journal of TROPICAL BIOLOGY & CONSERVATION

Vol. 16/2019

ISSN 1823-3902
E-ISSN 2550-1909




A JOURNAL OF THE INSTITUTE FOR TROPICAL BIOLOGY AND CONSERVATION
UNIVERSITI MALAYSIA SABAH

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Journal of

TROPICAL BIOLOGY & CONSERVATION

A journal of the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah

Cover image:
Lestes praevius - endemic to Borneo (a damselfly)
Lyriothemis biappendiculata - a dragonfly
(Photo credit: Choong Chee Yen)

Etlingera hamiguitanensis Naïve - Philippine endemic
Hornstedtia microcheila Ridl. - Philippine endemic
(Photo credit: Noe P. Mendez)

Date of issue: 2019-10-15

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Journal of Tropical Biology and Conservation 16: 1–8, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

Odonata Fauna of Imbak Canyon Conservation Area, Sabah

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Abstract

Dragonflies and damselflies, collectively known as Odonata, are an important component of fresh water ecosystems. The Odonata fauna of the Imbak Canyon Conservation Area (ICCA) was surveyed during the Batu Timbang Research Station Scientific Expedition on 16-26 August 2017. A total of 62 Odonata species from 13 families were recorded. The family Libellulidae had the highest number of species (27), and this was followed by Coenagrionidae (nine species), Calopterygidae (five species), Platycnemididae (five species) and Platystictidae (four species). The other families (Devadattidae, Chlorocyphidae, Euphaeidae, Lestidae, Philosinidae, Aeshnidae, Corduliidae and Macromiidae) were only represented by 1-3 species. Of the species recorded, 30 are new records for ICCA. The number of species recorded was high, indicating the high diversity of Odonata fauna of ICCA. These records were combined with the existing records in literature to produce a checklist. At present, 68 species from 15 families are known from ICCA.

Keywords: Biodiversity, Borneo, Checklist, Imbak Canyon Conservation Area, Odonata, Sabah

Introduction

Dragonflies and damselflies collectively known as Odonata, are one of the oldest groups of insects in the world. They first appeared on the earth in the Carboniferous Period around 300 million years ago. Dragonfly fossils with an age of 130 million years old do not differ much in morphology compared to the present dragonflies. Close to 6,000 Odonata species are distributed throughout the world (Dijkstra et al., 2013). In Malaysia, more than 400 species have been recorded, with over 160 species known from the state of Sabah (Orr, 2003, 2005; Choong et al., 2017).

Received 31 October 2018
Reviewed 19 March 2019
Accepted 10 April 2019
Published 15 October 2019

Generally, Odonata have vibrant colours on their body and sometimes their wings. They are active during the day on hot sunny days. Odonata are always associated with fresh water such as rivers, streams, ponds, lakes, waterfalls, canals, swamps, marshes and paddy fields. Fresh water bodies are essential habitats for Odonata larvae. Therefore, Odonata forms an important biological component for fresh water ecosystems. Both the larvae and adults are good hunters. Odonata larvae prey on aquatic insects, tadpoles, small fish and shrimps and the adults mostly prey on other flying insects. Many Odonata species are good biological indicators for water quality.

The Imbak Canyon Conservation Area (ICCA), a main part of larger Imbak Canyon, is located immediately to the north of Maliau Basin Conservation Area in the central part of Sabah. With a size of 27,599 ha, ICCA consists of complex rainforest habitats ranging from lowland dipterocarp forests to montane heath forests (Yayasan Sabah, 2014). The published Odonata records for Imbak Canyon come from three publications – Choong (2011), Dow & Orr (2012) and Chung et al. (2013). Choong (2011) recorded 38 species from Mount Kuli Research Station (within ICCA) and Chung et al. (2013) recorded 24 species from Sungai Imbak Forest Reserve. It is noted that the part of Sungai Imbak Forest Reserve surveyed by Chung et al. (2013) falls outside ICCA. On the other hand, Dow & Orr (2012) reported the type series of *Telosticta janeus* from ICCA. It is always of immense interest to carry out additional surveys of Odonata in different parts of Imbak Canyon to document the species richness of ICCA. We carried out a short sampling in ICCA on 16-26 August 2017. Here we present the results of the field survey together with the available literature records to produce a checklist of Odonata known from ICCA.

Methodology

The Odonata of ICCA was surveyed during Batu Timbang Research Station Scientific Expedition on 16-26 August 2017. The survey was done at two sites – Imbak Canyon Studies Centre (5°5’28.9682”N; 117°2’11.2567”E) on 17-21 August 2017 and Batu Timbang Campsite/Research Station (5°0’13.1444”N; 117°4’46.2944”E) on 23-25 August 2017. The survey was done at all types of aquatic habitats (streams, rivers, swamps, ponds, puddles, waterfalls etc.) at both the sites. Adult insects were caught using aerial net. Specimens collected were treated with acetone and then dried in silica gel. The identification of specimens down to species was done based on references and comparison with specimens from other places. The specimens are kept in Centre for Insect Systematics (Universiti Kebangsaan Malaysia) and the Sabah Forestry Department.

Results and Discussion

A total of 62 Odonata species from 13 families were recorded during the Batu Timbang Research Station Scientific Expedition (Table 1). The family Libellulidae had the highest number of species (27), and this was followed by Coenagrionidae (nine species), Calopterygidae (five species), Platycnemididae (five species) and Platystictidae (four species). The other families (Devadattidae, Chlorocyphidae, Euphaeidae, Lestidae, Philosinidae, Aeshnidae, Corduliidae and Macromiidae) were only represented by 1-3 species. A total of 54 species were recorded at Imbak Canyon Studies Centre and 35 species at Batu Timbang Campsite (Table 1). This disparity of the species number does not necessarily reflect the species richness of a particular site. It is most likely the consequence of a lower sampling effort at one site – five sampling days in Imbak Canyon Studies Studies and three sampling days in Batu Timbang Campsite.

Table 1. Odonata species recorded during Batu Timbang Research Station Scientific Expedition 2017. Data from Imbak Canyon Studies Centre is in column A and Batu Timbang Campsite is in column B. Records from Choong (2011) are in column C. # indicates an observation without a voucher specimen. * indicates a new record for ICCA.

No.	Species	A	B	C
	Family Lestidae			
*1	*Lestes praevius* Lieftinck, 1940	/		
2	*Orolestes wallacei* (Kirby, 1889)	/		/
	Family Platystictidae			
3	*Drepanosticta actaeon* Laidlaw, 1934		/	/
4	*Drepanosticta versicolor* (Laidlaw, 1913)	/	/	/
*5	*Protosticta* cf *kinabaluensis* Laidlaw, 1915		/	
6	*Telosticta janeus* Dow & Orr, 2012		/	/
	Family Calopterygidae			
7	*Neurobasis longipes* Hagen, 1887	#	/	/
8	*Vestalis amaryllis* Lieftinck, 1965	/	/	/
*9	*Vestalis amoena* Hagen in Selys, 1853	/		
10	*Vestalis anacolosa* Lieftinck, 1965	/	/	/
11	*Vestalis beryllae* Laidlaw, 1915		/	/
	Family Chlorocyphidae			
12	*Rhinocypha aurofulgens* Laidlaw, 1931		/	/
13	*Rhinocypha humeralis* Selys, 1873	/	/	/
	Family Devadattidae			
14	*Devadatta tanduk* Dow, Hämäläinen & Stokvis 2015	/	#	/

	Family Euphaeidae			
15	*Euphaea subcostalis* (Selys, 1873)	/	#	/
	Family Philosinidae			
16	*Rhinagrion elopurae* (McLachlan in Selys, 1886)	/		/
	Family Platycnemididae			
17	*Coeliccia nigrohamata* Laidlaw, 1918	/	#	/
18	*Coeliccia* cf *nemoricola* Laidlaw, 1912	/	#	/
19	*Coeliccia arcuata* Lieftinck, 1940	/	/	/
20	*Copera vittata* (Selys, 1863)	/	#	/
21	*Prodasineura hyperythra* (Selys, 1886)			/
22	*Prodasineura dorsalis* (Selys, 1860)			/
23	*Prodasineura hosei* (Laidlaw, 1913)			/
*24	*Prodasineura verticalis* (Selys, 1860)	/		
	Family Coenagrionidae			
*25	*Agriocnemis femina* (Brauer, 1868)	/		
*26	*Argiocnemis rubescens rubeola* Selys, 1877	/		
27	*Argiocnemis* sp.	/		/
*28	*Ceriagrion bellona* Laidlaw, 1915	/		
*29	*Pseudagrion microcephalum* (Rambur, 1842)	/		
30	*Stenagrion dubium* (Laidlaw, 1912)	/	/	/
31	*Teinobasis laidlawi* Kimmins, 1936	/	#	/
*32	*Teinobasis rajah* Laidlaw, 1912	/		
*33	*Xiphiagrion cyanomelas* Selys, 1876	/		
	Family Aeshnidae			
*34	*Anax panybeus* Hagen, 1867	/		
35	*Indaeschna grubaueri* (Förster, 1904)	/	#	/
*36	*Tetracanthagyna degorsi/brunnea* (female)		/	
	Family Gomphidae			
37	*Microgomphus chelifer* (Selys, 1858)			/
	Family Macromiidae			
38	*Macromia corycia* Laidlaw, 1922		/	/
	Family Synthemistidae			
39	*Idionyx* sp. (female)			/
	Family Corduliidae			
*40	*Epophthalmia vittigera* (Rambur, 1842)	/		
	Family Libellulidae			

*41	*Agrionoptera insignis* (Rambur, 1842)	/		
*42	*Agrionoptera sexlineata* Selys, 1879	/		
*43	*Brachydiplax chalybea* Brauer, 1868	/		
*44	*Camacinia gigantea* (Brauer, 1867)	#		
*45	*Cratilla lineata* (Brauer, 1878)	/	#	
46	*Cratilla metallica* (Brauer, 1878)	/	#	/
*47	*Diplacodes trivialis* (Rambur, 1842)	/		
*48	*Hylaeothemis clementia* Ris, 1909	/		
49	*Lyriothemis biappendiculata* (Selys, 1878)	/	#	/
50	*Lyriothemis cleis* Brauer, 1868			/
*51	*Nesoxenia lineata* (Selys, 1868)	/		
*52	*Neurothemis fluctuans* (Fabricius, 1793)	/	/	
*53	*Neurothemis ramburii* (Brauer, 1866)	/	#	
*54	*Neurothemis terminata* Ris, 1911	/	#	
55	*Orthetrum chrysis* (Selys, 1891)	/	#	/
56	*Orthetrum glaucum* (Brauer, 1865)	/	#	/
57	*Orthetrum pruinosum* schneideri Förster, 1903	/	/	/
*58	*Orthetrum sabina* (Drury, 1773)	/		
*59	*Orthetrum testaceum* (Burmeister, 1839)	/	#	
*60	*Pantala flavescens* (Fabricius, 1798)	/	#	
*61	*Rhyothemis triangularis* Kirby, 1889	/		
62	*Tetrathemis irregularis hyalina* Kirby, 1889	/		/
*63	*Tramea transmarina euryale* Selys, 1878	/		
64	*Trithemis aurora* (Burmeister, 1839)	/		/
65	*Trithemis festiva* (Rambur, 1842)	/	/	/
66	*Tyriobapta torrida* Kirby, 1889	/	#	/
67	*Zygonyx iris errans* Lieftinck, 1953		#	/
*68	*Zyxomma petiolatum* Rambur, 1842	#		
	Total number of species	**54**	**35**	**38**

Among the species recorded, *Orthetrum glaucum*, *Orthetrum testaceum*, *Neurothemis ramburii* and *Neurothemis terminata* were found abundantly at the open areas in Imbak Canyon Studies Centre.

On the other hand, the camp sites and the logging road in Batu Timbang

Figure 1. Some of the Odonata species photographed at Imbak Canyon Conservation Area. A: *Teinobasis rajah*, B: *Lestes praevius*, C: *Neurobasis longipes*, D: *Teinobasis laidlawi*, E: *Cratilla metallica*, F: *Cratilla lineata*, G: *Orthetrum testaceum*, and H: *Lyriothemis biappendiculata*.

Campsite were frequently visited by *Cratilla lineata*, *Cratilla metallica* and *O. glaucum*. Particularly interesting species recorded during the expedition were *Lestes praevius, Vestalis beryllae, Telosticta janeus, Rhinagrion elopurae* and *Protosticta* cf *kinabaluensis*. *Lestes praevius*, a new record for Malaysia, was found at Imbak Canyon Studies Centre. Orr (2003) mentioned that *L. praevius* was only known from east Borneo (Kalimantan), but postulated the possibility of its wider distribution. *Telosticta janeus* is a unique species endemic to Sabah, and it is only known to Danum Valley and Imbak Canyon (Choong, 2011; Dow & Orr, 2012), and Maliau Basin (Dow, personal communication). *Vestalis beryllae, Rhinagrion elopurae* and *P.* cf *kinabaluensis* are uncommon species and endemic to Borneo (Orr, 2003). Some of the Odonata species recorded during the expedition were photographed and are shown in Figure 1.

Of these 62 species recorded, 30 species are new records for ICCA (Table 1; marked with *). The high number of new records may indicate that the Odonata fauna of ICCA is still very much unexplored. It is also noted that five species (*Heliocypha biseriata, Rhinocypha stygia, Euphaea impar, Rhyothemis phyllis* and *Rhyothemis regia regia*) recorded from Sungai Imbak Forest Reserve by Chung et al. (2013) have not yet been recorded in ICCA. Some parts of the Sungai Imbak Forest Reserve are within ICCA. Furthermore, these species are not rare, and therefore there is a high possibility of their presence in ICCA. The species recorded from the current field survey were combined with the existing records by Choong (2011) from Mount Kuli Research Station to produce a checklist (Table 1). At present, 68 species from 15 families are known from ICCA. This represents 42% of the species known from Sabah, indicating the high diversity of Odonata in ICCA.

Conclusion

In general, ICCA is rich in Odonata fauna. Undoubtedly, it is a refuge for many species endemic to Borneo. Batu Timbang Research Station and Mount Kuli Research Station are not far from each other, and they are located at the southern part of ICCA. This shows that only a small part of the ICCA has been surveyed for Odonata so far. Therefore, further surveys in other parts of the conservation area are needed to produce a more comprehensive checklist for ICCA.

Acknowledgements

We are grateful to Yayasan Sabah for inviting us for the Batu Timbang Research Station Scientific Expedition, Edward Asrul Alimin Sinon, Brandon Don, Hardy Mangkawasa, Amshahri bin Agimin and other Yayasan Sabah rangers as well as Sabah Forestry Department staff who assisted in the field work.

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Journal of Tropical Biology and Conservation 16: 9–24, 2019
ISSN 1823-3902
E-ISSN 2550-1909

Research Article

A Preliminary Assessment of Insect Diversity in Imbak Canyon – Batu Timbang

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Abstract

This insect diversity survey by the Sabah Forestry Department team was carried out from 16th to 20th of August, 2017 in Imbak Canyon – Batu Timbang area. Nocturnal insect diversity was assessed through light-trapping while diurnal insects were documented through fruit-baited traps, sweep nets and forceps. The mean nocturnal insect species richness was 48 species while the mean abundance was 55 individuals. These values were affected by the presence of many wild honeybees, *Apis dorsata* and the unexpectedly wet weather. Some Bornean endemic species were recorded, which included moths, beetles, dragonflies and a butterfly species. The Bornean endemic butterfly, *Papilio acheron*, is a rare species documented during the survey. Interesting and iconic species recorded are the Malaysia national butterfly, *Trogonoptera brookiana* and the world's largest bush cricket, *Macrolyristes imperator*. These insect data provide salient information to enhance the conservation of the Batu Timbang forest. They will serve as baseline information for other insect research work in future. Threats, such as forest fire, fragmentation, illegal clearing of vegetation and poaching are likely to indirectly affect the insect fauna. As such, continuous monitoring and enforcement at strategic locations are important to minimize these threats.

Keywords: Insect diversity, Batu Timbang, Imbak Canyon, light trapping, fruit-baited traps, sweep nets, endemic species

Received 01 April 2019
Reviewed 26 June 2019
Accepted 02 July 2019
Published 15 October 2019

Introduction

Imbak Canyon Conservation Area (ICCA) is located to the north of Maliau Basin in central Sabah. The canyon, about 30,000 ha / 300 km^2 in size, consists of lowland rainforest vegetation with some lower montane vegetation, from 250-1,000 m a.s.l. Most, but not all, of the steep outer ridges are protected as Virgin Jungle Reserve (VJR), while the biodiversity-rich valley has been a Class I (Protection) Forest Reserve since 2009, set within the Yayasan Sabah Concession Area. The VJR and Forest Reserve combined, form the ICCA, with Gunung Kuli at 1,527 m as the highest point in the conservation area. Three expeditions have been organized in the past, in 2000 (Tampoi), 2004 (Kapur) and 2010 (Gg Kuli) respectively. These resulted in the discovery of new species, including *Dipterocarpus megacarpus*, a timber species from the Dipterocarpaceae family, and *Ceriscoides imbakensis*, a rare tree from the Rubiaceae family (Yayasan Sabah 2014). A new stick insect species, *Baculofractum shelfordi,* was discovered adjacent to the Imbak Waterfall (Bragg 2005).

Much is still unknown about this conservation area. As underscored in the ICCA Strategic Management Plan (2014-2023), biodiversity conservation and research are important components that should be given priority. Hence, a scientific expedition was organized from 16th to 26th of August 2017 to explore the biodiversity in the southern part of the conservation area, focusing on Batu Timbang Forest Reserve and its adjacent area. Batu Timbang is a VJR Forest Reserve, with limestone caves inhabited by swiftlets, encompassing 261 ha that was formerly gazetted under Gg Rara FR. Under the Sabah forest classification, VJR and Class VI FR are conserved strictly for forestry research purposes, including biodiversity and genetic conservation. Batu Timbang is one of the locations within the ICCA that has been earmarked for the establishment of a research station as specified in the ICCA Strategic Management Plan.

The base camp (N 05°00'14.8", E 117°04'36.4" at 375 m a.s.l.), was located beside Sg Lanap adjacent to Batu Timbang Forest Reserve on the south-eastern part and Gunung Kuli on the west. The vegetation in the base camp area is mainly lowland mixed dipterocarp forest. The entomology team from the Sabah Forestry Department participated in the first week of the expedition from 16th to 20th of August 2017. The insect diversity study carried out by Sabah Forestry Department was divided into three parts:

1. Nocturnal insect diversity as assessed through light-trapping.
2. Insects sampled through fruit-baited traps.
3. Diurnal insects sampled using sweep nets and forceps.

Materials and Methods

Insect sampling methods

Light trapping was used to sample nocturnal insects while fruit-baited traps, sweep nets and forceps were used to sample diurnal insects.

Light-trapping

Light trapping was used to sample nocturnal insects. The trap consists of a vertical white sheet (2 X 2 m) illuminated by a 250W mercury-lithium bulb (Figure 1). It was powered by a portable Yamaha ET950 generator. The trap was set up in an open area facing the forest reserve, from 7:00 to 9:00 p.m. A GPS (Model: Garmin GPSMAP 60CSx) was used to determine the coordinates of each sampling site. Temperature and humidity were taken with a digital hygrometer from Extech Instruments (model no. 445702) at 8:30 pm.

To evaluate diversity of the sampling area, insect species and individuals (≥ 5 mm) within the 1 X 1 m square of the white cloth were enumerated from 8:30 to 9:00 pm. This is a rapid biodiversity assessment method because by the end of the sampling time, species and individual numbers can be obtained, and the data can be used to calculate diversity indices, i.e. Shannon Wiener, Simpson and Fisher Alpha. This was done using the Species Diversity & Richness version IV (SDR 2006). This method is simple, fast and can be carried out by non-insect specialist. To avoid differences in sampling between nights, the same researcher was assigned to count the species and individual numbers throughout the sampling period, and across different sampling sites. Due to the rain, light-trapping was conducted at the base camp area for only two nights (Table 1).

Table 1. Light-trapping site at the base camp of Imbak Canyon – Batu Timbang.

Sampling site	Coordinates	Elevation (m)	Temp. (ºC)	Humidity (%)	Sampling date	Remarks
A	N 05°00'11.3" E 117°04'34.4"	363	24.7	75	18 August	Cloudy night
B	N 05°00'17.4" E 117°04'38.0"	364	22.7	86	19 August	Clear sky after rain in the late afternoon

Sampling using fruit-baited traps

The fruit-baited trap was about 100 cm in height and 30 cm in diameter, made of a nylon fabric cylinder with a plastic plate on top and another plate at the bottom baited with rotting bananas. Each trap was suspended from a tree branch (Figure 2). A total of six traps were set up along the Rafflesia Trail (Table 2). Traps were spaced at 80 m intervals along the trail. This sampling

Table 2. Fruit-baited traps (FBT) set up along the Rafflesia Trail in Batu Timbang.

FBT no.	Coordinates	Elevation (m)
1	N 05° 00'12.4" E 117° 04'32.4"	361
2	N 05° 00'10.5" E 117° 04'35.0"	359
3	N 05° 00'08.3" E 117° 04'35.7"	383
4	N 05° 00'04.4" E 117° 04'36.4"	416
5	N 05° 00'00.1" E 117° 04'36.4"	414
6	N 04° 59'55.9" E 117° 04'35.5"	388

method was based on the same apparatus used by Chey et al. (2014 & 2015). The traps were checked the next day (after 24 hours), and all the butterflies, moths and beetles within the traps were recorded.

Figure 1. Insect light-trapping in progress.

Figure 2. Setting-up a fruit-baited trap.

Sweep net and manual collection

Sweep nets were used to collect flying insects while other insects were sampled using fine forceps. Butterflies were put in triangle papers while other specimens were put in vials with 75% ethanol solution. Sampling was conducted along the trails established for the expedition. Three people were involved in this sampling from 8:00 am until 1:00 pm. Details of the daytime sampling sites are listed in Table 3.

Table 3. Daytime sampling sites in Batu Timbang.

Sampling site	Starting point coordinates	Ending point coordinates	Elevation (m)
1 (Rafflesia Trail)	N 05°00'11.3" E 117°04'34.4"	N 04°59'55.9" E 117°04'35.5"	363-388
2 (Sg Lanap Trail)	N 05°00'17.4" E 117°04'38.0"	N 05°00'09.8" E 117°04'34.4"	364-391

Insect specimens and identification

In this survey, we focussed on certain insect groups, i.e., butterflies, moths, beetles, dragonflies and damselflies. Only selected insect groups for further research work were sampled, so as to minimize the workload at the laboratory in preparing the specimens for identification. This is also one of the best practices adopted to minimize stress and disturbance to biodiversity, as pointed out by Costello et al. (2016) on field work ethics in biological research. Photographs were taken with DSLR Nikon D800E and other compact cameras to facilitate identification. Common insects were not sampled but photographs were taken as a record.

Selected specimens were dry-mounted and sorted to family and some to the genus and species level. Some of the identifications are still tentative. The specimens sampled from this study are deposited at the Forest Research Centre, Sepilok, Sabah. Dry-mounted specimens were identified based on the FRC Entomology Collection and various reference materials.

Specimens of dragonflies and damselflies were handed over to the expert, Dr Choong Chee Yen of Universiti Kebangsaan Malaysia, Bangi who also participated in the expedition while some butterfly specimens were given to students of Dr Homathevi Rahman of Universiti Malaysia Sabah who were documenting the butterfly fauna of Batu Timbang.

Results and Discussion

Nocturnal insect diversity as assessed through light-trapping

The nocturnal insect diversity is shown in Table 4. The mean nocturnal insect species richness was 48 species while the mean abundance was 55 individuals. The mean Shannon Index was 3.76 while Simpson Index was 99 and Fisher Alpha Index was 167.30. The value for the diversity indices was affected by the presence of many wild honeybees, *Apis dorsata*, at Site A. There was a *Koompassia excelsa* tree with wild honeybee nests near the camp. The presence and aggressive behaviour of the bees may have adversely affected the numbers of other insects. In enumerating the diversity values, honeybee abundance was not included as this species is social, and hence, individuals are not independent from each other, biasing calculation of diversity metrics. At Site B, the night wasp, *Provespa anomala*, was the most abundant species, with six individuals recorded during the enumeration time. Logistically, it was not feasible to carry out sampling far from the camp at night because of the extreme muddy road conditions.

