the similarity of species composition among sites. Other analyses were conducted using IBM Statistical Package for the Social Science (SPSS) Statistic version 26 software.

RESULTS & DISCUSSION

Family composition

Butterfly

A total of 412 individuals of butterflies representing 90 species, belonging to six families were recorded from all three forest types in Sandakan. Figure 2 shows the total number of species for each family combined from all three sites. Overall, Nymphalidae was the most speciose family, with a total of 34 species, followed by the Pieridae (19 species) and Lycaenidae (18 species).

From Table 2, Nymphalidae was the most abundant family in all three sites, with a relative abundance of 63.64% in MF, 69.10% in PF and 62.10% in DF. In MF, Pieridae was the second most abundant family (14.55%), followed by Riodinidae (9.09%). Interestingly, Riodinidae was not recorded in PF and DF. Riodinidae is a small family, previously classified under Lycaenidae, often with metallic markings on their wings. In PF, Hesperiidae (14.16%) and Pieridae (10.30%) were the second and third most abundant family respectively. In DF, Lycaenidae was the second most prominent family after Nymphalidae, with 24.19%.

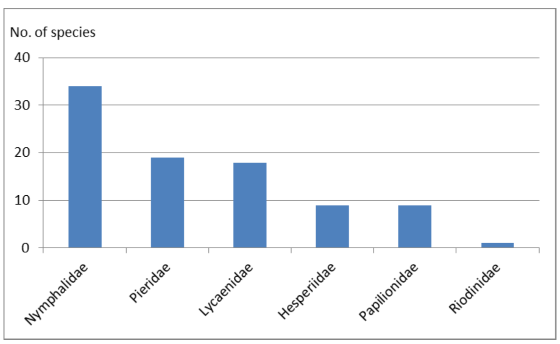

Figure 2. The total species recorded for each butterfly family from all sites.

Table 2. Relative abundance of each butterfly family from the different sites (MF=Mangrove Forest, PF=Plantation Forest & DF=Dipterocarp Forest).

Family	Relative abundance (%)		
	MF	**PF**	**DF**
Nymphalidae	63.64	69.10	62.10
Papilionidae	3.64	3.43	1.61
Lycaenidae	3.64	3.00	24.19
Hesperiidae	5.45	14.16	4.03
Pieridae	14.55	10.30	8.06
Riodinidae	9.09	0.00	0.00
Total	100	100	100

Dragonfly

In total, 247 individuals of dragonflies representing 44 species from nine families were recorded in this study, namely Gomphidae, Libellulidae, Calopterygidae, Chlorocyphidae, Coenagrionidae, Platystictidae, Platycnemididae, Protoneuridae and Philosinidae (Figure 3). A total of 121 individuals were true dragonfly (Anisoptera) and 126 individuals were damselflies (Zygoptera). There were only two families recorded under the suborder Anisoptera, namely Gomphidae and Libellulidae, while the other five families belong to the suborder Zygoptera.

The family Libellulidae dominated the dragonfly abundance in MF (84%) and PF (75.4%) but the population of Ceonagrionidae was the highest in DF with a relative abundance of 58.9% (Table 3). Eight families were recorded in DF, followed by five in PF and four in MF. Platystictidae was the only family not documented in DF.

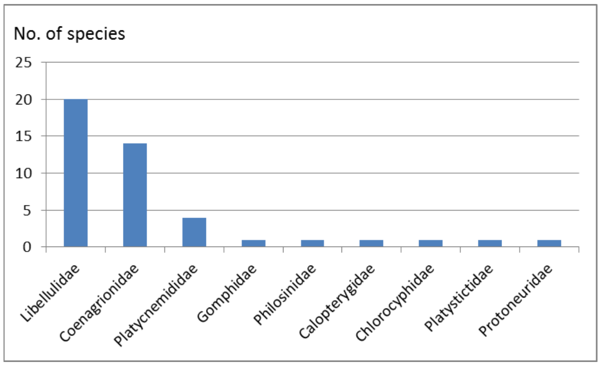

Figure 3. The total species recorded for each dragonfly family from all sites.

Table 3. Relative abundance of each dragonfly family from the different sites (MF=Mangrove Forest, PF=Plantation Forest & DF=Dipterocarp Forest).

Family	Relative abundance (%)		
	MF	**PF**	**DF**
Gomphidae	0.0	0.0	0.6
Libellulidae	84.0	75.4	33.6
Philosinidae	5.2	0.0	0.6
Coenagrionidae	0.0	12.8	58.9
Calopterygidae	0.0	0.0	0.6
Chlorocyphidae	5.2	0.0	1.8
Platycnemididae	5.2	5.6	3.4
Platystictidae	0.0	2.8	0.0
Protoneuridae	0.0	2.8	0.6
Total	100	100	100

Moth

A total of 211 individuals belonging to 12 families and 130 species were recorded throughout the samplings in the three forest types. Figure 4 shows the total number of species for each family combined from all three sites. Overall, Geometridae was the most speciose family, with a total of 49 species, followed by the Erebidae (28 species) and Crambidae (18 species).

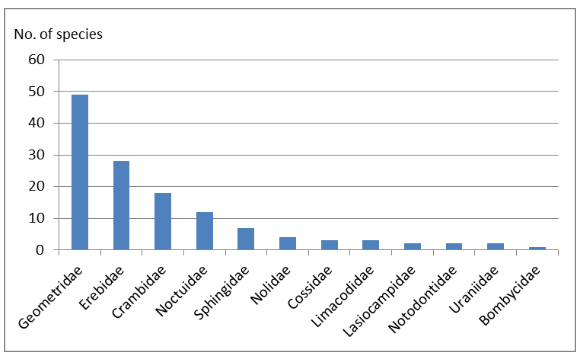

Figure 4. The total species recorded for each dragonfly family from all sites.

Table 4. Relative abundance of each moth family from the different sites (MF=Mangrove Forest, PF=Plantation Forest & DF=Dipterocarp Forest).

Family	Relative abundance (%)		
	MF	**PF**	**DF**
Crambidae	15.8	27.9	19.4
Cossidae	3.3	0.0	2.2
Bombycidae	1.6	0.0	0.0
Erebidae	9.8	14.3	20.5
Geometridae	46.6	28.6	35.6
Lasiocampidae	16.8	0.0	3.2
Limacodidae	3.3	0.0	1.1
Noctuidae	1.6	14.3	6.5
Nolidae	0.0	0.0	4.3
Notodontidae	0.0	0.0	2.2
Sphingidae	0.0	7.2	4.3
Uraniidae	1.6	7.1	1.1
Total	100	100	100

Eleven families were recorded in DF, nine in MF while six in PF (Table 4). Geometridae was the most frequently encountered family in the three sites, with relative abundance of 35.57% in DF, 47.56% in MF and 26.85% in PF. Erebidae was the second most abundant family in DF (20.50%) while it was Crambidae in MF (15.84%) and PF (27.92%) respectively.

Insect species richness, abundance and species-rank abundance distribution

The total number of species and individuals as well as the species rank-abundance curves for butterfly, dragonfly and moth in different forest types are shown in Figures 5-7. Butterfly species recorded from this study and their relative abundance are listed in Appendix 1 while dragonfly species are in Appendix II and moth species in Appendix III.

Total butterfly species richness was highest in DF, followed by PF and MF (Figure 5a). The higher abundance of butterflies in PF could be associated with the higher food source availability (nectar) as there were more flowering plants during the study (Figure 5b). The plantation forest is a more open area without much canopy cover, with an underground layer dominated with *Ageratum conyzoides* (Billygoat Weed), *Imperata cylindrica* (Lalang), *Ischaemum* spp. (Muraina Grass), *Musa laterita* and *Asystasia gangetica micrantha* (Chinese Violet) (Lee 2020). Ohwaki *et al*. (2017) suggested that increasing canopy openness can encourage the growth of herbaceous flowering plants. This means that there will be more nectar resources for adult butterflies. This is further supported by Nacua *et al*. (2015) who suggested that the open canopy area has higher butterfly abundance than in the closed-canopy forest. For the species-rank abundance distribution (Figure 5c), PF depicts the steepest curve, indicating the proliferation of two prominent species in the plantation, namely *Mycalesis horsfieldi hermana* and *Ypthima pandocus sertorius*, with 29 individuals repectively. These two species are known to be frequently encountered in the open grassy patches (Otsuka 2001 & Khew 2015), which are common in the plantation area.

For dragonflies, species and abundance were highest in DF, followed by PF and MF (Figures 6a & b). Generally, dragonflies prefer to stay near water with bushes on the fringe, so that both adult and larvae get source of food and places for roost (Mitra 1999). These study sites provided different types of microhabitats (water bodies) for the dragonflies. In DF, there were various ponds and streams, while the MF environment was surrounded by saline water bodies limited to robust dragonfly species, and for PF, the water bodies were confined to ditches and water puddles. The difference in water bodies' environment among the habitats affects dragonfly species richness and abundance (Orr 2003). In terms of species-rank abundance distribution (Figure 6c), DF has the steepest curve besides having the longest tail. The damselfly, *Ceriagrion cerinorubellum*, from the family Coenagrionidae scored the highest rank in the distribution curve in DF with 44 individuals followed by *Pseudagrion microcephallum* also from family Coenagrionidae with 33 individuals. Both *Ceriagrion cerinorubellum* and *Pseudagrion microcephallum* are common medium-sized damselflies distributed throughout Asia. Hence, it is not surprising to record such high abundance of these two species in the lowland dipterocarp forest in which they feed on smaller flying insects, especially mosquitoes.

In this study, both total moth species richness and abundance were highest in DF (Figures 7a &b). Results from past studies on moths conducted by Chey *et al*. (1997) and Intachat *et al*. (1999) have indicated that lowland dipterocarp forest is able to support high moth diversity due to its diverse plant species and good plant structures. The availability of food sources would reduce competition and tend to increase the survival rate of larvae. Comparatively, plant diversity in PF and MF is lower than DF. In the species-rank abundance distribution (Figure 7c), the micro moth from the family Crambidae, *Xanthomelaena schematias*, was the most abundant species with 11 individuals recorded in DF, rendered it the steepest curve among all the sites. This species was commonly found in the lowland forest, as it occurred during the three consecutive nights of light-trapping. It is interesting to note that MF also depicted a steep curve due to the high abundance of a large Lasiocampidae moth, *Suana concolor*, with nine individuals. Although it is a polyphagous species, the caterpillar is known to feed on the foliage on *Rhizophora apiculata* (Robinson *et al*. 2001), which is abundant in the Sepilok mangroves.

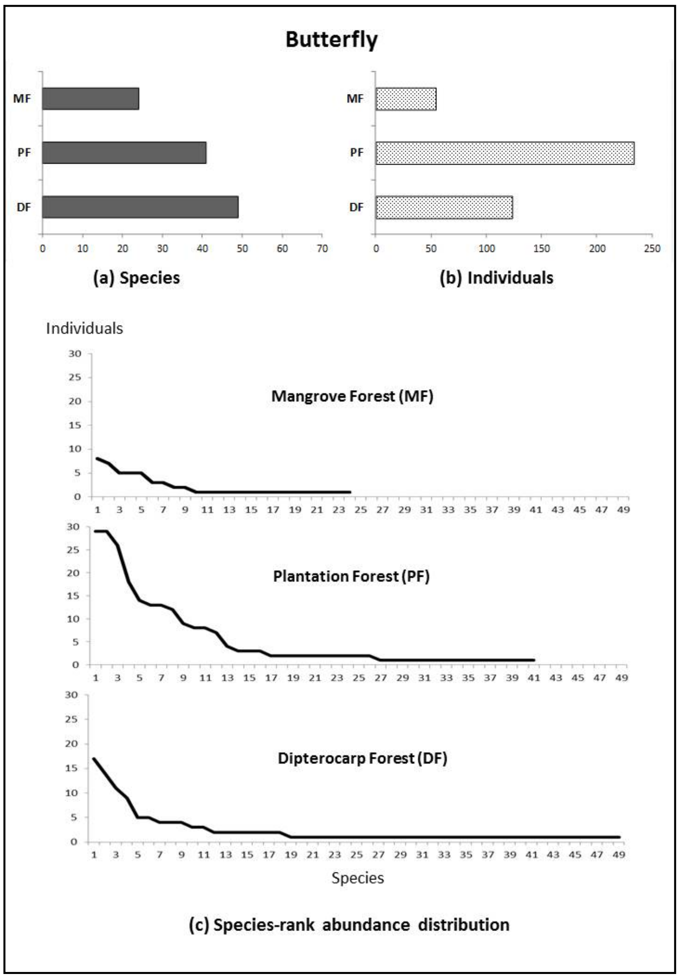

Figure 5. Total species richness and abundance, and species-rank abundance curve of butterflies in different forest sites. (MF=Mangrove Forest, PF=Plantation Forest & DF=Dipterocarp Forest).

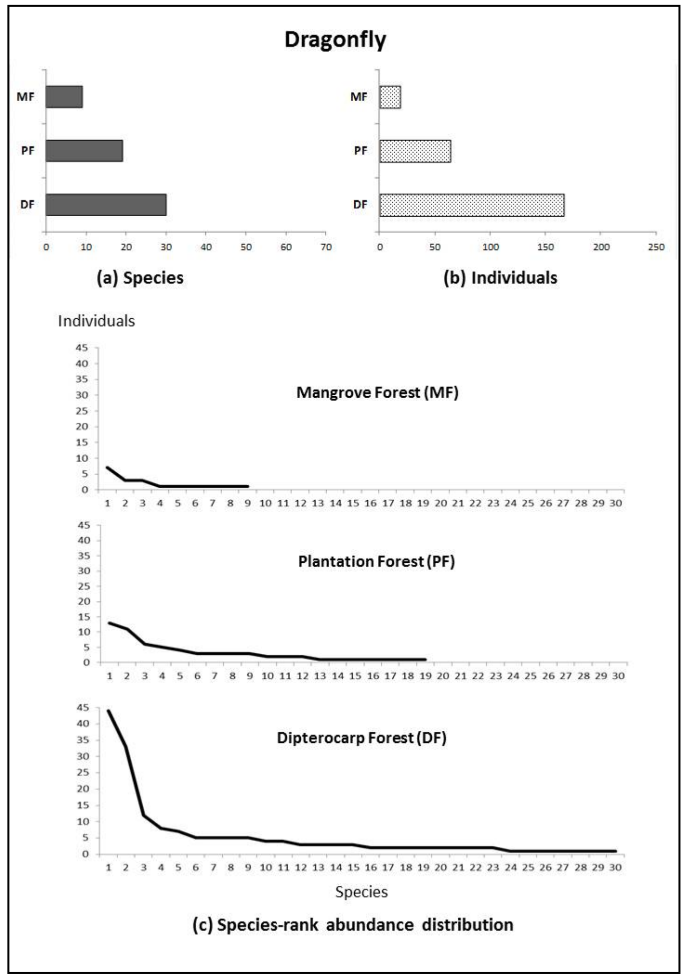

Figure 6. Total species richness and abundance, and species-rank abundance curve of dragonflies in different forest sites. (MF=Mangrove Forest, PF=Plantation Forest & DF=Dipterocarp Forest).

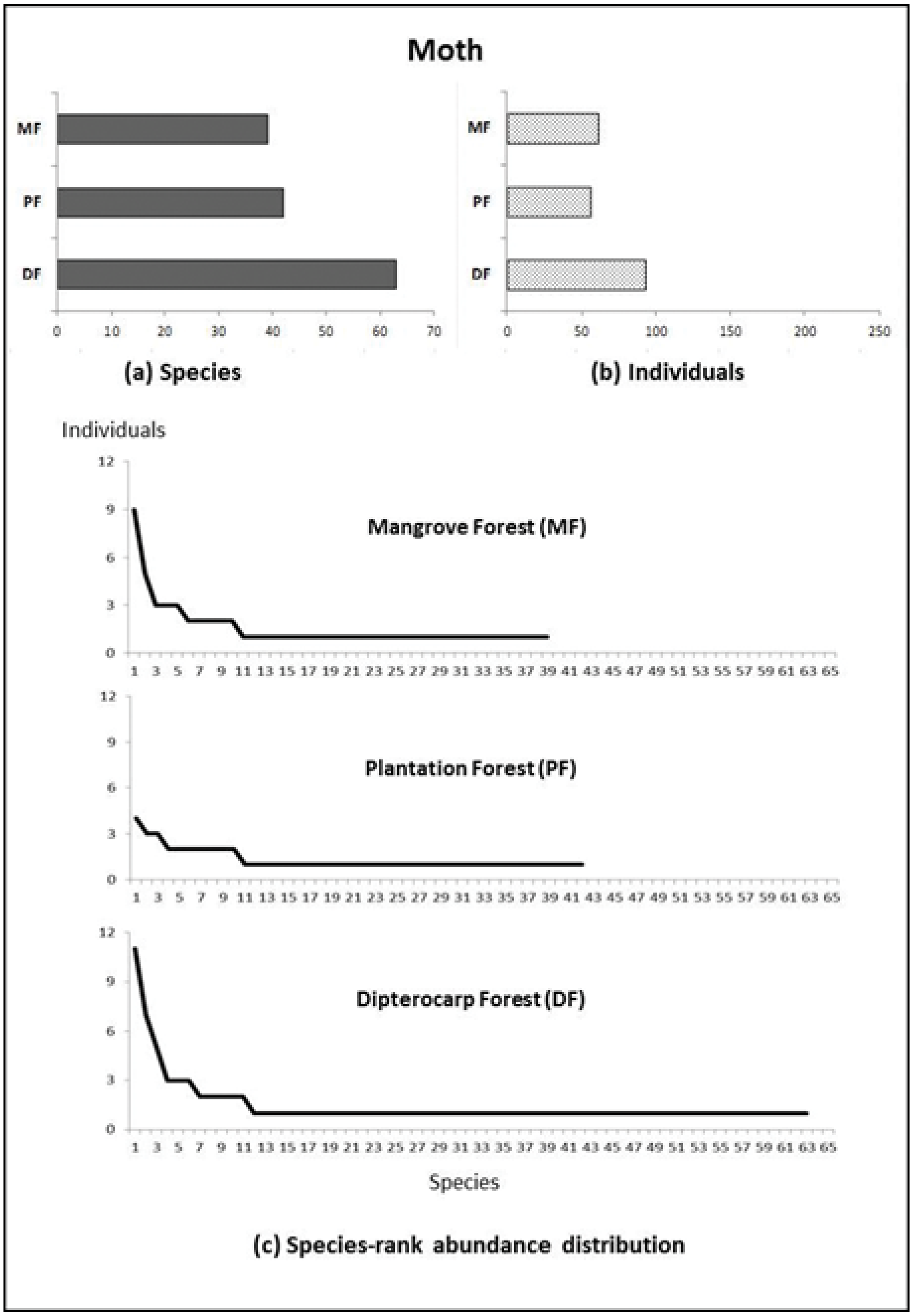

Figure 7. Total species richness and abundance, and species-rank abundance curve of moths in different forest sites. (MF=Mangrove Forest, PF=Plantation Forest & DF=Dipterocarp Forest).

Insect diversity

Diversity values of the three insect orders, as assessed with Shannon-Wiener's (H') and Simpson's Reciprocal indices (1/D) are shown in Table 5. For H', any value more than three is considered diverse in ecological studies (Magurran 2004). The moth values for all the habitat types are 3.40 and above, with the highest being DF, followed by PF, then MF. Hence, in general, these habitats are considered conducive for moths from the interpretation of Shannon Index. For butterflies, both DF and PF were above 3.0 while MF was below that value. Therefore, the diversity of butterflies in mangroves was lower than those in the lowland dipterocarp forest and plantation forest. It is not surprising because the mangrove habitat has low plant diversity for adult nectar source and larval host plants. Moreover, the mangrove forest is transitional between land and sea, fewer butterflies can withstand unfavourable environmental condition, such as high sunlight exposure and high salinity compared to the dense inland forest. All Shannon's values for dragonflies in the three sites were below 3.0. Hence, comparatively, the dragonfly fauna was not as diverse as moths and butterflies, and the dragonfly fauna was least diverse in the mangroves.

Comparatively, Simpson Reciprocal Index provided some differences in the interpretation as this index is heavily weighted towards the presence of abundant species and less sensitive to species richness (Magurran 2004). For butterflies, PF depicted the lowest value (15.52) because of two dominant species (with 29 individuals respectively) in the plantation as highlighted in the species abundance distribution. In contrast, PF shows the highest value for dragonflies (11.26) and moths (82.50) because this site did not record any prominent species with high abundance in comparison to DF and MF.

Table 5. Insect diversity among sites as assessed by Shannon-Wiener and Simpson Indices (DF=Dipterocarp Forest, MF=Mangrove Forest & PF=Plantation Forest).

Shannon-Wiener Index (H')			
Site	Butterfly	Dragonfly	Moth
MF	2.85	1.88	3.40
PF	3.04	2.57	3.64
DF	3.37	2.67	3.89
Simpson Reciprocal Index (1/D)			
Site	Butterfly	Dragonfly	Moth
MF	17.07	6.33	31.02
PF	15.52	11.26	82.50
DF	20.67	8.34	45.60

Insect similarity

Similarity of insect species composition between sites is reflected in Table 6 through Sorenson Similarity Index. DF & PF shared the highest value for butterflies (25.2%) and dragonflies (32.7%) while DF & MF depicted slightly higher similarity for moths, compared to DF & PF. In terms of vegetation, lowland dipterocarp forest shares more similarity with plantation forest. Mangrove vegetation is normally limited to certain plant species that can tolerate harsh environment. Moreover, the location of DF site is closer to PF site, as shown in Figure 1. Overall, the percentage of similarity for all sites by the three insect groups is considered low although all the three habitats are within the same district. Hence, fragmentation and land-use changes could have affected the insect assemblages, as such activities had adversely affected tropical biodiversity (e.g. Lawrence 2004 & Crooks *et al*. 2017).

Table 6. Insect similarity between sites as assessed by Sorenson Similarity Index (DF=Dipterocarp Forest, MF=Mangrove Forest & PF=Plantation Forest).

Site	No. of shared species	Sorenson Similarity (%)
Butterfly		
DF & MF	4	9.9
DF & PF	15	25.2
MF & PF	6	15.8
Dragonfly		
DF & MF	5	25.6
DF & PF	8	32.7
MF & PF	2	14.3
Moth		
DF & MF	7	13.7
DF & PF	7	13.3
MF & PF	2	4.9

The list of insect species that shared the same sites is shown in Table 7. Two species of nymphalid butterflies, *Ypthima baldus* and *Ypthima pandocus*, were found in all forest types. For dragonflies, *Neurothemis fluctuans* and *Orthetrum sabina* were recorded in all sites while for moths, *Comibaena attenuata* and *Glauconoe deductalis* occurred throughout the three sites.

Table 7. Insect species that shared the same forest types (DF=Dipterocarp Forest, MF=Mangrove Forest & PF=Plantation Forest).

DF-MF	DF-PF	MF-PF
Butterfly		
Ypthima baldus	*Ypthima baldus*	*Ypthima baldus*
Ypthima pandocus	*Ypthima pandocus*	*Ypthima pandocus*
Cirrochroa tyche	*Cupha erymanthis*	*Mycalesis mineus*
Junonia atlites	*Hypolimnas bolina*	*Taractrocera ardonia*
	Idea stolli	*Eurema hecabe*
	Junonia iphita	*Eurema sari*
	Junonia orithya	
	Mycalesis anapita	
	Parthenos sylvia	
	Papilio nephelus	
	Jamides pura	
	Koruthaialos rubecula	
	Koruthaialos sindu	
	Taractrocera ziclea	
	Leptosia nina	
Dragonfly		
DF-MF	**DF-PF**	**MF-PF**
Neurothemis fluctuans	*Neurothemis fluctuans*	*Neurothemis fluctuans*
Orthetrum sabina	*Orthetrum sabina*	*Orthetrum sabina*
Brachidiplax chalybea	*Neurothemis terminata*	
Rhinagrion elopurae	*Orthetrum chrysis*	
Rhinocypha humeralis	*Copera vittata*	
	Rhodothemis rufa	
	Ceriagrion cerinorubellum	
	Prodasineura humeralis	
Moth		
DF-MF	**DF-PF**	**MF-PF**
Comibaena attenuata	*Comibaena attenuata*	*Comibaena attenuata*
Glauconoe deductalis	*Glauconoe deductalis*	*Glauconoe deductalis*
Cleora determinata	*Tangala sexpunctalis*	
Tochara creberrima	*Xanthomelaena schematias*	
Hypochrosis binexata	Unidentified Sp. 1	
Ornithospila bipuncata	*Carriola ecnomoda*	
Lyssa menoetius	*Arthroschista hilaralis*	

Insects of conservation interest

Some Bornean endemic insects were recorded during the survey (Table 8). These are species or subspecies of conservation interest and can be included in preparation of high conservation value (HCV) reports to enhance the biodiversity conservation and protection effort in the study area. In this study, for example, the Bornean endemic damselflies, *Rhinagrion elopurae* and *Coeliccia* sp. were frequently encountered in the mangrove forest (see relative abundance in Appendix II). Hence, such data can support the protection of Sepilok mangroves.

Table 8. Bornean endemic insects recorded from different forest types in Sandakan, Sabah (DF=Dipterocarp Forest, MF=Mangrove Forest & PF=Plantation Forest).