During light-trapping, the temperature was between 22°C and 25°C with humidity relatively high, between 75 and 86% (Table 1). The distribution of insect species from the light-trapping sites (excluding wild honeybees) is reflected in the species-rank abundance curves in Figure 3.

Table 4. Insect diversity within a one-square-metre, as sampled through light-trapping in Imbak Canyon – Batu Timbang.

No.	Sampling site	Species	Ind.	Shannon	Simpson	Fisher Alpha
1.	A	49	56	3.81	118.46	187.47
2.	B	46	54	3.71	79.50	147.18
	Mean	47.5±2	55±1.4	3.76±0.07	99±27.60	167.30±28.50

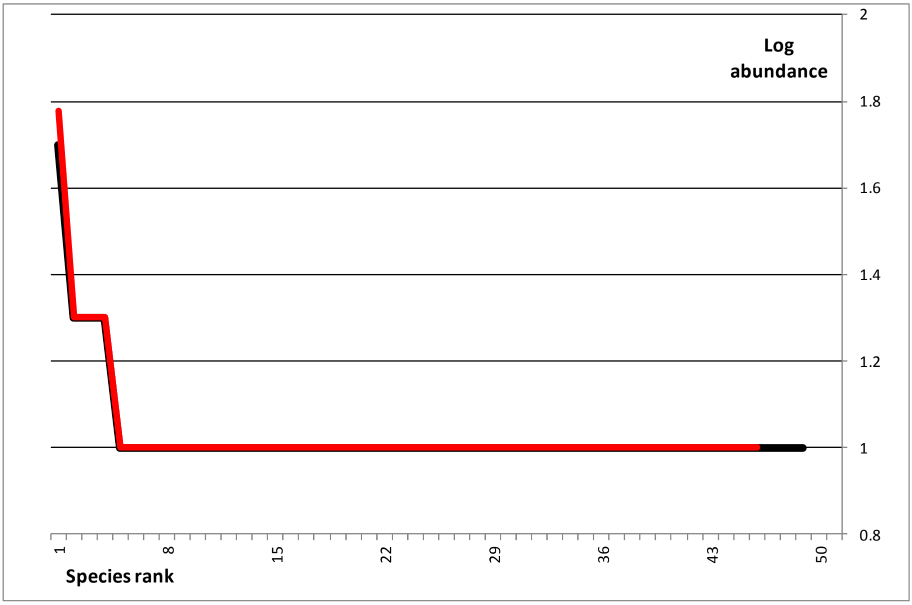

Figure 3. Species-rank abundance curves of light-trapping insect data in Imbak Canyon – Batu Timbang (Site A = black, Site B = red).

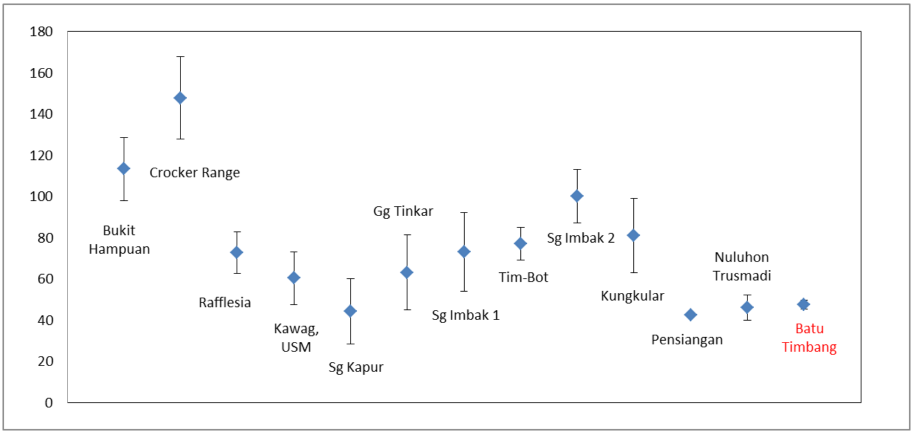

Figure 4a. Species number (±standard deviation) per night within one square metre as assessed through light-trapping in various forest reserves in Sabah.

When the nocturnal insect richness is compared with other forest reserves (Sabah Forestry Department's entomology data), Imbak Canyon - Batu Timbang insect richness (in red) appears to be low as shown in Figure 4a. In terms of nocturnal insect diversity, it is also moderately low (Figure 4b). Again, this could be due to the proliferation of the wild honeybees and the wet weather during the sampling period.

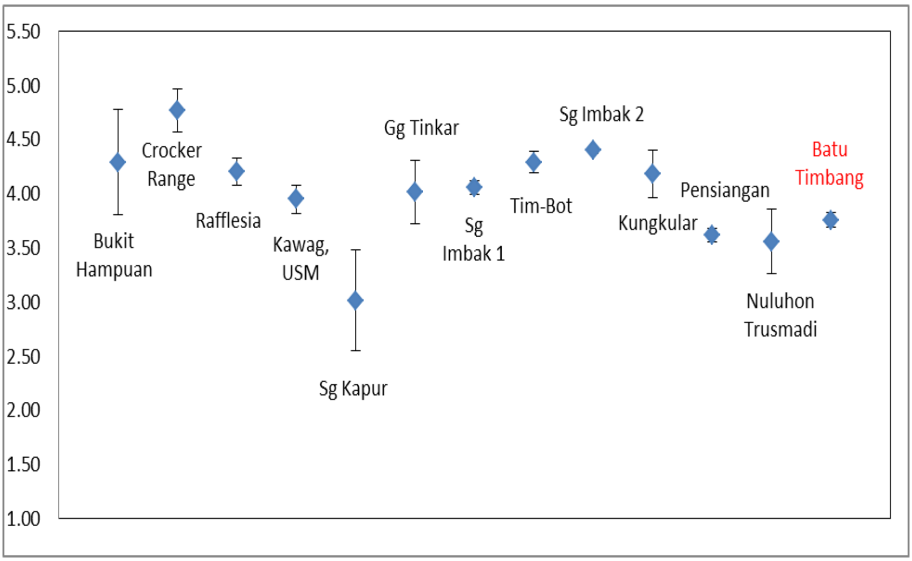

Figure 4b. Shannon Index (±standard deviation) per night within one square metre as assessed through light-trapping in various forest reserves in Sabah.

Table 5. Bornean endemic insect species from light-trapping in Imbak Canyon –Batu Timbang.

No.	Species	Author	Order	Family	Individuals
1	*Eulichas fasciolata*	Fairmaire	Coleoptera	Eulichadidae	1
2	*Chalcosoma moellenkampi*	Kolbe	Coleoptera	Scarabaeidae	2
3	*Amata prepuncta*	Holloway	Lepidoptera	Erebidae	1
4	*Panacra psaltria*	Jordan	Lepidoptera	Sphingidae	1

At least four Bornean endemic species were recorded from the base camp during light-trapping, as listed in Table 5. This information provides input towards recommendations on High Conservation Value Forest (HCVF) of the area, namely HCV 1.3 (WWF-Malaysia 2009). Endemism is one of the criteria that is highlighted as providing significant biodiversity values, hence, contributing towards forest conservation.

Eulichas fasciolata known as the Forest Stream Beetle, is common in the lowland mixed dipterocarp forests. However, not much is known about its ecology. It looks somewhat similar to a click beetle, with a distinctive scutellum with fine white hairs forming a circle and saw-toothed antennae (Bosuang et al. 2017). *Chalcosoma moellenkampi* or the Three-horned Beetle, is among the largest beetles in Borneo, measuring up to 115 mm in length. Although endemic, it is locally common in the lowlands, up to 1500 m in montane forests. *Amata prepuncta* is a wasp-like moth that normally prefers the understory of the lowland rainforest. *Panacra psaltria*, is one of the two hawkmoth species endemic to Borneo (Holloway 1998). It is found in dipterocarp forests, up to 900 m a.s.l.

Apart from endemic insects, the world's largest bush cricket, *Macrolyristes imperator*, was attracted to the light trap at Site A. This species has a wingspan of up to 274 mm and a body length (head to the tip of the forewings) of 150 mm (Chung 2002). The specimen sighted at Site A was a male, identified by a stridulatory organ at the base of the forewings. Four species of *Macrolyristes* have been described and all are found in the Malay Archipelago. The broad and serrated pronotum of the bush cricket is used in identification to differentiate among the four species.

Insects sampled using fruit-baited traps

Insects that were recorded feeding on rotting bananas in the fruit-baited traps are listed in Table 6. Some moths, butterflies and beetles were found in the

Table 6. Insects recorded from the fruit-baited traps in Imbak Canyon – Batu Timbang.

FBT no.	Species	Order, Family	Individuals
1	*Mycalesis orseis*	Lepidoptera, Nymphalidae	1
	Erebus caprimulgus	Lepidoptera, Erebidae	3
2	*Mycalesis orseis*	Lepidoptera, Nymphalidae	3
3	Curculionidae 5931	Coleoptera, Curculionidae	1
	Bocana 5933	Lepidoptera, Erebidae	2
4	*Ixorida* (*Mecinonota*) *regia bicolor*	Coleoptera, Scarabaeidae	1
	Erebus caprimulgus	Lepidoptera, Erebidae	1
5	*Ixorida* (*Mecinonota*) *regia bicolor*	Coleoptera, Scarabaeidae	1
	Sypnoides infernalis	Lepidoptera, Erebidae	1
	Bocana sp. 1 (5920)	Lepidoptera, Erebidae	1
	Bocana sp. 2 (5921)	Lepidoptera, Erebidae	1
6	*Erebus caprimulgus*	Lepidoptera, Erebidae	2

traps that were left overnight. One Bornean endemic subspecies of Flower Beetle, *Ixorida* (*Mecinonota*) *regia bicolor*, was recorded in two of the traps (FBT 4 & FBT5) while a Bornean endemic moth species, *Synoides infernalis*, was recorded from FBT5. The Purple Bushbrown butterfly, *Mycalesis orseis*, was the only butterfly sampled while *Erebus caprimulgus* was the commonest moth species recorded. Both *Mycalesis orseis* and *Erebus caprimulgus* are frequently attracted to rotting bananas (Chey 2015).

Diurnal insects sampled using sweep nets and forceps

Diurnal insects recorded from the base camp area include butterflies, dragonflies, damselflies and ants. However, they will not be discussed in depth in this report as Dr Choong Chee Yen of UKM reports on dragonflies and damselflies (Choong & Chung 2017) while Dr Homathevi Rahman and her students from UMS will report on the butterfly fauna. The Bornean endemic insect species recorded by the Forestry team while sampling along the trails at the base camp include the rare Bornean Mormon butterfly, *Papilio acheron* and a few damselfly species, namely *Rhinocypha aurofulgens*, *Drepanosticta actaeon* and *Devadatta tanduk*. The iconic Malaysia national butterfly, *Trogonoptera brookiana*, was sighted at the base camp and also along Sg. Lanap. A stick insect, *Acacus ?sarawacus*, was recorded from the Rafflesia Trail, adjacent to Sg. Lanap. Details of other insects recorded are listed in Appendices 1-5.

Threats and issues affecting insect diversity

Although ICCA is fully protected as Virgin Jungle Reserve (Class VI) and Protection Forest Reserve (Class I) under Sabah's forest classification, there are still various threats and issues which affect wildlife, including insects. Similar to that of other forest reserves, the major threats and issues that are affecting this reserve, as pointed out by Nilus et al. (2014), include forest fires, fragmentation, encroachment or illegal clearing of vegetation and poaching.

Of all these threats, forest fire is the most serious issue that can adversely affect insects within ICCA. A drought-induced fire on the forest may lead to the depletion of insect biomass, abundance and diversity that eventually changes species functions as well as alters the food chain. This has happened in the past in Sabah, e.g. Woods (1989) and reoccurs whenever there is a prolonged dry period. Therefore, monitoring during the dry season is crucial so that immediate action can be taken to reduce any forest fire incidences. Close cooperation and networking among relevant agencies, including capacity building, should be one of the main priorities in the management of any forest fire outbreak.

Forest fragmentation, encroachment or illegal clearing of vegetation and poaching are among threats that could indirectly affect the insect fauna in the long term. Having a ranger station in Batu Timbang would definitely enhance the enforcement in this part of the conservation area to curb any illegal activities. A station with radio communication and other basic facilities will also enable researchers to conduct long term research and monitoring work on wildlife, including insects.

A number of interesting Bornean endemic flora and fauna, including insects, were documented, even though this was just a two-week expedition. This indicates that the forest still harbours an interesting fauna, from the perspective of insects. Hence, the forest must continue to be protected and enhanced in terms of forest quality. Maintaining high forest quality is important for conserving rare taxa, especially for biodiversity conservation, while maintaining large forest areas is important for conserving common taxa, especially those that are important for conserving ecosystem functions.

Conclusion

Although the nocturnal insect diversity was not particularly high compared to other forest reserves, perhaps due to the proliferation of wild honeybees and unfavourable weather during the expedition, this pioneer data serves as baseline information for other research work in future. The endemic and interesting insect species recorded during the expedition provide information to enhance the conservation of this part of Imbak Canyon.

Any long-term threats are likely to indirectly affect the insect fauna. As such, continuous monitoring and enforcement at strategic locations are important to minimize these threats. Establishment of a ranger station and permanent research station would enable Yayasan Sabah and other relevant authorities to be more effective in managing this conservation area. This will ensure that the forest quality is improved in order to maintain the valuable biodiversity in the area, including insects.

Acknowledgements

Despite the unfavourable weather and logistical challenges, the expedition was successfully carried out, under the coordination of Dr Yap Sau Wai, who heads the Conservation and Environmental Management Division, Rita S. Galid, Dr Hamzah Tangki, Jadda Suhaimi and their team from Yayasan Sabah. We thank the then Chief Conservator of Forests, Datuk Sam Mannan, who was also the then chairman of the Imbak Canyon Management Committee; Deputy Chief Conservators, Frederick Kugan and Dr Lee Ying Fah, for their support in this expedition. We also wish to thank other entomologists who have contributed, namely Dr Choong Chee Yen, Dr Homathevi Rahman and Dr Francis Seow-Choen. Razy Japir, Nurul Aqidah and Dayang Fazrinah assisted in the insect list compilation. Dr Joan Pereira and Postar Miun provided some comments on the plants in this report. Viviannye Paul provided some insect photos from the second week of the expedition. The Sabah Forestry Department's participation in this expedition is an activity under the Heart of Borneo Initiative.

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Appendix 1. Butterfly list from Imbak Canyon - Batu Timbang (16-20 August, 2017).

No.	Species	Author	Family	Photo (IMB)	Remarks
1	*Paralaxita telesia ines*	Fruhstorfer	Riodinidae	DSCN5175, 5177	
2	*Trogonoptera brookiana brookiana*	Wallace	Papilionidae	DSC 0431	
3	*Papilio memnon memnon*	Linne	Papilionidae	DSC 0432	
4	*Cethosia hypsea hypsea*	Doubleday	Nymphalidae	Spotted	
5	*Mycalesis orseis borneensis*	Fruhstorfer	Nymphalidae	5963	
6	*Papilio acheron*	Grose-Smith	Papilionidae	5994, 5991	Endemic
7	*Mycalesis anapita fucentia*	Fruhstorfer	Nymphalidae	5785	
8	*Eurema* sp. 1		Pieridae	5805	
9	*Eurema* sp. 2		Pieridae	5958	

Appendix 2. Selected moths recorded from Imbak Canyon - Batu Timbang (16-20 August, 2017).

No.	Species	Author	Family	Subfamily	Photo no. (IMB)	Remarks
1	*Glyphodes canthusalis*	Walker	Crambidae		6057	
2	*Sylepte* sp.		Crambidae		6056	
3	Unidentified		Crambidae		5848	
4	Unidentified		Crambidae		5899	
5	*Drapetodes matulata*	Felder & Rogenhofer	Drepanidae	Drepaninae	6032	
6	*Tridrepana* sp.		Drepanidae		5894	
7	*Amata prepuncta*	Holloway	Erebidae	Arctiinae	spotted	Endemic
8	*Barsine rubricostata*	Herrich-Schaffer	Erebidae	Arctiinae	5839	
9	*Cyana* nr. *constifimbria*		Erebidae	Arctiinae	5844	
10	*Ischyja manlioides*	Prout	Erebidae	Catocalinae	5880	
11	*Ischyja manlioides*	Prout	Erebidae	Catocalinae	5890	
12	*Sypnoides infernalis*	Berio	Erebidae	Catocalinae	5919	Endemic
13	*Erebus caprimulgus*	Fabricius	Erebidae	Erebinae	5914a (female), 5927(male)	
14	*Bocana* sp. 1		Erebidae	Herminiinae	5920	
15	*Bocana* sp. 2		Erebidae	Herminiinae	5921	
16	*Cyana conclusa*	Walker	Erebidae	Lithosiinae	5883	
17	*Cyana determinata*	Walker	Erebidae	Lithosiinae	6037	

18	*Cyana malayensis*	Hampson	Erebidae	Lithosiinae	6035	
19	*Cyanaperornata*	Walker	Erebidae	Lithosiinae	5882	
20	Unidentified		Erebidae		5842	
21	Unidentified		Erebidae		6055	
22	*Bracca maculosa*	Walker	Geometridae	Ennominae	5850	
23	*Heterostegane* sp.	Guenee	Geometridae	Ennominae	6058	
24	Unidentified		Geometridae	Ennominae	5852	
25	*Pingasa rubicunda*	Warren	Geometridae	Geometrinae	6054	
26	*Pingasa ruginaria*	Guenee	Geometridae	Geometrinae	6033	
27	*Pingasa ruginaria*	Guenee	Geometridae	Geometrinae	6039	
28	*Pingasa venusta*	Warren	Geometridae	Geometrinae	6061	
29	Unidentified		Geometridae		6036	
30	*Setora* sp.		Limacodidae		5888	
31	*Carriola ecnomoda*	Swinhoe	Lymantriidae	Ennominae	6040	
32	*Arctornis* sp.		Lymantriidae		5855	
33	*Toxoproctis cosmia*	Collenette	Lymantriidae		5900	
34	Unidentified		Pyralidae		5889	
35	*Eupanacra busiris*	Walker	Sphingidae		6029	
36	*Panacra psaltria*	Jordan	Sphingidae		5863	Endemic
37	Unidentified		Unidentified		6060	

Appendix 3. Beetle list from Imbak Canyon - Batu Timbang (16-20 August, 2017).

No.	Species	Author	Family	Photo No. (IMB)	Remarks
1	*Rhabdoscelus* sp.		Curculionidae	DSCN5186	
2	*Alaus* sp.		Elateridae	5917	
3	*Lanelater* sp.		Elateridae	5856	
4	*Endomychus* sp.		Endomychidae	5696	
5	*Eumorphus* sp.		Endomychidae	5696	
6	*Eulichas fasciolata*	Fairmaire	Eulichadidae	5886	Endemic
7	*Pseudozaena orientalis*	Klug	Harpalidae	5847	
8	*Anomala* sp.		Scarabaeidae	5846	
9	*Chalcosoma atlas*	Linnaeus	Scarabaeidae	5579	
10	*Chalcosoma moellenkampi*	Kolbe	Scarabaeidae	spotted	Endemic
11	*Ixorida (Mecinonota) regia bicolor*	Kraatz	Scarabaeidae	DSCN5185a	Endemic subspecies

Appendix 4. Odonata list from Imbak Canyon - Batu Timbang (16-20 August, 2017)*.

No.	Species	Author	Family	Photo (IMB)	Remarks
1	*Vestalis* spp.	Selys, 1853	Calopterygidae	5630, 5750, 5753-5755, 5757-5758, 5770, 5959-5960, 5767	
2	*Rhinocypha aurofulgens*	Laidlaw, 1931	Chlorocyphidae	5773-5778	Endemic
3	*Rhinocypha humeralis*	Selys, 1873	Chlorocyphidae	5759-5760, 5995	
4	*Devadatta tanduk*	Dow et al., 2015	Devadattidae	5965-5966	Endemic
5	*Euphaea subcostalis*	Laidlaw, 1915	Euphaeidae	6006-6007, 5779-5780, 5822	
6	*Cratilla metallica*	Brauer,1878	Libellulidae	5824-5830	
7	*Neurothemis fluctuans*	Fabricus, 1993	Libellulidae	5799	
8	*Orthetrum chrysis*	Selys, 1891	Libellulidae	5807-5808	
9	*Orthetrum glaucum*	Brauer, 1865	Libellulidae	5637, 5640, 5804, 5981	
10	*Orthetrum pruinosum*	Burmeister, 1839	Libellulidae	5801, 5806, 56639(female)	
11	*Orthetrum testaceum*	Burmeister, 1839	Libellulidae	5802-5803	
12	*Trithemis festiva*	Rambur, 1842	Libellulidae	5809	
13	*Macromia* sp.		Macromiidae	5697-5699	
14	*Drepanosticta actaeon*	Laidlaw, 1934	Platystictidae	5795-5797, 5941	Endemic

*Choong Chee Yen has kindly identified some of the Odonata in this list.

Appendix 5. Other insects recorded from Imbak Canyon - Batu Timbang (16-20 August, 2017).

No.	Species	Author	Order	Family	Photo no. (IMB)	Remarks
1	*Leptogenys* nr *diminuta*	Smith	Hymenoptera	Formicidae	5814	
2	*Macrolyristes imperator*	Snellen van Vollenhoven	Orthoptera	Tettigoniidae	5872	
3	*Apis dorsata*	Fabricius	Hymenoptera	Apidae	5854	
4	*Provespa anomala*	Saussure	Hymenoptera	Vespidae	5840	
5	*Orientopsaltria padda*	Distant	Hemiptera	Cicadidae	6042	
6	*Dinomyrmex gigas*	Latreille	Hymenoptera	Formicidae	5861	
7	*Dundubia vaginata*	Fabricius	Hemiptera	Cicadidae	5857	
8	*Platylomia* nr *spinosa*	Fabricius	Hemiptera	Cicadidae	5892	
9	*Nevromus gloriosoi*	Liu, Hayashi & Yang	Megaloptera	Corydalidae	6051	
10	*Episyrphus?* sp.		Diptera	Syrphidae	6025	
11	*Neoperla* sp.		Plecoptera	Perlidae	5762	

12	*Acacus ?sarawacus*	Phasmida	Diapheromeridae	5790
13	*Calliphora* sp.	Diptera	Calliphoridae	5951

Journal of Tropical Biology and Conservation 16: 25–33, 2019
ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

Amphibians and Reptiles of Imbak Canyon Study Centre and Batu Timbang Camp

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Abstract
Amphibians and reptiles of Imbak Canyon Study Centre and Batu Timbang Camp have never been studied and this expedition was organised to produce an inventory of the species. The herpetofaunal animals were searched actively using the visual encounter survey method. A total of 84 specimens of amphibians and reptiles were obtained, comprising 75 amphibians and nine reptiles. The total number of species obtained during the expedition was 33 (26 amphibian species and 7 reptilian species). Twenty species were obtained from Batu Timbang Camp (BTC) and 21 species were recorded from Imbak Canyon Study Centre (ICSC). The updated compiled list of species of herpetofauna at ICCA is now 73 species (37 amphibian species and 36 reptilian species).

Keywords: herpetofauna, frogs, lizards, geckoes, snakes, biodiversity, vertebrate

Introduction
Herpetofauna has the highest diversity in tropical forests around the world and is a key component in the vertebrate fauna of the forest. For example, amphibians play a vital role in the food web as a major predator insectivore (Rebouças & Solé 2015). The study of ecology and zoology helped detect anthropogenic impacts on forest ecosystems. These studies contain information about the natural life history and habitat requirements of important taxa.

Received 01 March 2019
Reviewed 13 May 2019
Accepted 06 August 2019
Published 15 October 2019

For example, the study of the ecology of the species could identify changes in diet, 26seed dispersal, distribution patterns and behaviours, as a result of logging and how these changes affect animal populations and forest ecology (Meijaard et al. 2005). In the meantime, herpetofauna has been proven to be a suitable model for the study of human impact on the environment in complex biological systems (Ernst & Rodel 2005). These animals are very sensitive to environmental changes and require special habitat and microhabitat to survive, especially during breeding. Understanding patterns of biodiversity are vital to conservation management, and any decision on planning and development of an area, should be based on accumulated knowledge of the species groups that only come with more available data. Species lists are usually the basic data of biological inventory and are often employed when there are limited conservation resources (Mace 2004). However, species lists still represent information that can be used to assist management authority of the area, until more ecological studies can be associated with the lists. The objectives of this study were to identify as many species of amphibians and reptiles as possible at the Imbak Canyon Study Centre (ICSC) and at Batu Timbang camp.

Methodology

The interior of Imbak Canyon Conservation Area (ICCA) (approximately 30,000 ha) is covered with a continuous rainforest. The periphery of the area is inhabited by local communities. The main relief is a succession of hills and streams between 250 and 333 m, with the highest peak at 1120 m a.s.l. Temperatures in the lowland vary between 25°C to 35°C. The wet season is between November and April and the dry season is from May to October.

The study sites comprise the ICSC which includes forest trails after crossing the Ara hanging bridge and Kapur hanging bridge and roads towards the Imbak falls; and Batu Timbang camp about 27 km from the ICSC.

Sample collection

Reconnaissance surveys were conducted during the day to look for suitable sampling sites. Visual encounter surveys were conducted at night with the aid of headlights by a team of 3-4 observers, walking at a steady pace along a designated stream or trail for a prescribed time within the first two hours after nightfall from 2000h to 2200h. Frogs were observed with the naked eye with the aid of headlights in appropriate microhabitats, such as along the banks of streams and trails. All frog sightings and/or all calls heard at a distance of approximately 10 m on either side of the 1.5 km long centre-line were searched and animals sighted were captured by hand. All samples collected were placed into individual plastic bags and labelled accordingly.

At most, two voucher specimens were euthanized with Tricaine (Ethyl 3-aminobenzoate methanesulfonate salt), fixed in 10% formalin and transferred to 70% alcohol for storage. Before fixation, measurements of the specimens were taken with a Mitutoyo digimatic caliper to the nearest 0.1 mm. Parameters measured were snout-vent length (SVL), measured from the tip of the snout to the tip of the vent and tibia length (TL). Colour photographs were taken and liver tissue was extracted and stored in 95% ethanol prior to fixation and preservation. Taxonomic nomenclature follows Frost et al. (2011). All specimens are deposited at the Imbak Canyon Study Centre.

Results and Discussion

A total of 84 specimens of amphibians and reptiles was obtained, comprising 75 amphibians and nine reptiles (Figure 1). The amphians are represented by six families (Figure 2), Bufonidae (10 specimens), Dicroglossidae (18), Megophryidae (7), Microhylidae (3), Ranidae (19) and Rhacophoridae (18). The reptiles are represented by four families (Figure 3), Colubridae (one specimen), Gekkonidae (five specimens), Agamidae (one specimen), and Scincidae (two specimens).

The total number of species obtained during the expedition is 33 (26 amphibian species and 7 reptilian species) (Table 1). The updated compiled list of species of herpetofauna at ICCA is now 73 species (37 amphibian species and 36 reptilian species) (Yayasan Sabah 2014). Twenty species were obtained from Batu Timbang Camp (BTC) and 21 species were recorded from Imbak Canyon Study Centre (ICSC).

Based on the species list from Table 1, following the conservation status of the IUCN Red List of Threatened species, only one species is endangered, which is the Spiny Turtle; two species are Vulnerable (Malayan Flat-shelled Turtle and the King Cobra); and eight species are Near Threatened (Spiny Slender Toad, Long-fingered Slender Toad, Brown Slender Toad, Greater Swamp Frog, Lesser Swamp Frog, Dring's Slender Litter Frog, Green-spotted Rock Frog, Cinnamon Frog). There are 22 species that are not yet evaluated by the IUCN Red List and this is a major concern for the conservation status of these species.

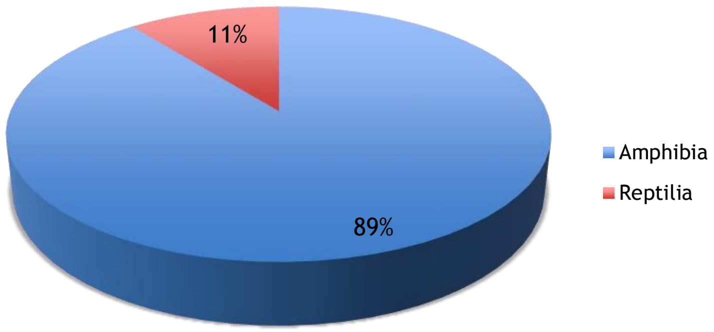

Figure 1. Relative abundance of amphibians and reptiles.

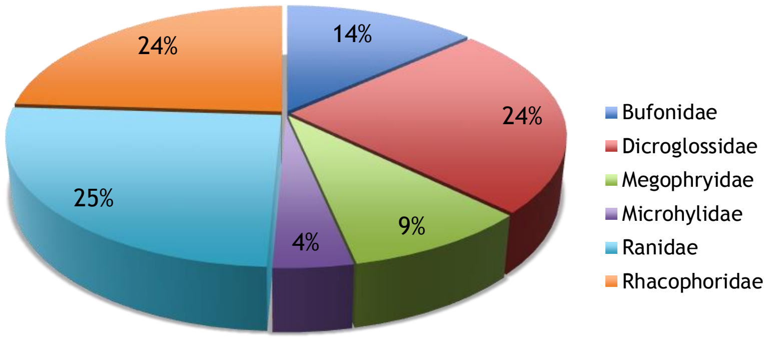

Figure 2. Amphibian relative abundance according to families.

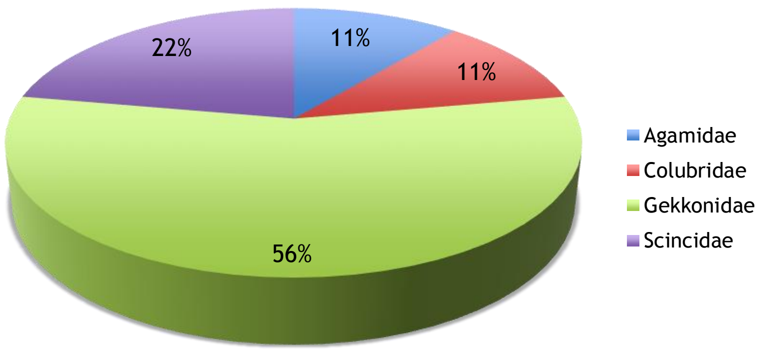

Figure 3. Reptilian relative abundance according to families.

Table 1. The updated compiled list of amphibian and reptile species at Imbak Canyon Conservation Area [ICSC=Imbak Canyon Study Centre, BTC=Batu Timbang Camp, YS=Yayasan Sabah (2014)].

No.	Class/Family/Species	Common Name	# Indiv.	ICSC	BTC	YS	Status
	Amphibia (75)						
	Bufonidae (10)						
1	*Ingerophrynus divergens*	Forest Toad	2		x	x	LC
2	*Rentapia hosii*	Hose's Tree Toad	2		x	x	LC
3	*Phrynoidis juxtasper*	Giant River Toad	3	x	x	x	LC
4	*Ansonia spinulifer*	Spiny Slender Toad	3	x		x	NT
5	*Ansonia longidigita*	Long-fingered Slender Toad				x	NT
6	*Ansonia leptopus*	Brown Slender Toad				x	NT
	Dicroglossidae (18)						
7	*Fejervarya limnocharis*	Grass Frog	1	x			LC
8	*Limnonectes ingeri*	Greater Swamp Frog	3		x		NT
9	*Limnonectes kuhlii*	Kuhl's Creek Frogs	8	x	x	x	LC
10	*Limnonectes leporinus*	Giant River Frog	5	x	x	x	LC
11	*Limnonectes paramacrodon*	Lesser Swamp Frog	1	x			NT
12	*Ingerana baluensis*	Dwarf Mountain Frog				x	LC
13	*Limnonectes finchii*	Rough Guardian Frog				x	NE
14	*Limnonectes palavanensis*	Smooth Guardian Frog				x	NE
	Megophryidae (7)						
15	*Leptolalax fritinniens*	Twittering Slender Litter Frog	5		x		NE
16	*Leptobrachella mjobergi*	Mjoberg's Dwarf Litter Frog	2		x	x	LC
17	*Megophrys nasuta*	Bornean Horned Frog			x	x	LC
18	*Leptolalax dringi*	Dring's Slender Litter Frog				x	NT
	Microhylidae (3)						
19	*Chaperina fusca*	Brown Thorny Frog	1		x	x	LC
20	*Metaphrynella sundana*	Tree Hole Narrow-mouthed Frog	2		x		LC
21	*Kalophrynus pleurostigma*	Rufous-sided Sticky Frog				x	LC
	Ranidae		19				
22	*Chalcorana raniceps*	White-lipped Frog	4	x	x	x	LC
23	*Meristogenys orphnocnemis*	Northern Torrent Frog	6		x	x	LC
24	*Odorrana hosii*	Poisonous Rock Frog				x	LC
25	*Pulchrana picturata*	Spotted Stream Frog	1	x	x		LC
26	*Pulchrana signata*	Striped Stream Frog	3	x		x	LC

27	*Staurois guttatus*	Black-spotted Rock Skipper	2		x	x	NE
28	*Staurois latopalmatus*	Rock Skipper	3		x	x	LC
29	*Staurois tuberilinguis*	Green-spotted Rock Frog				x	NT
	Rhacophoridae (18)						
30	*Kurixalus appendiculatus*	Frilled Tree Frog	1	x		x	LC
31	*Nyctixalus pictus*	Cinnamon Frog	1	x		x	NT
32	*Polypedates colletti*	Collett's Tree Frog	7	x		x	LC
33	*Polypedates leucomystax*	Four-lined Tree Frog	1	x			LC
34	*Polypedates macrotis*	Dark-eared Tree Frog	3	x			LC
35	*Polypedates otilophus*	File-eared Tree Frog		x		x	LC
36	*Rhacophorus nigropalmatus*	Wallace’s Flying Frog	2		x	x	LC
37	*Rhacophorus pardalis*	Harlequin Flying Frog	3	x	x	x	LC
	Reptilia (9)						
	Lacertilia						
	Agamidae (1)						
38	*Aphaniotis ornata*	Ornate Earless Agama	1	x			NE
39	*Draco melanopogon*	Black-bearded Gliding Lizard				x	NE
40	*Draco sumatranus*	Common Gliding Lizard				x	NE
41	*Gonocephalus bornensis*	Borneo Forest Dragon				x	NE
42	*Gonocephalus grandis*	Great Anglehead Lizard				x	LC
43	*Gonocephalus mjobergi*	Mjoberg's Anglehead Lizard				x	NE
44	*Phoxophrys cephalum*	Mocquard's Eyebrow Lizard				x	NE
	Gekkonidae (1)		5				
45	*Aeluroscalabotes felinus*	Cat Gecko	1		x		NE
46	*Cyrtodactylus cf. yoshii*	Yoshi's Bow-fingered Gecko	1	x		x	NE
47	*Cyrtodactylus ingeri*	Sabah Bow-fingered Gecko				x	NE
48	*Cyrtodactylus malayanus*	Borneo Bow-fingered Gecko				x	NE
49	*Cyrtodactylus pubisulcus*	Inger's Bow-fingered Gecko	1		x	x	NE
50	*Gekko mutilata*	Common Four-clawed Gecko	2	x			NE
51	*Gekko smithii*	Large Forest Gecko				x	LC
	Geoemydidae						

52	*Heosemys spinosa*	Spiny Turtle			x	EN
53	*Notochelys platynota*	Malayan Flat-shelled Turtle			x	VU
	Scincidae (2)					
54	*Tropidophorus brookei*	Brook's Keeled Skink	2	x	x	NE
55	*Eutropis rudis*	Rough Skink			x	NE
	Varanidae					
56	*Varanus salvator*	Water Monitor			x	LC
	Serpentine					
	Calamariidae					
57	*Calamaria suluensis*	Yellow-bellied Reed Snake			x	LC
	Colubridae (1)					
58	*Ahaetulla prasina*	Oriental Whipsnake			x	LC
59	*Asthenodipsas laevis*	Smooth Slug Snake	1	x		LC
60	*Boiga drapiezii*	White-spotted Cat Snake			x	LC
61	*Boiga nigriceps*	Black-headed Cat Snake			x	LC
62	*Gonyosoma oxycephalum*	Red-tailed Green Ratsnake			x	LC
63	*Lycodon effraenis*	Brown Wolf Snake			x	LC
64	*Lycodon subcinctus*	Malayan Banded Wolf Snake			x	LC
65	*Xenochrophis trianguligerus*	Triangle Keelback			x	NE
	Elapidae					
66	*Calliophis bivirgata*	Blue Coral Snake			x	NE
67	*Naja sumatrana*	Equatorial Spitting Cobra			x	LC
68	*Ophiophagus hannah*	King Cobra			x	VU
	Natricidae					
69	*Rhabdophis conspicillatus*	Red-belled Keelback			x	LC
	Viperidae					
70	*Trimeresurus borneensis*	Bornean pit viper			x	NE
71	*Tropidolaemus wagleri*	Wagler's Keeled Green Pit Viper			x	LC
	Pythonidae					
72	*Malayopython reticulatus*	Reticulated Python			x	NE
73	*Python breitensteini*	Bornean Short-tailed Python			x	LC
	Grand Total		*84*			

IUCN Red List of Threatened Species: **NE**=Not Evaluated, **DD**=Data Deficient, **LC**=Least Concern, **NT**=Near Threatened, **VU**=Vulnerable, **EN=**Endangered, **CR**=Critically Endangered, **EW**=Extinct in the Wild, **EX**=Extinct.

Acknowledgements

Several people contributed to the species list, especially Daicus Bellabut, Mahathir Ratig and Eddie Ahmad. We would like to acknowledge Universiti Kebangsaan Malaysia; Dr. Yap Sau Wai, acting group manager Conservation and Environmental Management Division, Sabah Foundation; Dr. Hamzah Tangki, the Manager of ICSC; and all the rangers of ICSC for their help and friendship during the expedition. The expedition was sponsored by Petronas and Sabah Foundation.

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Journal of Tropical Biology and Conservation 16: 35–39, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

Land and Freshwater Snails of Imbak Canyon Conservation Area (ICCA), Sabah, Northern Borneo

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Abstract

This paper presents the first checklist of land and freshwater snails of Imbak Canyon Conservation Area (ICCA) as part of the Imbak Crayon Scientific Expedition 2017. The specimens of land and freshwater snails were collected from six standard sampling plots and several random locations around Batu Timbang Basecamp between 17th and 19th August 2017. A total of 21 species of land snail and one species of freshwater snail are presented in a checklist. Currently, 24 species of land snails and one species of freshwater snail are recorded in ICCA, after including two species that were previously recorded but yet to be published.

Keywords: Borneo, Malaysia, Lahad Datu, Mollusca, lowland forest, Species diversity

Introduction

Imbak Canyon Conservation Area (ICCA), together with the Danum Valley Conservation Area and Maliau Basin Conservation Area are among the most important conservation areas in Sabah (Reynolds et al., 2011). Several malacological studies have been conducted in the tropical lowland rainforests, covering several localities across Sabah's mainland (Schilthuizen & Rutjes, 2001; Schilthuizen et al., 2002; Liew et al., 2008; Uchida et al., 2013), but there is hitherto no published information on the malacofauna diversity of the ICCA. Ten land snail species were recorded in ICCA based on specimens previously collected and deposited in the *BORNEENSIS* collection at Universiti Malaysia Sabah, but there is thus far no systematic survey on the snail diversity in Imbak Canyon and Maliau Basin. As part of the Imbak Canyon Scientific Expedition 2017, a systematic sampling and an opportunistic search for land and freshwater snails were carried out around the Batu Timbang Basecamp, which is located at the southwest of Imbak Canyon.

Received 21 February 2019
Reviewed 24 May 2019
Accepted 27 May 2019
Published 15 October 2019

Methodology

This sampling was conducted at Batu Timbang, located at the southwest of Imbak Canyon Conservation Area (ICCA), around 30km away from the Imbak Canyon Research Centre, between 17th August 2017 and 19th August 2017. We surveyed six standard plots (20m x 20m) along the three trails, namely West Summit Trail, Rafflesia Trail and Lanap Trail (Table 1). Snails encountered opportunistically around Batu Timbang were also collected. Land snail sampling and sorting were carried out according to methodology described in Phung et al. (2017) and Schilthuizen et al. (2003). Each specimen was identified to the lowest taxonomic level (or unit) possible by referring to the manuscript *Field Guide to the Land Snails of Sabah* (Vermeulen & Liew*in prep*), Vermeulen et al. (2015) and the *BORNEENSIS* collection in Universiti Malaysia Sabah. The specimens were catalogued and deposited in the BORNEENSIS collection at the Institute of Tropical Biology and Conservation, Universiti Malaysia Sabah (BOR/MOL 13315-13316, BOR/MOL 16618-13336, BOR/MOL 13339, BOR/MOL 13380-13402).

Table 1. Details and coordinates for all sampling locations.

Sampling locations	Latitude (°)	Longitude (°)
ICCA expedition, Batu Timbang Basecamp. Standard Plot No. 1 along West Summit Trail.	5.0041	117.0736
ICCA expedition, Batu Timbang Basecamp. Standard Plot No. 2 along West Summit Trail.	5.0028	117.0644
ICCA expedition, Batu Timbang Basecamp. Standard Plot No. 3 along West Summit Trail.	5.0048	117.0711
ICCA expedition, Batu Timbang Basecamp. Standard Plot No. 4. along Rafflesia Trail, beside waterfall.	4.9976	117.0791
ICCA expedition, Batu Timbang Basecamp. Standard Plot No. 5. along Rafflesia Trail.	4.9960	117.0769
ICCA expedition, Batu Timbang Basecamp. Standard Plot No. 6. along Lanap Trail.	5.0038	117.0802
ICCA expedition, random locations along Batu Timbang's river.	NA	NA
ICCA expedition, random locations along Batu Timbang Basecamp.	NA	NA
ICCA expedition, random locations along Batu Timbang 300 m from Lanap trail.	NA	NA

Results

The checklist reports a total of 21 land snail species belonging to 11 families and 19 genera (Table 2). *Clea* sp., a member of the family Nassariidae, was the only freshwater snail recorded during this expedition. Ariophantidae, with five species recorded, was the most species-rich family found in Imbak Canyon, followed by the family Trochomorphidae with three species recorded. Microsnails (size less than 5 mm) from genera *Microcystina*, *Charopa*, *Discocharopa*, *Diplommatina*, *Philalanka*, *Kaliella*, *Paralaoma*, accounted for approximately half of the total number of species documented in this study.

Table 2. Checklist of snails collected during Imbak Canyon Expedition 2017 at Batu Timbang. (#) indicates freshwater snail and (*) indicates land snail recorded previously.

Species	Standard Plot						Opportunistic search		
	1	2	3	4	5	6	River	Base Camp	300 m from Lanap trail
Ariophantidae									
**Hemiplecta humphreysiana* (Lea, 1841)				/		/			/
**Macrochlamys* sp.						/			
Microcystina microrhynchus Vermeulen, Liew & Schilthuizen, 2015			/	/	/	/			
Microcystina muscorum (Van Benthem Jutting, 1959)				/					
Vitrinula descrepignyi (Higgins, 1868)						/			
Camaenidae									
**Amphidromus martensi* (Boettger, 1894)				/		/			
Chloritis kinibalensis (Kobelt, 1894)								/	
Charopidae									
Charopa lissobasis Vermeulen, Schilthuizen & Liew, 2007		/		/	/	/			
Discocharopa aperta (von Möllendorff, 1888)			/	/		/			
Cyclophoridae									
Japonia sp.				/					
Opisthoporus sp.						/			
Diplommatinidae									
Diplommatina sp.			/	/		/			
Dyakiidae									
**Everettia paulbasintali* Liew, Schilthuizen & Vermeulen, 2009	/		/	/	/	/		/	
Endodontidae									

Philalanka kusana (Aldrich, 1889)			/		/			
Euconulidae								
Kaliella dendrophila (van Benthem Jutting, 1950)		/						
Kaliella microconus (Mousson, 1865)	/							
#Nassariidae								
Clea sp.						/		
Punctidae								
Paralaoma angusta Vermeulen, Liew & Schilthuizen, 2015		/						
Subulinidae								
Paropeas achatinaceum (Pfeiffer, 1846)			/	/				
Trochomorphidae								
**Bertia brookei* (Adams & Reeve, 1848)								/
**Geotrochus meristotrochus* Vermeulen, Liew & Schilthuizen, 2015			/	/	/			
**Videna metcalfei* (Pfeiffer, 1845)							/	

Discussion

This study adds 16 new records to the previously unpublished records of five land snail species in the ICCA. These new records are *Everettia paulbasintali*, *Japonia* sp., *Opisthoporus* sp., *Clea* sp., and *Chloritis kinibalensis* , *Microcystina microrhynchus*, *Microcystina muscorum*, *Vitrinula descrepignyi*, *Charopa lissobasis*, *Discocharopa aperta*, *Diplommatina* sp., *Philalanka kusana*, *Kaliella dendrophila*, *Kaliella microconus*, *Paralaoma angusta*, and *Paropeas achatinaceum*. Nevertheless, three species previously recorded in 2000 at the Transit Camp, *Leptopoma undatum* (BOR/MOL 231), *Everettia subconsul* (BOR/MOL 925,927,967,5272) and *Dyakia hugonis* (BOR/MOL 914), were not re-encountered during this study. The relatively large number of new records suggests that the current sampling efforts for snail fauna on ICCA are insufficient to be regarded as representative of the true land snail diversity. Therefore, this study which recorded a total of 24 species of land snails and one species of freshwater snail in ICCA to date should be treated as preliminary.

The species assemblage of ICCA is similar to the land snail fauna in the east coast of Sabah, namely, Lower Kinabatangan Valley, Danum Valley, Tawau Hills Park and Tabin Wildlife Reserve (Schilthuizen et al., 2003, 2005; Schilthuizen & Rutjes 2001). We recommend future studies to increase the geographical coverage and number of sampling plots so as to assess better the snail diversity in Imbak Canyon.

Acknowledgements

We thank Yayasan Sabah and their staff for excellent hospitality and logistics during fieldwork sampling. We also thank Elia Godoong, Miyabi Nakabayashi, PG Mohd Sahlan, Homathevi Rahman, and Amshahri for the help in collecting additional specimens from ICCA.

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Journal of Tropical Biology and Conservation 16: 41–60, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

Birds of Imbak Canyon Study Centre, Imbak Canyon Conservation Area, Tongod, Sabah, Malaysia

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Abstract
A survey on birds of the Imbak Canyon Study Centre was conducted from 16th to 26th August 2017, in every direction along the roads, forest trails through the understorey, i.e. whenever fine weather permitted. A total of 92 species of birds from 33 families were recorded. A total of 27 species are Near Threatened, one species is Vulnerable (Blue-headed Pitta), one species is not recorded (White-crowned Shama) and one species are Critically Endangered (Helmeted Hornbill). The present survey contributed nine new records to the area, updating the compiled list to 252 species, from the previous 243 species. The nine new records are Lesser Cuckoo Shrike, Red-bearded Bee-eater, Dark-sided Flycatcher, Yellow-vented Flowerpecker, Van Hasselt's Sunbird, Temminck's Sunbird, Yellow-bellied Prinia, Bold-striped Tit Babbler, and White-necked Babbler. All new records of the area are in the Least Concern category except the White-necked Babbler, which is Near Threatened.

Keywords: avian, Borneo, rainforest, Malaysia, biodiversity, vertebrate

Introduction
Management of protected areas for the conservation of species assemblages is a challenge due to the mounting loss of natural habitats and biodiversity. Although it is known that most vertebrate species need a large foraging area to survive and that they cannot be conserved only within a few protected areas (González-Maya et al., 2015), focusing conservation efforts to a few selected sites that do not have much human pressure is still much needed.