No.	Species	Family	Site	Remarks
Butterfly				
1.	*Acytolepis ripte*	Lycaenidae	DF	Endemic species
2.	*Taractrocera ziclea stella*	Hesperiidae	DF & PF	Endemic subspecies
3.	*Potanthus omaha maesina*	Hesperiidae	PF	Endemic subspecies
Dragonfly				
1.	*Rhinagrion elopurae*	Philosinidae	DF & MF	Endemic species
2.	*Prodasineura hyperythra*	Protoneuridae	DF	Endemic species
3.	*Coeliccia* sp.	Platycnemididae	MF	Many species in this genus are endemic to Borneo
4.	*Vestalis* sp.	Calopterygidae	DF	Some species in this genus are endemic to Borneo
Moth				
1.	*Hypochrosis waterstradti*	Geometridae	DF	Endemic species
2.	*Metaemene santubong*	Erebidae	MF	Endemic species

Insects with nature-based tourism potential

The diversity of insects in different habitat types can contribute to nature tourism. Like bird watching, butterfly and dragonfly watching are gaining momentum (Khew 2015 & Choong *et al*. 2018). These nature tourism related activities should be promoted as they can generate revenue for the state government and improve the livelihood of the local communities. This is one of the main thrusts in the Sabah Forest Policy (SFD 2018b). Such activities also promote nature appreciation and awareness. They can be incorporated as part of the activities for visitors at the Rainforest Discovery Centre, Sepilok Laut Reception Centre, as well as the Gum Gum Plantation Station. This study provides some baseline information for some insects with tourism potential. They are flagship and interesting species which are sought after by special interest tourists to photograph (Table 9). Budowski (1976) highlighted that nature tourism and environmental conservation can be a symbiotic relationship which leads to a better quality of life, provided that due consideration is given to the ecological principles that must guide resource-use.

Table 9. Selected insects with tourism potential recorded from different forest types in Sandakan, Sabah (DF=Dipterocarp Forest, MF=Mangrove Forest & PF=Plantation Forest).

No.	Species	Family	Site	Remarks
Butterfly				
1.	*Idea leuconoe*	Nymphalidae	MF	A fairly large butterfly, specific to mangroves.
2.	*Idea stolli*	Nymphalidae	DF & PF	A large butterfly, often spotted in pairs flying gracefully at the forest fringe.
3.	*Papilio memnon*	Papilionidae	DF & PF	A large butterfly with various forms of female with different colours.
4.	*Graphium antiphates*	Papilionidae	PF	A beautiful species with sword-like wing tail.
Dragonfly				
1.	*Ictinogomphus decoratus*	Gomphidae	DF	A large dragonfly, up to 7 cm in length.
2.	*Trithemis aurora*	Libellulidae	PF	A strikingly pink dragonfly, often sought after by photographers.
3.	*Rhinagrion elopurae*	Philosinidae	DF & MF	An endemic species found along small streams inside the forest.
4.	*Vestalis* sp.	Calopterygidae	DF	A beautiful damselfly with metallic green colour.
Moth				
1.	*Xyleutes strix*	Cossidae	DF	Borneo's largest Goat Moth, up to 6 cm in length.
2.	*Suana concolor*	Lasiocampidae	MF	Among the largest Lasiocampidae moths in which its caterpillar can measure up to 18 cm long.
3.	*Ambulyx pryeri* and other hawk moths	Sphingidae	DF & PF	Comparatively large moths with 'jet fighter'-like appearance.

Insects associated with plants

The study on insect assemblages can highlight some of the species that could be detrimental to the plants in the selected habitat types. This is baseline information that can minimize potential outbreaks and also assist in understanding the life cycle and ecology of the insects. Some larvae of butterfly and moth species are pests in forestry, but not dragonflies since they are predacious insects, even at the larval stage. Careful planning incorporating biodiversity would reduce the impact of insect pests (Gurr *et al*. 2012). Table 10 features selected insects that can cause damages to plants in the surveyed sites in this study.

Table 10. Selected insects that cause damages to plants in different forest types in Sandakan, Sabah (DF=Dipterocarp Forest, MF=Mangrove Forest & PF=Plantation Forest).

No.	Species	Family	Site	Remarks
Butterfly				
1.	*Chilades pandava*	Lycaenidae	DF	A serious pest of ornamental cycads (Chung 2012).
2.	*Eurema hecabe*	Pieridae	PF & MF	A serious defoliator of *Falcataria moluccana* (Chey 1996).
Moth				
1.	*Arthroschista hilaralis*	Crambidae	PF & DF	A serious pest of *Neolamarckia cadamba* (Chung *et al*. 2009)
2.	*Dysaethria quadricaudata*	Uraniidae	PF	A pest of *Neolamarckia cadamba* (Chung *et al*. 2012)

Diversity, conservation and habitat preference of insects in different forest types

The information on insect diversity in different forest types can contribute towards sustainable forest management in Sabah. The management takes into account environmental, social and economic sustainability approaches, through good forest governance and best practices to ensure forestry remains an integral and competitive land use in Sabah (SFD 2018b). Besides comparing biodiversity status of the forest from the perspective of insects, such data are essential in providing supporting information to enhance conservation, which are much needed in the preparation for forest management plans or conservation area management plans. Biodiversity documentation from different forest types is among one of the research components carried out by the Sabah Forestry Department. Through this documentation, rare, threatened and endangered species are identified and some of these will be used as key conservation target species for monitoring purposes in order to safeguard the integrity of the forest and well-being of the protected areas (Nilus *et al*. 2014). As recorded in the studies by Lee (2020), Bahudin (2020) and Hor (2020), species with conservation interest, such as the Bornean endemic insects, are often used for such purposes, e.g. Chung *et al*. (2016).

Comparison of insects in different habitat types enables us to gain a better understanding of the habitat preference of certain insects. Habitat specificity can be related to the availability and abundance of the host plant and food source (Koh 2007 & Schulze *et al*. 2010). Each habitat may have specific environment, conducive for certain insect species. Thus, that species can only be found in certain habitats. For example, a butterfly species recorded in this study is the Mangrove Tree Nymph, *Idea leuconoe*, which is only confined in the mangrove area, as the larva feeds on *Parsonsia* woody climbers (Apocynaceae) growing at forest margins or riverbanks (Rahman *et al*. 2015), making this species an indicator for mangrove area.

Another example is that the abundance of *Chilades pandava* (Cycad Blue Butterfly) in the Rainforest Discovery Centre could also be explained by the presence of the ornamental cycads which are the host plant. The butterflies were observed flying around the host plants. This species is a serious pest of cycads as the larvae feed on the young new shoots, which eventually stunted the plant growth and also decreased its aesthetic ornamental value (Chung 2012). *Chilades pandava* was originally confined within the region of India to South East Asia. With increasing cycad cultivation and trade, the butterfly has spread through migration or introduced to other places. In terms of habitat specificity, as long as the habitat has the required food sources, the insect species can survive.

Dragonflies observed in the mangrove forest were *Cratilla metallica, Agrinoptera insignis, Brachidiplax chalybea* and *Orthetrum sabina* from family Libellulidae. The larvae of these species can tolerate a wide variation in pH, temperature and chemical constitute of water. Hence, these are considered robust species that are able to adapt to such harsh environment in the mangrove forest.

For moths, *Dysaethria quadricaudata* and *Arthroschista hilaralis* were recorded to be abundant in plantation forest because of the presence of their host plant, *Neolamarckia cadamba*. Locally known as Laran, it is a fast-growing native species which is planted for its timber (Chung *et al*. 2009). The geometrid moths, *Comostola* spp. were found to be speciose and abundant in the plantation forest because this genus feeds on the foliage of *Terminalia* trees in the plot. Four species were recorded, with *Comostola laesaria* being the most abundant species.

To conserve an insect habitat, one important factor to be considered is the plant diversity of the habitat. High plant diversity is related to the high availability of food sources for the insect, which is a contributing factor for the highest diversity in the dipterocarp forest compared to plantation and mangrove forests as indicated in this study. To conserve certain insect species, one has to understand the host specificity as well as the tolerance level of the species in unique environment, such as the mangrove forest.

It is acknowledged that there were constraints and limitations while conducting this study as highlighted by Lee (2020), Bahudin (2020) and Hor (2020). The results procured from this study may provide an indication of the diversity of the respective insect groups in different forest types but such results may not be exhaustive mainly due to the limited sampling period as well as other biotic and abiotic factors.

CONCLUSION

From this study, it has shown that insect assemblages were different in various forest types. The similarity among species was low for all the insect orders surveyed in the three different forest sites. As expected, the lowland dipterocarp forest depicted the highest diversity of insects, as indicated by the Shannon-Wiener Index, for butterfly, dragonfly and moth, compared to the plantation and mangrove forests. Hence, high diversity of plants would indicate a high diversity of insects, mainly due to the availability and variety of food sources. However, the proliferation of certain species in a habitat would reduce the diversity of the area, as indicated by the Simpson Index and species abundance distribution in this study. This study has also identified the host specificity of certain insects in relation to certain plants in the studied forest types.

The study on insect assemblages has enabled species with conservation interest to be identified. Such information would contribute towards biodiversity conservation effort of the selected habitat or forest. Similarly, interesting insects that can be used to promote nature tourism were documented in this study. All the study sites are open to tourists, visitors and students. The Rainforest Discovery Centre is famed for its environmental education on the diversity in lowland rainforests. The Sepilok Laut Reception Centre promotes the importance of mangrove ecosystem while Gum Gum Plantation Station often receives visitors from various forestry stakeholders, especially academic institutions. Insect inventories, especially on butterflies and dragonflies, provide baseline information for insect watching, which potentially generates revenue for the local economy and state as well.

The abundance of certain insects causing damage to plants was noted in this study. These insects can be potentially pest species, especially in the plantation forest. Therefore, the findings from this study would enable the management to gain a better understanding of the insect assemblage in the monoculture and to conduct periodical monitoring to prevent any potential outbreak.

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Appendix 1. Checklist and relative abundance of butterfly species in three forest types (MF=Mangrove Forest, PF=Plantation Forest & DF=Dipterocarp Forest).

				Relative abundance (%)		
No.	**Family**	**Genus**	**Species**	**MF**	**PF**	**DF**
1	Papilionidae	*Graphium*	*agamemnon agamemnon*	0.0	0.9	0.0
2		*Graphium*	*antiphates itamputi*	0.0	0.9	0.0
3		*Graphium*	*sarpedon sarpedon*	0.0	0.4	0.0
4		*Graphium*	sp. 1	1.8	0.0	0.0
5		*Graphium*	sp. 2	1.8	0.0	0.0
6		*Papilio*	*helenus enganius*	0.0	0.4	0.0
7		*Papilio*	*karna carnathus*	0.0	0.4	0.0
8		*Papilio*	*memnon memnon*	0.0	0.0	0.8
9		*Papilio*	*nephelus albolineatus*	0.0	0.4	0.8
10	Pieridae	*Appias*	*libythea olferna*	0.0	1.3	0.0
11		*Appias*	*paulina athena*	0.0	0.4	0.0
12		*Eurema*	*hecabe hecabe*	1.8	5.6	0.0
13		*Eurema*	*sari sodalis*	1.8	0.9	0.0
14		*Eurema*	*nicevillei*	0.0	0.0	0.8
15		*Eurema*	sp. 1	0.0	0.0	0.8
16		*Eurema*	sp. 2	0.0	0.0	0.8
17		*Eurema*	sp. 3	0.0	0.0	0.8
18		*Eurema*	sp. 4	1.8	0.0	0.0
19		*Eurema*	sp. 5	1.8	0.0	0.0
20		*Eurema*	sp. 6	1.8	0.0	0.0
21		*Eurema*	sp. 7	1.8	0.0	0.0
22		*Leptosia*	*nina malayana*	0.0	0.9	0.8
23		*Gandaca*	*harina elis*	0.0	0.9	0.0
24		*Pareronia*	*valeria lutescens*	0.0	0.4	0.0
25		*Pareronia*	sp. 1	0.0	0.0	0.8
26		*Pareronia*	sp. 2	0.0	0.0	1.6
27		*Pareronia*	sp. 3	0.0	0.0	1.6
28		*Pareronia*	sp. 4	3.6	0.0	0.0
29	Nymphalidae	*Athyma*	*nefte subrata*	0.0	0.0	0.8
30		*Athyma*	sp. 1	0.0	0.0	0.8
31		*Charaxes*	*bernardus repititus*	0.0	0.0	0.8
32		*Cirrochroa*	*malaya calypso*	0.0	0.0	0.8
33		*Cirrochroa*	*satellita satellita*	9.1	0.0	4.0
34		*Cirrochroa*	*tyche thilina*	0.0	0.0	1.6
35		*Cupha*	*erymanthis erymanthis*	0.0	1.7	0.8
36		*Euploea*	*crameri crameri*	1.8	0.0	0.0
37		*Euthalia*	*kanda kanda*	0.0	0.0	0.8
38		*Hypolimnas*	*bolina philippensis*	0.0	5.2	0.8
39		*Idea*	*leuconoe chersonesia*	14.5	0.0	0.0
40		*Idea*	*stolli virgo*	0.0	0.4	13.7
41		*Idea*	sp. 1	1.8	0.0	0.0
42		*Idea*	sp. 2	1.8	0.0	0.0
43		*Ideopsis*	sp. 1	0.0	0.0	0.8
44		*Junonia*	*atlites atlites*	3.6	0.0	0.8

				Relative abundance (%)		
No.	**Family**	**Genus**	**Species**	**MF**	**PF**	**DF**
45		*Junonia*	*hedonia ida*	0.0	0.0	4.0
46		*Junonia*	*iphita viridis*	0.0	7.7	7.3
47		*Junonia*	*orithya metion*	0.0	0.9	0.8
48		*Mycalesis*	*anapita fucentia*	0.0	0.0	0.8
49		*Mycalesis*	*orseis borneensis*	0.0	3.4	2.4
50		*Mycalesis*	*horsfieldi hermana*	0.0	12.4	0.0
51		*Mycalesis*	*mineus macromalayana*	12.7	5.6	0.0
52		*Neorina*	*lowii lowii*	0.0	0.0	1.6
53		*Neptis*	*hylas soprata*	0.0	3.4	0.0
54		*Parantica*	*agleoides borneensis*	0.0	0.9	0.0
55		*Parthenos*	*sylvia borneensis*	0.0	3.9	11.3
56		*Polyura*	*athamas uraenus*	0.0	0.0	0.8
57		*Polyura*	*schreiber malayica*	1.8	0.0	0.0
58		*Ragadia*	*makuta umbrata*	0.0	0.0	0.8
59		*Tanaecia*	sp. 1	1.8	0.0	0.0
60		*Thaumantis*	*odana panwila*	0.0	0.0	0.8
61		*Ypthima*	*baldus selinuntius*	5.5	11.2	1.6
62		*Ypthima*	*pandocus sertorius*	9.1	12.4	3.2
63	Riodinidae	*Taxila*	*haquinus othrys*	9.1	0.0	0.0
64	Lycaenidae	*Acytolepis*	*ripte* *	0.0	0.0	3.2
65		*Arhopala*	sp. 1	0.0	0.0	0.8
66		*Arhopala*	sp. 2	1.8	0.0	0.0
67		*Chilades*	*pandava pandava*	0.0	0.0	8.9
68		*Curetis*	*sperthis*	1.8	0.0	0.0
69		*Curetis*	*santana malayica*	0.0	0.0	0.8
70		*Deudorix*	*epijarbes*	0.0	0.0	0.8
71		*Drupadia*	*theda umara*	0.0	0.0	3.2
72		*Jamides*	*aratus adana*	0.0	0.9	0.0
73		*Jamides*	*caeruleus caeruleus*	0.0	0.0	1.6
74		*Jamides*	*callistus mioae*	0.0	0.0	0.8
75		*Jamides*	*pura tenus*	0.0	0.0	0.8
76		*Jamides*	sp. 1	0.0	0.4	2.4
77		*Nacaduba*	*russelli*	0.0	0.4	0.0
78		*Nacaduba*	sp. 1	0.0	0.4	0.0
79		*Prosotas*	*nora superdates*	0.0	0.4	0.0
80		*Rapala*	*damona*	0.0	0.4	0.0
81		*Zizina*	*otis otis*	0.0	0.0	0.8
82	Hesperiidae	*Isma*	*iapis iapis*	0.0	0.9	0.0
83		*Koruthaialos*	*rubecula rubecula*	0.0	3.0	1.6
84		*Koruthaialos*	*sindu sindu*	0.0	0.9	0.8
85		*Pelopidas*	*mathias mathias*	0.0	1.3	0.0
86		*Potanthus*	*omaha maesina* *	0.0	6.0	0.0
87		*?Potanthus*	sp. 1	0.0	0.4	0.0
88		*Taractrocera*	*ardonia sumatrensis*	5.5	1.3	0.0
89		*Taractrocera*	*ziclea stella* *	0.0	0.4	0.8
90		*Tagiades*	*gana gana*	0.0	0.0	0.8

*Endemic species and subspecies

Appendix 2. Checklist and relative abundance of dragonfly species in three forest types (MF=Mangrove Forest, PF=Plantation Forest & DF=Dipterocarp Forest).

				Relative abundance (%)		
No.	**Suborder**	**Families**	**Species**	**MF**	**PF**	**DF**
1	Anisoptera	Gomphidae	*Ichtinogomphus decoratus*	0	0	1.2
2		Libellulidae	*Aethriamanta gracilis*	0	0	7.2
3			*Agrinoptera insignis*	15.7	0	0
4			*Brachydiplax chalybea*	15.7	0	1.2
5			*Camacinia gigantea*	0	0	0.6
6			*Cratilla metallica*	36.8	0	0
7			*Latherecista asiatica*	0	0	0.6
8			*Neurothemis fluctuans*	5.2	20.9	1.8
9			*Neurothemis terminata*	0	17.7	3
10			*Orthetrum chrysis*	0	8	4.8
11			*Orthetrum sabina*	5.2	4.8	1.2
12			*Pornothemis* sp.	5.2	0	0
13			*Rhodothemis rufa*	0	4.8	3
14			*Rhyothemis phyllis*	0	9.6	0
15			*Rhyothemis pygmaea*	0	0	1.2
16			*Rhyothemis triangularis*	0	0	2.4
17			*Tholymis tillarga*	0	3.2	0
18			*Trithemis aurora*	0	6.4	0
19			*Tyriobapta torrida*	0	0	4.2
20			*Urothemis signata insignata*	0	0	1.2
21			*Zyxomma petiolatum*	0	0	1.2
22	Zygoptera	Calopterygidae	*Vestalis* sp.	0	0	0.6
23		Chlorocyphidae	*Rhinocypha humeralis*	5.2	0	1.8
24		Coenagrionidae	*Prodasineura verticalis*	0	0	0.6
25			*Ceriagrion cerinobellum*	0	4.8	26.5
26			*Pseudagrion microcephalum*	0	0	19.2
27			*Archibasis melanocyana*	0	0	3
28			*Archibasis viola*	0	0	1.2
29			*Archibasis* sp.	0	0	2.4
30			Sp. 1	0	0	1.2
31			Sp. 2	0	0	3
32			Sp. 3	0	0	1.8
33			Sp. 4	0	1.6	0
34			Sp. 5	0	1.6	0
35			Sp. 6	0	1.6	0
36			Sp. 7	0	1.6	0
37			*Agriocnemis femina*	0	1.6	0
38		Philosinidae	*Rhinagrion elopurae**	5.2	0	0.6
39		Platycnemididae	*Copera vittata*	0	3.2	1.8
40			*Coeliccia* sp.	5.2	0	0
41			*Onychargia atrocyna*	0	1.6	0
42		Platystictidae	Sp. 1	0	1.6	0
43		Protoneuridae	*Prodasineura hyperthra**	0	0	0.6
44			*Prodasineura* sp.	0	4.8	0.6

*Endemic species

Appendix 3. Checklist and relative abundance of moth species in three forest types (MF=Mangrove Forest, PF=Plantation Forest & DF=Dipterocarp Forest).

No.	Family	Species	Relative abundance (%)		
			MF	PF	DF
1	Bombycidae	*Prismosticta tiretta*	1.64		
2	Cossidae	*Cossus verbeeki*	3.28		
3		*Cossus* sp. 1			1.08
4		*Xyleutes strix*			1.08
5	Crambidae	*Agrotera* sp. 1			1.08
6		*Arthroschista hilaralis*		3.57	1.08
7		*Bocchoris inspersalis*		1.79	
8		*Botyodes asialis*	1.64		
9		*Cirrhochrista fumipalpis*		1.79	
10		*Conogethes* sp. 1	1.64		
11		*Glauconoe deductalis*	4.92	5.38	2.15
12		*Herculia* sp. 1	4.92		
13		*Omiodes diemenalis*		1.79	
14		*Pitama hermesalis*		1.79	
15		*Rhimphalea astrigalis*			1.08
16		*Sameodes cancellalis*		1.79	
17		*Talanga sexpunctalis*		5.37	1.08
18		*Glyphodes bivitralis*	1.64		
19		*Xanthomelaena schematias*		3.57	11.83
20		Unidentified sp. 1		1.08	1.08
21		Unidentified s.p 2	1.08		
22	Erebidae	*Achaea serva*	1.64		
23		*Anomis prima*			1.08
24		*Anomis* sp. 1			1.08
25		*Anomis* sp. 2			1.08
26		*Arctornis discirufa*			1.08
27		*Arctornis* sp. 1	1.64		
28		*Arctornis* sp. 2		1.79	
29		*Asota heliconia*		1.79	
30		*Avatha rufiscripta*			1.08
31		*Bematha extensa*			1.08
32		*Bocula bifaria*	1.64		
33		*Carriola ecnomoda*		1.79	1.08
34		*Creatonotos transiens*		1.79	
35		*Cyana costifimbria*			1.08
36		*Cyana perornata*			1.08
37		*Cyana* sp. 1		1.79	
38		*Daddala quadrisignata*			1.08
39		*Lyclene reticulata*			1.08
40		*Lyclene* sp. 1			1.08
41		*Metaemene santubong**	1.64		
42		*Metaemene* sp. 1		1.79	
43		*Oeonistis altica*		1.79	
44		*Somena similis*			1.08
45		*Tamba mnionomera*		1.79	
46		*Tigrioides leucanioides*	1.64		
47		*Tochara creberrima*	1.64		2.15
48		*Ugia disjungens*			3.23
49		*Ugia viridior*			1.08
50	Geometridae	*Antitrygodes divisaria*		1.79	
51		*Argyrocosma* sp. 1	1.64		
52		*Argyrocosma* sp. 2		1.79	
53		*Cassyma quadrinata*	1.64		
54		*Catoria olivescens*	1.64		
55		*Celerena signata*			1.08
56		*Cleora alienaria*	1.64		
57		*Cleora determinata*	3.28		1.08
58		*Cleora injectaria*	1.64		
59		*Cleora repetita*	3.28		
60		*Comibaena attenuata*	4.92	3.57	5.38
61		*Comostola laesaria*		3.57	
62		*Comostola meritaria*		1.79	
63		*Comostola orestias*		1.79	
64		*Comostola pyrrhogona*		1.79	
65		*Comostolodes dialitha*			1.08
66		*Dysphania transducta*			1.08
67		*Ectropidia fimbripedata*	8.2		
68		*Eucyclodes albisparsa*	1.64		
69		*Eucyclodes semialba*		1.79	
70		*Eumelea biflavata*	1.64		
71		*Godonela translineata*	1.64		
72		*Heteralex rectilineata*			1.08
73		*Hypochrosis binexata*	1.64		3.23
74		*Hypochrosis pyrrhophaeata*			1.08
75		*Hypochrosis waterstradti**			1.08
76		*Hypomecis costaria*	3.28		
77		*Hypomecis* sp. 1	1.64		
78		*Hyposidra picaria*			1.08
79		*Hyposidra talaca*			1.08
80		*Idaea* sp. 1	1.64		
81		*Ornithospila bipuncata*	1.64		2.15
82		*Ornithospila* sp1		1.79	
83		*Ornithospila succinta*	1.64		
84		*Ozura* sp. 1			1.08
85		*Pingasa lariaria*		1.79	
86		*Probithia imprimata*	1.64		
87		*Protuliocnemis biplagiata*			7.53
88		*Scopula* sp. 1			1.08
89		*Syngonorthus subpunctatus*	1.64		
90		*Tanaorhinus rafflesii*			1.08
91		*Thalassodes* sp. 1		1.79	
92		*Visiana sordidata*		1.79	
93		*Zythos strigata*			1.08
94		*Pingasa rubimontana*			1.08
95		*Cleora* sp. 1		1.79	
96		Unidentified sp. 3			1.08
97		Unidentified sp. 4			1.08
98		Unidentified sp. 5		1.79	
99	Lasiocampidae	*Suana concolor*	14.76		
100		Unidentified sp. 6			3.23
101	Limacodidae	*Cania* sp. 1	1.64		
102		*Parasa* sp. 1	1.64		
103		*Scopelodes* sp. 1			1.08
104	Noctuidae	*Anuga fida*			1.08
105		*Calymniops convergens*			1.08
106		*Chalciope mygdon*		1.79	
107		*Chrysodeixis illuminata*		3.57	
108		*Claterna cydonia*			2.15
109		*Ischyja hemiphae*			1.08
110		*Ischyja marapok*			1.08
111		*Leucania yu*		1.79	
112		*Saroba maculicosta*	1.64		
113		Unidentified sp. 7		1.79	
114		Unidentified sp. 8		1.79	
115		Unidentified sp. 9		1.79	
116	Nolidae	*Nola lucidalis*			1.08
117		*Pterogonia nubes*			1.08
118		*Tathothripa continua*			1.08
119		Unidentified sp. 6			1.08
120	Notodontidae	*Cerasana anceps*			1.08
121		*Gangarides vardena*			1.08
122	Sphingidae	*Ambulyx pryeri*			1.08
123		*Ambulyx subocellata*			1.08
124		*Daphnusa ocellaris*		1.79	
125		*Elibia dolichus*			1.08
126		*Hippotion rosetta*		1.79	
127		*Theretra boisduvalii*			1.08
128		*Theretra latreillei*		3.57	
129	Uraniidae	*Lyssa menoetius*	1.64		1.08
130		*Dysaethria quadricaudata*		7.14	

*Endemic species

An illustrated preliminary checklist of inland fishes from the Kabili-Sepilok Forest Reserve, Sandakan, Sabah

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Abstract. A total of 48 fish species from 24 families were recorded in the present survey. Of these 48 species, fourteen (14) species were endemic to Borneo (*Anguilla borneensis, Barbodes sealei, Nematabramis everetti, Rasbora* cf. *hubbsi, R. pycnopeza, R.* cf. *semilineata, Theriodes sandakanensis, Hemibagrus baramensis, Neosthetus geminus, Macrognathus keithi, Betta ocellata, Nandus prolixus, Channa baramensis* and *Dichomyctere kretamensis*). Three (3) species were recorded for the first time in Sabah (*Clarias batrachus, Neostethus geminus* and *Periophthalmus malaccensis*) and three (3) were non-native (*Barbonymus gonionotus*, *Pangasianodon hypophthalmus* and *Oreochromis niloticus*), as they were obtained from an artificial pond located in the Rainforest Discovery Centre (RDC), all of which are probably introduced for angling purposes. Two species have been categorized as Critically Endangered (CR) and Vulnerable (VU), *Pandaka pygmaea* and *Anguilla borneensis,* which would require proper measures for species conservation.