Imbak Canyon Conservation Area is largely free of human settlements and is covered with a continuous primary rain forest. The nearest village is Kg. Langga (4 km away), while the nearest town is Telupid, approximately 94 km from the Imbak Canyon Study Centre (ICSC). There are a number of satellite camps in

Received 08 March 2019
Reviewed 10 June 2019
Accepted 21 June 2019
Published 15 October 2019

ICCA, with facilities to accommodate the most basic needs of a small group of researchers. A scientific expedition was conducted at Batu Timbang Camp (BTC), which is approximately 10 km from ICSC. This camp is the nearest to the Batu Timbang Forest Reserve, under the jurisdiction of the Sabah Forestry Department. BTC has never been surveyed before, and thus, this expedition was to obtain representative samples of biodiversity, and to support planning for long-term conservation. Hot spots of species richness, rarity or endemism may be identified and used to define priority areas (Reid, 1998).

Birds are often used as indicator taxa because they are well known, the most conspicuous of all fauna, easy to census, charismatic and they have been found to be among the best surrogate taxa for biodiversity in conservation area planning (Reyers et al., 1999). The total bird species in Malaysia is 785, of which 51 species are endemic (47 species are endemic to Sabah and Sarawak). A total of 48 species, unfortunately, are globally threatened species (35 in Peninsular Malaysia and 30 species in Sabah and Sarawak). This report covers the presence of birds at the ICSC, a previously undocumented area.

Method

Study Area

The interior of ICCA (approximately 30,000 ha) is covered with a continuous rain forest, sparsely inhabited, almost only along its borders. The main relief is a succession of hills and streams between 250 and 333 m, with the highest peak at 1,120 m a.s.l. Temperature in the lowland varies between 25°C to 35°C. The wet season is between November and April and the dry season is from May to October.

Study sites

The study sites comprise the ICSC, which include the forest trails after crossing the Ara hanging bridge and Kapur hanging bridge; viewpoints at ICSC, verandah of the dining hall, and roads towards Imbak Falls.

Census technique

At the ICSC, daily surveys were conducted from 16 to 26th August 2017, in every direction along the roads, forest trails through the understorey, mainly within a 500m radius, i.e. whenever fine weather permitted. All locally existing forest trails, as well as clearings associated with rivers were covered. Speed of walking was very slow (<1 km/h), with frequent stops, recording and photographing every bird, seen or heard, that was perched within 25 m of the walking line at any height. Only species records were used, the number of individuals involved at each record was most often difficult to assess. All diurnal (Falconiforms) and nocturnal (Strigiforms), migrants, occasional, vagrant or resident species were included in the surveys. Photos were taken with two set-ups: 1) Nikon D500 with a AF-S Nikkor 300mm f/4E PF ED VR lens and AF-S teleconverter TC-14E III and 2) Nikon D500 with a AF-S Nikkor 200-500mm f/5.6 E ED VR lens.

Results and Discussion

A total of 92 species from 33 families were recorded during the expedition (Table 1). Timaliidae has the highest species richness, with 12 species, followed by Pycnonotidae (10 species) and Nectariniidae (8 species). The rest of the families range in species number between one and six species (Figure 1).

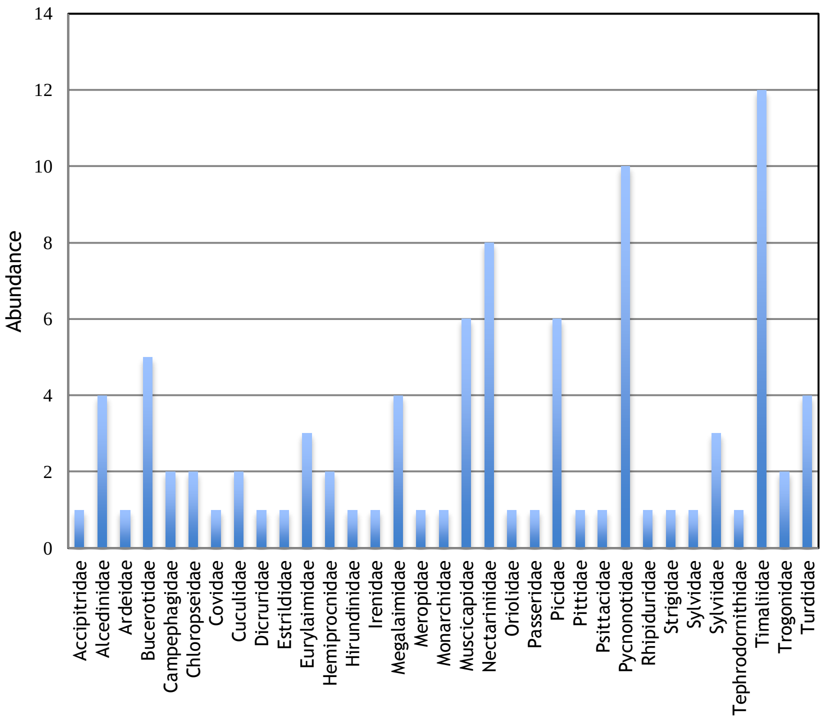

Figure 1. Total count of species of birds recorded during the expedition according to families.

Table 1. The checklist of bird species at Imbak Canyon Study Centre during the expedition.

No	Family	Common Name	Scientific Name	Local Name	Status	Location	ID Method
1	Accipitridae	Oriental Honey Buzzard	*Pernis ptilorhynchus torquatus*	Lang Lebah	LC	ICSC	dark-pale morphs
2	Alcedinidae	Banded Kingfisher	*Lacedo pulchella*	Pekaka Riang Rimba	LC	Ara Bridge	Calls
3	Alcedinidae	Malay Blue-banded Kingfisher	*Alcedo peninsulae peninsulae*	Pekaka Bukit	NT	Imbak Falls Trail	Photo
4	Alcedinidae	Collared Kingfisher	*Todiramphus chloris*	Pekaka Sungai	LC	ICSC	Photo
5	Alcedinidae	Rufous-backed Kingfisher	*Ceyx rufidorsus*	Pekaka Api	LC	Imbak Falls Trail	Photo
6	Ardeidae	Great-billed Heron	*Ardea sumatrana*	Pucung Lembu	LC	ICSC	Photo
7	Bucerotidae	Black Hornbill	*Anthracoceros malayanus*	Enggang Gatal Birah	NT	ICSC	Sighting (M & F)
8	Bucerotidae	Bushy-crested Hornbill	*Anorrhinus galeritus*	Enggang Belukar	LC	Main Access Rd	Sighting
9	Bucerotidae	Helmeted Hornbill	*Rhinoplax vigil*	Enggang Tebang Mentua	**CR**	Imbak Falls Rd	Calls
10	Bucerotidae	Rhinoceros Hornbill	*Buceros rhinoceros*	Enggang Badak	NT	Main Access Rd	Sighting
11	Bucerotidae	Wrinkled Hornbill	*Rhabdotorrhinus corrugatus*	Enggang Berkedut	NT	Main Access Rd	Sighting
12	Campephagidae	Black-winged Flycatcher Shrike	*Hemipus hirundinaceus*	Rembah Batu	LC	Kapur Bridge	Photo
13	Campephagidae	Lesser Cuckoo Shrike	*Coracina fimbriata*	Sewah Kecil	LC	Main Access Rd	Photo (F)
14	Chloropseidae	Common Iora	*Aegithina tiphia*	Kunyit Kacat	LC	Imbak Falls Rd	Photo
15	Chloropseidae	Lesser Green Leafbird	*Chloropsis cyanopogon*	Daun Kecil	NT	ICSC	Photo (M & F)
16	Covidae	Slender-billed Crow	*Corvus enca*	Gagak Paruh Lampai	LC	ICSC	Sighting
17	Cuculidae	Chestnut-breasted Malkoha	*Phaenicophaeus curvirostris*	Cenok Birah	LC	Main Access Rd	Sighting
18	Cuculidae	Raffles's Malkoha	*Phaenicophaeus chlorophaeus*	Cenok Kerak	LC	Main Access Rd	Sighting

(*Continue on next page*)

Table 1. Continued

No	Family	Common Name	Scientific Name	Local Name	Status	Location	ID Method
19	Dicruridae	Greater Racket-tailed Drongo	*Dicrurus paradiseus*	Cecawi Anting-anting	LC	Main Access Rd	Sighting
20	Estrildidae	Dusky Munia	*Lonchura fuscans*	Pipit Hitam	LC	ICSC	Photo
21	Eurylaimidae	Black-and-yellow Broadbill	*Eurylaimus ochromalus*	Takau Hitam Kuning	NT	Ara Bridge	Photo
22	Eurylaimidae	Banded Broadbill	*Eurylaimus javanicus*	Takau Rimba	NT	Ara Bridge	Photo
23	Eurylaimidae	Green Broadbill	*Calyptomena viridis*	Takau Selawit	NT	Ara Bridge	Photo (M & F)
24	Hemiprocnidae	Grey-rumped Treeswift	*Hemiprocne longipennis*	Layang-layang Jambul Kelabu	LC	ICSC	Photo
25	Hemiprocnidae	Whiskered Treeswift	*Hemiprocne comata*	Layang-layang Jambul Kecil	LC	ICSC	Photo
26	Hirundinidae	Pacific Swallow	*Hirundo tahitica*	Sualo Batu	LC	ICSC	Photo
27	Irenidae	Asian Fairy-bluebird	*Irena puella*	Dendang Gajah	LC	Imbak Falls Rd	Sighting (M & F)
28	Megalaimidae	Blue-eared Barbet	*Psilopogon duvaucelii*	Takor Akar	LC	ICSC	Sighting
29	Megalaimidae	Gold-whiskered Barbet	*Psilopogon chrysopogon*	Takor Jambang Emas	LC	Ara Bridge	Photo
30	Megalaimidae	Red-throated Barbet	*Psilopogon mystacophanos*	Takor Raya	NT	Imbak Falls Rd	Sighting
31	Megalaimidae	Yellow-crowned Barbet	*Psilopogon henricii*	Takor Mahkota Kuning	NT	ICSC	Sighting
32	Meropidae	Red-bearded Bee-eater	*Nyctyornis amictus*	Berek-Berek Janggut Merah	LC	Kapur Bridge	Photo
33	Monarchidae	Black-naped Monarch	*Hypothymis azurea*	Sambar Uban Hitam	LC	Imbak Falls Rd	Photo (M & F)
34	Muscicapidae	Asian Paradise Flycatcher	*Terpsiphone affinis*	Sambar Ekor Panjang	LC	Imbak Falls Rd	Sighting
35	Muscicapidae	Dark-sided Flycatcher	*Muscicapa sibricia*	Sambar Sibiria	LC	Ara Bridge	Photo
36	Muscicapidae	Rufous-chested Flycatcher	*Ficedula dumetoria*	Sambar Dada Oren	LC	Kapur Bridge	Photo (F)

(Continue on next page)

Table 1. Continued

No	Family	Common Name	Scientific Name	Local Name	Status	Location	ID Method
37	Muscicapidae	Yellow-vented Flowerpecker	*Dicaeum chrysorrheum*	Sepah Puteri Rimba	LC	Imbak Falls Rd	Photo
38	Muscicapidae	Yellow-rumped Flowerpecker	*Prionochilus xanthopygius*	Sepah Puteri Acang Bilang	LC	ICSC	Photo (M & F)
39	Muscicapidae	Yellow-breasted Flowerpecker	*Prionochilus maculatus*	Sepah Puteri	LC	ICSC	Photo M & F)
40	Nectariniidae	Little Spiderhunter	*Arachnothera longirostra*	Kelicap Jantung	LC	Kapur Bridge	Photo
41	Nectariniidae	Thick-billed Spiderhunter	*Arachnothera crassirostris*	Kelicap Jantung Paruh Tebal	LC	Kapur Bridge	Photo
42	Nectariniidae	Plain Sunbird	*Anthreptes simplex*	Kelicap Kelabu	LC	Kapur Bridge	Photo
43	Nectariniidae	Red-throated Sunbird	*Anthreptes rhodolaema*	Kelicap Pinang	NT	Imbak Falls Rd	Photo
44	Nectariniidae	Van Hasselt's Sunbird	*Leptocoma brasiliana*	Kelicap Nibong	LC	Imbak Falls Rd	Photo
45	Nectariniidae	Purple-naped Sunbird	*Hypogramma hypogrammicum*	Kelicap Rimba	LC	Kapur Bridge	Photo (F)
46	Nectariniidae	Ruby-cheeked Sunbird	*Chalcoparia singalensis*	Kelicap Belukar	LC	Main Access Rd	Photos (M & F)
47	Nectariniidae	Temminck's Sunbird	*Aethopyga temminckii*	Kelicap Merah	LC	Imbak Falls Rd	Photo (M & F)
48	Oriolidae	Dark-throated Oriole	*Oriolus xanthonotus*	Dendang Senja	NT	Imbak Falls Rd	Photo (M & F)
49	Passeridae	Eurasian Tree-sparrow	*Passer montanus*	Ciak Urasia	LC	ICSC	Photo
50	Picidae	Rufous Piculet	*Sasia abnormis*	Belatok Kecil	LC	Kapur Bridge	Photo
51	Picidae	Buff-rumped Woodpecker	*Meiglyptes grammithorax*	Belatuk Awan	LC	Main Access Rd	Photo
52	Picidae	Checker-throated Woodpecker	*Picus mentalis*	Belatuk Ranting	NT	Main Access Rd	Photo
53	Picidae	Olive-backed Woodpecker	*Dinopium rafflesii*	Belatuk Rimba	NT	Main Access Rd	Photo (M & F)
54	Picidae	White-bellied Woodpecker	*Dryocopus javensis*	Belatuk Gajah	LC	Main Access Rd	Photo

(Continue on next page)

Table 1. Continued

No	Family	Common Name	Scientific Name	Local Name	Status	Location	ID Method
55	Picidae	Grey-and-buff Woodpecker	*Hemicircus sordidus*	Belatuk Punggoh	LC	ICSC	Sighting
56	Pittidae	Blue-headed pitta	*Hydrornis baudii*	Pita Kepala Biru	VU	Kapur Bridge	Photo (F)
57	Psittacidae	Blue-crowned Hanging Parrort	*Loriculus galgulus*	Bayan Serindit	LC	ICSC	Sighting
58	Pycnonotidae	Grey-cheeked Bulbul	*Alophoixus bres*	Merbah Sampah	LC	Ara Bridge	Photo
59	Pycnonotidae	Yellow-bellied Bulbul	*Alophoixus phaeocephalus*	Merbah Perut Kuning	LC	Imbak Falls Rd	Photo
60	Pycnonotidae	Buff-vented Bulbul	*Iole crypta*	Merbah Riang	NT	Ara Bridge	Photo
61	Pycnonotidae	Hairy-backed Bulbul	*Tricholestes criniger*	Merbah Bulu Tengkok Panjang	LC	Kapur Bridge	Photo
62	Pycnonotidae	Cream-vented Bulbul	*Pycnonotus pseudosimplex*	Merbah Mata Putih	LC	Imbak Falls Rd	Photo
63	Pycnonotidae	Grey-bellied Bulbul	*Pycnonotus cyaniventris*	Merbah Kelabu	NT	Ara Bridge	Photo
64	Pycnonotidae	Puff-backed Bulbul	*Pycnonotus eutilotus*	Merbah Coklat Berjambul	NT	Kapur Bridge	Photo
65	Pycnonotidae	Asian Red-eyed Bulbul	*Pycnonotus brunneus*	Merbah Mata Merah	LC	Kapur Bridge	Photo
66	Pycnonotidae	Spectacled Bulbul	*Pycnonotus erythropthalmos*	Merbah Kecil	LC	Kapur Bridge	Photo
67	Pycnonotidae	Yellow-vented Bulbul	*Pycnonotus goiavier*	Merbah Kapur	LC	ICSC	Photo
68	Rhipiduridae	Pied Fantail	*Rhipidura javanica*	Sambar Murai Gila	LC	ICSC	Photo
69	Strigidae	Brown Wood Owl	*Ninox scutulata*	Hantu Betemak	LC	ICSC	Photo
70	Sylvidae	Yellow-bellied Prinia	*Prinia flaviventris*	Perenjak Padi	LC	ICSC	Photo
71	Sylviidae	Dark-necked Tailorbird	*Orthotomus astrogularis*	Perenjak Belukar	LC	Imbak Falls Rd	Photo (M & F)
72	Sylviidae	Ashy (Red-headed) Tailorbird	*Orthotomus ruficeps*	Perenjak Kelabu	LC	ICSC	Photo

(Continue on next page)

Table 1. Continued

No	Family	Common Name	Scientific Name	Local Name	Status	Location	ID Method
73	Sylviidae	Rufous-tailed Tailorbird	*Orthotomus sericeus*	Perenjak Rimba	LC	ICSC	Photo
74	Tephrodornithidae	Rufous-winged Philentoma	*Philentoma pyrhoptera*	Sambar Paya	LC	Kapur Bridge	Photo
75	Timaliidae	Bold-striped Tit Babbler	*Macronous bornensis*	Rimba berjalur	LC	ICSC	Photo
76	Timaliidae	Fluffy-backed Tit Babbler	*Macronous ptilosus*	Rimba Pong-pong	NT	Imbak Falls Rd	Sighting
77	Timaliidae	Chestnut-winged Babbler	*Stachyris erythroptera*	Rimba merbah sampah	LC	ICSC	Photo
78	Timaliidae	Rufous-fronted Babbler	*Stachyridopsis rufifrons*	Rimba Api	LC	Kapur Bridge	Photo
79	Timaliidae	Grey-headed Babbler	*Stachyris poliocephala*	Rimba Kepala Kelabu	LC	Imbak Falls Rd	Photo
80	Timaliidae	White-necked Babbler	*Stachyris leucotis*	Rimba Rembang	NT	Ara Bridge	Sighting
81	Timaliidae	Moustached Babbler	*Malacopteron magnirostre*	Rimba Bermisai	LC	Imbak Falls Rd	Sighting
82	Timaliidae	Rufous-crowned Babbler	*Malacopteron magnum*	Rimba Tua Besar	NT	Imbak Falls Rd	Photo
83	Timaliidae	Scaly-crowned Babbler	*Malacopteron cinereum*	Rimba Tua Kecil	LC	Imbak Falls Rd	Photo
84	Timaliidae	Sooty-capped Babbler	*Malacopteron affine*	Rimba Tinjau Belukar	NT	Imbak Falls Rd	Photo
85	Timaliidae	Striped Wren-babbler	*Kenopia striata*	Rimba Tanda Hujan	NT	Kapur Bridge	Photo
86	Timaliidae	Brown Fulvetta	*Alcippe brunneicauda*	Rimba Murai Coklat	NT	Imbak Falls Rd	Photo
87	Trogonidae	Diards Trogon	*Harpactes diardii*	Kesumba Bukit	NT	Imbak Falls Trail	Calls

(Continue on next page)

Table 1. Continued

No	Family	Common Name	Scientific Name	Local Name	Status	Location	ID Method
88	Trogonidae	Scarlet-rumped Trogon	*Harpactes duvaucelii*	Kesumba Puteri	NT	Main Access Rd	Photo
89	Turdidae	Chestnut-naped Forktail	*Enicurus ruficapillus*	Murai Cegar	NT	Imbak Falls Trail	Sighting
90	Turdidae	Oriental Magpie Robin	*Copsychus saularis*	Murai Kampung	LC	ICSC	Photo (M & F)
91	Turdidae	White-crowned Shama	*Copsychus striklandii*	Murai Rimba Dahi Putih	**NE**	Kapur Bridge	Photo
92	Turdidae	Rufous-tailed Shama	*Copsychus pyrropygus*	Murai Rimba Ekor Kuning	NT	Ara Bridge	Sighting

IUCN Red List of Treatened Species: **NE**=Not Evaluated, **DD**=Data Deficient, **LC**=Least Concern, **NT**=Near Threatedned, **VU**=Vulnerable, **EN=**Endangered, **CR**=Critically Endagered, **EW**=Extinct in the Wild, **EX**=Extinct.; F=Female, M=Male

The overall bird species richness is high, with a large proportion of species that are Least Concern in the conservation status of the IUCN Red List v. 3.1 (67% or 62 species). Approximately 33% of the total species recorded is under some pressure of threat (Figure 2). A total of 27 species are Near Threatened, one species is Vulnerable (Blue-headed Pitta), one species is Not Evaluated (White-crowned Shama) and one species is Critically Endangered (Helmeted Hornbill).

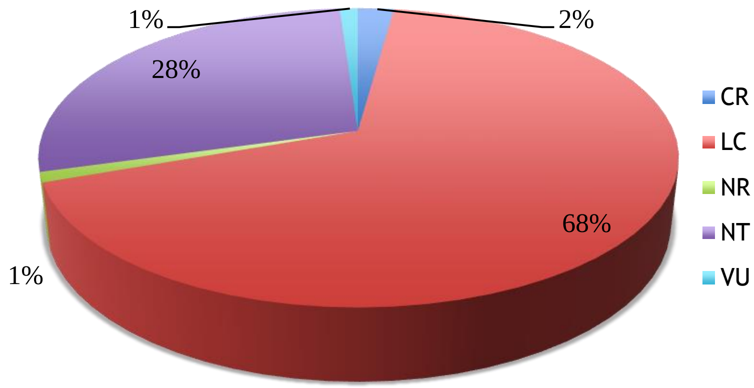

Figure 2. Percentages of bird species recorded during the 2017 expedition according to the conservation status of the IUCN Red Data List of Threatened Species.

Table 2 shows the compilation of bird data from the latest expedition and five previous surveys in ICCA (Yayasan Sabah 2014). The total species of birds documented by Yayasan Sabah (2014) (after some data clean-up, i.e. duplication) is 243 species (originally 245 species). The present survey contributed nine new records to the area, updating the compiled list to 252 species. The nine new records are Lesser Cuckoo Shrike, Red-bearded Bee-eater, Dark-sided Flycatcher, Yellow-vented Flowerpecker, Van Hasselt's Sunbird, Temminck's Sunbird, Yellow-bellied Prinia, Bold-striped Tit Babbler, and White-necked Babbler. All new records of the area are Least Concern except the White-necked Babbler, which is Near Threatened. It is important to note that since the last update by Yayasan Sabah (2014), the Short-tailed Babbler (*Trichastoma malaccensis*) has become Near Threatened from Least Concern (BirdLife

International, 2016) due to increased rate of forest loss owing partly to illegal logging and land conversion in the Sundaic lowlands. The Straw-headed Bulbul (*Pycnonotus zeylanicus*) has also since become Endangered from Vulnerable, due to illegal trading and forest loss. It has been reported that the average price for wild-caught bird is USD483 (A. Miller *in litt*. 2016). From the latest compiled data, 32% of the total of 252 are in some level of threat: two species are Critically Endangered, one species is Endangered (Straw-headed Bulbul), 71 species are Near Threatened, nine species are Vulnerable, and one species is Not Evaluated (White-crowned Shama). The rest of the species (170 species) are in the Least Concern category (Table 2).

ICCA and Maliau Basin Conservation Area (MBCA) are characterised as being lowland and montane rainforests, while Danum Valley Conservation Area (DVCA) as being largely montane (Yayasan Sabah 2014). The diverse range of natural vegetation communities and habitats (five types) defined for ICCA by Ong et al. (2000) shelter a high biodiversity with many endemic and threatened species, as well as potential new records of new species. The mosaic of different natural habitats, specific communities and patch sizes result in high diversity, population sizes and species composition of birds. Case in point is the bird surveys conducted during this expedition. Although sampling during the expedition was nine days, there were only three full days of good weather that actually permitted productive fieldwork that produced most of the bird species in the list of 92 in total. Many of the species were photographed as evidence of presence. These records and materials will be useful for Yayasan Sabah to manage the ICCA.

Table 2. Updated compilation of data from the 2017 expedition and Yayasan Sabah (2014)

No.	Family	Common Name	Scientific Name	Status	Note
1	Accipitridae	Oriental Honey Buzzard	*Pernis ptilorhynchus torquatus*	LC	2014/ 2017
2	Accipitridae	Bat Hawk	*Macheiramphus alcinus*	LC	2014
3	Accipitridae	Lesser Fish Eagle	*Haliaeetus humilis*	NT	2014
4	Accipitridae	Grey-headed Fish Eagle	*Haliaeetus ichthyaetus*	NT	2014
5	Accipitridae	Crested-serpent Eagle	*Spilornis cheela*	LC	2014
6	Accipitridae	Mountain (Kinabalu) Serpent Eagle	*Spilornis kinabaluensis*	VU	2014
7	Accipitridae	Besra	*Accipiter virgatus*	LC	2014
8	Accipitridae	Crested Goshawk	*Accipiter trivirgatus*	LC	2014
9	Accipitridae	Black Eagle	*Ictinaetus malaiensis*	LC	2014
10	Accipitridae	Rufous-bellied Eagle	*Lophotriorchis kienerii*	LC	2014
11	Accipitridae	Wallace's Hawk Eagle	*Nisaetus nanus*	VU	2014
12	Aegithinidae	Green Iora	*Aegithina viridissima*	NT	2014
13	Alcedinidae	Banded Kingfisher	*Lacedo pulchella*	LC	2014/ 2017
14	Alcedinidae	Malay Blue-banded Kingfisher	*Alcedo peninsulae*	NT	2014/ 2017
15	Alcedinidae	Collared Kingfisher	*Todiramphus chloris*	LC	2014/ 2017
16	Alcedinidae	Rufous-backed Kingfisher	*Ceyx rufidorsus*	LC	2014/ 2017
17	Alcedinidae	Stork-billed Kingfisher	*Pelargopsis capensis*	LC	2014
18	Alcedinidae	Blue-eared Kingfisher	*Alcedo meninting*	LC	2014
19	Alcedinidae	Oriental dwarf Kingfisher	*Ceyx erithaca*	LC	2014
20	Alcedinidae	Rufous-collared Kingfisher	*Actenoides concretus*	NT	2014
21	Anhingidae	Oriental Darter	*Anhinga melanogaster*	NT	2014
22	Apodidae	Glossy Swiftlet	*Collocalia esculenta*	LC	2014
23	Apodidae	Edible-nest Swiftlet	*Aerodramus fuciphagus*	LC	2014
24	Apodidae	Brown-backed Needletail	*Hirundapus giganteus*	LC	2014
25	Apodidae	Silver-rumped Spinetail	*Rhaphidura leucopygialis*	LC	2014
26	Apodidae	House Swift	*Apus nipalensis*	LC	2014
27	Ardeidae	Great-billed Heron	*Ardea sumatrana*	LC	2014/ 2017
28	Ardeidae	Little Heron	*Butorides striata*	LC	2014
29	Ardeidae	Malayan Night Heron	*Gorsachius melanolophus*	LC	2014
30	Bucerotidae	Black Hornbill	*Anthracoceros malayanus*	NT	2014/ 2017
31	Bucerotidae	Bushy-crested Hornbill	*Anorrhinus galeritus*	LC	2014/ 2017
32	Bucerotidae	Helmeted Hornbill	*Rhinoplax vigil*	CR	2014/

					2017
33	Bucerotidae	Rhinoceros Hornbill	*Buceros rhinoceros*	NT	2014/ 2017
34	Bucerotidae	Wrinkled Hornbill	*Rhabdotorrhinus corrugatus*	NT	2014/ 2017
35	Bucerotidae	Wreathed Hornbill	*Rhyticeros undulatus*	LC	2014
36	Bucerotidae	Oriental Pied Hornbill	*Anthracoceros albirostris*	LC	2014
37	Bucerotidae	White-crowned Hornbill	*Berenicornis comatus*	NT	2014
38	Campephagidae	Black-winged Flycatcher Shrike	*Hemipus hirundinaceus*	LC	2014/ 2017
39	Campephagidae	Lesser Cuckoo Shrike (F)	*Coracina fimbriata*	LC	2017
40	Campephagidae	Scarlet Minivet	*Pericrocotus speciosus*	LC	2014
41	Campephagidae	Fiery Minivet	*Pericrocotus igneus*	NT	2014
42	Caprimulgidae	Large-tailed Nightjar	*Caprimulgus macrurus*	LC	2014
43	Caprimulgidae	Malaysian-eared Nightjar	*Caprimulgus macrurus*	LC	2014
44	Cettiidae	Yellow-bellied Warbler	*Abroscopus superciliaris*	LC	2014
45	Chloropseidae	Common Iora	*Aegithina tiphia*	LC	2014/ 2017
46	Chloropseidae	Lesser Green Leafbird	*Chloropsis cyanopogon*	NT	2014/ 2017
47	Columbidae	Large Green Pigeon	*Treron capellei*	VU	2014
48	Columbidae	Thick-billed Green Pigeon	*Treron curvirostra*	LC	2014
49	Columbidae	Little Green Pigeon	*Treron olax*	LC	2014
50	Columbidae	Jambu Fruit Dove	*Ptilinopus jambu*	NT	2014
51	Columbidae	Green Imperial Pigeon	*Ducula aenea*	LC	2014
52	Columbidae	Common Emerald Pigeon	*Chalcophaps indica*	LC	2014
53	Columbidae	Spotted Dove	*Spilopelia chinensis*	LC	2014
54	Columbidae	Pink-necked Green Pigeon	*Treron vernans*	LC	2014
55	Coraciidae	Dollarbird	*Eurystomus orientalis*	LC	2014
56	Corvidae	Bornean Black Magpie	*Platysmurus leucopterus aterrimus*	NT	2014
57	Corvidae	Large-billed Crow	*Corvus macrorhynchos*	LC	2014
58	Corvidae	Crested Jay	*Platylophus galericulatus*	NT	2014
59	Corvidae	Bornean Treepie	*Dendrocitta cinerascens*	LC	2014
60	Covidae	Slender-billed Crow	*Corvus enca*	LC	2014/ 2017
61	Cuculidae	Chestnut-breasted Malkoha	*Phaenicophaeus curvirostris*	LC	2014/ 2017
62	Cuculidae	Raffles's Malkoha	*Phaenicophaeus chlorophaeus*	LC	2014/ 2017
63	Cuculidae	Greater Coucal	*Centropus sinensis*	LC	2014

64	Cuculidae	Lesser Coucal	*Centropus bengalensis*	LC	2014
65	Cuculidae	Short-toed Coucal	*Centropus rectunguis*	VU	2014
66	Cuculidae	Bornean Ground Cuckoo	*Carpococcyx radiceus*	NT	2014
67	Cuculidae	Plaintive Cuckoo	*Cacomantis merulinus*	LC	2014
68	Cuculidae	Violet Cuckoo	*Chrysococcyx xanthorhynchus*	LC	2014
69	Cuculidae	Banded Bay Cuckoo	*Cacomantis sonneratii*	LC	2014
70	Cuculidae	Indian Cuckoo	*Cuculus micropterus*	LC	2014
71	Cuculidae	Moustached Hawk-cuckoo	*Hierococcyx vagans*	NT	2014
72	Cuculidae	Hodgson's (Malaysian) Hawk-cuckoo	*Hierococcyx nisicolor*	LC	2014
73	Cuculidae	Oriental Cuckoo	*Cuculus optatus*	LC	2014
74	Cuculidae	Rusty-breasted Cuckoo	*Cacomantis sepulcralis*	LC	2014
75	Cuculidae	Little Bronze Cuckoo	*Chrysococcyx minutillus*	LC	2014
76	Cuculidae	Square-tailed Drongo-cuckoo	*Surniculus lugubris*	LC	2014
77	Cuculidae	Black-bellied Malkoha	*Phaenicophaeus diardi*	NT	2014
78	Cuculidae	Chestnut-bellied Malkoha	*Phaenicophaeus sumatranus*	NT	2014
79	Dicaeidae	Scarlet-breasted Flowerpecker	*Prionochilus thoracicus*	NT	2014
80	Dicaeidae	Crimson-breasted Flowerpecker	*Prionochilus percussus*	LC	2014
81	Dicaeidae	Orange-bellied Flowerpecker	*Dicaeum trigonostigma*	LC	2014
82	Dicaeidae	Yellow-breasted Flowerpecker	*Prionochilus maculatus*	LC	2014
83	Dicruridae	Greater Racket-tailed Drongo	*Dicrurus paradiseus*	LC	2014/ 2017
84	Dicruridae	Crow-billed Drongo	*Dicrurus annectans*	LC	2014
85	Dicruridae	Bronzed Drongo	*Dicrurus aeneus*	LC	2014
86	Dicruridae	Spangled Drongo	*Dicrurus bracteatus*	LC	2014
87	Estrildidae	Dusky Munia	*Lonchura fuscans*	LC	2014/ 2017
88	Eupetidae	Rail Babbler	*Eupetes macrocerus*	NT	2014
89	Eurylaimidae	Black-and-yellow Broadbill	*Eurylaimus ochromalus*	NT	2014/ 2017
90	Eurylaimidae	Banded Broadbill	*Eurylaimus javanicus*	NT	2014/ 2017
91	Eurylaimidae	Green Broadbill	*Calyptomena viridis*	NT	2014/ 2017
92	Eurylaimidae	Black-and-red Broadbill	*Cymbirhynchus macrorhynchos*	LC	2014
93	Eurylaimidae	Dusky Broadbill	*Corydon sumatranus*	LC	2014
94	Falconidae	Bornean (White-fronted) Falconet	*Microhierax latifrons*	NT	2014
95	Hemiprocnidae	Grey-rumped Treeswift	*Hemiprocne longipennis*	LC	2014/ 2017
96	Hemiprocnidae	Whiskered Treeswift	*Hemiprocne comata*	LC	2014/

					2017
97	Hirundinidae	Pacific Swallow	*Hirundo tahitica*	LC	2014/ 2017
98	Hirundinidae	Barn Swallow	*Hirundo rustica*	LC	2014
99	Irenidae	Asian Fairy-bluebird	*Irena puella*	LC	2014/ 2017
100	Lybiidae	Black-throated Barbet	*Tricholaema melanocephala*	LC	2014
101	Megalaimidae	Blue-eared Barbet	*Psilopogon duvaucelii*	LC	2014/ 2017
102	Megalaimidae	Gold-whiskered Barbet	*Psilopogon chrysopogon*	LC	2014/ 2017
103	Megalaimidae	Red-throated Barbet	*Psilopogon mystacophanos*	NT	2014/ 2017
104	Megalaimidae	Yellow-crowned Barbet	*Psilopogon henricii*	NT	2014/ 2017
105	Megalaimidae	Red-crowned Barbet	*Psilopogon rafflesii*	NT	2014
106	Megalaimidae	Golden-naped Barbet	*Psilopogon pulcherrimus*	LC	2014
107	Megalaimidae	Brown Barbet	*Caloramphus fuliginosus*	LC	2014
108	Megalaimidae	Mountain Barbet	*Psilopogon monticola*	LC	2014
109	Meropidae	Red-bearded Bee-eater	*Nyctyornis amictus*	LC	2017
110	Meropidae	Blue-throated Bee-eater	*Merops viridis*	LC	2014
111	Monarchidae	Black-naped Monarch	*Hypothymis azurea*	LC	2014/ 2017
112	Muscicapidae	Asian Paradise Flycatcher	*Terpsiphone affinis*	LC	2014/ 2017
113	Muscicapidae	Dark-sided Flycatcher	*Muscicapa sibricia*	LC	2017
114	Muscicapidae	Rufous-chested Flycatcher (F)	*Ficedula dumetoria*	LC	2014/ 2017
115	Muscicapidae	Yellow-vented Flowerpecker	*Dicaeum chrysorrheum*	LC	2017
116	Muscicapidae	Yellow-rumped Flowerpecker	*Prionochilus xanthopygius*	LC	2014/ 2017
117	Muscicapidae	Yellow-breasted Flowerpecker	*Prionochilus maculatus*	LC	2014/ 2017
118	Muscicapidae	White-rumped Shama	*Copsychus malabaricus*	LC	2014
119	Muscicapidae	White-crowned Forktail	*Enicurus leschenaulti*	LC	2014
120	Muscicapidae	Asian Brown Flycatcher	*Muscicapa dauurica*	LC	2014
121	Muscicapidae	Verditer Flycatcher	*Eumyias thalassinus*	LC	2014
122	Muscicapidae	Blue-and-white Flycatcher	*Cyanoptila cyanomelana*	LC	2014
123	Muscicapidae	Pale Blue Flycatcher	*Cyornis unicolor*	LC	2014
124	Muscicapidae	Malaysian Blue Flycatcher	*Cyornis turcosus*	NT	2014
125	Muscicapidae	Bornean Blue Flycatcher	*Cyornis superbus*	LC	2014
126	Muscicapidae	Red-throated Flycatcher	*Ficedula albicilla*	LC	2014
127	Muscicapidae	Grey-chested Jungle Flycatcher	*Cyornis umbratilis*	NT	2014

128	Muscicapidae	Eyebrowed Jungle Flycatcher	*Vauriella gularis*	LC	2014
129	Muscicapidae	Rufous-chested flycatcher	*Ficedula dumetoria*	LC	2014
130	Muscicapidae	Brown-chested Jungle Flycatcher	*Cyornis brunneatus*	VU	2014
131	Muscicapidae	Sunda Blue Flycatcher	*Cyornis caerulatus*	VU	2014
132	Nectariniidae	Little Spiderhunter	*Arachnothera longirostra*	LC	2014/ 2017
133	Nectariniidae	Thick-billed Spiderhunter	*Arachnothera crassirostris*	LC	2014/ 2017
134	Nectariniidae	Plain Sunbird	*Anthreptes simplex*	LC	2014/ 2017
135	Nectariniidae	Red-throated Sunbird	*Anthreptes rhodolaema*	NT	2014/ 2017
136	Nectariniidae	Van Hasselt's Sunbird	*Leptocoma brasiliana*	LC	2017
137	Nectariniidae	Purple-naped Sunbird (F)	*Hypogramma hypogrammicum*	LC	2014/ 2017
138	Nectariniidae	Ruby-cheeked Sunbird	*Chalcoparia singalensis*	LC	2014/ 2017
139	Nectariniidae	Temminck's Sunbird	*Aethopyga temminckii*	LC	2017
140	Nectariniidae	Yellow-eared Spiderhunter	*Arachnothera chrysogenys*	LC	2014
141	Nectariniidae	Long-billed Spiderhunter	*Arachnothera robusta*	LC	2014
142	Nectariniidae	Spectacled Spiderhunter	*Arachnothera flavigaster*	LC	2014
143	Nectariniidae	Grey-breasted Spiderhunter	*Arachnothera modesta*	LC	2014
144	Nectariniidae	Brown-throated Sunbird	*Anthreptes malacensis*	LC	2014
145	Nectariniidae	Olive-backed Sunbird	*Cinnyris jugularis*	LC	2014
146	Nectariniidae	Crimson Sunbird	*Aethopyga siparaja*	LC	2014
147	Oriolidae	Dark-throated Oriole	*Oriolus xanthonotus*	NT	2014/ 2017
148	Passeridae	Eurasian Tree-sparrow	*Passer montanus*	LC	2014/ 2017
149	Pellorneidae	White-chested Babbler	*Trichastoma rostratum*	NT	2014
150	Pellorneidae	Sooty-capped Babbler	*Malacopteron affine*	NT	2014
151	Pellorneidae	Ferruginous Babbler	*Trichastoma bicolor*	NT	2014
152	Pellorneidae	Short-tailed Babbler	*Trichastoma malaccensis*	**NT (LC)**	2014
153	Pellorneidae	Black-capped Babbler	*Pellorneum capistratum*	LC	2014
154	Pellorneidae	Abbott's Babbler	*Malacocincla abbotti*	LC	2014
155	Pellorneidae	Bornean Wren Babbler	*Ptilocichla leucogrammica*	VU	2014
156	Pellorneidae	Black-throated Wren Babbler	*Napothera atrigularis*	NT	2014
157	Pellorneidae	Horsfield's Babbler	*Malacocincla sepiaria*	LC	2014
158	Phasianidae	Great Argus	*Argusianus argus*	NT	2014
159	Phasianidae	Ferruginous Partridge	*Caloperdix oculeus*	NT	2014
160	Phasianidae	Crested Wood Partridge	*Rollulus rouloul*	NT	2014

161	Phasianidae	Chestnut-necklaced Partridge	*Arborophila charltonii*	NT	2014
162	Phylloscopidae	Arctic Warbler	*Phylloscopus borealis*	LC	2014
163	Picidae	Rufous Piculet	*Sasia abnormis*	LC	2014/2017
164	Picidae	Buff-rumped Woodpecker	*Meiglyptes grammithorax*	LC	2014/2017
165	Picidae	Checker-throated Woodpecker	*Picus mentalis*	NT	2014/2017
166	Picidae	Olive-backed Woodpecker	*Dinopium rafflesii*	NT	2014/2017
167	Picidae	White-bellied Woodpecker	*Dryocopus javensis*	LC	2014/2017
168	Picidae	Grey-and-buff Woodpecker	*Hemicircus sordidus*	LC	2014/2017
169	Picidae	Great Slaty Woodpecker	*Mulleripicus pulverulentus*	VU	2014
170	Picidae	Crimson-winged Woodpecker	*Picus puniceus*	LC	2014
171	Picidae	Rufous Woodpecker	*Micropternus brachyurus*	LC	2014
172	Picidae	Maroon Woodpecker	*Blythipicus rubiginosus*	LC	2014
173	Picidae	Orange-backed Woodpecker	*Reinwardtipicus validus*	LC	2014
174	Picidae	Buff-necked Woodpecker	*Meiglyptes tukki*	NT	2014
175	Pittidae	Blue-headed pitta	*Hydrornis baudii*	VU	2014/2017
176	Pittidae	Black-crowned Pitta	*Erythropitta ussheri*	NT	2014
177	Pittidae	Blue-winged Pitta	*Pitta moluccensis*	LC	2014
178	Pittidae	Giant Pitta	*Hydrornis caeruleus*	NT	2014
179	Pittidae	Hooded Pitta	*Pitta sordida*	LC	2014
180	Pittidae	Bornean Banded Pitta	*Hydrornis schwaneri*	LC	2014
181	Pityriaseidae	Bornean Bristlehead	*Pityriasis gymnocephala*	NT	2014
182	Psittacidae	Blue-crowned Hanging Parrot	*Loriculus galgulus*	LC	2014/2017
183	Psittacidae	Long-tailed Parakeet	*Psittacula longicauda*	NT	2014
184	Psittacidae	Blue-rumped Parrot	*Psittinus cyanurus*	NT	2014
185	Pycnonotidae	Grey-cheeked Bulbul	*Alophoixus bres*	LC	2014/2017
186	Pycnonotidae	Yellow-bellied Bulbul	*Alophoixus phaeocephalus*	LC	2014/2017
187	Pycnonotidae	Buff-vented Bulbul	*Iole crypta*	NT	2014/2017
188	Pycnonotidae	Hairy-backed Bulbul	*Tricholestes criniger*	LC	2014/2017
189	Pycnonotidae	Cream-vented Bulbul	*Pycnonotus pseudosimplex*	LC	2014/2017
190	Pycnonotidae	Grey-bellied Bulbul	*Pycnonotus cyaniventris*	NT	2014/2017
191	Pycnonotidae	Puff-backed Bulbul	*Pycnonotus eutilotus*	NT	2014/2017
192	Pycnonotidae	Asian Red-eyed Bulbul	*Pycnonotus brunneus*	LC	2014/2017

193	Pycnonotidae	Spectacled Bulbul	*Pycnonotus erythropthalmos*	LC	2014/2017
194	Pycnonotidae	Yellow-vented Bulbul	*Pycnonotus goiavier*	LC	2014/2017
195	Pycnonotidae	Black-headed Bulbul	*Pycnonotus atriceps*	LC	2014
196	Pycnonotidae	Asian Red-eyed Bulbul	*Pycnonotus brunneus*	LC	2014
197	Pycnonotidae	Olive-winged Bulbul	*Pycnonotus plumosus*	LC	2014
198	Pycnonotidae	Streaked Bulbul	*Ixos malaccensis*	NT	2014
199	Pycnonotidae	Ochraceous Bulbul	*Alophoixus ochraceus*	LC	2014
200	Pycnonotidae	Black-crested Bulbul	*Pycnonotus flaviventris*	LC	2014
201	Pycnonotidae	Black-and-white Bulbul	*Pycnonotus melanoleucos*	NT	2014
202	Pycnonotidae	Scaly-breasted Bulbul	*Pycnonotus squamatus*	NT	2014
203	Pycnonotidae	Straw-headed Bulbul	*Pycnonotus zeylanicus*	**EN (VU)**	2014
204	Pycnonotidae	Finsch's Bulbul	*Alophoixus finschii*	NT	2014
205	Rhipiduridae	Pied Fantail	*Rhipidura javanica*	LC	2014/2017
206	Rhipiduridae	Spotted Fantail	*Rhipidura perlata*	LC	2014
207	Stenostiridae	Grey-headed Flycatcher	*Culicicapa ceylonensis*	LC	2014
208	Stenostiridae	White-tailed Blue Flycatcher	*Elminia albicauda*	LC	2014
209	Strigidae	Brown Wood Owl	*Strix leptogrammica*	LC	2014/2017
210	Strigidae	Collared-scops Owl	*Otus lettia*	LC	2014
211	Strigidae	Oriental Scops Owl	*Otus sunia*	LC	2014
212	Strigidae	Buffy Fish Owl	*Ketupa ketupu*	LC	2014
213	Strigidae	Brown Hawk-owl (Boobook)	*Ninox scutulata*	LC	2014
214	Sturnidae	Common Hill Myna	*Gracula religiosa*	LC	2014
215	Sturnidae	Velvet-fronted Nuthatch	*Sitta frontalis*	LC	2014
216	Sturnidae	Asian Glossy Starling	*Aplonis panayensis*	LC	2014
217	Sylvidae	Yellow-bellied Prinia	*Prinia flaviventris*	LC	2017
218	Sylviidae	Dark-necked Tailorbird	*Orthotomus astrogularis*	LC	2014/2017
219	Sylviidae	Ashy (Red-headed) Tailorbird	*Orthotomus ruficeps*	LC	2014/2017
220	Sylviidae	Rufous-tailed Tailorbird	*Orthotomus sericeus*	LC	2014/2017
221	Tephrodornithidae	Rufous-winged Philentoma	*Philentoma pyrhoptera*	LC	2014/2017
222	Tephrodornithidae	Large Woodshrike	*Tephrodornis virgatus*	LC	2014
223	Tephrodornithidae	Maroon-breasted Philentoma	*Philentoma velata*	NT	2014
224	Timaliidae	Bold-striped Tit Babbler	*Macronous bornensis*	LC	2017
225	Timaliidae	Fluffy-backed Tit Babbler	*Macronous ptilosus*	NT	2014/2017
226	Timaliidae	Chestnut-winged Babbler	*Stachyris erythroptera*	LC	2014/2017

227	Timaliidae	Rufous-fronted Babbler	*Stachyridopsis rufifrons*	LC	2014/2017
228	Timaliidae	Grey-headed Babbler	*Stachyris poliocephala*	LC	2014/2017
229	Timaliidae	White-necked Babbler	*Stachyris leucotis*	NT	2017
230	Timaliidae	Moustached Babbler	*Malacopteron magnirostre*	LC	2014/2017
231	Timaliidae	Rufous-crowned Babbler	*Malacopteron magnum*	NT	2014/2017
232	Timaliidae	Scaly-crowned Babbler	*Malacopteron cinereum*	LC	2014/2017
233	Timaliidae	Sooty-capped Babbler	*Malacopteron affine*	NT	2014/2017
234	Timaliidae	Striped Wren-babbler	*Kenopia striata*	NT	2014/2017
235	Timaliidae	Brown Fulvetta	*Alcippe brunneicauda*	NT	2014/2017
236	Timaliidae	Chestnut-rumped Babbler	*Stachyris maculata*	NT	2014
237	Timaliidae	Black-throated Babbler	*Stachyris nigricollis*	NT	2014
238	Timaliidae	Pin-Striped Tit-babbler	*Macronus gularis*	LC	2014
239	Timaliidae	Chestnut-backed Scimitar Babbler	*Pomatorhinus montanus*	LC	2014
240	Trogonidae	Diard's Trogon	*Harpactes diardii*	NT	2014/2017
241	Trogonidae	Scarlet-rumped Trogon	*Harpactes duvaucelii*	NT	2014/2017
242	Trogonidae	Red-naped Trogon	*Harpactes kasumba*	NT	2014
243	Trogonidae	Cinnamon-rumped Trogon	*Harpactes orrhophaeus*	NT	2014
244	Trogonidae	Orange-breasted Trogon	*Harpactes oreskios*	LC	2014
245	Turdidae	Chestnut-naped Forktail	*Enicurus ruficapillus*	NT	2014/2017
246	Turdidae	Oriental Magpie Robin	*Copsychus saularis*	LC	2014/2017
247	Turdidae	White-crowned Shama	*Copsychus striklandii*	NA	2014/2017
248	Turdidae	Rufous-tailed Shama	*Copsychus pyrropygus*	NT	2014/2017
249	Upupidae	Hoopoe	*Upupa epops*	LC	2014
250	Vireonidae	White-bellied Yuhina	*Erpornis zantholeuca*	LC	2014
251	Zosteropidae	Chestnut-crested Yuhina	*Yuhina everetti*	LC	2014
252	Zosteropidae	Everett's White-eye	*Zosterops everetti*	LC	2014

IUCN Red List of Treatened Species: **NE**=Not Evaluated, **DD**=Data Deficient, **LC**=Least Concern, **NT**=Near Threatedned, **VU**=Vulnerable, **EN=**Endangered, **CR**=Critically Endagered, **EW**=Extinct

Acknowledgements

Several people contributed to the species list, especially Daicus Bellabut, Mahathir Ratig and Eddie Ahmad. We would like to acknowledge Universiti Kebangsaan Malaysia; Dr. Yap Sau Wai, acting group manager Conservation and Environmental Management Division, Sabah Foundation; En. Hamzah Tangki, the manager of ICSC; and all the rangers of ICSC for their help and friendship during the expedition, especially Edward Asrul Alimin Sinon and Brandon Don. The expedition was sponsored by Petronas and Yayasan Sabah.