Keywords: Aquatic biodiversity, pisces, Southeast Asia, freshwater, mangrove

INTRODUCTION

Inland fishes live in various types of environments, including disturbed areas, primary forests, rivers, lakes, swamps and mangroves. There are more than 600 freshwater fish recorded in Malaysia with more than 300 species in Peninsular Malaysia (Lim & Tan 2002, Md Shah *et al.* 2009), 249 species in Sarawak (Kottelat & Whitten 1996) and 150 species in Sabah (Ng *et al.* 2017). Despite these figures, the inland fish in Sabah are still relatively underexplored and understudied. This was due to lack of knowledge on the diversity of freshwater ichthyofauna in the region, and active continual research (Chong *et al.* 2010).

The initial freshwater fish studies in Borneo were carried out by Pieter Bleeker in 1850-60s. While in North Borneo, the first paper of primary freshwater fishes was cited from Vaillant (1893) where five were recorded from Mount Kinabalu. Further research on freshwater in Sabah were then reported by Boulenger (1894, 1899), Regan (1906), Seale (1910), Hora (1932), de Beaufort (1933), Herre (1933, 1940a, 1940b), Fowler (1941), Brittan (1954) and (Inger & Chin 2002). Yet, the most comprehensive collections and taxonomic studies were documented by Inger & Chin (1962), with a subsequent supplementary chapter by Chin, published in 2002. Several other regional collections have been reported in Kinabalu Park (Samat 1990), Danum Valley (Martin-

Smith & Tan 1998), Klias and Binsulok (Ishak *et al.* 2000), Crocker Range (Rahim *et al.* 2002), Ulu Tungud (Ahmad & Nek 2006). Maliau Basin (Sade & Biun 2012), Sandakan Bay (Manjaji-Matsumoto *et al.* 2016) and Kalabakan Forest Reserve (Wilkinson & Tan 2018).

The Kabili-Sepilok Forest Reserve (KSFR; 5.83919ºN, 117.9474193ºE) is a 4,294 ha Class VI Virgin Jungle Reserve and is situated about 23km from Sandakan town, Eastern Sabah. There are no large rivers (more than 4m wide) in KSFR. The region occupies a large part of the drainage of the Kabili, Sepilok Kecil and Sepilok Besar Rivers, which flow south into Sandakan Bay. The drainage from the north side and the eastern side of Kabili-Sepilok Forest Reserve are flowing to Gum-Gum Besar River and Seguntor River respectively. The Reserve is fringing with the Sepilok Mangrove Forests (1,235 ha) and contiguous with Elopura Mangrove Forest Reserve which extends around Sandakan Bay.

Although many terrestrial forms of wildlife have been surveyed (e.g. mammals and birds), some in great detail, the diversity of in-stream life has remained largely neglected. Earlycollections and descriptions of freshwater fish of KSFR by Inger & Chin (2002) had recorded 22 species. An updated preliminary checklist is presented herein based on literature and recent opportunistic collections conducted during a five-day workshop conducted in May 2019.

MATERIALS AND METHODS

The fishes were sampled in four tributaries flowing from the Kabili-Sepilok Forest Reserve and in mangrove swamps area at the Sepilok Laut Reception Centre (SLRC) mainly based on a five-day workshop conducted from 14th to 18th of May 2019 (Figure 1). Fish specimens were obtained using push nets, scoop nets, hand nets, cast nets, gill nets, angling and fish traps. Sampling sites were randomly selected and were limited to the accessibility. Specimens were fixed in 10% formalin solution and later transferred to 75% ethanol for long-term storage. Fish species were identified using Inger & Chin (1962), Kottelat *et al.* (1993) and Kottelat (2013). All of the specimens collected have been deposited in the Forest Research Centre (FRC), Sabah, Malaysia; and the Zoological Reference Collection (ZRC) in the Lee Kong Chian Natural History Museum, National University of Singapore, Singapore.

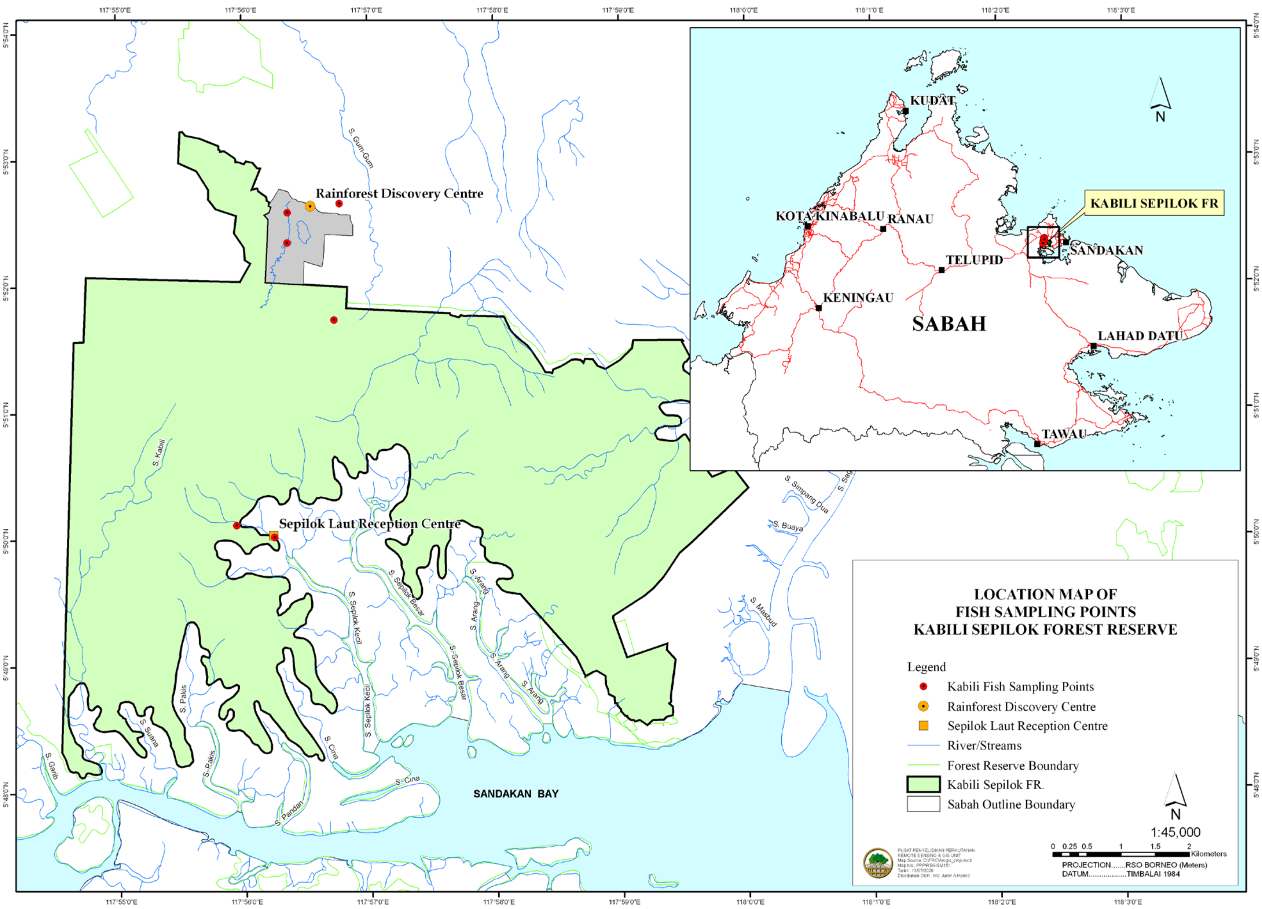

Figure 1. Top right box shows the location of KSFR, left is sampling locations within the Kabili-Sepilok catchment area.

RESULTS

The inland fish fauna of Kabili-Sepilok Forest Reserve comprise of 48 species from 38 genera, 24 families and 8 orders. The order Perciformes, with 30 species in 12 families, is the most diverse, followed by Cypriniformes (7 species, 4 families), Siluriformes (4 species, 3 families), Atheriniformes (2 species), Beloniformes (2 species). Whilst Anguilliformes, Synbranchiformes and Tetraodontiformes all have one species recorded in the area respectively. Of the 48 species, 14 species were endemic to Borneo (*Anguilla borneensis, Barbodes sealei, Nematabramis everetti, Rasbora* cf. *hubbsi, R. pycnopeza, R.* cf. *semilineata, Theriodes sandakanensis, Hemibagrus baramensis, Neosthetus geminus, Macrognathus keithi, Betta ocellata, Nandus prolixus, Channa baramensis* and *Dichomyctere kretamensis*), three species were recorded for the first time in Sabah, *Clarias batrachus, Neostethus geminus* and *Periophthalmus malaccensis*; and three were non-native species (*Barbonymus gonionotus*, *Pangasionodon hyophthalmus* and *Oreochromis niloticus*), located in an artificial pond.

The freshwater fishes of Kabili-Sepilok Forest Reserve were first documented by Inger & Chin (1962) with 22 species, which includes: *Anguilla borneensis* (Anguillidae), *Puntius sealei* (currently *Barbodes sealei*) (Cyprinidae), *Nematabramis everetti, Rasbora sumatrana* (currently *Rasbora pcynopeza*) (Danionidae), *Acanthopthalmus sandakanensis* (currently *Theriodes sandakanensis*) (Cobitidae), *Hemibagrus baramensis* (Bagridae), *Clarias leiacanthus* (Clariidae), *Dermogenys pusillus* (currently *Dermogenys bispina*) (Zenarchopteridae), *Mastacembelus keithi* (currently *Macrognathus keithi*) (Mastacembelidae), *Ambassis interrupta* (Ambassidae), *Toxotes jaculatrix* (Toxotidae), *Nandus nebulosus* (currently *Nandus prolixus*) (Nandidae), *Eleotris melanosoma* (Eleotridae), *Brachygobius kabiliensis* (Gobiidae), *Ophicephalus melanosoma* (currently *Channa baramensis*) (Channidae), *Betta ocellata* and *Trichopodus trichopterus* (Osphronemidae). At least five species of inland fishes were originally described from this area. They are: *Theriodes sandakanensis* (Cobitidae), *Macrognathus keithi* (Mastacembelidae), *Brachygobius kabiliensis*, *Eugnathogobius kabilia* (Gobiidae) and *Nandus prolixus* (Nandidae).

At least 52% of the species are yet to be assessed by the IUCN Red List of Threatened Species which indicate that most species are inadequately known or no data is available to determine their risk of extinction or extent of threat (Ng *et al.* 2017). The catadromous species, *Anguilla borneensis* is listed as Vulnerable (VU), *Pandaka pygmae* is identified as Critically Endangered (CR) species, while the others are listed as Least Concern (LC) and Data Deficient (DD) (Appendix I).

Order Anguilliformes

Family Anguillidae

***Anguilla borneensis* Popta, 1924**

Anguilla borneensis – Inger & Chin, 1962: 39 (Sepilok Forest Reserve).

Remarks. A catadromous species; adults inhabit freshwaters or estuaries, migrating to the sea to spawn. Bornean endemic species.

Order Cypriniformes

Family Cyprinidae

***Barbodes sealei* (Herre, 1933) – Topotypic**

Barbus elongatus Seale, 1910c: 265, pl. 2 fig. 1 (type locality: Malaysia: Borneo: Sabah: Sandakan).

Barbodes sealei Herre, 1933b: 3 (replacement name for *Barbus elongatus* Seale, 1910a: 265).

Puntius sealei – Inger & Chin, 1962: 73 (Sepilok Forest Reserve).

Remarks. This Bornean endemic species is the most common cyprinid species around KSFR and is found in all streams.

***Barbonymus gonionotus* (Bleeker, 1849)**

Remarks: This is a common fish used extensively for aquaculture.

Family Danionidae

***Nematabramis everetti* Boulenger, 1894**

Nematabramis everetti – Inger & Chin, 1962 (Sepilok Forest Reserve).

***Rasbora* cf. *hubbsi* Brittan, 1954**
Remarks. Bornean endemic species. More intensive survey and a larger series is required for identification.

***Rasbora pycnopeza* Wilkinson & Tan 2018**

Rasbora sumatrana – Inger & Chin, 1962 (Sungei Kabili, Sepilok Forest Reserve).

Remarks: A recently described species of *Rasbora,* previously identified as *R. sumatrana.* Originally described from Brantian drainage, Kalabakan Forest Reserve (see Wilkinson & Tan, 2018, for more details).

***Rasbora* cf. *semilineata* Weber & de Beaufort, 1916**
Remarks: This species was caught from the back mangroves. More intensive survey work will reveal the true extent of its distribution.

Family Cobitidae

***Theriodes sandakanensis* (Inger & Chin, 1962) - Topotypic**

Acanthophthalmus sandakanensis – Inger & Chin, 1962: 120, fig. 54E (Sepilok Forest Reserve).

Lepidocephalichthys sandakanensis – Kottelat *et al.*, 1993: 82, Pl. 28.

Remarks: Muddy pool and stream inhabitant. Originally described from KSFR and endemic to Borneo. Previously placed in the genus *Pangio* until Kottelat (2012) diagnosed it as a new genus *Theriodes* and making it monotypic.

Order Siluriformes

Family Bagridae

***Hemibagrus baramensis* (Regan, 1906)**

Mystus baramensis – Inger & Chin, 1962: 139 (Sungei Kabili).

Family Clariidae

***Clarias batrachus* (Linnaeus, 1758)**
Remarks: *Clarias batrachus* is a new record for Sabah.

***Clarias leiacanthus* Bleeker, 1851**

Clarias teysmanni – Inger & Chin, 1962: 131 (Sungei Kabili, Sepilok Forest Reserve).

Family Pangasiidae

***Pangasianodon hypophthalmus* (Sauvage, 1878)**
Remarks: This is common species used for aquaculture.

Order Atheriniformes

Family Phallostethidae

***Neostethus borneensis* Herre 1939 - Topotypic**
Neostethus borneensis Herre, 1939: 143
Remarks: This is only found in small streams in the back mangroves. When first observed in-situ, it resembles *Oryzias* from the dorsal view, the short body and wide head.

***Neostethus geminus* Parenti, 2014**
Remarks. New record for Sabah and Bornean endemic. This species was obtained from the main channel in the mangroves swimming near the water surface.

Order Beloniformes

Family Zenarchopteridae

***Dermogenys bispina* Meisner & Collette, 1998**
Dermogenys pusillus – Inger & Chin, 1962: 152 (Sungei Kabili).
Remarks. A small-sized schooling species which is viviparious, mostly abundant in slightly brackish water, swimming near the surface along the banks.

***Zenarchopterus buffonis* (Valenciennes, in Cuvier & Valenciennes, 1847)**
Remarks. Can be sighted from Sepilok Laut Reception Centre swimming just below the water's surface. Inhabits brackish habitats, such as river estuaries and mangroves.

Order Synbranchiformes

Family Mastacembelidae

***Macrognathus keithi* (Herre, 1940) - Topotypic**
Mastacembelus keithi Herre, 1940a: 24, pl. 19; Inger & Chin, 1962: 35 (Kabili).
Remarks. This species is originally described from KSFR and Bornean endemic.

Order Perciformes

Family Ambassidae

***Ambassis interrupta* Bleeker, 1853**

***Ambassis kopsii* Bleeker, 1858**

Family Leiognathidae

***Nuchequula blochii* (Valenciennes, in Cuvier & Valenciennes, 1835)**

Family Toxotidae

***Toxotes jaculatrix* (Pallas, in Schlosser, 1767)**
Remarks. Known as archerfish which hunts mainly by shooting droplets of water from its mouth to hit terrestrial insects perched on overhanging vegetation. Inhabits mangrove swamps and can be sighted from SLRC.

Family Scatophagidae

***Scatophagus argus* (Linne, 1766)**
Remarks: This is based on a sighting at the SLRC.

Family Nandidae

***Nandus prolixus* Chakrabarty, Oldfield & Ng, 2006 – Topotypic**
Nandus prolixus Chakrabarty, Oldfield & Ng, 2006: 52
(Sandakan: Sepilok drainage).
Remarks. This species inhabits slow moving water and most of the time hiding amongst submerged leaf litter. Bornean endemic species.

Family Cichlidae

***Oreochromis niloticus* (Linnaeus, 1758)**
Remarks: This species is recorded based on sightings at the artificial lake at the Rainforest Discovery Centre. An introduced species.

Family Butidae

***Butis humeralis* (Valenciennes, in Cuvier & Valenciennes, 1837)**

Family Eleotridae

Eleotris melanosoma

Eleotris melanosoma – Inger & Chin, 1962: 169 (Sepilok Forest Reserve).

***Oxyeleotris marmorata* Bleeker, 1853**

Family Gobiidae

***Brachygobius kabiliensis* Inger, 1958 - Topotypic**

Brachygobius kabiliensis Inger, 1958: 110, fig. 19; Inger & Chin, 1962: 180 (Sungei Kabili).

Remarks: This dimunitive species (less than 2 cm length) with distinctive black and yellow bars inhabit mangrove habitats in slow flowing water and tide pools.

***Eugnathogobius illota* (Larson, 1999)**

***Eugnathogobius kabilia* (Herre, 1940) - Topotypic**

Vaimosa kabilia Herre, 1940a: 19, pl. 14.

Remarks. This is not a commonly reported species. Here the topotypic material is illustrated.

***Eugnathogobius* cf. *siamensis* (Fowler, 1934)**

***Glossogobius sandakanensis* Inger, 1957 - Topotypic**

Glossogobius sandakanensis Inger, 1957: 393, fig. 6.

***Gobiopterus chuno* (Hamilton, 1822)**

***Gobiopterus panayensis* (Herre, 1944)**

***Hemigobius* cf. *hoeveni* (Bleeker, 1851)**

***Hemigobius melanurus* (Bleeker, 1849)**

Note: This is based on a sighting along the boardwalk at the Sepilok Laut Reception Centre. This species can be found in tide pools or slow-moving waters, typically hanging in the water column head-up motionless.

***Mugilogobius rambaiae* (Smith, 1945)**

***Pandaka pygmaea* (Herre, 1927)**

Remarks. This species has been listed as Critically Endangered in 1996 by IUCN Red List.

***Periophthalmus malaccensis* (Eggert, 1935)**
Remarks. This species is a new record for Sabah. The first record of *P. malaccensis* from Borneo is from Brunei (Polgar, 2016). They have evolved techniques that allow them to breathe out of water through their adapted gills.

***Pseudogobius javanicus* (Bleeker, 1856)**

***Redigobius chrysosoma* (Bleeker, 1874)**

***Stigmatogobius pleurostigma* (Bleeker, 1849)**

Family Anabantidae

***Anabas testudineus* (Bloch, 1792)**

Family Osphronemidae

***Betta ocellata* (de Beaufort, 1933) - Topotypic**
Betta ocellata de Beaufort, 1933: 35.
Betta unimaculata – Inger & Chin, 1962: 158 (Sungei Kabili, Sepilok Forest Reserve).
Remarks. Inger & Chin (1962) has documented this fish inhabiting waterways above waterfalls. The original site where this species was described from is from Bettotan, 23.6 km away from Sepilok.

***Trichopodus trichopterus* (Pallas, 1770)**

Family Channidae

***Channa baramensis* (Steindachner, 1901)**
Ophicephalus melanosoma – Inger & Chin, 1962: 154 (Sepilok Forest Reserve).

***Channa striata* (Bloch, 1793)**

Order Tetraodontiformes

Family Tetraodontidae

***Dichomyctere kretamensis* (Inger, 1953)**
Tetraodon kretamensis Inger, 1953: 149, fig. 27
Remarks. This pufferfish is rather common in the mangroves, swimming near the water surface feeding on invertebrates as it follows the ebbing tide out (pers. obs.).

DISCUSSION

In this survey, the fishes caught were categorized into primary and secondary freshwater fish species. The primary freshwater fish species have little or no salt tolerance and are more confined to freshwaters while secondary freshwater fish species are those whose family members are salt-tolerant or originate from predominantly marine fish families (Zakaria-Ismail 1994). The primary freshwater fish consisted of *Barbodes sealei, Nematabramis everetti, Rasbora* cf. *hubbsi, Rasbora pycnopeza, Rasbora* cf. *semilineata, Theriodes sandakanensis, Hemibagrus baramensis, Nandus prolixus*, *Trichopodus trichopterus* and *Betta ocellata.* Meanwhile, most species in the order of Perciformes namely Ambassidae, Eleotridae, Leognathidae, Toxotidae, Scatophagidae Butidae, Eleotridae, Gobiidae and Tetraodontidae which were found in the mangrove swamp forests are categorized as secondary freshwater fish as they are more common in brackish and marine habitats although a few species can be found in pure freshwater (Kottelat *et al.* 1993).

The forest streams provide suitable habitats and breeding sites for most of the fishes especially the Cyprinids as a variety of microhabitats such as dead branches and submerged leaf litter are abound. For the mangrove areas, although the sampling was done in one day, the number of species caught was quite high as compared to the study of Inger & Chin (1962). Nevertheless, longer sampling period may reveal more species, especially members of the family Gobiidae as this species is common in muddy mangroves. The presence of the catadromous species *Anguilla borneensis* in KSFR provides evidence that the streams are connected to the sea. The species migrate to the sea to spawn but spends their adult life in fresh water (Aoyama 2009, Kamal & Ng 2017).

Meanwhile, RDC pond was dominated by the introduced species *Barbonymus gonionotus*, *Pangasionodon hyophthalmus* and *Oreochromis niloticus* for aquaculture. Alien species are considered to be one of the major threats to local freshwater biodiversity and has adverse impacts on the habitats and ecosystems as some species could be very aggressive, and preys on smaller native species. In Malaysia, many alien species had been introduced but there is still lack of comprehensive study on species composition, ecology, habitat preference and relative abundance (Khairul 2012, Khairul *et al.* 2013). Hence, precautionary approach to prevent the invasive species from escaping into the waterways is important.

The level of disturbance in this lowland dipterocarp forest reserve is moderate despite being located adjacent to oil palm plantations, orchards and villages. Nevertheless, several appropriate measures should be implemented in order to prevent incursions into the reserve's waterways by the locals. First, comprehensive monitoring and surveillance in the area should be done by well-trained wildlife research unit to eradicate illegal fishing activities in the forest reserve. Second, protecting and conserving the inland fish by maintaining or restoring of floodplains which serve as important spawning and nursery grounds, as well as important sources of food for fishes. Third, placing 'no fishing' signage along the streams and lastly, increasing the community-level responsibility and raising awareness and education of the importance of inland fish.

CONCLUSIONS

The Kabili-Sepilok Forest Reserve contains good ichthyofaunal diversity in both streams and mangrove habitats. Despite only limited time and area surveyed, the results are significant and the area should be protected for species survival. Future sampling covering the area with consideration of wet season may reveal more species, especially members of the family Gobiidae. To maintain the richness of the aquatic ecosystem, continuous monitoring of the area is needed.

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Appendix I. Checklist of fishes from Kabili-Sepilok Forest Reserve, Sabah, Malaysia.