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Journal of Tropical Biology and Conservation 16: 61–72, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

Screening for Antibiotic-Producing Bacteria from Imbak Canyon Conservation Area (ICCA)

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Abstract

Antibiotic resistance is an escalating threat to public health. Therefore, there is an urgent need for new antibiotics. This study aims to screen for antibiotic-producing microorganisms from the forest soil of Batu Timbang. Soil samples were collected, diluted and spread plated onto 1/5 Nutrient Agar (NA) and Actinomycete Isolation Agar (AIA) for the isolation of antibiotic-producing microorganisms. A total of 180 bacterial isolates were screened for their antibiotic-producing ability, and ten were tested positive for inhibitory activity against one or more test pathogens via agar overlay assay (*Staphylococcus aureus* ATCC BAA-1717, *Enterococcus faecalis* ATCC 700802, and *Acinetobacter baumannii* ATCC BAA-1605). Ten bacterial isolates were subjected to 16S rRNA gene amplification and gene sequence analysis. The isolates were identified to be closely related to the genus *Variovorax*, *Streptomyces*, *Kitasatospora*, *Chromobacterium*, *Burkholderia*, *Pseudomonas* and *Massilia*. Three isolates (*Variovorax* sp. A5, *Variovorax* sp. A6 and *Kitasatospora* sp. H8) are potentially novel as these isolates form a different clade from their respective closely related species via phylogenetic tree analysis using reference sequences obtained from GenBank/EMBL/DDBJ databases. The antibiotics produced by the bacterial isolates might potentially be new, as novel species might possess unique biosynthetic gene clusters to produce new compounds. Nevertheless, further taxonomic identification and antibiotic isolation work is required. This study has revealed the potential of antibiotic discovery from Batu Timbang (Imbak Canyon Conservation Area) and its implications in tackling antibiotic resistance.

Keywords: Antibiotic, antibiotic-resistance, bio-prospecting, conservation, forest soil, Imbak Canyon Conservation Area

Received 16 March 2019
Reviewed 17 July 2019
Accepted 26 July 2019
Published 15 October 2019

Introduction

The extensive usage of antibiotics in the past few decades has caused an increase in the emergence and prevalence of antibiotic-resistant bacteria (ARB) (Holmes et al., 2016). The emergence of antibiotic-resistant bacteria is an inevitable event, as it is an evolutionary process during antibiotic therapy due to selection pressure. Some examples of ARB are methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and *Acinetobacter baumannii* (Martens & Demain, 2017).

S. aureus is a major example of ARB that causes life-threatening infections. The first line therapy for *S. aureus* infection is beta-lactam antibiotics such as penicillin, cephalosporin and methicillin (Loubet et al., 2018). The emergence of methicillin-resistant *S. aureus* (MRSA) strains essentially indicates that these strains are resistant to all currently available beta-lactam antibiotics. Consequently, infections caused by MRSA must be treated with other non-beta lactam antibiotics such as vancomycin, linezolid and daptomycin (Kaur and Chate, 2015). However, studies have reported the emergence of *S. aureus* strains resistant to these non-beta lactam antibiotics, suggesting that *S. aureus* has gained resistance even to the last few options of antibiotic therapy (Silva et al., 2019; Yang et al., 2018).

Enterococci are another example of ARB which are known to be difficult to treat due to their intrinsic resistance and ability to acquire resistance determinants (Ong et al., 2017). Enterococci are currently ascendant nosocomial pathogens to cause bacteremia, nosocomial urinary tract and wound infections. Vancomycin has been consistently being relied on for the treatment of infections caused by multidrug-resistant enterococci (O'Driscoll and Crank, 2015). As vancomycin is used widely to treat MRSA and other gram-positive bacterial infections, certain enterococci species such as *Enterococcus faecalis* and *Enterococcus faecium* have gained resistance towards vancomycin, hence limiting the treatment options to other antibiotics, for instance linezolid, daptomycin, quinupristin/dalfopristin, and tigecycline (Ahmed and Baptiste, 2018).

A similar pattern of antibiotic resistant can be seen in gram negative bacteria such as *Acinetobacter baumannii*. This bacterium is resistant to most antibiotics that are currently being used to treat gram-negative bacterial infections globally (Asif et al., 2018). Despite this, the current progress on the development of new antibiotics is slow due to biological and pharmacological drawbacks (Brown &

Wright, 2016). Thus, there is a dire need to discover new antibiotics to treat ARB infections (Martens & Demain, 2017).

One strategy to look for new antibiotics is by exploring untapped resources such as the deep-sea sediments and pristine forests (Wang et al., 2013; Ong et al., 2016). Microorganisms from these environments have always been the main source of new antibiotics, as they might have evolved to develop unique gene clusters for the biosynthesis of new compounds. When the competition is intense, microorganisms will tend to produce compounds such as antibiotics to secure their niches (Ong et al., 2016). As a result, the attempt to prospect for new antibiotics from unexplored environments remains a great research opportunity. A similar approach was adopted by Lo et al., (2000) and resulted in the positive isolation of actinomycetes from dipterocarp rainforest soils in Borneo. These actinomycetes possess various biological activities such as anti-cancer properties (Yip et al., 2010) and *Mycobacterium* isocitrate lyase inhibitor (Shin et al., 2009). Hence, by using the same technique, the objectives of this study were to isolate and to identify potential antibiotic-producing bacteria from the forest soil of Imbak Canyon Conservation Area (ICCA).

Methods and materials

Soil sampling

Soil samples were collected mainly in three localities: (1) the ICCA, as well as the area designated for the Batu Timbang scientific expedition which include the (2) Rafflesia track and (3) Lanap track. Each localities was sampled randomly six times with each sampling site being at least 100 m apart. The scientific expedition was carried out from 16 to 26 August 2017. The leave litters and top soils (~ 5 mm) were first removed, and approximately 50 g of soil samples were collected using sterile spatulas into sterile 50 mL Falcon tubes. The Falcon tubes with soil samples were then kept at room temperature in sealed polyethylene bags. All samples were transported to Monash University Malaysia for processing immediately after the expedition. Prior to processing, large roots and stones were first removed, followed by soil dilutions and cultivation experiments.

Isolation of bacteria and Actinomycetes

One gram of each soil sample was immersed in 0.85% (w/v) saline and serially diluted up to 10^{-8}. The diluents were then spread plated onto 1/5 NA (Merck, Germany) containing 100 µg/mL cycloheximide for the isolation of bacteria (Ong et al., 2015), and onto AIA (Becton Dickinson, UK) for the isolation of Actinomycete (Rashad et al., 2015). All media plates were incubated at 30°C aerobically for 5 days. Ten representative colonies were randomly selected using the Harrison's disc method. These isolates were re-streaked onto their respective media and incubated at 30°C aerobically for 5 days to obtain pure cultures.

Isolation and screening for antibiotic-producing bacteria

The soil isolates were patched onto their respective plates by using sterile toothpicks and incubated at 30°C aerobically for 5 days. The inhibitory activity of the soil isolates against the test pathogen was assayed using agar overlay assay. The test pathogens that were used in this study include *Staphylococcus aureus* ATCC BAA-1717, *Enterococcus faecalis* ATCC 700802, and *Acinetobacter baumannii* ATCC BAA-1605. Briefly, 9 mL of 0.9% (w/v) tryptic soy agar (Merck, Germany) containing 100 µL of test pathogen strains (adjusted to 2.0 McFarland standard OD_{625} 0.32-0.40, approximately 6×10^8 CFU/mL) was overlaid on top of the patched plate and incubated at 37°C aerobically for 18-24 hours. The presence of a halo zone around the patched isolate indicates antibiotic activity against the test pathogen strains. The screening was carried out in triplicate and the annular radiuses of the inhibition zones were measured using a ruler.

DNA extraction, amplification and phylogenetic analysis

Microbial isolates with positive antibiotic activity were subjected to 16S rRNA gene sequence amplification and phylogenetic analysis. DNA extraction was performed by suspending bacterial colonies in 50 µL of sterile distilled water in PCR tubes and heated at 100°C for 3 min. The suspension was then centrifuged at 13000 × *g* for one min. The supernatant containing the bacterial DNA was transferred into a new PCR tube and used as DNA template for the following PCR reaction. The 16S rRNA gene sequences of the isolates were amplified using the universal primers 63F (5' – CAG GCC TAA CAC ATG CAA GTC – 3') and 1387R (5' – GGG CGG WGT GTA CAA GC – 3') (Marchesi et al., 1998). The PCR was set up as follow: 5 µL of DNA extract, 10 µL of 5 × MyTaq Red Reduction Buffer, 5 µM of forward primer, 5 µM of reverse primer and 1.25 U of MyTaq DNA polymerase. The reaction volume will be made up to 50 µL using sterile filtered milliQ water (Millipore, Germany). The PCR included an initial denaturation step at 95°C for 1 min, followed by 30 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 45 sec, and elongation at 72°C for 45 sec. PCR products were separated on a 1.5% (w/v) agarose gel in TAE buffer and the bands were visualized with 1 × GelRed. The 16S rRNA gene sequences of the isolates were aligned with sequences of closely related type strains retrieved from the GenBank/EMBL/DDBJ databases using CLUSTAL-X software (Thompson et al., 1997). The alignment was manually verified and adjusted prior to the construction of phylogenetic tree using the neighbor-joining (Saitou & Nei, 1987) algorithm with the MEGA version 6.0 software (Tamura et al., 2011). The stability of the resultant tree topologies was evaluated by using the bootstrap resampling method (Felsenstein, 1985). The evolutionary distance for the neighbour-joining algorithm was computed using the Kimura's two-parameter model (Kimura, 1980).

Results and Discussion

A total of ten out of 180 isolates were tested positive for antibiotic activity against one or more test pathogens, as determined via agar overlay assay (Figure 1).

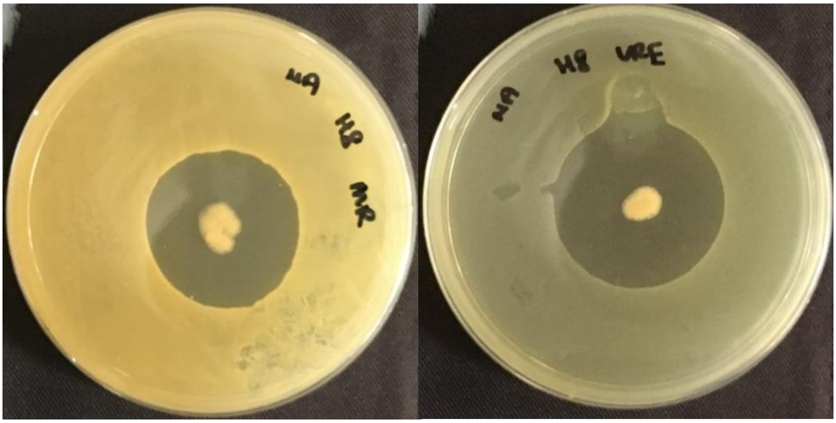

Figure 1. Antibiotic activity of isolate H8 against *S. aureus* ATCC BAA-1717 (left) and *E. faecalis* ATCC 700802 (right).

Staphylococcus aureus ATCC BAA-1717 is an opportunistic pathogen that is resistant to many conventional beta-lactam antibiotics, including methicillin, and has been reported to cause an epidemic in the USA (Tenover and Goering, 2009). Pathogens that are resistant to methicillin are resistant to most of the currently available beta-lactam antibiotics, hence limiting the treatment option to the last resort antibiotics such as vancomycin or daptomycin. Eight bacterial isolates (A5, A6, B8, D4, D7, E4, H2, and H8) were able to inhibit *S. aureus* ATCC BAA-1717 and could be potentially important species that can be further studied (Table 1).

Table 1. Annular radius of inhibition against three different test pathogens

Soil isolates	Annular radius of inhibition (mm)		
	S. aureus ATCC BAA-1717	*E. faecalis* ATCC 700802	*A. baumanii* ATCC BAA-1605
A5	2.7 ± 1.2	NA	NA
A6	2.7 ± 1.2	NA	NA
B8	6.0 ± 2.6	3.7 ± 1.5	NA
D4	2.3 ± 0.6	2.3 ± 0.6	NA
D7	3.3 ± 1.5	2.0 ± 1.0	NA
E4	1.7 ± 0.6	1.7 ± 0.6	NA
F1	NA	NA	7.0 ± 1.0
E9	NA	NA	4.7 ± 0.6
H2	14.3 ± 1.5	9.7 ± 1.2	NA
H8	14.0 ± 1.0	12.3 ± 2.1	NA

Values are reported as mean annular radius of inhibition zone ± SD in mm (n=3). NA = no activity.

Apart from this, isolates B8, D4, D7, E4, H2, and H8 could inhibit the growth of *Enterococcus faecalis* ATCC 700802, which is a pathogen known for its ability to resist the action of vancomycin (Ahmed and Baptiste, 2018). This bacterium is difficult to treat as it is intrinsically resistant to many classes of antibiotic. Vancomycin-resistant enterococci account for one-third of the enterococcal healthcare-associated infections in the USA and for more than 20% of such infections in certain European countries (Balli et al., 2014).

Lastly, two isolates (F1 and E9) were able to inhibit the growth of *Acinetobacter baumannii* ATCC BAA-1605. *A. baumannii* can cause complications such as wound infections, urinary tract infections, and meningitis (Peleg et al., 2008). It is particularly pathogenic due to its intrinsic resistance mechanisms towards many antibiotics (Cerceo et al., 2016). Since the isolates obtained showed positive antibiotic activity against the three clinically-relevant pathogens, it was of our interest to carry out further work to determine their identities. This was achieved through 16S rRNA gene sequence amplification, followed by cross-referencing with the National Centre of Biotechnology Institute (NCBI) database. Seven out of the ten isolates had more than 99% gene sequence similarity to their corresponding top-hits, indicating that these isolates could belong to the same genus and species, but of a different strain. On the other hand, three soil isolates – A5, A6, and H8 showed gene sequence similarity with less than 99%, suggesting that these isolates could potentially be novel bacterial species (Table 2). Novel microorganisms thriving in the environment might develop unique biosynthesis gene clusters giving rise to potentially novel compounds (Imhoff et al., 2011). These compounds with antibiotic activity could be useful in the future to treat ARB infections. However, further work which include a complete analysis on the phenotypic, genotypic, and chemotaxonomic of these isolates, as well as the isolation of the antibiotics are warranted.

Table 2. Location, top-hit, and percentage similarity of the 10 antibiotic-producing soil isolates

Isolates	Location (track)	Top-hit	Similarity (%)
A5	Rafflesia	*Variovorax guangxiensis* GXGD002	98.84
A6	Rafflesia	*Variovorax guangxiensis* GXGD002	98.76
B8	Lanap	*Streptomyces xanthocidicus* NBRC 13469	99.29
D4	IC centre	*Kitasatospora cystarginea* JCM 7356	99.84
D7	IC centre	*Kitasatospora cystarginea* JCM 7356	99.84
E4	IC centre	*Chromobacterium vaccinii* MWU205	99.60
F1	Rafflesia	*Burkholderia ubonensis* CIP 107078	99.60
E9	IC centre	*Pseudomonas nitritireducens* WZBFD3-gA2	99.76
H2	Rafflesia	*Massilia violacea* CAVIO	99.20
H8	Rafflesia	*Kitasatospora cheerisanensis* KCTC 2395	98.52

Since isolate A5, A6, and H8 showed percentage similarity lower than 99%, phylogenetic trees using neighbor joining based on 16S rRNA gene sequences were constructed to reveal the relationship of these isolates with published species of the known genus. The phylogenetic trees are shown in Figure 2 and Figure 3. Both isolate A5 and A6 were closely related to *Variovorax gossypii* JM310 and *Variovorax guangxiensis* GXGD002 (Figure 2).

Variovorax is a group of gram-negative bacteria that can tolerate high concentrations of toxic metals. Moreover, this group of bacteria are known to degrade organic pollutants, hence have been suggested for biotechnological use (Chen et al., 2013; Satola et al., 2012). However, the antibiotic-producing property of *Variovorax* species has not been described thus far. Isolate H8 was found to be closely related to *Kitasatospora cheerisanensis* KCTC395 (Figure 3). *Kitasatospora* are gram-positive bacteria, which belonged to the group of rare-actinomycetes. Members of *Kitasatospora* are known to produce antimicrobial compounds such as talosins (Yoon et al., 2006) and fuzanins (Aida et al., 2009). Nevertheless, isolate H8 could be producing a different type of compound with antibiotic activity. This is because it forms a distinct arm from its nearest relatives, hence signifying that it might be a novel species. This is an advantage because novel species tend to possess new gene clusters for the biosynthesis of new compounds. Nonetheless, further work on the species identification and isolation of the antibiotics are warranted.

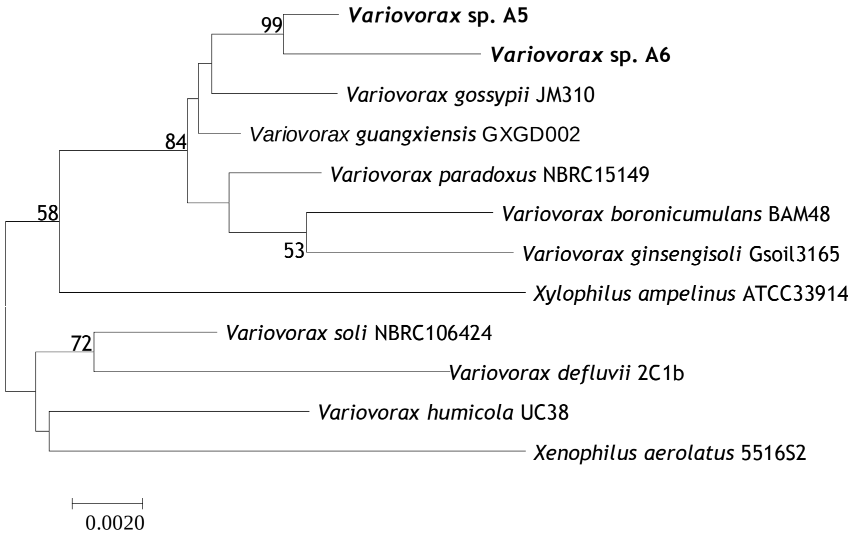

Figure 2. Neighbour-joining tree based on 16S rRNA sequences showing relationship between isolate A5 and A6 with their representatives related taxa. Bootstrap values (>50%) based on 1000 resampled datasets are shown at branch nodes. Bar, 2 substitutions per 1000 nucleotide positions.

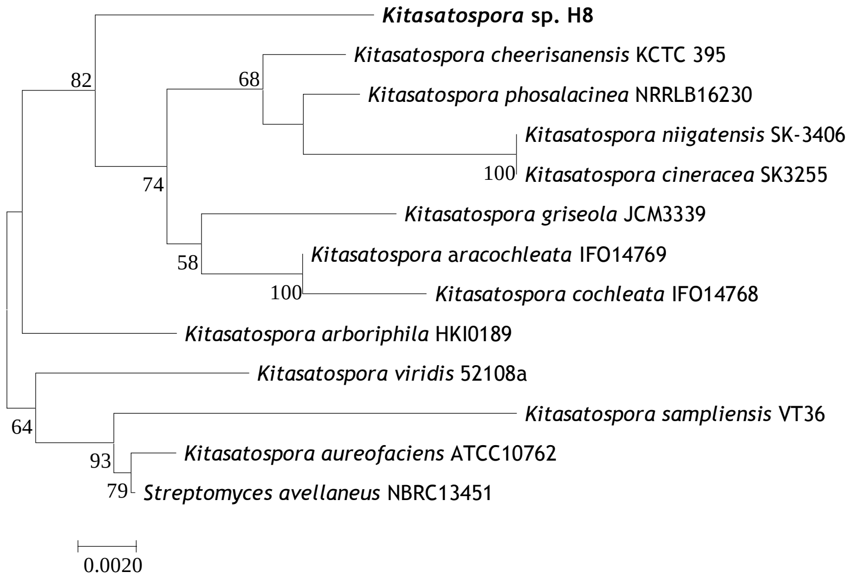

Figure 3. Neighbour-joining tree based on 16S rRNA sequences showing relationship between isolate H8 with their representatives related taxa. Bootstrap values (>50%) based on 1000 resampled datasets are shown at branch nodes. Bar, 2 substitutions per 1000 nucleotide positions.

Conclusions

The forest soils of Batu Timbang were found to be reservoirs for potentially novel bacteria with antibiotic producing ability. Three isolates – A5 and A6, as well as H8 were identified which belong to the genus *Variovorax* sp., and *Kitasatospora* sp., respectively. Preliminary work have shown that these isolates, along with their antibiotics could potentially be novel, hence, further work is required to fully determine their identities as well as to isolate the antibiotics. This study highlights the potential of antibiotic discovery from Imbak Canyon Conservation Area and its possible implications in addressing the issue of antibiotic resistance.

Acknowledgement

The Conservation and Environmental Management Division (CEMD) of Yayasan Sabah and Sabah Forestry Department are greatly acknowledged for the invitation to participate in the recent Imbak Canyon Conservation Area (ICCA) Batu Timbang Scientific Expedition 2017. The authors also thank Sabah Biodiversity Centre (SaBC) and Department of Agriculture Sabah for the access and export licenses as well as the phytosanitary certificate. Mr Asni bin Bulot (senior forest ranger) is acknowledged for his guidance and aid in specimen collection during the scientific expedition.

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Journal of Tropical Biology and Conservation 16: 73–77, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

Fruit flies of Batu Timbang Forest Within Imbak Canyon Conservation Area, Sabah, Malaysia (Diptera: Tephritidae)

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Abstract

A survey of fruit fly fauna was carried out at Batu Timbang Research Station, (Imbak Canyon Conservation Area – ICCA), Telupid, Sabah from 17th to 20th of August 2017. Fruit flies were collected using aerial net and bottle traps containing an attractant (Methyl Eugenol (ME) and Cue-lure (CUE)) at Lanap Trail, Rafflesia Trail and base camp. A total of 77 fruit flies belonging to *Bactrocera* were collected. This includes six species and three morphospecies, of which *Bactrocera tau* was the dominant species (56 individuals). This is the first study on fruit fly species at Imbak Canyon Conservation Area.

Keywords: Fruit fly, Diversity, Batu Timbang, ICCA, Sabah

Introduction

The family Tephritidae, is an important group of insects as it contains many pests of agricultural fruits and vegetables, such as *Bactrocera dorsalis*, *B. papayae*, *B. carambolae*, *B. oleae*, *Rhagoletis pomonella* and *Anastrapha ludens*. There are also many beautiful bamboo shoot flies classified under Tephritidae which are susceptible to environmental and habitat destruction. Morphologically, Tephritids, except for *Bactrocera* spp., possess attractive, colourful markings and spotted or banded wings (Chua, 2010) with body length varying from 2 mm to over 20 mm. Worldwide there are about 4,550 described tephritid species from 500 genera (Jackson et al., 2011), of which about 200 species are recorded as pests.

Larvae of tephritids feed on phyto-materials and can be classified into two categories, frugivorous and non-frugivorous. Frugivorous larvae feed on fleshy fruits while non-frugivorous larvae feed on plant parts other than fruit (Christenson & Foote, 1960). Mature female oviposits eggs on ripening host fruit. The larva hatches and feeds on the fleshy fruit which eventually causes direct damage to the fruit. The fruit is further damaged by penetration of microorganisms and decomposers through the opening holes leading to the early fall of fruit (Uchôa, 2012), causing economic losses to farmer.

Information on the presence of fruit fly species and diversity is useful in implementing integrated pest management (IPM) on pests. The diversity of fruit flies would also indicate roughly the extent of forest tree destruction in an area, especially trees that bear fruits for the fruit flies, and thus indirectly the loss of plant diversity. Studies on forest fruit flies also give an idea of potential agricultural pests found in such an area. This paper presents a preliminary list of fruit fly species collected from Batu Timbang Forest, Imbak Canyon Conservation Area (ICCA).

Methodology

A fruit fly survey was carried out at Batu Timbang Research Station, (Imbak Canyon Conservation Area – ICCA), Telupid, Sabah from 17th to 20th of August 2017. These flies were collected individually using aerial net or trapped by baited bottle traps at three sites namely Lanap Trail, Rafflesia Trail and base camp. The bait was prepared with cotton roll, attractants and insecticide (Malathion). Two kinds of parapheromones: Methyl Eugenol (ME), 1,2-dimethoxy-4-(2-propenyl) and Cue-lure (CUE), 4-(p-hydroxyphenyl)-2-butanone acetate, were used as attractants.

A total of 10 baited bottle traps were hung on trees above 2m and left overnight, to trap the fruit flies. As for manual collection, a small amount of attractant lure, ME or CUE were smeared on top of leaves randomly along the trails to attract fruit flies. The attracted flies were collected manually using aerial net or with the aid of transparent plastic bottles. All specimens were sorted, dry mounted and identified on the basis of morphological characters, detailed in Drew & Hancock (1994) and specimens were housed at BORNEENSIS at the Institute for Tropical Biology and Conservation (ITBC), Universiti Malaysia Sabah.

Results and Discussion

A total of nine species of fruit flies belonging to a single genus *Bactrocera* were recorded (Table 1) in this survey. The most abundant species is the *Bactrocera* *tau* with 56 individuals (73%), followed by *Bactrocera* sp 15 (9%) and *Bactrocera nigrotibialis* (6%). The rest of the species were caught in relatively smaller numbers. *Bactrocera* sp A is a newly recorded species in comparison to previous studies conducted within Tunku Abdul Rahman Park and islands of Tun Mustapha Park (Banggi, Balambangan and Malawali islands).

Table 1. Fruit fly species recorded from Batu Timbang Forest

Family	Species	Number of Individuals
Tephritidae	*Bactrocera carambolae*	1
	Bactrocera fusctibia	1
	Bactrocera nigrotibialis	5
	Bactrocera papayae	3
	Bactrocera sp.7	1
	Bactrocera tau	56
	Bactrocera sp.15	7
	Bactrocera merapiensis	1
	Bactrocera sp A	2
	Total	**77**

Overall this result is similar to that of previous studies, where the genus *Bactrocera* is dominant and well presented, due to the endemic distribution (Drew 2004). Almost all of the fruit fly species collected from this site have been recorded from Tunku Abdul Rahman National Park and Banggi, Balambangan and Malawali islands. This study includes fruit flies species recorded for the first time from Imbak Canyon Conservation Area. Most of the species recorded here are common species in South East Asia. *Bactrocera papayae*, *B. tau* and *B. carambolae* are known as common species. The less common species recorded are *B. fuscitibia*, *B. nigrotibialis*, and *B. merapiensi*.

Bactrocera papayae, and *B. carambolae*, are highly polyphagous pests that share similar host plants (Allwood et al., 1999). They are among the major economic pests (Plant Health Australia, 2011) and recorded as the most abundant species in Sarawak and Peninsular Malaysia (Kuei et al., 2013; Yong et al., 2010a, 2010b). These two species have economic importance and are also found in abundance in agro-forested locations of southern Thailand (Danjuma et al., 2013).

Considering the small number of species and the relatively small number of individuals caught, it would appear either that the Tephritid fauna of Batu Timbang Forest is rather poor or the period of trapping (three days) is not long enough to get a good representative catch. The disturbed habitat within the survey area might have also contributed to the low diversity of fruit flies. A more intensive study would enable to determine the right answer.

Conclusion

This study includes fruit flies species recorded for the first time from Imbak Canyon Conservation Area. The fruitflies of Batu Timbang Forest is dominated by the genus *Bactrocera* and represented by common species such as *Bactrocera papayae*, *B. tau* and *B. carambolae* which are known to be agricultural pests. Future research should be emphasized for fruit fly diversity and its relative natural enemies to provide more information on agricultural pest species for better pest management.

Acknowledgements

We would like to thank Yayasan Sabah, Petronas, UMS and all staff members for all the arrangement, assistants and facilities provided.

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Journal of Tropical Biology and Conservation 16: 79–100, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

Taxanomic Composition and Conservation Status of Plants in Imbak Canyon, Sabah, Malaysia

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Abstract
A study of plant diversity and their conservation status was conducted in Batu Timbang, Imbak Canyon Conservation Area (ICCA), Sabah. The study aimed to document plant diversity and to identify interesting, endemic, rare and threatened plant species which were considered high conservation value species. A total of 413 species from 82 families were recorded from the study area of which 93 taxa were endemic to Borneo, including 10 endemic to Sabah. These high conservation value species are key conservation targets for any forested area such as ICCA. Proper knowledge of plant diversity and their conservation status is vital for the formulation of a forest management plan for the Batu Timbang area.

Keywords: Vascular plant, floral diversity, endemic, endangered, Borneo

Introduction
The earth as it is today has a lot of important yet beneficial natural resources such as tropical forests. Tropical forests are one of the world's richest ecosystems, providing a wide range of important natural resources comprising vital biotic and abiotic components (Darus, 1982). These forests are classified into various types such as lowland forest, mixed dipterocarp forest (MDF), peat swamp forest, mangrove forest, hill forest and heath forest, providing crucial habitats for a diverse array of plants and animals that interact with each other and in providing a healthy ecosystem.

Received 01 March 2019
Reviewed 08 August 2019
Accepted 18 September 2019
Published 15 October 2019

Borneo as the second largest tropical island in the world is a biodiversity hotspot region that harbours approximately 15,000 varieties of plant species of which c. 3000 species are trees (MacKinnon et al., 1996). According to Whitmore (1984), most of the tree species are found in lowland rainforests with species of Dipterocarpaceae usually dominating the forest tree community.

The need for studies of the forest community especially in Borneo's tropical forests is due to species richness and endemism that require greater attention mainly for conservation purposes. Borneo was once connected through land bridges with the Asian mainland together with Java and Sumatra (Morley, 2000). However, glaciation events that occured during the Pleistocene period had separated the island from the mainland and seemed to have influenced the presence of tree flora in Borneo today (Slik et al. 2003).

However, Borneo is today projected to see socio-economic development and to be part of the aspirations of a high-income nation. This continuously creates pressures on the biodiversity of its tropical rainforest and problems to the environment. Apart from this, habitat fragmentation, invasive alien species, pollution, increasing competition for land, and climate change are among the threats to the vast amount of biodiversity (Ministry of Natural Resource and Environment, 2016) especially to the floral and faunal communities. Many species are trying to survive amidst development transition, while some are not known about due to lack of discovery. Therefore, monitoring biological diversity is essential for sustainable forest management. According to Noss (1999), the challenge lies in defining sound and practical biodiversity monitoring systems that deliver the scientific basis for sustainable forest management. The use of inventories on biodiversity for forest management operations is a common way of gathering information on the composition, diversity of tree species and species-rich communities (Suratman, 2012) while Pesiu et al. (2016) strongly believe that to understand such complex ecological interaction requires basic baseline studies of the tree community.

The surrounding forest of Batu Timbang Research Station, Imbak Canyon Conservation Area, is classified as a typical tropical rainforest of Borneo. Apart from its importance in representing the surrounding forest ecosystems in the region, it is also an important catchment system for several major rivers in the state. In this paper, we present the tree species inventory data gathered from the latest scientific expedition to Batu Timbang, Imbak Canyon Conservation Area in 2017. Our aims are to record and list plant resources and to identifythe conservation status of plant species.

Methodology

Batu Timbang is within Imbak Canyon Conservation Area, located over 140 kilometers south east of Kota Kinabalu or 145 km south west of Sandakan. It is geographically located between latitude 04° 54' 21"to 05° 11' 53" N and longitude 116° 49' 28" to 117° 06' 08" E within the Imbak Canyon Forest Reserve, a Class I Protection Forest Reserve in the middle part of Sabah (Figure 1). The area has experienced timber extraction in the past, except for the steep area on the western side towards Kuli peak. The topography of Batu Timbang is mostly hilly with very steep slopes and the highest point is Kuli peak about 1400 m above sea level at the west of the base camp. The area at the base camp is currently dominated by secondary forest (*Macaranga* spp.).

Prior to the field survey, various maps including soil map, satellite image (world map), and natural vegetation map were obtained for field survey planning. From the maps, areas of interest were identified and located. The field surveys were conducted from 21 to 25 August, 2017. All plant species and trees ≥10 cm diameter at breast height (dbh) were recorded from 6 of 0.1 ha circle plots which were established in various forest conditions (Table 1). Plant specimens with reproductive parts were collected (including voucher specimens) and deposited at the Sandakan Herbarium (SAN). Collecting and preserving plant specimens follow Bridson et al., (1992). The common plant species were identified directly to species level in the field by means of their distinctive field characteristics. For those that could not be readily identified, voucher specimens were collected for subsequent determination at SAN. The voucher specimen collections were oven-dried to 55° C for several days before determining their identities.

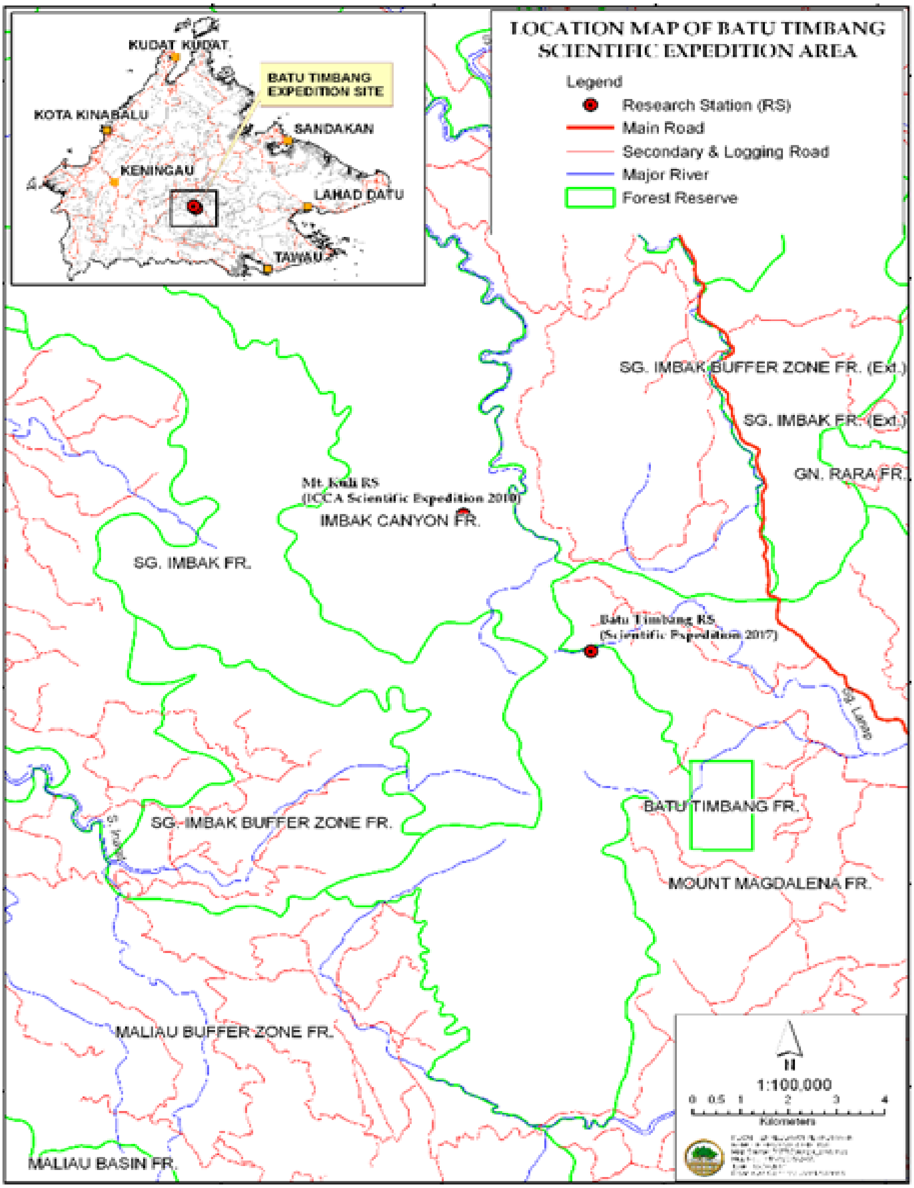

Figure 1. Location map of Batu Timbang, Imbak Canyon Conservation Area, Sabah, Malaysia.

Table 1. Plot numbers and their corresponding geographical position points for the forest assessment and rapid plant diversity inventory in ICCA-Batu Timbang Imbak Canyon Conservation Area, Sabah, Malaysia.

Plot No	Soil Association	Forest Formation	Coordinates	Altitude (m)
1	Maliau	Lowland mixed dipterocarp forest and kerangas forest	4°59'50.0"N, 117°04'38.8"E	515
2	Maliau	Upland mixed dipterocarp and kerangas forest	4°59'57.9"N, 117°04'29.1"E	624
3	Maliau	Upland mixed dipterocarp and kerangas forest	5°00'03.8"N, 117°04'04.8"E	777
4	Maliau	Lower Montane kerangas forest	5°00'1.2"N, 117°03'43.5"E	1099
5	Maliau	Lowland disturbed mixed dipterocarp forest	5°00'06.4"N, 117°04'41.5"E	366
6	Maliau	Lowland disturbed mixed dipterocarp forest	5°00'03.6"N, 117°04' 40.2"E	413

All specimens were sorted according to morphospecies and attempted for identification to species level by cross-referencing with the existing specimens in the herbarium related flora references such as Tree Flora of Sabah and Sarawak (Soepadmo & Wong, 1995; Soepadmo et al., 1996; Soepadmo & Saw, 2000; Soepadmo et al., 2002; Soepadmo et al., 2004; Soepadmo et al., 2007; Soepadmo et al., 2011; Soepadmo et al., 2014), The Bamboo of Sabah (Soetjani, 1992) and The Rattan of Sabah (Dransfield,1984). Plant classification of the Angiosperm Group is based on Stevens (2001) onwards. Some plant specimens could not be identified to species level due to incomplete specimens.

For plant conservation status, we used the IUCN Red list of Threatened species (IUCN, 2019), Malaysia Plant Red List, Peninsular Malaysian Dipterocarpaceae (Chua *et al*., 2010), Sabah Forest Enactment (Sabah Forest Department, 1968), Sabah Wildlife Conservation Enactment (Sabah Wildlife Department, 1997) and CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora, 2018).

Results and discussion

Based on the tree enumeration and voucher specimens, a total of 413 taxa from 82 families were recorded from the study area. Details on the overall checklist of plant species recorded was shown in Appendix I. The most species rich family is Dipterocarpaceae with 42 species followed by Lauraceae and Euphorbiaceae with 23 and 21 species respectively. The other family that has more than ten species are Annonaceae, Clusiaceae, Meliaceae, Myrtaceae, Ebenaceae, Rubiaceae, Phyllantaceae, Fagaceae and Malvaceae. There are 29 plant families that are only represented by one species as shown in Table 2. In terms of biological types, the highest number of family recorded was trees with 54 families followed by herbs and climbers with 11 families. Ferns and shrubs each with 9 and 3 families respectively while grass, leucophytes, palm and saprophyte were only represented by one family. The number of taxa recorded with respect to each biological type was shown in Table 3.

Table 2. The number of genera and species for each respective family recorded in Batu Timbang Imbak Canyon Conservation Area (ICCA).

Family	No of genera	No of species
Dipterocarpaceae	6	42
Lauraceae	8	23
Euphorbiaceae	10	21
Annonaceae	9	17
Clusiaceae	3	17
Meliaceae	4	16
Myrtaceae	2	16
Ebenaceae	1	15
Rubiaceae	12	15
Phyllanthaceae	4	13
Fagaceae	3	11
Malvaceae	7	11
Arecaceae	5	10
Zingiberaceae	7	9
Achariaceae	1	7
Burseraceae	2	7
Fabaceae	4	7
Sapotaceae	2	7
Anacardiaceae	3	6
Lecythidaceae	2	6
Melastomataceae	5	6
Polygalaceae	1	6
Primulaceae	4	6
Araceae	4	5
Celastraceae	2	5
Lamiaceae	2	5
Myristicaceae	3	5
Convolvulaceae	1	4
Cornaceae	2	4
Pandanaceae	2	4
Podocarpaceae	4	4
Proteaceae	2	4
Actinidiaceae	1	3
Hymenophyllaceae	2	3
Orchidaceae	3	3
Pentaphylacaceae	1	3
Pteridaceae	2	3
Symplocaceae	1	3
Dilleniaceae	1	2
Dryopteridaceae	1	2
Elaeocarpaceae	1	2
Gesneriaceae	2	2
Magnoliaceae	1	2

Marantaceae	2	2
Poaceae	2	2
Polypodiaceae	2	2
Rhamnaceae	1	2
Rutaceae	1	2
Salicaceae	1	2
Sapindaceae	2	2
Selaginellaceae	1	2
Stemonuraceae	2	2
Tectariaceae	2	2
Vitaceae	1	2
Araucariaceae	1	1
Begoniaceae	1	1
Casuarinaceae	1	1
Combretaceae	1	1
Connaraceae	1	1
Costaceae	1	1
Cyatheaceae	1	1
Dichapetalaceae	1	1
Ericaceae	1	1
Flagellariaceae	1	1
Gentianaceae	1	1
Hypoxidaceae	1	1
Juglandaceae	1	1
Lindasaeaceae	1	1
Lygodiaceae	1	1
Menispermaceae	1	1
Moraceae	1	1
Olacaceae	1	1
Oleaceae	1	1
Oleandraceae	1	1
Piperaceae	1	1
Putranjivaceae	1	1
Rafflesiaceae	1	1
Rosaceae	1	1
Sabiaceae	1	1
Simaroubaceae	1	1
Tetramelaceae	1	1
Theaceae	1	1
Urticaceae	1	1
Total	189	413

Table 3. Number of plant taxa classified by biological type from Batu Timbang, ICCA, Sabah, Malaysia.

	T	Sh	h	c	g	f	l	pt	sa
Families	54	3	11	11	1	9	1	1	1
Genera	122	6	24	16	2	13	1	3	1
Species	324	8	27	30	2	16	2	3	1

* Biological type: t=tree; c=climber; sh=shrub; h=herb; g=grass, f=fern; ep=epiphyte; l=lycophyte; pt=palm tree; sa= saprophytic

Of the 413 taxa that have been recorded, 93 taxa are endemic to Borneo, including 10 taxa endemic to Sabah (Appendix I). These Sabah and Borneo endemics were recorded from the recent survey areas/plots. In terms of legal protection, two endemic plants *(Durio acutifolius* and *Shorea macrophylla*) are protected under the Sabah Forest Enactment (Sabah Forest Department, 1968) while six (*Caryota no*, *Boesenbergia gracilipes*, B. *pulchella*, *Burbidgea schizochella*, *Plagiostachys parva* and *Rafflesia tengku-adlinii*) through the Sabah Wildlife Conservation Enactment (Sabah Wildlife Department, 1997). All the Sabah endemic plants that were recorded from Batu Timbang are also found in other places in Sabah.

The IUCN Red List Categories and Criteria were designed for global taxon assessments. There may be differences between the Malaysian Plant Red List and the IUCN Red List, and in such cases, the Malaysian Red Data Book should always take precedence. Conservation status in parenthesis are based on the Malaysian Red List. There are six plant species that are listed as Vulnerable (VU), four Endangered (EN) and four are Critically Endangered (CR) from the Batu Timbang area (Appendix I). In terms of legal protection, only two threatened species (Eusideroxylon zwageri and Shorea macrophylla) are protected under state law (Sabah Forest Enactment 1968).

In terms of legal protection, only two threatened species (*Eusideroxylon zwageri* and *Shorea macrophylla*) are protected under state law (Sabah Forest Department, 1968). Three taxa, namely *Rafflesia tengku-adlinii*, *T. diepenhorstii* and *T. lanceolarium* are under Schedule 1, part II, are classified as Totally Protected Plant Species. There were 15 plant taxa, comprising nine species of gingers, one *Caryota*, one *Rhododendron*, one *Podocarpus*, and three orchids that fall under Schedule 2, part II, Protected Plant Species while three species are listed under CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) from the orchid family (Appendix I). There are 25 plant species that are prohibited under the Sabah Forest Enactment (1968), mostly fruits trees and threatened tree species (Appendix I).

Imbak Canyon Conservation Area provides a wide range of plant resources and this can be seen by the high number of plant species that were previously recorded in Imbak Canyon Conservation Area (Appendix 1). A provisional checklist in de Kok and Utteridge (2010) based on the collections on databases in the Herbarium of the Sabah Forest Department and the Royal Botanic Gardens Kew, recorded a total of 470 plant species from 82 families. Based on comparison with the checklist in de Kok and Utteridge (2010), this study has recorded a total of 297 additional plant species (species identified until species level) with 62 species being shared species or have been recorded in previous studies.

Meanwhile, previous expeditions in Mount Kuli by Suratman et al. (2011) recorded 153 tree species from 46 families, Sugau et al. (2011) on the study of Dipterocarpaceae recorded a total 42 species. Rosea et al. (2011) has reported a total of 109 species of orchids in ICCA while Chong et al. (2011) on the study of *Begonia* recorded eight species. Shim et al. (2011) on the study of ferns recorded a total of 104 taxa from 21 families.

Conclusion

The forests in Batu Timbang area contain highly diverse and high conservation value plant species. It is recommended that forest monitoring should be done and is a prerequisite tool to measure ecological integrity and functionality of this conservation area, with support of protection and environmental awareness campaign programmes. This paper lays a foundation in studies in the future on the floral species richness and diversity in our tropical rainforest as well as for forest succession.

Acknowledgements

We would like to express our deep appreciation to Yayasan Sabah especially to Dr. Yap Sau Wai, Mrs. Roselia and staff for administrative and logistic support throughout the survey.We also thank the Chief Conservator of Forests, Deputy Chief Conservator of Forests (FSP), Deputy Chief Conservator of Forests (R&D), Institute of Tropical Biodiversity and Sustainable Development and School of Marine and Environmental Science, Universiti Malaysia Terengganu, for their continuous support. Also thank to En. Jumri Abd. Hamid for providing maps and the staff of the Systematic Botany section from Sabah Forest Department are gratefully acknowledged for their hard work in the field.