No	Order/Family	Species	IUCN
1	**Anguillidae**	*Anguilla borneensis* *	VU
2	**Cyprinidae**	*Barbodes sealei* *	NA
3		*Barbonymus gonionatus*	LC
4	**Cobitidae**	*Theriodes sandakanensis* *	NA
5	**Danionidae**	*Nematabramis everetti*	NA
6		*Rasbora* cf. *hubbsi*	NA
7		*Rasbora pycnopeza*	NA
8		*Rasbora* cf. *semilineata*	NA
9	**Bagridae**	*Hemibagrus baramensis* *	NA
10	**Clariidae**	*Clarias batrachus*	LC
11		*Clarias leiacanthus*	LC
12	**Pangasiidae**	*Pangasianodon hypophthalmus*	LC
13	**Phallostethidae**	*Neostethus borneensis*	NA
14		*Neostethus geminus* *	NA
15	**Zenarchopteridae**	*Dermogenys bispina*	NA
16		*Zenarchopterus buffonis*	NA
17	**Mastacembelidae**	*Macrognathus keithi* *	NA
18	**Ambassidae**	*Ambassis interrupta*	LC
19		*Ambassis kopsii*	NA

20	**Leiognathidae**	*Nuchequula blochii*	NA
21	**Toxotidae**	*Toxotes jaculatrix*	LC
22	**Scatophagidae**	*Scatophagus argus*	LC
23	**Nandidae**	*Nandus prolixus* *	NA
24	**Cichlidae**	*Oreochromis niloticus*	LC
25	**Butidae**	*Butis humeralis*	NA
26	**Eleotridae**	*Eleotris melanosoma*	LC
27		*Oxyeleotris marmorata*	LC
28	**Gobiidae**	*Brachygobius kabiliensis*	LC
29		*Eugnathogobius illotus*	LC
30		*Eugnathogobius kabilia*	LC
31		*Eugnathogobius* cf. *siamensis*	LC
32		*Glossogobius sandakanensis*	NA
33		*Gobiopterus chuno*	DD
34		*Gobiopterus panayensis*	NA
35		*Hemigobius* cf. *hoeveni*	NA
36		*Hemigobius melanurus*	NA
37		*Mugilogobius rambaiae*	LC
38		*Pandaka pygmaea*	CR
39		*Periophthalmus malaccensis*	NA
40		*Pseudogobius javanicus*	NA
41		*Redigobius chrysosoma*	LC
42		*Stigmatogobius pleurostigma*	NA
43	**Anabantidae**	*Anabas testudineus*	DD
44	**Osphronemidae**	*Betta ocellata* *	LC
45		*Trichopodus trichopterus*	LC
46	**Channidae**	*Channa baramensis* *	NA
47		*Channa striata*	LC
48	**Tetraodontidae**	*Dichomyctere kretamensis* *	NA

Note: CR - Critically endangered, VU - Vulnerable, DD - Data deficient, LC - Least Concern, NA - Not available, *indicate endemic to Borneo

Appendix II. An illustrated checklist figures.

ANGUILLIDAE

Anguilla borneensis - ca. 140 mm SL

CYPRINIDAE

Barbodes sealei - 76.0 mm SL

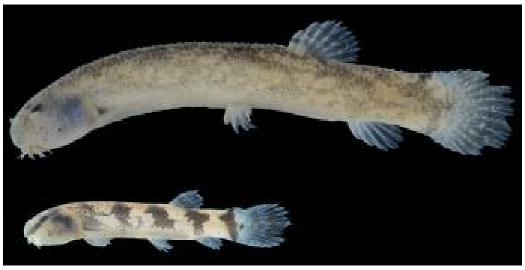
Nematabramis everetti - 84.0 mm SL

Rasbora cf. *hubbsi* - ca. 33.0 mm SL

Rasbora pycnopeza - ca. 70.0 mm SL

Rasbora cf. *semilineata* - male above 30.8 mm SL, female below 29.5 mm SL

COBITIDAE



Theriodes sandakanensis - 37.1 mm SL male, 13.3 mm SL juvenile

BAGRIDAE

Hemibagrus baramensis - ca. 100 mm SL

CLARIIDAE

Clarias leiacanthus - 115.0 mm SL

Clarias batrachus - 125.5 mm SL

PHALLOSTETHIDAE

Neostethus borneensis - male above 16.0 mm SL, female below 14.9 mm SL

Neostethus geminus - male above 25.2 mm SL, female below 22.3 mm SL

ZENARCHOPTERIDAE

Dermogenys bispina - male above 32.0 mm SL, female below 41.8 mm SL

Zenarchopterus buffonis - 80.0 mm SL

MASTACEMBELIDAE

Macrognathus keithi - 160.0 mm SL

AMBASSIDAE

Ambassis interrupta - 48.5 mm SL

Ambassis kopsii - 51.5 mm SL

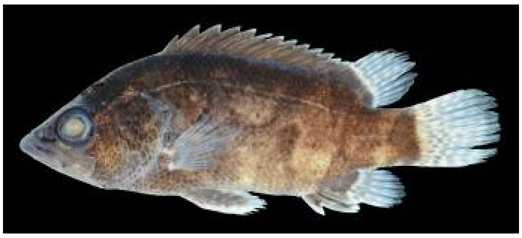
LEIOGNATHIDAE

Nuchequula blochi - 35.7 mm SL

TOXOTIDAE

Toxotes jaculatrix - 130.5 mm SL

NANDIDAE

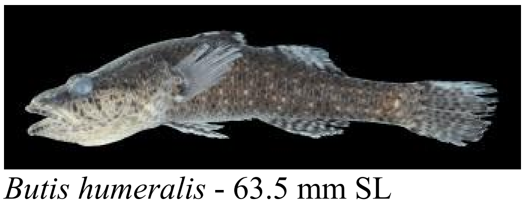


Nandus prolixus - 69.8 mm SL

CICHLIDAE

Oreochromis niloticus - 76.0 mm SL

BUTIDAE



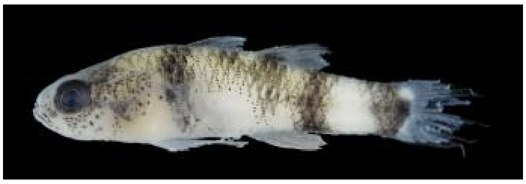
Butis humeralis - 63.5 mm SL

ELEOTRIDAE

Eleotris melanosoma - 56.7 mm SL

Oxyeleotris marmorata - 144.0 mm SL

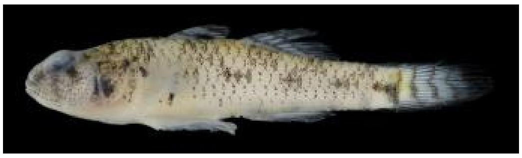
GOBIIDAE



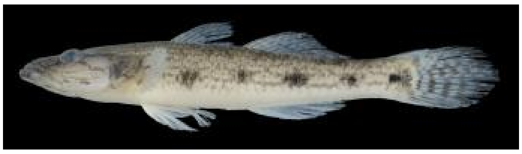
Brachygobius kabiliensis - 11.9 mm SL

Eugnathogobius illota - 32.0 mm SL

Eugnathogobius kabilia - male above 36.7 mm SL, female below 35.2 mm SL



Eugnathogobius cf. *siamensis* - 22.5 mm SL



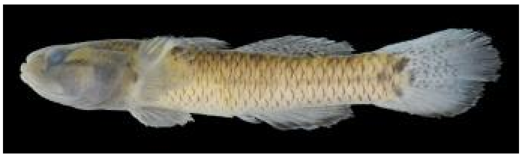
Glossogobius sandakanensis - 89.5 mm SL

Gobiopterus chuno - male above 20.0 mm SL, female below 15.9 mm SL

Gobiopterus panayensis - male above 17.2 mm SL, female below 16.0 mm SL

Hemigobius cf. *hoeveni* - male above 27.4 mm SL, female below 25.9 mm SL

Hemigobius melanurus - 3 individuals hanging vertically at water surface (typical behaviour), in-situ



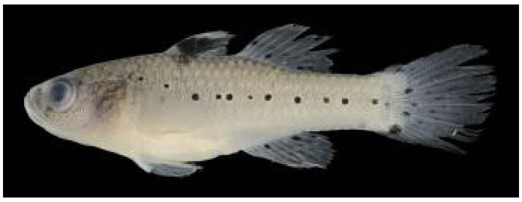
Mugilogobius rambaiae - 36.0 mm SL

Pandaka pygmaea - 9.6 mm SL

Periophthalmus malaccensis - 57.4 mm SL

Pseudogobius javanicus - 28.5 mm SL

Redigobius chryososoma - 28.5 mm SL



Stigmatogobius pleurostigma - 27.3 mm SL

ANABANTIDAE

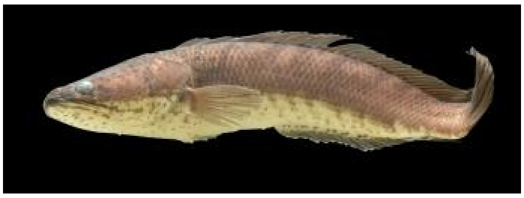
Anabas testudineus - 82.0 mm SL

OSPHRONEMIDAE

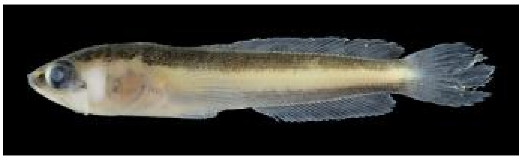
Betta ocellata - male above 55.0 mm SL, female below 63.6 mm SL

Trichopodus trichopterus - 76.7 mm SL

CHANNIDAE



Channa striata - 270 mm SL



Channa striata - 23.0 mm SL juvenile

TETRAODONTIDAE

Dichotomyctere kretamensis - 38.9 mm SL (dorsal and lateral views)

Dichotomyctere kretamensis - 54.4 mm SL (dorsal and lateral views)

Preliminary anuran checklist of Kabili-Sepilok Forest Reserve, Sabah

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Abstract. Tropical anurans are currently experiencing massive global decline as a result of habitat degradation, disease and climate change. Forest fragments within disturbed areas often provide an important refuge, supporting high levels of amphibian diversity. Unfortunately, a lack of baseline diversity data undermines potential conservation efforts in many fragmented forests. Here, we carried out a baseline anuran inventory within the Kabili-Sepilok Forest Reserve, in Sabah, Malaysian Borneo. This study aimed to provide useful baseline information for future studies on anurans in Kabili-Sepilok FR, as well as to contribute to our current knowledge of anuran diversity and distribution in Sabah. Specimens were surveyed by using Visual Encounter Survey (VES) method with the help of a torchlight to pinpoint sample locations; and whenever necessary, hand-grabbing technique. Throughout the study, 29 anurans from six families were recorded including 12 Bornean endemic species. The small forest fragments such as this can serve as viable habitats for anurans in the lowland rainforest in Sabah.

Keywords: anuran survey, Visual Encounter Survey (VES), Kabili-Sepilok Forest Reserve

INTRODUCTION

The global amphibian decline is a well-documented phenomenon, with up to 40% of species threatened with extinction (Stuart *et al*. 2004). Due to their environmental and physiological limitations, tropical anurans are highly sensitive to changes in their environment and are thus highly susceptible to habitat loss and degradation (Gibbon *et al*. 2000). The island of Borneo contains both high anuran diversity (over 180 species), and heavy levels of deforestation and habitat degradation (Gaveau *et al*. 2014). While the central regions of the island consist of protected areas and sustainably managed landscapes, the lowland areas of eastern Sabah comprise a mosaic of forest fragments and oil palm plantations (Stuebig *et al*. 2015). Forest fragments in such mosaics can support high anuran diversity (Riemann *et al*. 2015).

This study was conducted within the Kabili-Sepilok FR under the administration of the Sabah Forestry Department. Kabili-Sepilok FR was first gazetted on 2nd February 1931 and was later gazetted as a Class VI or Virgin Jungle Forest Reserve on 14th of March 1984. The reserve is located on 5° 49’ N/ 117° 57’ E in the district of Sandakan and comprises a range of disturbed and virgin lowland dipterocarp forest. Kabili-Sepilok FR is the site of several nature tourism and education centres such as, the Rainforest Discovery Centre (RDC), Sepilok Orang-utan Centre and the Bornean Sun Bear Conservation Centre. A checklist of the anurans of Kabili-Sepilok FR may thus enhance the value of Sepilok as a centre for nature-based tourism and environmental education. The objectives of this study were to produce the checklist of anurans in Kabili-Sepilok FR, to provide useful baseline information to build future studies on anurans in that area, as well as to contribute to our current knowledge of anuran diversity and the distribution in Sabah. Determining the anuran diversity of small forested areas is paramount for ensuring the protection of forest patches which may not support large mammalian populations, but can serve as valuable sites of anuran diversity in disturbed landscapes.

MATERIALS & METHODS

Sampling methods

A 10-day sampling was conducted in October and November, 2017 at the Rainforest Discovery Centre, Sepilok B&B and MY Nature Resort next to Kabili-Sepilok FR. All Visual Encounter Survey (VES) were conducted between 19:00 hrs and 22:00 hrs to coincide with the peak anuran activity period (Inger *et al*. 2017). As many Bornean amphibian breeding cues are triggered by rainfall, all samplings were conducted within 48 h of rain to maximise detections of calling and breeding anurans (Duellman & Trueb 1994). VES was carried out along established forest trails, small, heavily silted streams, waterfalls and small lakes. Areas along these locations, such as leaf litter piles, tree branches, stumps, puddles, small streams, waterfalls and tree holes were searched for individuals (Muslim 2017, Yong *et al.* 2013) with the help of torchlight to pinpoint their location. Hand-grabbing technique was used to catch reachable individuals (Yong *et al.* 2013).

Anuran identification

All specimens visually detected were identified to species and their respective microhabitat and GPS locations were recorded. Amphibians were identified to species based on Inger *et al*. (2017). All previously undetected species were captured, placed in a clear plastic bag, and transported to indoor controlled conditions for specimen photography (Kueh 2006). Following photo documentation, the specimens were released at the original site of capture.

Manual collection

Some representative individuals were put inside transparent plastic bags for preservation purpose. The individuals were euthanized, fixed in 10% formalin and then preserved in 70% ethanol (Ehwan *et al.* 2018). The voucher specimens were deposited at the Forest Research Centre, Sepilok.

RESULTS AND DISCUSSION

During the 10-day sampling period (30 h sampling effort), a total of 29 species belonging to six families, were recorded in the Kabili-Sepilok FR (Table 1). The richness documented herein represents 16.1% of Borneo's total anuran diversity. Twelve Bornean endemic species were recorded during the survey, namely *Limnonectes ingeri*, *Limnonectes leporinus*, *Limnonectes finchi*, *Occidozyga baluensis*, *Hylarana megalonesa*, *Hylarana raniceps, Kalophrynus meizon, Metaphrynella sundana, Microhyla borneensis, Polypedates otilophus, Rhacophorus rufipes,* and *Leptobrachium abbotti*. In addition, the finding of this survey show that there were four species of frogs from two families that were categorized as

"Near Threatened" (NT) by the International Union for Conservation of Nature (IUCN). The four species were *Limnonectes ingeri* (Greater Swamp Frog), *Occidozyga baluensis* (Seep Frog), *Nyctixalus pictus* (White Spotted Tree Frog), and *Rhachophorus rufipes* (Red-legged Tree Frog). The anurans encountered herein were a mixture of species that strictly inhabit primary forest as well as those may thrive in disturbed sites or even co-exist with humans. The mixture of habitat types in RDC and its surroundings may have contributed to the diversity of anuran species of different life-history traits and ecological niches.

It is acknowledged that the methods and time-frame over which the data were collected only allowed for enumeration of species richness. More surveys throughout the year will be required to detect elusive species and those with seasonal activity patterns. Additional methods such as pitfall trapping and drift fences may also be conducted for amphibians that are difficult to detect using traditional methods (such as small burrowing Microhylidae). This preliminary findings can be used to promote nature tourism in the conservation area. Bornean endemic and interesting anuran species, such as the *Polypedates otilophus, Metaphrynella sundana, Rhacophorus rufipes,* and *Limnonectes leporinus* can attract nature lovers. This fact eventually can be harnessed as a baseline and supplementary information for future research and also is able to be assessed as an additional product of nature tourism known as Anuran Tourism, which potentials can be measured by several criteria (Kueh 2005).

Table 1. List of frog and toad species in Kabili-Sepilok FR.

NO.	FAMILY	SPECIES		BORNEAN ENDEMIC	IUCN STATUS*
		Scientific name	**Common name**		
1	Dicroglossidae	*Limnonectes kuhlii*	Kuhl's Creek Frog	No	LC
2		*Limnonectes ingeri*	Greater Swamp Frog	Yes	**NT**
3		*Limnonectes leporinus*	Giant River Frog	Yes	LC
4		*Limnonectes finchi*	Rough Guardian Frog	Yes	LC
5		*Limnonectes palavanensis*	Smooth Guardian Frog	No	LC
6		*Occidozyga sumatrana*	Yellow-bellied Puddle Frog	No	LC
7		*Occidozyga baluensis*	Seep Frog	Yes	**NT**
8		*Fejervarya limnocharis*	Grass Frog	No	LC
9	Ranidae	*Hylarana erythraea*	Green Paddy Frog	No	LC
10		*Hylarana megalonesa*	White-lipped Stream Frog	Yes	NE
11		*Hylarana raniceps*	Jade-backed Stream Frog	Yes	LC
12		*Hylarana nicobariensis*	Cricket Frog	No	LC
13		*Hylarana picturata*	Spotted Stream Frog	No	LC
14		*Hylarana glandulosa*	Rough-sided Frog	No	LC
15	Bufonidae	*Duttaphrynus melanostictus*	Common Sunda Toad	No	LC
16		*Ingerophrynus divergens*	Crested Toad	No	LC
17	Mycrohylidae	*Kalophrynus meizon*	Bornean Sticky Frog	Yes	NE
18		*Metaphrynella sundana*	Hole-tree Frog	Yes	LC
19		*Microhyla borneensis*	Bornean Narrow mouthed Frog	Yes	LC
20		*Chaperina fusca*	Saffron-bellied Frog	No	LC
21	Rhacophoridae	*Nyctixalus pictus*	White Spotted Tree Frog	No	**NT**
22		*Polypedates leucomystax*	Four-lined Tree Frog	No	LC
23		*Polypedates coletti*	Collett's Tree Frog	No	LC
24		*Polypedates macrotis*	Dark-eared Tree Frog	No	LC
25		*Polypedates otilophus*	File-eared Tree Frog	Yes	LC
26		*Rhacophorus pardalis*	Harlequinn Tree Frog	No	LC
27		*Rhacophorus rufipes*	Harlequinn Tree Frog	Yes	**NT**
28		*Kurixalus appendiculatus*	Fringed Tree Frog	No	LC
29	Megophryidae	*Leptrobrachium abboti*	Lowland Large-eyed Litter Frog	Yes	LC

***NT**= Near Threatened, LC= Least Concern, NE= Not Evaluated

CONCLUSION

As a conclusion, it is noted that Kabili-Sepilok FR is home to a variety of anuran species and likely more, had the sampling period been conducted over a longer period. The recorded Bornean endemic anuran species provide salient information to enhance the conservation of Kabili-Sepilok FR. Continuous monitoring and enforcement within the reserve are important to minimize threats and adverse issues. This will ensure that the forest quality would improve in order to maintain its interesting biodiversity, including anurans.

ACKNOWLEDGEMENTS

Special thanks to Dr. Robert C. Ong, Head of Forest Research Centre, Sepilok for his support and encouragement throughout this study. We also thank Zulfazli bin Mokhtar and Dorilyne Daniel for rendering their assistance in the field. We are also grateful to Mr. Paul Yambun Imbun of the Sabah Parks for species identification.

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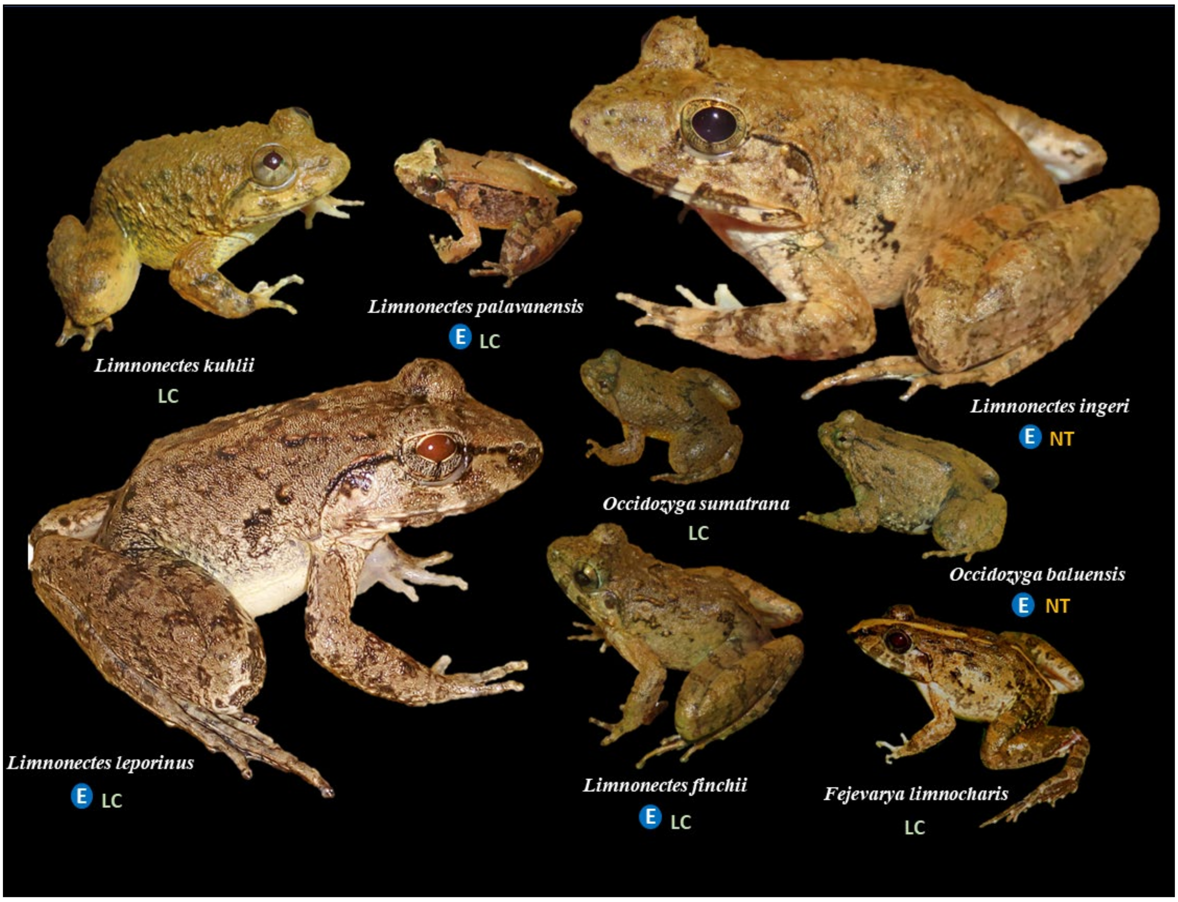

Plate 1. Family: Dicroglossidae (E= Endemic, NT= Near Threatened, LC= Least Concern).

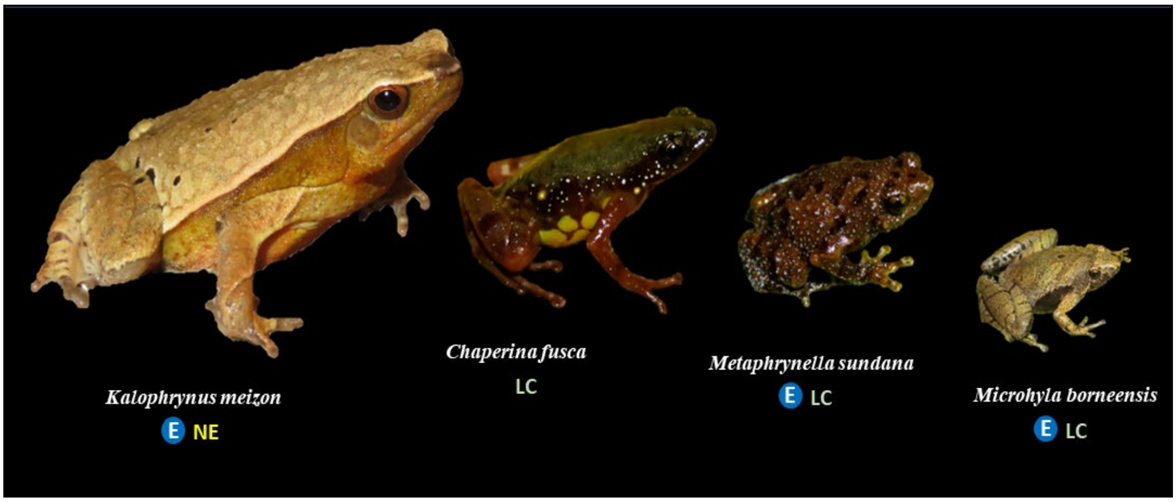

Plate 2. Family: Mycrohylidae (E= Endemic, LC= Least Concern, NE= Not Evaluated).

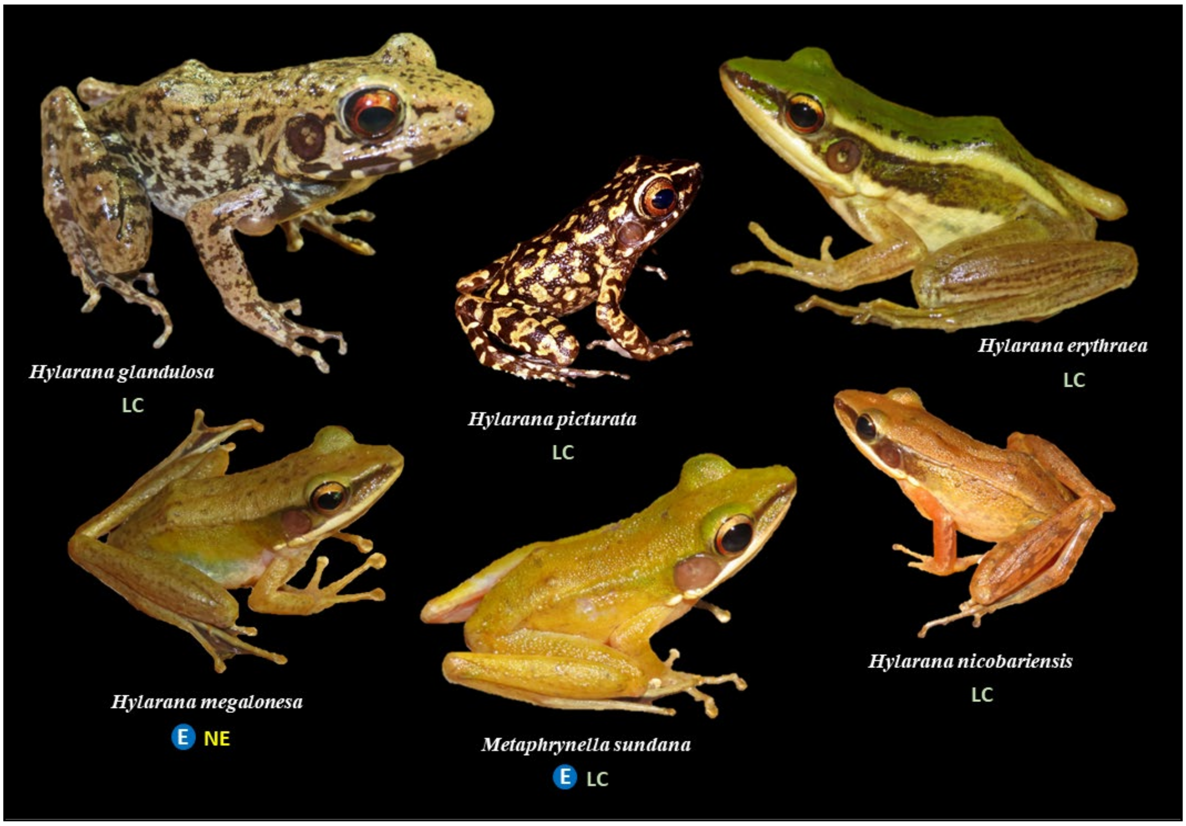

Plate 3. Family: Ranidae (E= Endemic, LC= Least Concern, NE= Not Evaluated).

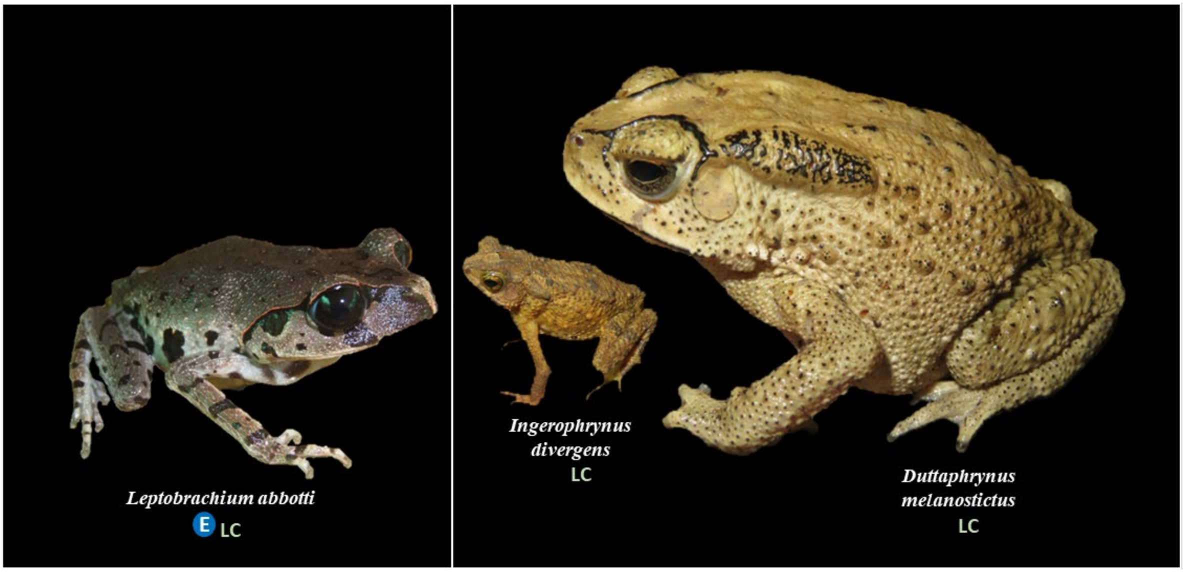

Plate 4. Family: Megophryidae (left), Bufonidae (right) (E= Endemic, LC= Least Concern).

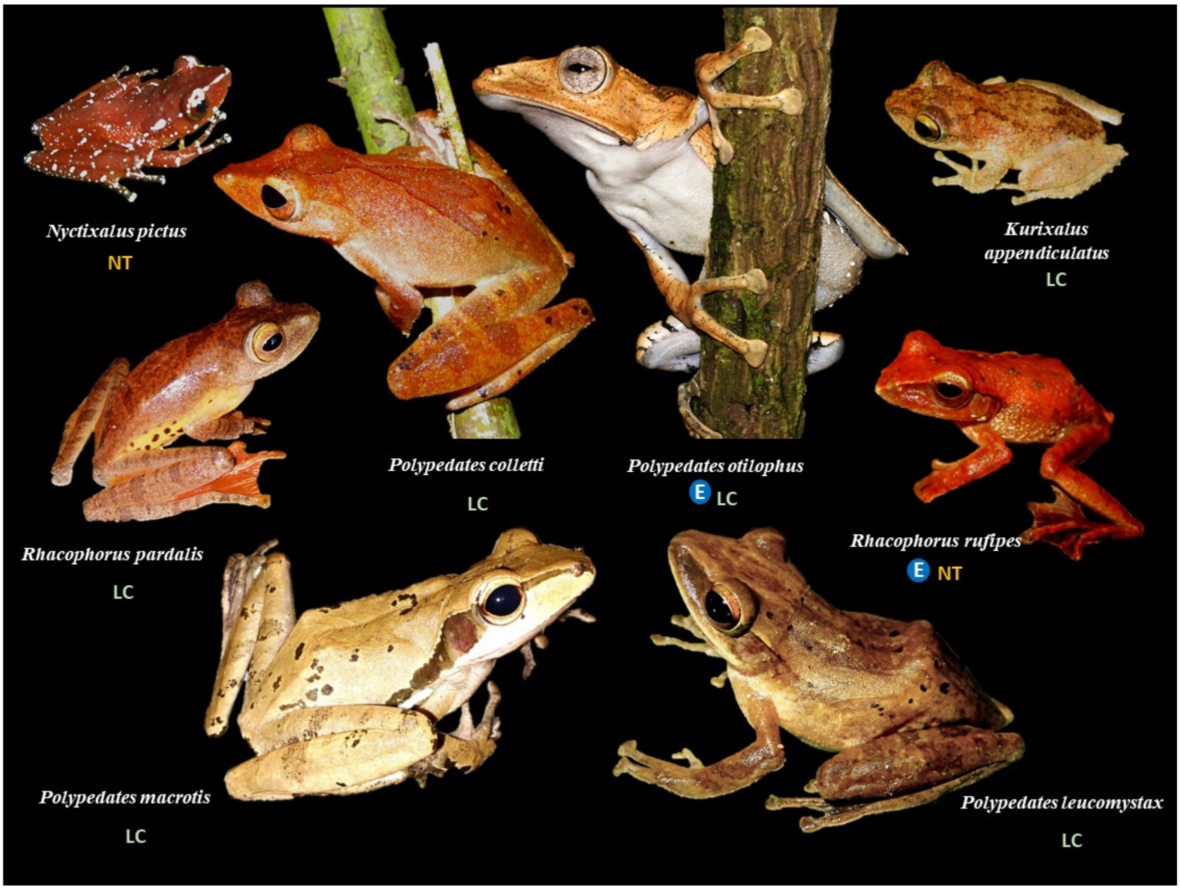

Plate 5. Family: Rhacophoridae ((E= Endemic, NT= Near Threatened, LC= Least Concern).

Evaluating the vegetative growth rates of *Etlingera coccinea* (Zingiberaceae) in smallholder agroforestry farms in Sabah, Malaysia

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Abstract. The increasing demand for *Etlingera coccinea* (Tuhau) as vegetables and other Tuhau-based products requires sufficient supply of the young shoots in Sabah, Malaysia. This paper presents the vegetative growth habits, and productivity of young Tuhau shoots under different management regimes in smallholder farms practising agrisilvicultural systems. Twelve rectangular plots measuring 5 m x 3 m which comprised of three plots of managed with tree(s) (MT), three plots of managed without tree (MNT), three plots of non-managed with tree(s) (NMT), and three plots of non–managed without tree (NMNT). The number of stalks and shoots and growth attributes data were collected every month from July 2017 until September 2018. Multiple regression showed that the type of plots and weather data are important factors that can affect the vegetative growth of Tuhau. The five principal growth stages of Tuhau are shoot development, leaf development, stalk elongation, development of vegetative rhizome and senescence.

Keywords: Agroforestry, *Etlingera coccinea*, number of new shoots, vegetative growth

INTRODUCTION

Etlingera coccinea (Blume) S. Sakai & Nagam of the Zingiberaceae family is locally known as Tuhau among the local people of Sabah, Malaysia. Traditionally, Tuhau leaves are used for making rice parcels, and the sheath is used to make handicrafts (Poulsen 2006). Tuhau was also known to remediate illness such as stomach ache, food poisoning and gastric problems (Shahid-Ud-Daula *et al*. 2015). Tuhau is most sought after for its pith, especially in the food cottage industry. Among many of the Tuhau based products that were developed includes pickles, Tuhau paste, Tuhau floss, Tuhau jam, and body scrub and soap made from Tuhau extract. The variety and versatility of Tuhau products have increased the demand for raw Tuhau.

The Tuhau plant proliferates naturally in the wild through rhizome and seeds but has poor performance in vegetative and reproductive growth and population spread (Jualang *et al*. 2015). Tuhau can be found in the gaps or shade in the forests, and near water sources such as streams with sandy or clayey soils with distances ranging around 30 up to 1150 m (Poulsen 2006). Relying only on the wild for harvesting Tuhau shoots is not productive, time-consuming and not sustainable. Alternatively, local people had been planting Tuhau traditionally within their homesteads and farms at a relatively small scale. The small-scale production of Tuhau raw material can only sustain small cottage industries and can limit the potential growth for commercialisation. Past research on Tuhau for the vast majority covered issues related to its chemical composition and its active ingredients, and Tuhau propagation via tissue culture method.

The growth, shoot development and flowering of Tuhau mentioned as *E. punicea* at Sabah Agriculture Park, Tenom, Sabah, Malaysia was hampered in old Tuhau clumps, improper management practices and the lack of sunlight and moisture (Yursi & Tsan 2011). The presence of large trees at the agriculture park competed with the Tuhau plants for growth requirements. Pests also damaged Tuhau plants. Tuhau showed better growth performance under shaded condition compared to non-shaded area in a small-scale trial at the arboretum of Universiti Malaysia Sabah (Ade Esah Azzahra 2019). Tuhau famous sister species, *E. elatior* (Kantan), took up to 22 days to form the first leaf whereas the inflorescence shoot emerged after 70 days from the similar rhizome as the leafy shoot (Choon & Ding 2016).

Our current research documented the vegetative growth stages from the emergence of its buds up to senescence. The number of new young shoots and the vegetative growth were recorded at two smallholder farms practicing different agrisilvicultural system, at different locations and ages. This research intends to provide the basis for improving Tuhau production under productive traditional agroforestry practices.

MATERIALS & METHODS

Study site

The study was conducted from July 2017 until September 2018. The first study site is a 15-year old smallholder home garden farm at Kg. Kokol, Manggatal located at N6° 00' 19.2", E116° 12' 20.94" with an elevation of 733–738 m. The second site is a five-year old smallholder multilayer tree garden farm at Kg. Marakau, Ranau located at N5° 57' 43.5", E116° 43' 34.8" with an elevation of 697–703 m. The study site is shown in Figure 1. Manggatal is a sub-district on the outskirt of the capital city Kota Kinabalu. Undulating lands characterise Ranau district with valley plain. Both sites are part of the west coast of the state of Sabah, Malaysia. At Kg. Kokol, the average total monthly rainfall was 48.8–583.9 mm, the total monthly number of rainy days was 9–24 days, the average monthly temperature was 26.5–28.9 °C and the mean relative humidity was 67.0–85.2 % throughout the observation period. At Kg. Marakau, the average total monthly rainfall was 99.6–437.6 mm, the average total monthly number of rainy days was 8-26 days, the average monthly temperature was 23.7–25.1 °C and the mean relative humidity was 73.5–88.0 %. Tuhau plants were planted at both sites.

At Kg. Kokol, the species of tree within the farm nearing to the plots are *Lansium domesticum* (Langsat), *Garcinia* sp. (Mangosteen), *Alstonia angustiloba* (Pulai), and *Litsea* spp. The tree crown spread ranged from 1.05–7.3 m and diameter at breast height (dbh) of 5.6–28.6 cm. *Litsea* spp. was the most abundant species and had the largest tree crown and dbh followed by Pulai tree. There were also *Ananas* sp. (Pineapple), *Arenga* *unditifolia* (*Polod* palm), *Etlingera elatior* (Torch ginger) and *Cymbopogon citratus* (Lemon grass). At Kg. Marakau, the species of tree within the farm nearing to the plots are *Neolamarckia cadamba* (*Laran*), *Durio zibethinus* (Durian) and *Garcinia* sp. (Mangosteen). The tree crown spread ranged from 6.35–47.75 m and dbh of 6.6–62.6 cm. The Laran trees are the most abundant and had the largest crown spread and dbh. There were also *Citrus microcarpa* (Calamondin), and *Areca catechu* (Betel nut).

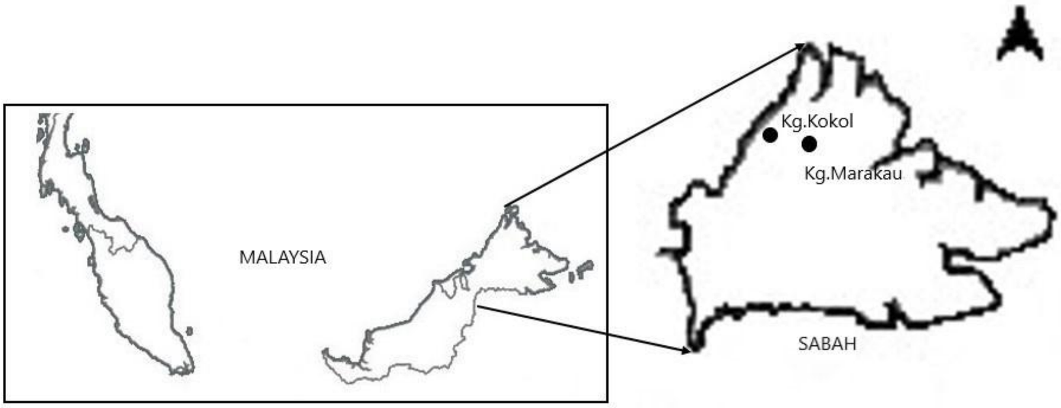

Figure 1. The location of Tuhau agroforestry farms at Kg. Kokol, Manggatal and Kg. Marakau, Ranau.

Plot establishment and data collection

At each site, a total of 12 rectangular plots measuring 5 m x 3 m were established based on Complete Randomized Design (CRD) comprising six managed and six non–managed plots. The farm owner managed the plots following traditional practices which include regular harvesting of young shoots, slashing of senesced leaf stalks and manual weeding. Non-managed plots are devoid of any management practices. Each 'management' plot was sub–divided into three subplots with tree(s) and three subplots without tree. Therefore, the plot treatments at each site were: (i) managed with tree(s) (MT), (ii) managed without tree (MNT), (iii) non–managed with tree(s) (NMT), and (iv) non–managed without tree (NMNT). At Kg. Kokol, Manggatal, there was a total of 218 Tuhau clumps with an average distance between 1.02 – 1.30 m. At Kg. Marakau, Ranau, there was a total of 175 Tuhau clumps, with an average distance between clumps was 0.97 – 1.24 m.

In an initial inventory in July 2017, the Tuhau clumps, stalks and shoots were counted, tagged, and the initial growth parameters of the tagged shoots i.e. length and number of leaves were recorded. The Tuhau clump refers to an individual Tuhau plant which arises from a rhizome and consist of several leafy shoots. The total length of the Tuhau stalk refers to the longest leafy stalk of a clump and was measured from the base (above ground) to the tip of the stalk. The final inventory was conducted 15 months after the first inventory in September 2018.

The vegetative growth stages were observed for the young shoots counted, tagged and designated in the first inventory. The growth parameters (i.e. length and the number of leaves) of the tagged shoots were also recorded monthly from July 2017 until September 2018. The vegetative growth stages of Tuhau were recorded and then described based on the general scale of the Biologische Bundesanstalt, Bundessortenamt and Chemical industry (BBCH) scale (Meier, 2001, pp. 9-12). The principal growth stages and secondary growth stages are given a number from 0-9 respectively to indicate the progression of the Tuhau growth stages. The principal growth stage is the identifiable main phases of plant development which is represented by the first number in the double-digit BBCH code. The secondary growth stage is the progress of plant growth of each principal growth stage which is the second number in the double-digit BBCH code.

The number of stalks and the number of new shoots that emerged within every plot were recorded monthly from July 2017 until September 2018. The monthly average weather data i.e. the amount of rain, number of rainy days, temperature and relative humidity during the study period were obtained from the Malaysian Meteorological Department for regression analysis.

Data analysis

The Relative Growth Rate (RGR) of length was calculated based on Hoffman & Poorter (2002) with modification. The formula used is as follows:

$$RGR\ of\ length = \frac{L_2 - L_1}{t_2 - t_1}$$

Where;

L_1 = Length at initial month

L_2 = Length at subsequent month

t_1 = Time at initial month

t_2 = Time at subsequent month

The number of new stalks and new shoots, vegetative growth and the RGR of length in the different management regimes were analysed using one-way ANOVA. The relationship between the number of stalks, new shoots, the vegetative growth and the RGR of length with the meteorological data were analysed using stepwise multiple regression analysis. The analyses were done using R software version 3.6.1 (R Core Team 2019).

RESULTS

Number of stalks

At Kg. Kokol, Manggatal, the monthly average number of Tuhau stalks was 391 stalks in the managed plots and 306 stalks in the non–managed plots (Figure 2). The plots without tree had a greater number of stalks compared to the plots with tree(s). At Kg. Marakau, Ranau, the monthly average number of Tuhau stalks was 256 stalks in the managed plots and 147 stalks in the non–managed plots (Figure 3). There was a higher number of stalks in MNT plots at both sites. For Kg. Kokol, Manggatal, there were significant differences in the number of stalks between the plots throughout the observation period ($p<0.05$). The post hoc comparisons using Tukey HSD showed that the NMT plots were generally significantly different from MNT plots in 2017, whereas the NMNT plots were significantly different from MT plots in 2018. At Kg. Marakau, Ranau, there were significant differences in the number of stalks between the plots in August to October 2017 ($p<0.05$). Tukey HSD showed that the NMNT plots were significantly different from the MNT plots.

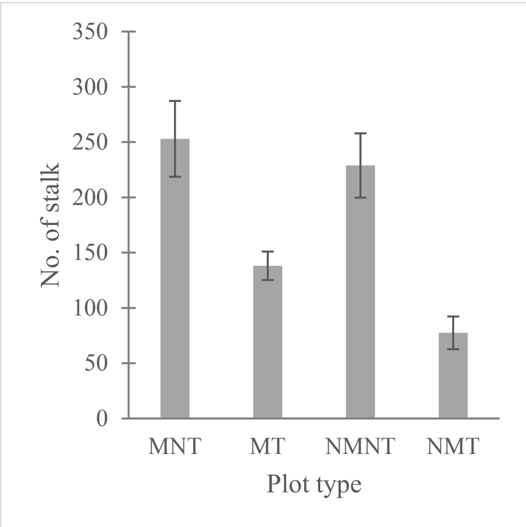

Figure 2. Monthly average number of Tuhau stalks at Kg. Kokol, Manggatal.

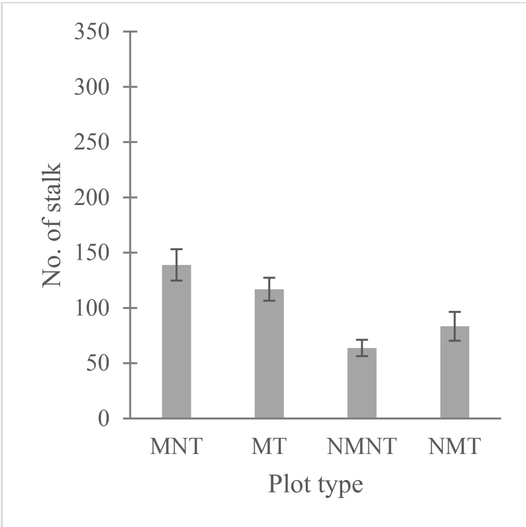

Figure 3. Monthly average number of Tuhau stalks at Kg. Marakau, Ranau.

(Note: MNT= Managed plots without tree, MT= Managed plots with tree, NMNT= Non-managed plots without tree, NMT= Non-managed plots with tree)

Number of new shoots

The monthly average number of Tuhau new shoots was 30 shoots in the managed plots and 18 shoots in the non–managed plots at Kg. Kokol, Manggatal (Figure 4). At Kg. Marakau, Ranau the monthly average number of Tuhau new shoots were 20 shoots in the managed plots and eight shoots in the non–managed plots (Figure 5). The number of Tuhau new shoots was higher in the managed plots than in the non–managed plots at both sites. Management activities such as manual weeding and removing old and unhealthy stalk were favourable for the production of Tuhau shoots. Generally, there was a higher number of shoots in the plots without tree(s). There were fewer Tuhau clumps near or around the evergreen *Garcinia* sp. at both sites. However, there were more Tuhau clumps near the *Neolamarckia cadamba* tree at Kg. Marakau.

For Kg. Kokol, Manggatal, the ANOVA showed significant differences in the number of Tuhau shoots between the plots from July to December 2017 ($p<0.05$). The post hoc comparisons using Tukey HSD test showed that the MNT plots generally were significantly different from the NMNT plots. For Kg. Marakau, Ranau, there was a significant difference in the number of Tuhau shoots ($p<0.05$) from May to September 2018. The Tukey HSD test showed that MNT plots were generally significantly different from the NMT and NMNT plots.

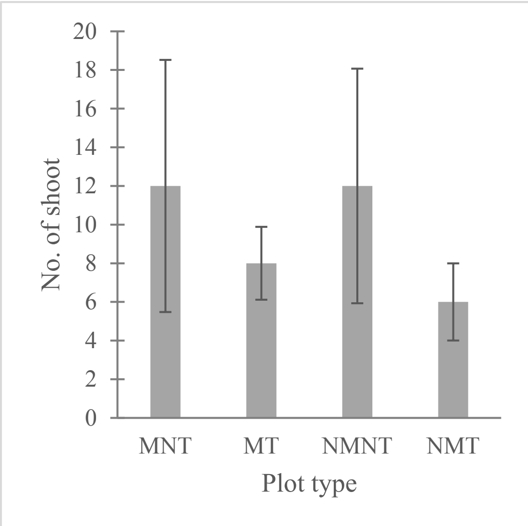

Figure 4. Monthly average number of Tuhau shoots at Kg. Kokol, Manggatal.

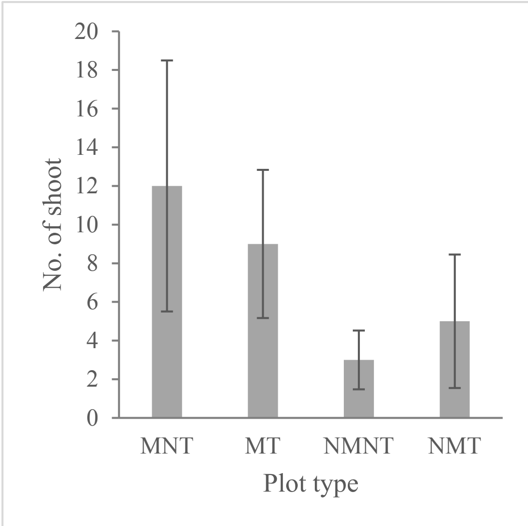

Figure 5. Monthly average number of Tuhau shoot at Kg. Marakau, Ranau.

(Note: MNT= Managed plots without tree, MT= Managed plots with tree, NMNT= Non-managed plots without tree, NMT= Non-managed plots with tree)

Vegetative growth

Total length

At Kg. Kokol, the ANOVA showed no significant difference between all types of plots throughout the observation period. At Kg. Marakau, only in July 2017 which is during the initial observation showed a significant difference in the total length between the plots. The Tukey HSD test showed that MT plots were significantly different from MNT and NMNT plots. By the end of the study period, the longest Tuhau at Kg. Kokol was observed in the NMT plots (4.68 m) whereas the longest Tuhau at Kg. Marakau was observed in the MT plots (4.32 m) (Table 1).

Number of leaves

The ANOVA showed that there were significant differences in the number of leaves between the plots after August 2018 at both sites. Based on the Tukey HSD test, the NMT plots were significantly different from the other plots at Kg. Kokol. At Kg.

Marakau, the NMT plots was significantly different from the MNT plots. The highest number of leaves at Kg. Kokol was 20 leaves in the NMT plots whereas the highest number of leaves at Kg. Marakau was 24 leaves in the MNT plots (Table 1).

Table 1. Summary of Tuhau growth characteristics under different types of plots at Kg. Kokol, Manggatal and Kg. Marakau, Ranau.

Site	Plot	Average total length (m)			Average no. of leaf		
		Jul 2017	Feb 2018	Sep 2018	Jul 2017	Feb 2018	Sep 2018
Kg. Kokol	MNT	0.27a	3.22a	4.28a	0	14a	12a
	MT	0.29a	3.54a	4.44a	0	15a	11a
	NMNT	0.28a	3.27a	4.32a	0	15a	11a
	NMT	0.26a	2.99a	4.68a	0	13a	20a
Kg. Marakau	MNT	0.23a	3.12a	4.23a	0	15a	24a
	MT	0.1b	3.28a	4.32a	0	16a	20ab
	NMNT	0.16a	-	-	0	-	-
	NMT	0.2ab	3.05a	4.00a	0	16a	12b

(Note: MNT= Managed plots without tree, MT= Managed plots with tree, NMNT= Non-managed plots without tree, NMT= Non-managed plots with tree)
Values with the same letters are not significantly different at $p < 0.05$.

Vegetative growth stages

The emergence of the Tuhau shoot from the rhizome marks the start of the vegetative phase. Tuhau shoot is reddish, greenish or brownish in colour and sometimes may appear spiky due to the apex of the sheaths protruding outwards. At the shoot stage, the Tuhau base did not produce a bulbous structure. The leaf development started with the emergence of the first small leaf followed by the emergence of cigar–like leaves that unfolded one after another. It took 25 days and 56 days for the shoot to develop two to three leaves at Kg. Kokol and at Kg. Marakau respectively. The leaves grew closer together as the Tuhau plant matured. After three months (90 days), the bulbous base of the Tuhau stalk was visible and started to develop a vegetative rhizome. The commencement of the leaf senescence occurred at 214 days and 237 days (6 months after the first inventory) at Kg, Kokol and Kg, Marakau respectively, usually indicated by the wilting of the lowermost leaves of the leaf stalk. At the end of the 15 months observation period, 50% of the leaves on each leaf stalk had wilted while other leaves had yellow discolouration. Although the leaf stalk began to senesce, new leaf emerged on the leaf stalk based on monthly observation. Leaf stalk that completely senesced were those attacked by pests.

The vegetative growth stages of Tuhau categorized and described based on the BBCH scale (Meier 2001). There are five principal growth stages of Tuhau. Principal growth stage 0 is the bud development (Figure 6); stage 1 is for leaf development (Figure

7); stage 3 is stalk elongation (Figure 8); stage 4 is the development of a vegetative organ (Figure 9), and stage 9 is the senescence (Figure 10). According to the BBCH scale (Meier 2001), each of the principal growth stages can be sub-categorized into secondary growth stages and described based on the specific growth developments. The principal growth stages and secondary growth stages of Tuhau are depicted in Figure 6 to Figure 9, with respective descriptions.

Figure 6. Tuhau principal growth stage 0 - Bud development. Stage 09, Emergence of shoot through the soil surface.

Figure 7. Tuhau principal growth stage 1 - Leaf development. A: Stage 10, first small true leaf visible, one cigar leaf visible; B: Stage 11, leaf unfolded; Stage 12, two leaves unfolded; C: Stage 13, three leaves unfolded; D: Stage 19, nine or more leaves unfolded.

Figure 8. Tuhau principal growth stage 3 - Stalk elongation. A: Stage 31, stalk 10% of final length; B: Stage 31, stalk 20% of final length; C: Stage 33, stalk 30% of final length; D: Stage 39, maximum stalk length reached.

Figure 9. Tuhau principal growth stage 4 - Development of vegetative organ. Stage 40, the vegetative rhizome begins to develop.

Figure 10. Tuhau principal growth stage 9 - Senescence. A: Stage 93, lowermost leaves begin to wilt; B: Stage 95, 50% of leaves wilted.

Relative Growth Rate (RGR)

Figure 11 and Figure 12 showed the trend of RGR of Tuhau length under different types of plots at Kg. Kokol, Manggatal and Kg. Marakau, Ranau. The RGR of length at Kg. Kokol generally follows the rainfall amount and the number of rainy days trend. There was an increase in the RGR of length during the high rainfall amount and frequent rainy days and *vice versa*, The RGR of length at Kg. Marakau generally showed a similar trend with the rainfall amount and relative humidity. The RGR of length increase when the rainfall amount and relative humidity increase and *vice versa*. The RGR of length value was higher during the early growth of Tuhau and decreased as the Tuhau matured. The growth spike after May 2018 at both sites was due to the reduction and death of some Tuhau stalks that were tagged for observation. The RGR of length indicated that Tuhau at Kg. Kokol generally had faster growth compared to the Tuhau at Kg. Marakau during the early growth stages. In August 2017, the highest value of RGR of Tuhau length at both sites was 0.0346 m day^{-1} in the NMT plots at Kg. Kokol, and 0.0252 m day^{-1} in NMNT plots at Kg. Marakau (Table 2). In September 2018, almost all plots showed no increase in growth rate. ANOVA showed no significant difference in the RGR of length between all type of plots throughout the observation period (p>0.05).

Table 2. Summary of Tuhau Relative Growth Rate of length under different types of plots at Kg. Kokol, Menggatal and Kg. Marakau, Ranau.

Site	Plot	RGR of length (m day^{-1})		
		Aug 2017	Feb 2018	Sep 2018
Kg. Kokol	MNT	0.0211a	0.0055a	0.0000a
	MT	0.0236a	0.0092a	0.0029a
	NMNT	0.0283a	0.0039a	0.0000a
	NMT	0.0346a	0.0137a	0.0000a
Kg. Marakau	MNT	0.0107a	0.0056a	0.0019a
	MT	0.0136a	0.0071a	0.0000a
	NMNT	0.0252a	-	-
	NMT	0.0138a	0.0047a	0.0000a

(Note: MNT= Managed plots without tree, MT= Managed plots with tree, NMNT= Non-managed plots without tree, NMT= Non-managed plots with tree)
Values with the same letters are not significantly different at $p < 0.05$.

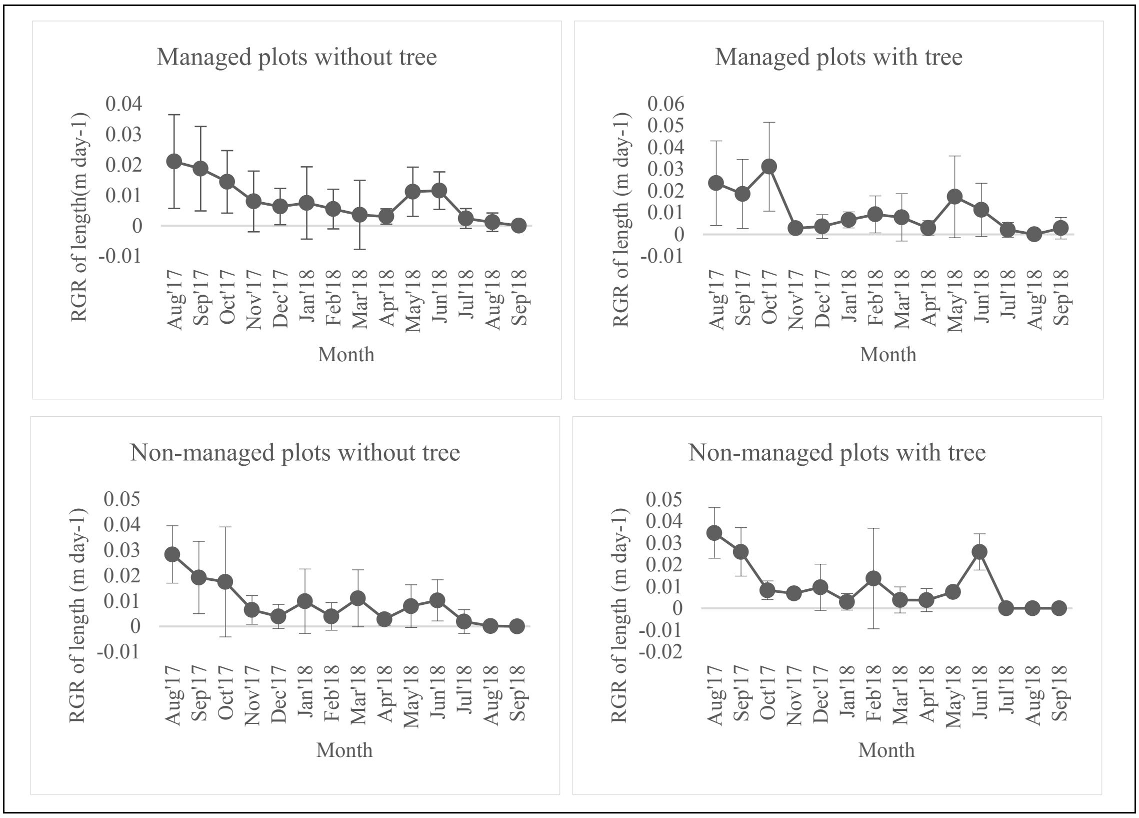

Figure 11. Relative Growth Rate of Tuhau length at Kg. Kokol.

Figure 12. Relative Growth Rate of Tuhau length at Kg. Marakau.

Multiple regression analysis

Number of stalks

Table 3 and Table 4 showed the multiple regression model of the number of stalks at Kg. Kokol, Manggatal and Kg. Marakau, Ranau respectively. The plot type was designated as the factor, and the meteorological data designated as the variable in the regression analysis. Both sites had a significant (p<0.05) multiple regression models with adjusted R^2 of 0.91 at Kg. Kokol and 0.90 at Kg. Marakau respectively. The significant predictor variables of the number of stalks at Kg. Kokol is the type of plots and the number of rainy days. The significant predictor variables of the number of stalks at Kg. Marakau is the type of plots and relative humidity.

Table 3. Multiple regression model of the number of stalks at Kg. Kokol, Menggatal.

Adjusted R-square (R^2) = 0.91***

No. of stalk (MNT) = 298.10*** – 2.71(No. of rainy day)***

No. of stalk (MT) = 298.10*** – 2.71(No. of rainy day)*** – 114.8(MT)***

No. of talk (NMNT) = 298.10*** – 2.71(No. of rainy day)*** – 24.07(NMNT)***

No. of stalk (NMT) = 298.10*** – 2.71(No. of rainy day)*** – 175.47(NMT)***

(Note: *** : p < 0.001. MNT= Managed plots without tree, MT= Managed plots with tree, NMNT= Non-managed plots without tree, NMT= Non-managed plots with tree)

Table 4. Multiple regression model of the number of stalks at Kg. Marakau, Ranau.

Adjusted R-square (R^2) = 0.90***

No. of stalk (MNT) = –386.77** – 0.02(Rainfall amount) + 1.18(Relative humidity)*

No. of stalk (MT) = –386.77** – 0.02(Rainfall amount) + 1.18(Relative humidity)* – 22.00(MT)***

No. of stalk (NMNT) = –386.77** – 0.02(Rainfall amount) + 1.18(Relative humidity)* – 75.13(NMNT)***

No. of stalk (MT) = –386.77** – 0.02(Rainfall amount) + 1.18(Relative humidity)* – 55.53(NMT)***

(Note: * : p < 0.05, ** : p < 0.01, *** : p < 0.001. MNT= Managed plots without tree, MT= Managed plots with tree, NMNT= Non-managed plots without tree, NMT= Non-managed plots with tree)

Number of shoots

Table 5 and Table 6 showed the multiple regression model of the number of shoots at Kg. Kokol, Manggatal and Kg. Marakau, Ranau respectively. The plot type was designated as the factor, and the meteorological data designated as the variable in the regression analysis. Both sites had a significant (p<0.05) multiple regression models with adjusted R^2 of 0.70 at Kg. Kokol and 0.37 at Kg. Marakau respectively. At Kg. Kokol, the type of plots, temperature and relative humidity are the significant predictor variables for the number of shoots. For Kg. Marakau, only the type of plots had a significant effect on the number of shoots.

Table 5. Multiple regression model of the number of shoots at Kg. Kokol, Manggatal.

Adjusted R-square (R^2) = 0.70***

No. of shoot (MNT) = 215.69*** – 5.58(Temperature)*** – 0.50(Relative humidity)*

No. of shoot (MT) = 215.69*** – 5.58(Temperature)*** – 0.50(Relative humidity)* – 13.07(MT)***

No. of shoot (MT) = 215.69*** – 5.58(Temperature)*** – 0.50(Relative humidity)* – 9.20(NMNT)***

No. of shoot (MT) = 215.69*** – 5.58(Temperature)*** – 0.50(Relative humidity)* – 15.73(NMT)***

(Note: * : $p < 0.05$, *** : $p < 0.001$. MNT= Managed plots without tree, MT= Managed plots with tree, NMNT= Non-managed plots without tree, NMT= Non-managed plots with tree)

Table 6. Multiple regression model of the number of shoots at Kg. Marakau, Ranau.

Adjusted R-square (R^2) = 0.37***

No. of shoot (MNT) = 12.55*** – 0.003(Rainfall amount)

No. of shoot (MT) = 12.55*** – 0.003(Rainfall amount) – 3.27(MT)*

No. of shoot (NMNT) = 12.55*** – 0.003(Rainfall amount) – 9.00(NMNT)***

No. of shoot (NMT) = 12.55*** – 0.003(Rainfall amount) – 6.53(NMT)***

(Note: * : $p < 0.05$, *** : $p < 0.001$. MNT= Managed plots without tree, MT= Managed plots with tree, NMNT= Non-managed plots without tree, NMT= Non-managed plots with tree)

Vegetative growth

Table 7 and Table 8 showed the multiple regression model of the Tuhau growth parameters at Kg. Kokol, Manggatal and Kg. Marakau, Ranau respectively. The plot type was designated as the factor, and the meteorological data designated as the variable in the regression analysis. The regression models of the growth parameters of Tuhau at both sites were significant different ($p<0.05$). At Kg. Kokol, the number of rainy days has a significant effect on the total length, the number of leaf and RGR of length. The regression output showed that, the type of plots at Kg. Kokol had an insignificant effect on the total length, the number of leaf and RGR of length. At Kg. Marakau, the rainfall amount had a significant effect on the total length and number of leaves. Only the number of leaves is significantly affected by temperature. The number of rainy day is the significant predictor for the RGR of length. The regression output also showed that the type of plots at Kg. Marakau is one of the predictor variables for the total length and number of leaves.

Table 7. Multiple regression model of the growth of Tuhau at Kg. Kokol, Manggatal.

Adjusted R^2 = 0.24***

Total length = 10.34*** – 0.10(No. of rainy day)* – 0.07(Relative humidity)

Adjusted R^2 = 0.16***

No. of leaf = 21.31*** – 0.61(No. of rainy day)***

Adjusted R^2 = 0.39***

RGR of length = –0.009* + 0.0008(No. of rainy day)* + 0.00002(Rainfall amount)

(Note: *** : $p < 0.001$, * : $p < 0.05$)

Table 8. Multiple regression model of the growth of Tuhau at Kg. Marakau, Ranau

Adjusted R^2 = 0.28**

Total length (MNT) = –17.16 – 0.01(Rainfall amount)** + 0.87(Temperature)

Total length (MT) = –17.16 – 0.01(Rainfall amount)** + 0.87(Temperature) + 0.09(MT)

Total length (NMNT) = –17.16 – 0.01(Rainfall amount)** + 0.87(Temperature) – 1.52(NMNT)*

Total length (NMT) = –17.16 – 0.01(Rainfall amount)** + 0.87(Temperature) – 0.04(NMT)

Adjusted R^2 = 0.29***

No. of leaf (MNT) = –112.3 – 0.03(Rainfall amount)** + 5.44(Temperature)*

No. of leaf (MT) = –112.3 – 0.03(Rainfall amount)** + 5.44(Temperature)* – 0.47(MT)

No. of leaf (NMNT) = –112.3 – 0.03(Rainfall amount)** + 5.44(Temperature)* – 9.43(NMNT)*

No. of leaf (NMT) = –112.3 – 0.03(Rainfall amount)** + 5.44(Temperature)* – 2.20(NMT)

Adjusted R^2 = 0.26***

RGR of length = –0.0043 + 0.0006(No. of rainy day)***

(Note: *** : $p < 0.001$, ** : $p < 0.01$, * : $p < 0.05$. MNT= Managed plots without tree, MT= Managed plots with tree, NMNT= Non-managed plots without tree, NMT= Non-managed plots with tree)

DISCUSSION

At Kg. Kokol, the number of stalks and shoots was affected by the type of plots (management and presence of tree) and weather. Management practices such as the removal of old and unhealthy stalks encouraged the translocation of photoassimilates for the development of new vegetative buds until it developed leaves to conduct its own photosynthesis. In *Etlingera elatior*, the inflorescences relied on the stalks as a nutrient source for its development (Choon & Ding 2016). The higher number of stalks in managed plots is related to the higher number of shoots in managed plots. The increasing number of Tuhau stalks act as the sources of nutrient supply whereas the Tuhau shoot is an active sink organ which demands an adequate supply of growth sources (Choon & Ding 2016).

Low productivity of Tuhau shoots in the plots with tree(s) could be influenced by the presence of different tree species. The differing tree species have different tree architecture, morphology and habit present different competition pressure on the proliferation of Tuhau plant. At Kg. Marakau, the NMT plots had a higher number of stalks and shoots compared to the NMNT plots. The fallen branches of *N. cadamba* injured the Tuhau stalks and was replaced by new growth which mimics the management practices of removing old stalks. There were lesser Tuhau stalks and shoots near the *Garcinia* sp. tree at both sites. The dense foliage of *Garcinia* sp. tree causes heavy shading and its short heights hinder the growth of Tuhau near or underneath. Tuhau is a tall shrub plant that grows in the forest gaps (Poulsen 2006, Poulsen 2007), thus thrives with some exposure to sunlight. Even though there are several studies reporting the allelopathic potential of *Garcinia* spp. (Dhanya & Benny 2016, Md. Mahfuzur *et al*. 2019), there is no evidence that the Tuhau at both sites were affected by the allelopathic properties of the *Garcinia* sp. The Tuhau growth was most likely affected by reduced exposure to sunlight under *Garcinia* sp.

At both sites, other tree species did not hinder the growth of Tuhau near or underneath the tree. Fertilizer was not applied to the Tuhau plant. But, stalks that were cut down or senesce were left on the ground to decompose and thus contributed to the fertility of the soil. The tree(s) on the farms are more deep-rooted compared to the creeping Tuhau. Thus, the root system of Tuhau and the tree(s) are non–competitive or may have complementary root interaction. According to Umrani & Jain (2010), indigenous trees within the agroforestry system did not cause a negative effect on the crop yield. Further study is required to evaluate the root competition for growth resources between Tuhau and other tree species. Certain tree-crop interaction may cause adverse effects such as competition and allelopathy but suitable combinations lead to a positive complementary effect (Toppo & Toppo 2019).

The growth of Tuhau at both Kg. Kokol and Kg. Marakau generally showed no significant differences between the plots. Management alone does not influence the growth (total length, number of leaves, RGR of length) of Tuhau. The RGR of Tuhau length was higher during the early growth which decreased as it matures, which was similarly demonstrated in the RGR of length in *C. longa (*Rao *et al.* 2006) and *C. xanthorrhiza* (Bambang & Samanhudi 2011). Higher RGR of length during the early growth signifies an active growing phase of Tuhau. The decrease of Tuhau RGR of length signifies the leaf stalk maturity and new generative phase that produces new tillers. The little or no increase in the RGR of length after 12 months observation indicates the decrease of photosynthesis rates due to the senescence process. At Kg. Kokol, the number of rainy days, relative humidity and rainfall amount are the important variables affecting the growth of Tuhau. At Kg. Marakau, the plot type, rainfall amount, temperature and number of rainy days are the important variables affecting the growth of Tuhau. We found that the increment of Tuhau total length was high during the less frequent rainy day and less humidity. This finding was not in agreement with Atiyong *et al.* (2018) where the yield of *Z. officinale* increased as the rainfall amount increased. The effect of tree shade within the site, resulted in a lower temperature, minimized soil evaporation which creating environment unique to the sites. The positive effect of temperature on the growth of Tuhau was similar to *C. longa* which reported increased sprouting and growth at higher temperatures (Ishimine *et al.* (2004). In this study, Tuhau growth was observed in a single growing phase and is not able to provide the response of Tuhau against long-term fluctuating weather. The growth characteristics of Tuhau did fluctuate based on the fluctuations of the 15 months meteorological data and in different months.

It was not possible to determine the age of the individual clumps, nor to determined which stalk belong to which clumps without digging out the rhizome (Poulsen 2006). The stalks were considered as a clump if the stalks were less than 50 cm apart (Poulsen 2006). However, there was a huge difference in the period of Tuhau farm establishment between the two sites (15- and 5-years old smallholder agroforestry farm). The farm at Kg. Kokol was comparatively larger and older than at Kg. Marakau. Thus, there are more Tuhau plants or clumps at Kg. Kokol than at Kg. Marakau. However, both sites cannot be statistically compared.

The frequency of harvesting Tuhau shoots at Kg. Kokol was thrice per month or every 10 days). Tuhau shoots were harvested at least once a month at Kg. Marakau. We assumed that the higher harvesting frequency actively increases the translocation of photoassimilates to develop vegetative bud. The farmers usually harvested Tuhau stalks when it reaches one and a half meters to be sold at the open-air market. The time required for a young shoot to reach harvestable length is 60 days (two months) at Kg. Kokol, and up to 120 days (four months) at Kg. Kokol. Therefore, the growth rate of Tuhau is faster at Kg. Kokol than Kg. Marakau. In the future, a planting trial to determine the relationship between age of clumps and frequency of harvesting to the vegetative growth performance of Tuhau is recommended. The growth performance of Tuhau on different soil types is not studied. The soil type is clayey but soil analysis is required. Poulsen (2006) reported that Tuhau can be found near water sources on sandy or clayey soils.

CONCLUSION

In this study, the number of Tuhau shoots and stalks can be improved through good management practices by removing old and unhealthy stalks and weeding. The tree-crop interaction in relation to space and shading should be taken into considerations when integrating Tuhau under or near tree canopies. The type of plots (management and presence of trees) and meteorological conditions are important factors that affected the vegetative growth of Tuhau. The principal and secondary growth stages were identified and described from shoot establishment until senescence stage. Based on this study, the knowledge gaps regarding Tuhau growth and productivity is identified and recommended for further study. Long-term observation over several growth cycles of Tuhau is required to verify the relationship between growth performance and meteorological data. The study on vegetative growth of Tuhau under the specific shade condition is recommended because the optimum light intensity requirement of Tuhau is not known. The optimum harvestable size of Tuhau is also recommended for future study.

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Influence of grinding method on the precision of multi-element analysis of plant materials

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Abstract. The precision of analytical measurements is contingent upon appropriate sample preparation, particularly the sample comminution steps that involve direct manipulation of the raw materials. Here, we evaluated the effects of sample comminution using two commercial grinders with respect to the repeatability of multi-element analysis of plant materials. Wood and leaf samples were ground using a cutting mill and a Wiley mill to pass through a 2-mm sieve, following which an aliquot was subsampled for ball-milling using a mortar grinder. The latter served as an objective standard for repeatability for the former methods. The samples were analysed for total K, Na, Ca, Mg, P, Al, Mn, and Fe on an inductively coupled plasma-optical emission spectrometer. The repeatability of K, Ca, Mg, P, and Mn were all within the acceptable range of coefficient of variation (CV) while Na, Al, and Fe exhibited significant uncertainty (CV > 10%) and were thus excluded in further statistical tests. As expected, the mean CV indices were the lowest in samples prepared using mortar grinder compared to cutting mill and Wiley mill. However, most of the differences were insignificant (all $p > 0.0125$) except in the wood sample ground using Wiley mill that yielded more variable measurements ($p = 0.007$). The differences in repeatability with respect to grinding methods can be attributed to heterogeneity in particle size that influences representative subsampling of the test samples. The choice of grinding method may therefore affect the precision of multi-element analysis of plant samples, particularly fibrous woody matrices.

Keywords: Analytical chemistry, sample preparation, elemental analysis

INTRODUCTION

Within the past decade, single-element analytical instruments such as atomic absorption spectroscopy have been gradually supplanted by analytical tools employing inductively coupled plasma (ICP)-spectrometry such as ICP-mass spectrometry (ICP-MS) and ICP-optical emission spectrometry (ICP-OES). These instruments are becoming increasingly ubiquitous as they enable rapid, multi-element chemical fingerprinting of virtually any sample. However, errors during sample preparation, decomposition, and spectrometric analysis may invalidate the results due to the high sensitivity of ICP-spectrometry (Hansen *et al.* 2013). Sample preparation is a particularly significant factor as it involves direct manipulation of the raw material into homogeneous forms amenable to attacks by chemicals and reagents. Moreover, this process is arguably the most time-consuming and labour intensive in the whole analytical pipeline, thus constituting a major bottleneck in any analytical laboratory. To further complicate matters, the techniques and instruments employed in sample preparation may vary between laboratories, which warrant optimization and validation of the in-house protocol.

In forestry, reliable analytical measurements of environmental samples are essential towards formulating sound decisions involving many facets of forestry and forestry-related industries. For example, in establishing the importance of leaving logging residues (i.e., fresh litter, woody debris, and tree bark) during log harvesting in minimising the impact of nutrient loss (Daimun *et al.* 2006). Environmental samples, especially in solid forms, are characterised by their complexity and heterogeneity that pose numerous challenges during the sample preparation steps and laboratory analysis. Here, we conducted a test on the effects of sample comminution towards the precision of multi-element analysis of plant sample matrices on an ICP-OES platform. Sample comminution refers to one of the discrete steps during sample preparation in which the materials are reduced in size through grinding, milling, sieving, and subsampling of test samples (Allen 2003). We sought to answer the question of how much variability in the measurements can be linked to comminution methods. To this end, we compared the repeatability of two heavy-duty commercial grinders: SM 2000 Heavy-duty Cutting Mill (Retsch, Haan, Germany) and ED-5 Wiley Mill (Thomas Scientific, NJ, USA).

"Repeatability" is used herein as a measure of variability in repeated measurements on the same sample under the same condition (Taylor & Kuyatt, 1994). We included a ball-milling treatment on subsamples from the ground materials using an RM 200 Mortar Grinder (Retsch) that served as a yardstick for the repeatability of the grinders. This is because ball-milling generally results in finer particle size and improved homogeneity (Schepers *et al.* 1989) that are prerequisites for adequate subsampling.

MATERIALS & METHODS

Materials, reagents & apparatus

The materials used in laboratory analysis consisted of Kapur Merah (*Dryobalanops becarii*) leaves collected from a single tree at the Forest Research Centre (FRC) Lungmanis Experimental Plantation Plot in Beluran, Sabah and an authenticated Sedaman (*Macaranga pruinosa*) wood sample provided in kind by the FRC Wood Science Section. Sample digestion was carried out using J.T. Baker concentrated (95–98%) sulfuric acid (analytical-grade) and 30% hydrogen peroxide (Avantor, PA, USA) and R&M Chemicals lithium sulphate monohydrate (R&M Marketing, Essex, UK). Inductively coupled plasma was generated using high-purity (99.999%) HiQ Argon Ar 5.0 gas (Linde Malaysia, Selangor DE, Malaysia). Calibration standards for spectrometric measurements was prepared using an equimolar (*ca.* 1,000 mg/L per element) PlasmaCAL Multi-Element Standard (SCP Science, Quebec, Canada). Preparations and dilutions of samples and reagents were carried out using ultrapure water obtained from Barnstead MicroPure ST water purification system (ThermoScientific, Langenselbold, Germany) and all glassware were washed in 1% HCl acid bath prior.

Instrumentation

The SM 2000 Heavy-duty Cutting Mill has a 1.5 kW drive equipped with a six-disc rotor that rotates at 1,390 rpm. The discs consist of spirally arranged metal plates made of heavy-metal free stainless steel, each with three knives that cut in sequence. The ED-5 Wiley Mill features a 1 hp heavy-duty motor and provides cutting speeds of 1,140 rpm. Shearing action is provided by four stainless steel knives on a revolving parallel section rotor working against four adjustable knives that are bolted onto the circumference of the cutting chamber.

The RM 200 Mortar Grinder was fitted with stainless steel mortar and pestle and polyurethane scraper. The mortar rotates at 100 rpm and the pressure applied equals the weight of the pestle combined with an adjustable spring pressure acting on its axis. Sample digestion was carried out using an open-vessel Velp digestion/mineralization system consisting of a DK42 Heating Digester and SMS Scrubber (Velp Scientifica, Lombardy, Italy). The ICP-OES platform comprise a SpectroArcos FHX22 (Spectro Analytical Instruments, Kleve, Germany) with dual-view optics integrated with an ASX 560 autosampler unit (Teledyne Cetac, NE, USA).

Sample preparation, digestion, and spectrometric analysis

Laboratory analyses were performed at the Chemistry Laboratory in FRC Sepilok, Sandakan. The primary samples were air-dried at 50°C to constant mass. The wood sample was nicked into toothpick-sized chips using a stainless-steel cleaver to facilitate grinding. The samples were ground to pass through a 2-mm sieve fitted onto each grinder, following which a subsample was further comminuted by ball-milling without sieving. Grinding usually took around 2 min to complete while ball-milling was done in 2 cycles, 2 min each. In-between samples, all grinding and sieving apparatus were cleaned by blasting with compressed air (110 psi, 6.0 cfm) to minimise cross-contamination. A subsample from each treatment was oven-dried at 105°C to estimate the percentage moisture content of the sample.

Six technical replicates (0.1 ± 0.01 g per replicate) per treatment were digested using a hydrogen peroxide-sulfuric acid procedure for ecological samples adapted from Allen (1993). The mixture was heated at 370°C for 1 h and the digestate was diluted to a final volume of 50 mL. Two analytical blanks and a duplicate of an internal quality control (IQC) sample were included in each digestion batch. Additionally, a random sample from a previous digestion batch was included in successive batches to account for inter-batch variability.

Determination of total K (analytical wavelength = 769.896 nm), Na (589.592 nm), Ca (396.847 nm), Mg (279.553 nm), P (178.202 nm), Al (167.078 nm), Mn (257.611 nm), and Fe (238.204 nm) was performed in a single run on the ICP-OES in radial view mode. Calibration curves were plotted spanning a linear range of 0, 1, 3, 6 and 12 mg/L for all elements (all $r > 0.9999$). The limit of detection (LoD) was estimated based on three repeated measurements of the corresponding analytical blanks and is predefined as the upper 99.7% confidence limit (CL; equal to 3×SD) of the mean intensity of signals produced in the blanks (IUPAC 1976). Inter-batch data were deemed acceptable if the IQC sample measurements were within a pre-established 95% CL (equal to 2×SD; data not shown). The results were reported as corrected values on the basis of oven-dry mass.

Experimental design & data analysis

The grinding and sieving treatments were treated as a matched pair and the corresponding ball-milling treatment was nested within each main treatment. Measures of central tendencies (mean and median) were calculated based on replicated measurements while spread about these values was estimated as the unbiased standard deviation (SD), 95% CL, and interquartile range. The intra-assay percentage coefficient of variation (CV; Lewontin 1966), also referred to as percentage relative standard deviation (RSD), was used as the main metric of repeatability. Repeated measurements of an element were considered repeatable if the CV does not exceed 10% for concentrations below 0.1 mg/g and 7% if they are greater than 0.1 mg/g (Senila *et al.* 2014). We compared differences in the pooled multi-element CV between treatments using paired *t*-test at significance threshold (α) of 0.05 with Bonferroni correction for multiple pairwise comparisons. Statistical tests and data visualizations were implemented in R v3.6.3 (R Core Team 2020).

RESULTS

The location and spread of the measurements in the leaf and wood samples prepared using four comminution techniques are summarised in Table 1. The relative abundance of elements in the leaf sample decreased from K > Ca > Mg > P > Mn > Fe > Na > Al. A similar pattern was observed in the wood sample except Mg was more abundant than Ca. The distribution of of most elements were approximately normally distributed based on inspection of the quantile-quantile plots (results not shown). Therefore, the 95% CL of each element was calculated assuming an underlying *t*-distribution. Sodium, Al, and Fe exhibited significantly high variability relative to the mean with CV indices ranging between 16.87 and 1,613.69% in spite of having low-to-moderate SD of only up to 0.033 mg/L. Meanwhile, the CV of K, Ca, Mg, P, and Mn were more conservative with a range of distribution between 0.25 and 4.28%. Given the disproportionately large dispersion in the repeated measurements of Na, Al, and Fe, we excluded these elements in the statistical tests.

Table 1. Summary statistics of total elements in leaf and wood samples prepared using four comminution methods (all $n = 6$). The mean, standard deviation, and 95% confidence limits are in mg/g and the coefficient of variation is in %.

Leaf sample								
	Cutting Mill, 2-mm sieve				**Wiley Mill, 2-mm sieve**			
	Mean	SD	95% CL	CV	Mean	SD	95% CL	CV
K	5.919	0.095	5.819 – 6.019	1.61	6.212	0.117	6.089 – 6.335	1.88
Na	0.032	0.033	-0.003 – 0.066	103.55	0.006	0.016	-0.010 – 0.023	245.56
Ca	3.922	0.047	3.873 – 3.972	1.20	4.431	0.075	4.353 – 4.509	1.68
Mg	1.228	0.017	1.210 – 1.246	1.38	1.273	0.014	1.258 – 1.288	1.09
P	0.566	0.014	0.551 – 0.580	2.42	0.598	0.008	0.590 – 0.606	1.28
Al	-0.002	0.006	-0.008 – 0.005	359.33	0.001	0.008	-0.008 – 0.009	1,613.69
Mn	0.364	0.004	0.360 – 0.368	1.08	0.357	0.007	0.350 – 0.364	1.84
Fe	0.034	0.006	0.028 – 0.040	16.87	0.038	0.007	0.031 – 0.045	17.65
	Cutting Mill, ball-milled				**Wiley Mill, ball-milled**			
	Mean	SD	95% CL	CV	Mean	SD	95% CL	CV
K	6.204	0.048	6.154 – 6.254	0.77	6.288	0.056	6.230 – 6.347	0.89
Na	0.026	0.029	-0.005 – 0.056	113.95	-0.009	0.011	-0.020 – 0.003	127.53
Ca	3.963	0.036	3.925 – 4.001	0.91	4.314	0.053	4.258 – 4.370	1.23
Mg	1.263	0.016	1.246 – 1.279	1.28	1.277	0.007	1.269 – 1.285	0.56
P	0.596	0.006	0.590 – 0.602	0.99	0.612	0.008	0.603 – 0.620	1.28
Al	-0.004	0.006	-0.011 – 0.002	146.44	-0.004	0.002	-0.006 – -0.001	56.33
Mn	0.367	0.002	0.365 – 0.370	0.61	0.373	0.003	0.370 – 0.376	0.77
Fe	0.033	0.007	0.025 – 0.040	22.76	0.047	0.014	0.032 – 0.061	29.95
Wood sample								
	Cutting Mill, 2-mm sieve				**Wiley Mill, 2-mm sieve**			
	Mean	SD	95% CL	CV	Mean	SD	95% CL	CV
K	2.512	0.017	2.495 – 2.529	0.66	2.389	0.070	2.315 – 2.462	2.92
Na	0.046	0.030	0.015 – 0.078	64.45	0.008	0.019	-0.012 – 0.027	237.79
Ca	0.520	0.006	0.514 – 0.526	1.09	0.506	0.011	0.494 – 0.517	2.19
Mg	1.345	0.009	1.335 – 1.355	0.70	1.306	0.025	1.280 – 1.332	1.92
P	0.128	0.003	0.125 – 0.131	2.29	0.116	0.005	0.111 – 0.121	4.28
Al	-0.009	0.004	-0.013 – -0.004	46.54	-0.005	0.005	-0.010 – 0.000	103.38
Mn	0.067	0.002	0.065 – 0.068	2.81	0.063	0.001	0.062 – 0.064	2.24
Fe	0.015	0.008	0.007 – 0.024	54.36	0.029	0.011	0.017 – 0.040	37.48
	Cutting Mill, ball-milled				**Wiley Mill, ball-milled**			
	Mean	SD	95% CL	CV	Mean	SD	95% CL	CV
K	2.564	0.013	2.550 – 2.578	0.52	2.472	0.035	2.436 – 2.509	1.40
Na	0.020	0.009	0.010 – 0.029	44.52	-0.012	0.005	-0.017 – -0.006	45.60
Ca	0.526	0.002	0.524 – 0.527	0.29	0.531	0.006	0.525 – 0.537	1.09
Mg	1.332	0.009	1.323 – 1.341	0.65	1.322	0.012	1.310 – 1.334	0.87
P	0.127	0.003	0.124 – 0.131	2.66	0.127	0.005	0.121 – 0.132	3.92
Al	-0.007	0.002	-0.009 – -0.004	32.40	-0.009	0.002	-0.011 – -0.007	19.50
Mn	0.067	0.001	0.066 – 0.067	0.77	0.066	0.000	0.065 – 0.066	0.62
Fe	0.022	0.021	0.000 – 0.044	93.27	0.026	0.006	0.019 – 0.033	24.69

Sample comminution using cutting mill and Wiley mill and passed through 2-mm sieve yielded broadly distinctive multi-element profiles in the plant matrices (Figure 1). Differences between the two methods were most pronounced with respect to the comparative recovery of K, Ca, Mg, and P, wherein contrasting trends were observed between the leaf and wood samples. In the former, grinding using cutting mill resulted in lower recovery of the macroelements relative to Wiley mill, while the reverse was observed in the latter. It is also worth noting that there was little to no overlap in the interquartile range and 95% CL of the macroelements in each sample type. The trend in Mn concentration was more consistent in that the relative recovery was higher in samples ground using cutting mill in both the leaf and wood samples.

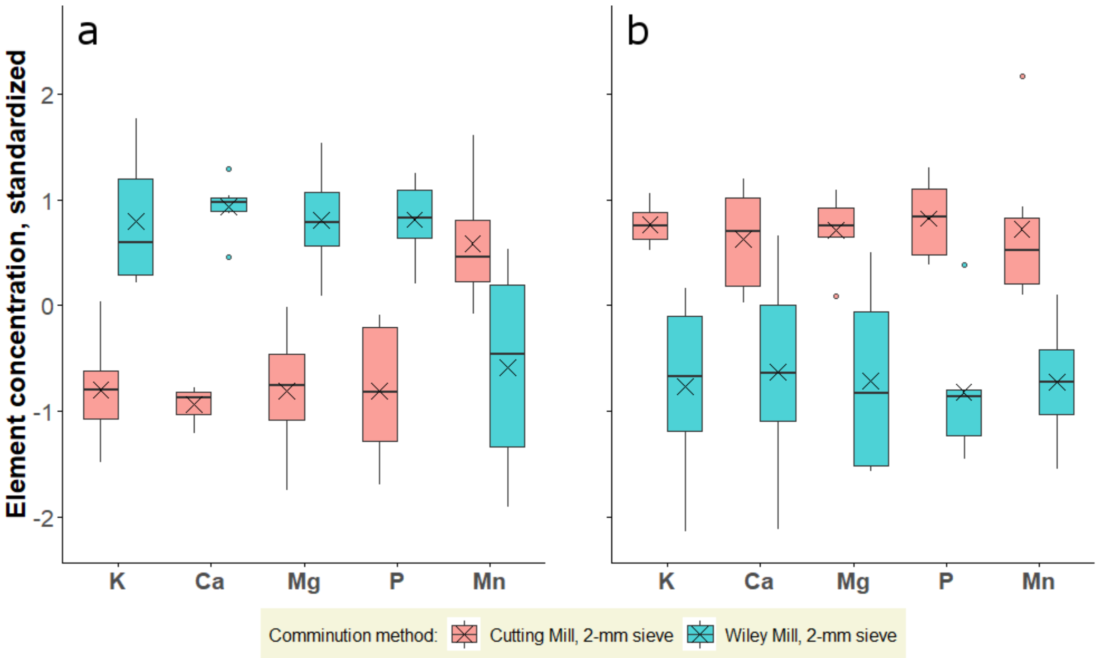

Figure 1. Relative concentrations (*Z*-score standardised) of K, Ca, Mg, P, and Mn in (a) leaf and (b) wood samples ground using Cutting Mill and Wiley Mill and passed through 2-mm sieve (both $n = 6$). Circles denote outlying observations and crosses denote the mean.

The distribution of mean CV indices according to comminution treatment are juxtaposed in Figure 2. In the leaf sample, the pooled CV was equal ($p = 0.961$) between grinding techniques with mean (± SD) of 1.54 ± 0.53% using cutting mill and 1.56 ± 0.35% using Wiley mill. Wood sample ground using Wiley mill appeared to have more variable multi-element profile with mean (± SD) CV of 2.71 ± 0.95% against 1.51 ± 0.98% using cutting mill. However, this difference was also not statistically significant ($p = 0.072$). The variability in ball-milled samples were lower by up to 3.8-fold but most of these differences were not significant ($p > 0.0125$) except in the wood sample ground using Wiley mill and its corresponding ball-milled aliquot ($p = 0.007$). Comparisons between ball-milled test samples showed that there was no difference in mean CV in either the leaf ($p = 0.857$) or wood ($p = 0.075$) sample.

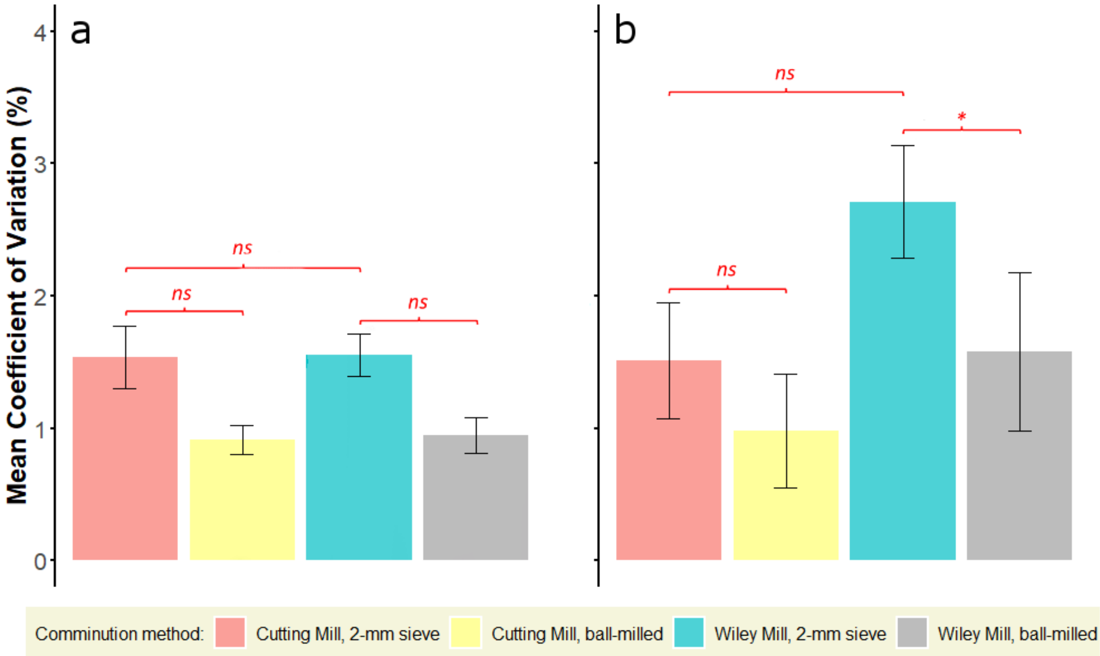

Figure 2. Mean coefficient of variation (%) of K, Ca, Mg, P, and Mn in (a) leaf and (b) wood samples prepared using the four comminution methods (all n = 6). Error bars denote the standard error of the means. Mean difference ^{ns}not significant and *significant at α-value of 0.0125 after correction for multiple pairwise comparisons.

DISCUSSION

Repeated measurements of over half of the elements were relatively consistent and were dispersed below the maximum limits of repeatability with CV below 7% for elements with concentrations exceeding 0.1 ppm (all macroelements and Mn in the leaf sample) and below 10% for Mn in the wood sample, in which the concentration was below 0.1 ppm (Table 1). Current literature on the repeatability of ICP-OES methods for analysis of plant samples predominantly pertain to medicinal plants, particularly in the evaluation of mineral elements of nutritional and toxicological significance. In these assessments, the cut-off CV of individual elements typically range from 10% (Senila *et al.* 2014, Júnior *et al.* 2017) to 20% (USP-NF 2015). The higher cut-off value predefined in the latter was most likely due to concerns over heavy-metal impurities in drugs and herbal preparations that typically occur in trace amounts. A higher CV threshold may be necessary for low-concentration elements as the repeatability of any analysis decreases greatly with decreasing concentration (Horwitz *et al.* 1980) such as that observed for Na, Al, and Fe in this study.

Influence of Comminution Method

The grinding techniques differed in two respects namely, relative recovery of the macroelements and mean variability in harder sample type. Although we did not explicitly test for statistical significance (due to the small sample size), the general lack of overlap in the 95% CL and interquartile range of K, Ca, Mg, and P between grinding methods, irrespective of sample type, may indicate that these discrepancies were potentially significant (Table 1 and Figure 1). The reason for this pattern is unclear and so far, we have not encountered any literature reporting similar observations. The difference in macroelement concentrations associated with grinding techniques represent another aspect of precision namely, reproducibility, which is a measure referring to consistency in the results of analysis under different conditions (Taylor & Kuyatt 1994). Unfortunately, comparative analysis on reproducibility was beyond the scope of this experiments as a larger sample size for each sample type is needed to adequately test the proportional bias between the methods.

Differences in the relative recovery of elements and variability in the repeated measurements seemed to be mutually exclusive. Despite discrepancies in mean concentration of individual elements, estimates of mean CV for samples comminuted using cutting mill and Wiley mill were broadly equal across the leaf and wood samples (both $p > 0.0125$; Fig. 2). Based on these results alone, we could conclude that the two methods yielded equally repeatable measurements. However, comparing the mean CV of the ground samples and their ball-milled aliquots suggested that Wiley mill (with parallel section rotor) may be suitable for soft plant samples but may not be appropriate for harder and fibrous sample matrices especially woody tissues. Meanwhile, cutting mill (with six-disc rotor) is suitable in preparing plant samples across the two sample types tested herein as the precision using this grinding technique is the closest to ball-milling.

The observed differences in precision between comminution methods are due to their influence towards uniformity in particle size distribution, which had been noted previously in plant and soil samples (Schepers *et al.* 1989, Allan *et al.* 1999, Milller *et al.* 2012). Although we did not analyse for the particle-size distribution in the Kapur and Sedaman samples, we observed that grinding using Wiley mill resulted in coarser particles in both leaf and wood samples relative to cutting mill. An additional test of particle-size distribution was performed using leaf and wood samples from three timber species that were available in the laboratory (unpublished data). The results confirmed that the grinding techniques yielded significantly distinct particle-size distributions in both leaf ($\chi^2 = 26.492$, $p = 0.005$) and wood ($\chi^2 = 54.810$, $p = 0.000$) samples, regardless of species and wood density. There was notably a higher proportion of particles over 2.0 mm with Wiley mill (Figure 3). Non-uniform particle size makes subsampling of test samples (especially in small amounts to reduce reagent consumption) difficult as particles in different size classes tend to become vertically stratified during handling.

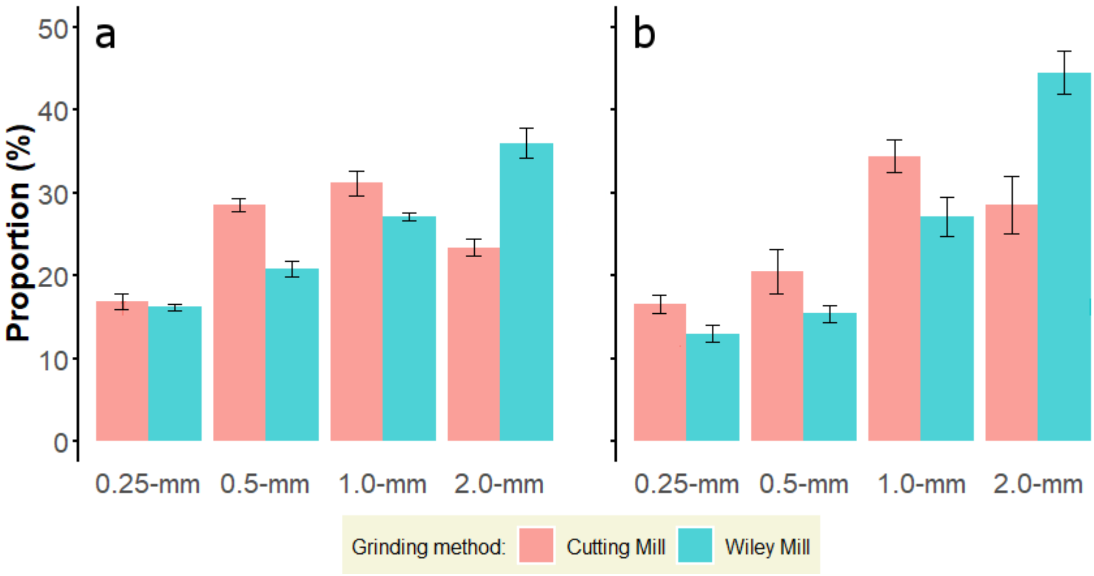

Figure 3. Pooled particle-size distribution (%) in (a) leaf and (b) wood samples from Laran (*Neolamarckia cadamba*), Urat Mata Daun Lichin (*Parashorea malaanonan*), and Keruing (*Dipterocarpus* sp.) ground using cutting mill and Wiley mill (source: unpublished data). Error bars denote the standard error of the pooled sample means.

The higher diversity of particle size in samples ground using Wiley mill appeared to have had a less discernible influence on the analytical precision of the leaf sample. However, there was a significant effect on the repeatability of the wood sample measurements that we attributed to sample hardness. After ball-milling of the wood sample ground using Wiley mill, we noticed presence of relatively large clumps of wood fibres that were not observed in other ball-milled aliquots (Fig. 4). This suggests less efficient ball-milling of the wood subsample ground using Wiley mill that was reflected in its larger mean CV (Figure 2b). A similar observation was noted earlier by Schepers *et al.* (1989, p. 954) who noted that "even with ball-milling, some plant material may tend to fractionate into powder and fibrous portions". Apart from sample type, we may also add grinding technique as another factor giving rise to this tendency for plant samples to fractionate during ball milling.

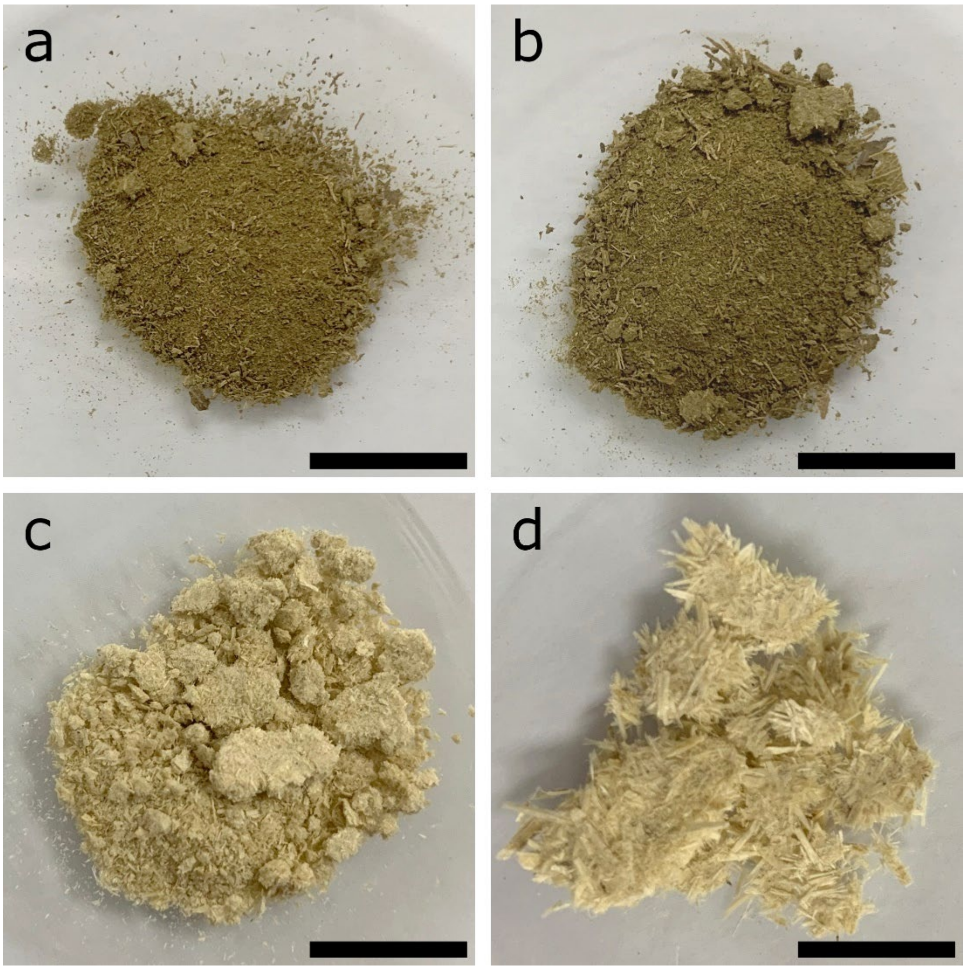

Figure 4: Ball-milled aliquots of *Neolamarckia cadamba* leaf (top row) and wood (bottom row) samples ground using (a, c) cutting mill and (b, d) Wiley mill showing presence of powder and fibrous clumps that were prominent in the wood sample. Scale bar equivalent to 10 mm.

Notes on measurement uncertainties

The lack of repeatability of Na, Al, and Fe were independent of the comminution methods. The concentrations of Na and Al were generally below or near the LoD (0.027 and 4.949×10^{-4} mg/g for Na and Al, respectively) that increased the likelihood of false positives (i.e., background signal mistaken as analyte signal) and subsequently led to significant variability. Both elements are considered nonessential elements in most plants, especially Al that was almost consistently below the LoD (Tbl. 1). Even though the concentration of Fe in the samples were higher than its LoD (2.244×10^{-3} mg/g), there was still a significant intra-assay variability. Inherent Fe contamination in the primary sample was a likely explanation for this observation. Reliable determination of Fe in environmental plant tissue samples is notoriously difficult due to Fe deposition from air-borne dust particles in the field (Jones & Wallace 1992). Subsequently, it had been suggested that the determination of Fe in environmental plant samples may require a specialised sample preparation protocol which includes a washing pretreatment step to reduce background noise caused by extraneous Fe (e.g. Procopiou & Wallace 1982, Wallace *et al.* 1982a 1982b). Stainless steel parts of grinders may also be a source of Fe contamination (Allan *et al.* 1999). However, uncertainties in the Fe measurements herein precluded further examination into potential Fe contamination associated with the grinding and milling instruments.

CONCLUSION

We demonstrated how different grinding techniques may affect the analytical precision of multi-element analysis of leaf and wood sample matrices. Although the measurements of K, Ca, Mg, P, and Mn were within the acceptable range of repeatability in all comminution methods, the performance of the grinding instruments differed when the overall elemental profiles were compared. Ball-milling provided the best precision in both sample type, followed by cutting mill and Wiley mill. Cutting mill was discernibly better than Wiley mill for woody sample type, indicating that the former is more optimal for comminution of a wider range of plant sample types. In addition to a larger sample size, future works may also include concurrent analysis of other factors relevant to the precision of multi-element analysis of plant matrices such as particle-size distribution of the ground samples and structural carbohydrate composition of the materials.

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A preliminary note on phenology and animal feeding of *Sterculia cordata* var. *montana*

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Sterculia is a genus of flowering plants that belongs to the family Malvaceae. The genus was named after Sterculius, the god of odour in Roman mythology. This is in reference to the strong and unpleasant smell of the flower of this genus. The genus comprises more than 200 species that are widespread in tropical and sub-tropical forests globally. Of these, 17 species are found in Sabah including our study subject, *Sterculia cordata*, locally known as *Kelumpang*. This species has been recorded in Malaysia Sumatera, Peninsular Malaysia, Java and the Philippines. The specific epithet 'cordata' was derived from its heart-shaped or cordate leaf base (Plate 1). It is a deciduous tree that can grow up to 40 meters in height and 70 cm in diameter (Soepadmo *et al.* 2011). When the seed pod ripens, it turns red and dehisces in the center, exposing the black seeds within (Plate 2).

Sterculia cordata is a species with multiple uses, for example the wood is harvested for commercial purposes such as light interior construction, veneer and plywood. Aside from that the seeds are edible (after cooking), and rich in oil content, once fried its flavour was described by some to be similar to that of peanuts (Lemmans *et al.* 1995). Researchers have explored the potential of *S. cordata* for medicinal properties. The study by Kader *et al.* (2018) investigated the antioxidant activities of various concentrations of ethanolic extracts of *S. cordata* leaves. This study showed that the leaves of *S. cordata* contain natural antioxidant agents (e.g. flavonoids and phenols) and the extracts are found to have significant antioxidant and antimicrobial activities. This suggests that this species could be incorporated as an antioxidant agent in the development of drugs, especially against drug-resistant microorganisms and pathogens. In another study by Kader *et al.* (2017) it showed that ethanolic leaves extracts of *S. cordata* are also capable of dissolving blood clots in an *in-vitro* clot lysis test. Both studies emphasized further investigation of *S. cordata* should be done to fully exploit its medical use potential.

In terms of taxonomy, there are two varieties of this species described by Tandra namely, the cordata and montana varieties (Soepadmo *et al.* 2011). These two varieties are distinguishable from the calyx lobe and seed size; var. *cordata* has diverging calyx lobes (not joined at the apex) while var. *montana* has converging calyx lobes (joined at the apex) and the seeds of var. *cordata* (1.1–1.3 cm long, 0.5–0.6 cm in diameter) are smaller than the seeds of var. *montana* (2–2.5 cm long, 1.6–1.8 cm in diameter). To date, the var. *montana* is known to be native to Borneo and the Philippines. Based on herbarium records, the species is found to be widespread in many districts of Sabah as shown in Figure 1. Most collections in Sabah and Sarawak were var. *montana* (Soepadmo *et al.* 2011).

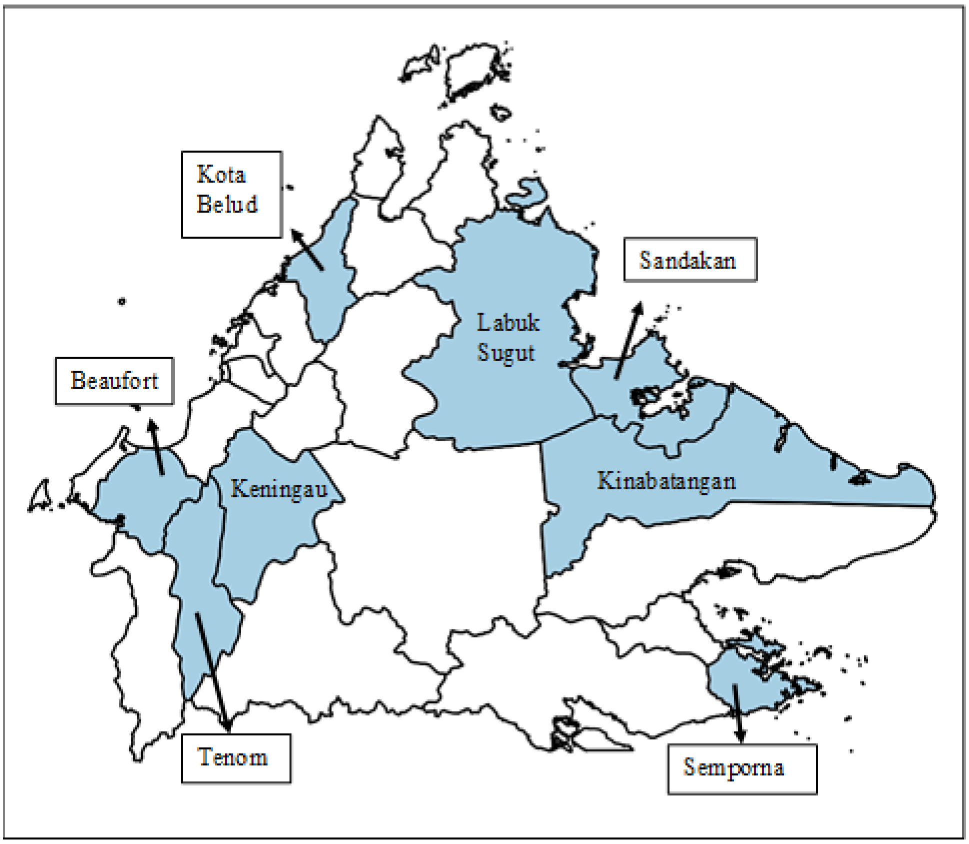

Figure 1. Highlighted area indicates the distribution of *Sterculia cordata* var. *montana* in Sabah.

A *S. cordata* var. *montana* tree located next to the entrance of Forest Research Centre Guesthouse in Sepilok was observed to have started fruiting in February 2020. Hence, a preliminary phenological observation was conducted at FRC Guesthouse from February until July. The fruiting season started at the end of February and lasted until the end of March. Post fruiting, shedding of leaves started in the middle of March and took around three weeks before the tree became bald (Plate 3). The flushing of new leaves then took place and it took about a month (21st March to 22nd April) for the tree to regain its foliage (Plate 4). The aim of this note is to report on the wildlife observed feeding on the seeds of *S. cordata* during the fruiting season. Through a systematic review of literature, our observation seems to be the first record to date in which the following animal species were found feeding on *S. cordata* seeds.

1. Ear-spot squirrel (*Callosciurus adamsi*)

Ear-spot squirrel (*Callosciurus adamsi*) is endemic to Borneo, mainly inhabiting lowland forests (Plate 5). The species has been spotted in *Gmelina arborea* and *Acacia mangium* plantations in Sarawak, suggesting that this species is likely to be able to adapt in disturbed habitat. Despite the adaptability of this species towards habitat changes, it is listed as Near Threatened under the IUCN Red List of Threatened Species as its habitat preference is confined to lowland forest where deforestation was severe (Kennerly & Meijaard 2016). Other than that, seeds and fruits are the main food sources of the ear-spot squirrels which allow them to play a role in seed dispersal (Payne *et al.* 1985). Although the species is not listed, past literature recorded that squirrels do feed on the seeds of *S. cordata* (Veevers-Carte 1984). Although the information on predation of this species is unclear, other closely related species from the same family serve as prey for many carnivores such as snakes suggesting this species could also play an important role in the food web of the forest ecosystems (Payne *et al.* 1985).

2. Common Hill Myna (*Gracula religiosa*)

A group of Common Hill Myna (*Gracula religiosa*) was spotted feeding on the tree as well (Plate 6). Common Hill Myna is listed as 'Least Concern' under IUCN Threatened Species List as this species has an extremely large range (BirdLife International 2018a). This species occurs in east and north-east India, southern China, South-east Asia including Borneo (Phillipps& Phillipps 2011). However, the population is decreasing due to deforestation throughout the species' range. Additionally, this species was a popular pet due to its ability to mimic noises and human speech. In the 1990s, this species was traded heavily and impacted the population significantly in Thailand (Feare & Craig 1998). This species was included in CITES Appendix III by Thailand and then uplisted to CITES Appendix II in 1997 upon requests by the Philippines and Netherlands. Common Hill Myna feeds on fruits and seeds and the seeds will be regurgitated, suggesting the species might assist in seed dispersal (BirdLife International 2018b). Common Hill Myna had also been reported to feed on *Sterculia villosa* which is distributed from northeast India to Thailand (Datta & Rawat 2008).

3. Hornbills

There were two hornbill species spotted feeding on the *S. cordata* seeds, namely Black Hornbill (*Anthracoceros malayanus*) and Oriental Pied Hornbill (*Anthracoceros albirostris*). Hornbills are frugivores and feed on a rich diversity of fruits including *S. cordata.* (Veevers-Carte 1984, Naniwadekar *et al.* 2019). Due to their high fruit consumption, hornbills are considered as important seed dispersal agents in Asian rainforests and referred to as 'farmer of forest' (Kitamura 2011). Additionally, hornbills are highly mobile species with large home ranges that allow a farther distance of seed-dispersal (Naniwadekar *et al.* 2019). For instance, the Black Hornbill is known for its hours-long continuous flight time and has the potential for long-distance seed dispersal which makes it an effective seed dispersal agent. Besides, hornbills have high fruit consumption and feed on rich diversity of fruits underlying their potential of dispersing a wide range of fruit species (Kitamaru *et al.* 2014, Kitamaru 2011). Despite their importance in seed-dispersal, many hornbill species are threatened by hunting pressure and habitat loss.

3a. Black Hornbill (*Anthracoceros malayanus*)

A female Black Hornbill (*Anthracoceros malayanus*) was spotted feeding upon the seeds of *S. cordata* (Plate 7). The species of hornbill is known to have a distribution range spanning Thailand, Peninsular Malaysia, Borneo and Indonesia (Phillipps & Phillipps 2011). Black Hornbill is a lowland specialist and mainly occurs in lowland primary dipterocarp forest, secondary forest, occasionally can be encountered in modified habitats such as plantations. This species is listed as 'Vulnerable' as the species is suspected to undergo a large reduction in population. The decline of the population is due to the high rate of deforestation at lowland forests, resulting inhabitat loss that is worsened by high hunting pressure (BirdLife International 2018a). Black Hornbills are also known to consume many other species of fruits and seeds (more than 50 species) including *Durio graveolens* (Nakashima *et al.* 2007).

3b. Oriental-Pied Hornbill (*Anthracoceros albirostris*)

The Oriental Pied Hornbill (*Anthracoceros albirostris*) (Plate 8) is a common hornbill species and has a wider range than Black Hornbill, occurring from Northeast India to Southern China and throughout Southeast Asia (Phillipps & Phillipps 2011). The population has been impacted by the trading of fledglings in Thailand and Peninsular Malaysia, habitat loss and hunting pressure. However, this species has strong adaptability to degraded habitat and a broad range of food preferences that consume more than 100 species of fruits. A study showed that Oriental Pied Hornbill feeds on *Sterculia* species such as *S. balanghas* except for *S. cordata* (Kitamaru *et al.* 2002). Ismail *et al*. (2015) reported observing several pairs of Oriental Pied Hornbill utilizing abandoned clay jars as nesting habitats in an agricultural area in Sungai Panjang, Sabak Bernam, Malaysia. The studies also recorded breeding activities of these hornbill pairs and being able to find food from nearby palm oil plantations and orchards, suggesting this species could adapt well in modified habitats (Ismail *et al.* 2015, Rahman *et al.* 2019). Hence, this species is listed as 'Least Concern' under the IUCN List of Threatened Species (BirdLife International 2016).

The findings of this observation could serve as preliminary records on the granivores species and the phenological phases of *S. cordata* var. *montana* (mainly leaf fall and leaf flushing). The fruit trees of *Sterculia* genus are among the food sources of frugivorous animals, including some striking hornbills. This species could be planted for its recreational values and become an attraction for bird watching. Other than that, Sofiah & Soejono (2020) reported that *S. cordata* has high rainfall interception and reduces throughfall, implying that it is suitable for soil and water conservation. Hence, it is recommended that further investigation could be conducted to ascertain the suitability of *S. cordata* in restoration programmes of degraded areas.

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The authors are very grateful to Dr. Joan T. Pereira, who supervised the phenological observation. Special thanks to Ms. Eyen Khoo, who oversaw the writing of this paper, and her constructive comments on the draft is also acknowledged. The authors thank Mr. Chong Yi Yao and Mr. Amirul Mukminin for helping in the identification of the animals.

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Plate 1. Leaves of *Sterculia cordata* var. *montana* with heart-shaped leaf base.

Plate 2. Ripen fruits of *Sterculia cordata* var. *montana.*

Plate 3. The bald tree with newly-produced leaves.

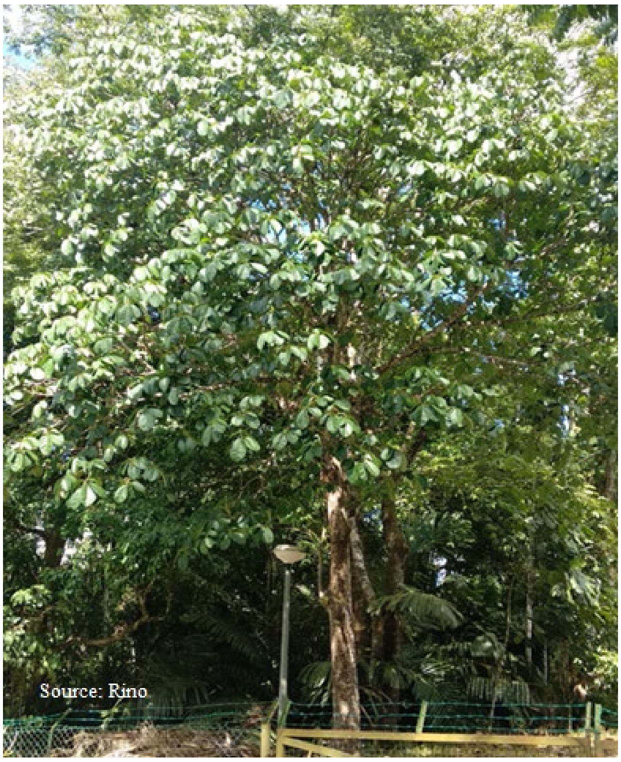


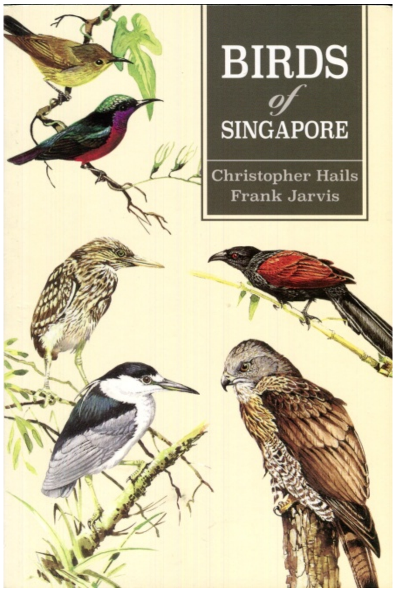
Plate 4. The tree fully flushed with leaves.

Plate 5. Sighting of Ear-spot squirrel (*Callosciurus adamsi*) after feeding on seeds.

Plate 6. Common Hill Myna feeding on the seeds.

Plate 7. A female Black Hornbill (*Anthracoceros malayanus*) resting on the tree.

Plate 8. A pair of Oriental Pied Hornbill.



Birds of Singapore by Christopher Hails & Frank Jarvis. Published by Marshall Cavendish International (Asia) Pte Ltd. 2018. Pp 168. ISBN 9-789814-794473.

Reviewed by E. Khoo

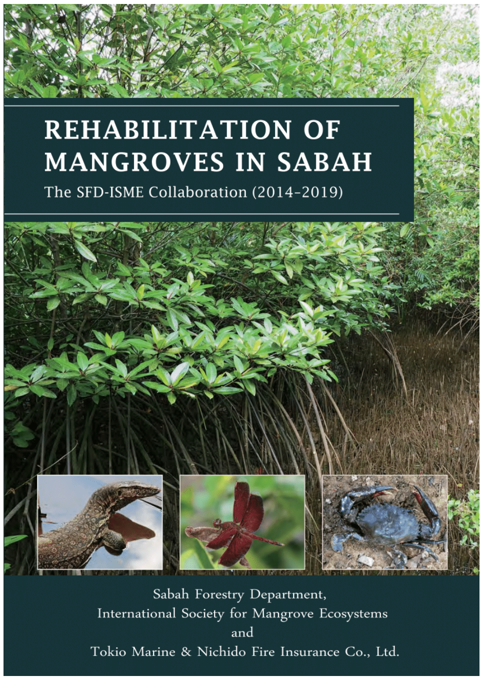
In a recent visit to "The Art of Birding Exhibition" in Singapore Botanic Gardens, among the artworks that were on display were paintings by the late Frank Jarvis and some short notes by Chris Hails that were extracted from their book "Birds of Singapore". The exquisite and immense attention paid to the details of the artworks have led to the instant purchase of a copy of their book.

After going through the book, it was easy to see why a paperback reprint (2018) of their book was done even though 30 years have passed since it was first published in 1987. Its simplicity in presenting the essential background knowledge and the book's rare usage of jargons, makes it a good book to start in introducing the topic to any casual person or beginner who is venturing into the birding scene. The authors have put in great thoughts into weaving a story on how such a small island as Singapore can possess such species diversity, through the recounting of the roles of the various habitat types, climate, island's location, migration patterns and *etc*.

To ease a beginner into identifying birds, some simple but useful tips on how to go about, where and what physical features to look out for in the identification process were given, such as the various tail types or beak shapes and how these features are influences by the species' habits. One would have thought that such notes would provide some rather dry reading, but this was not the case as the authors have injected their writings with great humour, at the same time presenting the useful information and the science behind it.

The following is an example of the authors humour: "*So do not despair if your office is on the 21*st *floor at Shenton Way: look out of the window and you have the opportunity for eyeball to eyeball contact with a wealth of Singapore birdlife still waiting to be properly documented and understood.*" Although it does not provide an updated bird lists for Singapore, of which the authors have stated clearly in their book, it is felt that such writing does stimulate a reader to want to know more about the subject. On top of that coupled with the beautiful artworks of the late Frank Jarvis, it does make one want to find out when the next bird chirps by the window, what species could it be that is hanging out there.

BOOK REVIEW



Rehabilitation of Mangroves in Sabah: The SFD-ISME Project (2014-2019) by Joseph Tangah, Arthur Y.C. Chung, Shigeyuki Baba, Hung Tuck Chan & Mio Kezuka. Published by Sabah Forestry Department (SFD), International Society for Mangrove Ecosystems (ISME) and Tokio Marine & Nichido Fire Insurance Co., Ltd., 2020. Pp 58. ISBN: 978-4-906584-20-8.

Reviewed by V. Paul

Rehabilitation of Mangroves in Sabah: The SFD-ISME Project (2014-2019) is the continuation of the previous publication of *Rehabilitation of Mangroves in Sabah: The SFD-ISME Project (2011-2014).* In the Introduction, the book provides a synopsis of mangroves in Sabah and an overview of Phase two of the SFD-ISME project.

The first book let the readers take an insight regarding the first phase of the mangrove project (2011-2014) with more than 150 ha of mangroves planted paved the way for the International Exchange Programme on Coastal Resources, a collaboration between SFD and the Tropical Biosphere Research Center (TBRC) of University of the Ryukyus in Okinawa, Japan. Meanwhile, through this second project publication, the readers will be inspired by the successful planting of ~200ha of degraded mangrove forests in 8 project sites within 8 forest reserves of 7 forestry districts. Besides that, readers are able to get to know the strategic approaches adopted by the Project Steering Committee (PSC), and four cases studies at Sungai Tokio Marine (Kunak), Sungai TBRC (Lahad Datu), Ex-OP FELDA (Kalabakan) and Pulau ISME (Beaufort). Achievements and calendar of activities are part of the spotlights of this book. When reading this book, I as a Sabahan can feel a sense of pride where Sabah was chosen for this collaborative endeavour with a well-known international agency such as ISME.

This educational, comprehensive and perfectly-illustrated book is indeed informative to people from all walks of life who might be interested to know more about mangroves. Looking forward for more success stories of SFD-ISME collaboration for the 3rd phase of the project, 2019-2024!

"Mangroves, salt marshes and sea grass lock away carbon at up to five times the rate of tropical forests." – Frances Beinecke

GUIDE TO CONTRIBUTORS

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