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Appendix I. List of vascular plant species recorded from Batu Timbang, ICCA. The species are arranged by family in alphabetical order

Species	Family	G	H	End	IUCN/ Malaysia Red List	SFDpro	SWCE	CITES
Hydnocarpus borneensis	Achariaceae	Ad	t	Borneo	NE	No	No	No
Hydnocarpus cf. *woodii*	Achariaceae	Ad	t			No	No	No
Hydnocarpus polypetalus	Achariaceae	Ad	t	Not	NE	No	No	No
Hydnocarpus sp.	Achariaceae	Ad	t			No	No	No
Hydnocarpus subfalcatus	Achariaceae	Ad	t	Not	NE	No	No	No
Hydnocarpus sumatranus	Achariaceae	Ad	t	Not	NE	No	No	No
Hydnocarpus woodii	Achariaceae	Ad	t	Not	NE	No	No	No
Saurauia agamae	Actinidiaceae	Ad	t	Sabah	NE	No	No	No
Saurauia oblancifolia	Actinidiaceae	Ad	t	Borneo	NE	No	No	No
Saurauia strigosa	Actinidiaceae	Ad	t	Not	NE	No	No	No
Gluta cf. *oba*	Anacardiaceae	Ad	t			No	No	No
Gluta rugulosa	Anacardiaceae	Ad	t	Borneo	NE	No	No	No
Gluta sp.	Anacardiaceae	Ad	t			No	No	No
Gluta wallichii	Anacardiaceae	Ad	t	Not	NE	No	No	No
Mangifera cf. *macrocarpa*	Anacardiaceae	Ad	t			Yes	No	No
Swintonia cf. *schwenkii*	Anacardiaceae	Ad	t			No	No	No
Artabotrys cf. *suaveolens*	Annonaceae	Ad	c			No	No	No
Enicosanthum grandifolium	Annonaceae	Ad	t	?	?	No	No	No
Fissistigma latifolium	Annonaceae	Ad	c	Not	NE	No	No	No
Friesodielsia glauca	Annonaceae	Ad	c	Not	NE	No	No	No
Indet.	Annonaceae	Ad						
Orophea alba	Annonaceae	Ad	t	Borneo	NE	No	No	No
Orophea myriantha	Annonaceae	Ad	t	Borneo	NE	No	No	No
Orophea rubra	Annonaceae	Ad	t	Borneo	NE	No	No	No
Polyalthia cauliflora	Annonaceae	Ad	t	Not	NE	No	No	No
Polyalthia insignis	Annonaceae	Ad	t	Not	NE	No	No	No
Polyalthia microtus	Annonaceae	Ad	t	Not	NE	No	No	No
Polyalthia sp.	Annonaceae	Ad	t					
Polyalthia sumatrana	Annonaceae	Ad	t	Not	NE	No	No	No
Popowia odoardi	Annonaceae	Ad	t	Borneo	NE	No	No	No
Sagaraea sp	Annonaceae	Ad	t			No	No	No
Sageraea lanceolata	Annonaceae	Ad	t	Not	NE	No	No	No
Uvaria cf. *littoralis*	Annonaceae	Ad	t			No	No	No
Alocasia denudata	Araceae	Am	h	Not	NE	No	No	No
Amorphophallus pendulus	Araceae	Am	h	Borneo	NE	No	No	No
Raphidophora maingayi	Araceae	Am	h	Not	NE	No	No	No

Scindapsus rupestris	Araceae	Am	h	Not	NE	No	No	No
Scindapsus sp.	Araceae	Am	h			No	No	No
Agathis lenticula	Araucariaceae	G	t	Sabah	VU	No	No	No
Calamus javensis	Arecaceae	Am	c	Not	NE	No	No	No
Calamus kiahii	Arecaceae	Am	c	Borneo	NE	No	No	No
Calamus marginatus	Arecaceae	Am	c	Not	NE	No	No	No
Calamus pogonacanthus	Arecaceae	Am	c	Borneo	NE	No	No	No
Caryota no	Arecaceae	Am	pt	Borneo	NE	No	Yes	No
Korthalsia echinometra	Arecaceae	Am	c	Not	NE	No	No	No
Korthalsia furtadoana	Arecaceae	Am	c	Borneo	NE	No	No	No
Korthalsia rigida	Arecaceae	Am	c	Not	NE	No	No	No
Licuala campestris	Arecaceae	Am	pt	Borneo	NE	No	No	No
Pinanga lepidota	Arecaceae	Am	pt	Borneo	NE	No	No	No
Begonia rotundibracteata	Begoniaceae	Ad	h	Sabah	NE	No	No	No
Dacryodes cf. *longifolia*	Burseraceae	Ad	t			Yes	No	No
Dacryodes cf. *rugosa*	Burseraceae	Ad	t			Yes	No	No
Dacryodes incurvata	Burseraceae	Ad	t	Not	NE	Yes	No	No
Dacryodes rostrata	Burseraceae	Ad	t	Not	LC	Yes	No	No
Dacryodes sp.	Burseraceae	Ad	t			Yes	No	No
Santiria apiculata	Burseraceae	Ad	t	Not	LC	Yes	No	No
Santiria oblongifolia	Burseraceae	Ad	t	Not	NE	Yes	No	No
Gymnostoma sumatranum	Casuarinaceae	Ad	t	Not	NE	No	No	No
Indet.	Celastraceae	Ad	t			No	No	No
Lophopetalum glabrum	Celastraceae	Ad	t	Borneo	NE	No	No	No
Lophopetalum sp.	Celastraceae	Ad	t			No	No	No
Salacia korthalsiana	Celastraceae	Ad	t	Not	NE	No	No	No
Salacia sp.	Celastraceae	Ad	t			No	No	No
Calophyllum blancoi	Clusiaceae	Ad	t	Not	NE	No	No	No
Calophyllum cf. *biflorum*	Clusiaceae	Ad	t			No	No	No
Calophyllum cf. *gracilipes*	Clusiaceae	Ad	t			No	No	No
Calophyllum nodusum	Clusiaceae	Ad	t	Not	NE	No	No	No
Calophyllum sp.	Clusiaceae	Ad	t			No	No	No
Calophyllum sp1	Clusiaceae	Ad	t			No	No	No
Calophyllum sp2	Clusiaceae	Ad	t			No	No	No
Calophyllum venulosum	Clusiaceae	Ad	t	Not	NE	No	No	No
Garcinia cf. *parvifolia*	Clusiaceae	Ad	t			No	No	No
Garcinia desrousseauxii	Clusiaceae	Ad	t	Borneo	NE	No	No	No
Garcinia maingayi	Clusiaceae	Ad	t	Not	LC	No	No	No
Garcinia parvifolia	Clusiaceae	Ad	t	Not	NE	No	No	No
Garcinia rostrata	Clusiaceae	Ad	t	Not	NE	No	No	No
Garcinia sp.	Clusiaceae	Ad	t			No	No	No
Garcinia tetragonus	Clusiaceae	Ad	t	Not	NE	No	No	No

Garcinia venulosa	Clusiaceae	Ad	t	Not	NE	No	No	No
Kayea borneensis	Clusiaceae	Ad	t	Borneo	NE	No	No	No
Terminalia phellocarpa	Combretaceae	Ad	t	Not	NE	No	No	No
Connarus euphlebius	Connaraceae	Ad	t	Not	NE	No	No	No
Erycibe praecipua subsp. *borneensis*	Convolvulaceae	Ad	c	Sabah	NE	No	No	No
Erycibe sp.	Convolvulaceae	Ad	c			No	No	No
Erycibe stapfiana	Convolvulaceae	Ad	c	Not	NE	No	No	No
Erycibe stenophylla	Convolvulaceae	Ad	c	Borneo	NE	No	No	No
Alangium javanicum var. *ebenaceum*	Cornaceae	Ad	t	Not	LC	No	No	No
Alangium sp.	Cornaceae	Ad	t			No	No	No
Mastixia cuspidata	Cornaceae	Ad	t	Not	NE	No	No	No
Mastixia sp.	Cornaceae	Ad	t			No	No	No
Costus globosus	Costaceae	Am	h	Not	NE	No	No	No
Cyathea ramispina	Cyatheaceae	F	f	Borneo	NE	No	No	No
Dichapetalum grandifolium	Dichapetalaceae	Ad	t	Borneo	NE	No	No	No
Tetracera akara	Dilleniaceae	Ad	c	Not	NE	No	No	No
Tetracera korthalsii	Dilleniaceae	Ad	c	Not	NE	No	No	No
Dipterocarpus applanatus	Dipterocarpaceae	Ad	t	Borneo	CR	No	No	No
Dipterocarpus caudiferus	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Dipterocarpus hasseltii	Dipterocarpaceae	Ad	t	Not	CR (VU)	No	No	No
Dipterocarpus kunstleri	Dipterocarpaceae	Ad	t	Not	CR (NT)	No	No	No
Dryobalanops keithii	Dipterocarpaceae	Ad	t	Borneo	CR	No	No	No
Dryobalanops lanceolata	Dipterocarpaceae	Ad	t	Borneo	EN	No	No	No
Hopea ferruginea	Dipterocarpaceae	Ad	t	Not	CR (LC)	No	No	No
Hopea sp.	Dipterocarpaceae	Ad	t					
Parashorea tomentella	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Shorea agamii	Dipterocarpaceae	Ad	t	Borneo	EN	No	No	No
Shorea argentifolia	Dipterocarpaceae	Ad	t	Borneo	EN	No	No	No
Shorea cf. *hopeifolia*	Dipterocarpaceae	Ad	t					
Shorea cf. *micans*	Dipterocarpaceae	Ad	t					
Shorea confusa	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Shorea faguetiana	Dipterocarpaceae	Ad	t	Not	EN (LC)	No	No	No
Shorea fallax	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Shorea ferruginea	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Shorea flaviflora	Dipterocarpaceae	Ad	t	Borneo	CR	No	No	No
Shorea havilandii	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Shorea hopeifolia	Dipterocarpaceae	Ad	t	Not	CR (NT)	No	No	No
Shorea johorensis	Dipterocarpaceae	Ad	t	Not	CR (NT)	No	No	No
Shorea laevis	Dipterocarpaceae	Ad	t	Not	LC (NT)	No	No	No
Shorea leprosula	Dipterocarpaceae	Ad	t	Not	EN (LC)	No	No	No
Shorea macrophylla	Dipterocarpaceae	Ad	t	Borneo	VU	Yes	No	No
Shorea monticola	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Shorea obscura	Dipterocarpaceae	Ad	t	Borneo	EN	No	No	No
Shorea ovalis	Dipterocarpaceae	Ad	t	Not	EN (NT)	No	No	No
Shorea ovata	Dipterocarpaceae	Ad	t	Not	EN (NT)	No	No	No

Shorea parvifolia	Dipterocarpaceae	Ad	t	Not	NE (LC)	No	No	No
Shorea parvistipulata	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Shorea patoiensis	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Shorea platyclados	Dipterocarpaceae	Ad	t	Not	EN (NT)	No	No	No
Shorea rubra	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Shorea scorbiculata	Dipterocarpaceae	Ad	t	Not	NE (LC)	No	No	No
Shorea sp.	Dipterocarpaceae	Ad	t			No	No	No
Shorea symingtonii	Dipterocarpaceae	Ad	t	Sabah	CR	No	No	No
Shorea venulosa	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Vatica cf. *rassak*	Dipterocarpaceae	Ad	t			No	No	No
Vatica oblongifolia	Dipterocarpaceae	Ad	t	Borneo	NE	No	No	No
Vatica odorata subsp. *mindanaensis*	Dipterocarpaceae	Ad	t	Not	NE	No	No	No
Vatica sp.	Dipterocarpaceae	Ad	t			No	No	No
Vatica umbonata	Dipterocarpaceae	Ad	t	Not	LC	No	No	No
Bolbitis heteroclita	Dryopteridaceae	F	f	Not	NE	No	No	No
Bolbitis sp.	Dryopteridaceae	F	f			No	No	No
Diospyros sp.	Ebenaceae	Ad	t			No	No	No
Diospyros sp 1	Ebenaceae	Ad	t			No	No	No
Diospyros sp 2	Ebenaceae	Ad	t			No	No	No
Diospyros buxifolia	Ebenaceae	Ad	t	Not	NE	No	No	No
Diospyros cf. *elliptifolia*	Ebenaceae	Ad	t			No	No	No
Diospyros curanii	Ebenaceae	Ad	t	Not	NE	No	No	No
Diospyros foxworthyii	Ebenaceae	Ad	t	Not	LC	No	No	No
Diospyros frutescens	Ebenaceae	Ad	t	Not	NE	No	No	No
Diospyros macrophylla	Ebenaceae	Ad	t	Not	NE	No	No	No
Diospyros mindanaensis	Ebenaceae	Ad	t	Not	NE	No	No	No
Diospyros oligantha	Ebenaceae	Ad	t	Borneo	NE	No	No	No
Diospyros perfida	Ebenaceae	Ad	t	Borneo	NE	No	No	No
Diospyros pilosanthera	Ebenaceae	Ad	t	Not	NE	No	No	No
Diospyros subrhomboidea	Ebenaceae	Ad	t	Not	NE	No	No	No
Diospyros sumatrana	Ebenaceae	Ad	t	Not	NE	No	No	No
Elaeocarpus cf. *pendunculatus*	Elaeocarpaceae	Ad	t			No	No	No
Rhododendron cf. *durionifolium*	Ericaceae	Ad	t			No	Yes	No
Elaeocarpus sp.	Elaeocarpaceae	Ad	t			No	No	No
Agrostistachys longifolia	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Blumeodendron concolor	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Blumeodendron kurzii	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Blumeodendron sp.	Euphorbiaceae	Ad	t			No	No	No
Blumeodendron tokbrai	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Koilodepas laevigatum	Euphorbiaceae	Ad	t	Borneo	NE	No	No	No

Koilodepas longifolium	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Macaranga glandibracteolata	Euphorbiaceae	Ad	t	Borneo	NE	No	No	No
Macaranga hypoleuca	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Mallotus lackeyi	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Mallotus penangensis	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Mallotus stipularis	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Neoscortechinia cf. *forbesii*	Euphorbiaceae	Ad	t			No	No	No
Neoscortechinia philippinensis	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Neoscortechinia sp	Euphorbiaceae	Ad	t			No	No	No
Neoscortechinia sumatrensis	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Omphalea sargentii	Euphorbiaceae	Ad	c	Not	NE	No	No	No
Ptychopyxis arborea	Euphorbiaceae	Ad	t	Borneo	NE	No	No	No
Ptychopyxis sp.	Euphorbiaceae	Ad	t			No	No	No
Spathiostemon javensis	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Sumbaviopsis albicans	Euphorbiaceae	Ad	t	Not	NE	No	No	No
Bauhinia endertii	Fabaceae	Ad	c	Borneo	NE	No	No	No
Bauhinia kockina var. *kockiana*	Fabaceae	Ad	c	Not	NE	No	No	No
Caesalpinia sappan	Fabaceae	Ad	c	Not	LC	No	No	No
Ormosia bancana	Fabaceae	Ad	t	Not	NE	No	No	No
Spatholobus cf. *gyrocarpus*	Fabaceae	Ad	c			No	No	No
Spatholobus hirsutus	Fabaceae	Ad	c	Not	NE	No	No	No
Spatholobus macropterus	Fabaceae	Ad	c	Not	NE	No	No	No
Castanopsis hypophoenicea	Fagaceae	Ad	t	Not	NE	Yes	No	No
Lithocarpus cantleyanus	Fagaceae	Ad	t	Not	NE	Yes	No	No
Lithocarpus elegans	Fagaceae	Ad	t	Not	NE	Yes	No	No
Lithocarpus ferrugineus	Fagaceae	Ad	t	Not	NE	Yes	No	No
Lithocarpus gracilis	Fagaceae	Ad	t	Not	NE	Yes	No	No
Lithocarpus lucidus	Fagaceae	Ad	t	Not	NE	Yes	No	No
Lithocarpus pseudokunstleri	Fagaceae	Ad	t	Not	NE	Yes	No	No
Lithocarpus sp.	Fagaceae	Ad	t			Yes	No	No
Quercus argentata	Fagaceae	Ad	t	Not	NE	No	No	No
Quercus lineata	Fagaceae	Ad	t	Not	NE	No	No	No
Quercus sp.	Fagaceae	Ad	t				No	No
Flagellaria indica	Flagellariaceae	Am	c	Not	NE	No	No	No
Utania spicata	Gentianaceae	Ad	t	Not	NE	No	No	No
Cyrtandra sarawakensis	Gesneriaceae	Ad	h	Borneo	NE	No	No	No
Henckelia amoena	Gesneriaceae	Ad	h	Borneo	NE	No	No	No

Cephalomanes javanicum	Hymenophyllaceae	F	f	Not	NE	No	No	No
Crepidomanes bipunctatum	Hymenophyllaceae	F	f	Not	NE	No	No	No
Vandenboschia maxima	Hymenophyllaceae	F	f	Not	LC	No	No	No
Curculigo latifolia	Hypoxidaceae	Am	h	Not	NE	No	No	No
Engelhardia serrata	Juglandaceae	Ad	t	Not	NE	No	No	No
Teijsmanniodendron bogoriense	Lamiaceae	Ad	t	Not	NE	No	No	No
Teijsmanniodendron glabrum	Lamiaceae	Ad	t	Not	NE	No	No	No
Teijsmanniodendron smilacifolium	Lamiaceae	Ad	t	Borneo	NE	No	No	No
Teijsmanniodendron sp.	Lamiaceae	Ad	t					
Vitex vestita	Lamiaceae	Ad	t	Not	NE	No	No	No
Actinodaphne borneensis	Lauraceae	Ad	t	Borneo	NE	No	No	No
Actinodaphne cf. *borneensis*	Lauraceae	Ad	t			No	No	No
Actinodaphne glomerata	Lauraceae	Ad	t	Not	NE	No	No	No
Alseodaphne oblanceolata	Lauraceae	Ad	t	Not	NE	No	No	No
Beilschmedia assamica	Lauraceae	Ad	t	Not	NE	No	No	No
Beilschmedia lucidula	Lauraceae	Ad	t	Not	NE	No	No	No
Beilschmedia tawaensis	Lauraceae	Ad	t	Not	NE	No	No	No
Beilschmiedia sp.	Lauraceae	Ad	t					
Caryodaphnopsis tonkensis	Lauraceae	Ad	t	Not	NE	No	No	No
Cryptocarya ferrea var. *ferrea*	Lauraceae	Ad	t	Not	NE	No	No	No
Cryptocarya griffithianum var. *strictifolia*	Lauraceae	Ad	t	Not	NE	No	No	No
Cryptocarya teysmanniana	Lauraceae	Ad	t	Not	NE	No	No	No
Eusideroxylon zwageri	Lauraceae	Ad	t	Not	VU	Yes	No	No
indet 1	Lauraceae	Ad	t					
indet 2	Lauraceae	Ad	t					
Litsea cylindrocarpa	Lauraceae	Ad	t	Not	NE	No	No	No
Litsea ficoidea	Lauraceae	Ad	t	Not	NE	No	No	No
Litsea firma	Lauraceae	Ad	t	Not	NE	No	No	No
Litsea fulva	Lauraceae	Ad	t	Not	NE	No	No	No
Litsea grandis	Lauraceae	Ad	t	Not	NE	No	No	No
Litsea oppositifolia	Lauraceae	Ad	t	Borneo	NE	No	No	No
Litsea sp.	Lauraceae	Ad	t					
Phoebe macrophylla	Lauraceae	Ad	t	Not	NE	No	No	No
Barringtonia cf. *lancelata*	Lecythidaceae	Ad	t					
Barringtonia curranii	Lecythidaceae	Ad	t	Not	NE	No	No	No
Barringtonia lanceolata	Lecythidaceae	Ad	t	Borneo	NE	No	No	No
Barringtonia sarcostachys	Lecythidaceae	Ad	t	Not	NE	No	No	No

Barringtonia scortechinii	Lecythidaceae	Ad	t	Not	NE	No	No	No
Planchonia valida	Lecythidaceae	Ad	t	Not	NE	No	No	No
Lindsaea parasitica	Lindasaeaceae	F	f	Not	NE	No	No	No
Lygodium circinatum	Lygodiaceae	F	f	Not	NE	No	No	No
Magnolia bintuluensis	Magnoliaceae	Ad	t	Not	NE	No	No	No
Magnolia gigantifolia	Magnoliaceae	Ad	t	Not	NE	No	No	No
Brownlowia stipulata	Malvaceae	Ad	t	Not	NE	No	No	No
Byttneria reinwardtii	Malvaceae	Ad	t	Not	NE	No	No	No
Durio grandiflorus	Malvaceae	Ad	t	Borneo	VU	Yes	No	No
Heritiera elata	Malvaceae	Ad	t	Not	NE	No	No	No
Heritiera sumatrana	Malvaceae	Ad	t	Not	NE	No	No	No
Microcos hirsuta	Malvaceae	Ad	t	Not	NE	No	No	No
Microcos sp.	Malvaceae	Ad	t					
Microcos triflora var longipetiolata	Malvaceae	Ad	t	Borneo	NE	No	No	No
Pterospermum elongatum	Malvaceae	Ad	t	Not	NE	No	No	No
Pterospermum subpeltatum	Malvaceae	Ad	t	Borneo	NE	No	No	No
Sterculia rubiginosa	Malvaceae	Ad	t	Not	NE	No	No	No
Phacelophrynium aurantium	Marantaceae	Am	h	Borneo	NE	No	No	No
Phrynium pubinerve	Marantaceae	Am	h	Not	NE	No	No	No
Memecylon scolopacium	Melastomataceae	Ad	t	Borneo	NE	No	No	No
Memecylon sp.	Melastomataceae	Ad	t					
Anerincleistus quintuplinervis	Melastomataceae	Ad	sh	Not	NE	No	No	No
Oxyspora beccarii	Melastomataceae	Ad	sh	Borneo	NE	No	No	No
Pternandra rostrata	Melastomataceae	Ad	t	Not	NE	No	No	No
Sonerila borneensis	Melastomataceae	Ad	h	Borneo	NE	No	No	No
Aglaia cf. *macrocarpa*	Meliaceae	Ad	t					
Aglaia cf. *teysmannia*	Meliaceae	Ad	t					
Aglaia edulis	Meliaceae	Ad	t	Not	NT	No	No	No
Aglaia elliptica subsp. *clementis*	Meliaceae	Ad	t	Not	LC	No	No	No
Aglaia grandis	Meliaceae	Ad	t	Not	NT	No	No	No
Aglaia korthalsii	Meliaceae	Ad	t	Not	NT	No	No	No
Aglaia odoratissima	Meliaceae	Ad	t	Not	LC	No	No	No
Aglaia sp.	Meliaceae	Ad	t					
Aglaia tomentosa subsp. *tomentosa*	Meliaceae	Ad	t	Not	LC	No	No	No
Aphanamixis borneensis	Meliaceae	Ad	t	Not	NE	No	No	No
Chisocheton sarawakanus	Meliaceae	Ad	t	Not	NE	No	No	No
Chisocheton sp.	Meliaceae	Ad	t					
Dysoxylum crytobotryum	Meliaceae	Ad	t	Not	NE	No	No	No

Dysoxylum excelsum	Meliaceae	Ad	t	Not	NE	No	No	No
Dysoxylum parasiticum	Meliaceae	Ad	t	Not	NE	No	No	No
Dysoxylum sp.	Meliaceae	Ad	t					
Tinospora merrilliana	Menispermaceae	Ad	c	Not	NE	No	No	No
Ficus sumatrana	Moraceae	Ad	t	Not	NE	No	No	No
Gymnacranthera farquhariana	Myristicaceae	Ad	t	Not	NE	No	No	No
Knema korthalsii	Myristicaceae	Ad	t	Not	NE	No	No	No
Knema latericia	Myristicaceae	Ad	t	Not	NE	No	No	No
Knema latericia var. *albifolia*	Myristicaceae	Ad	t	Borneo	NE	No	No	No
Myristica cinnamomea	Myristicaceae	Ad	t	Not	LC	No	No	No
Syzygium bankense	Myrtaceae	Ad	t	Not	NE	No	No	No
Syzygium castaneum	Myrtaceae	Ad	t	Not	NE	No	No	No
Syzygium cephalophorum	Myrtaceae	Ad	t	Borneo	NE	No	No	No
Syzygium cf. *palembanica*	Myrtaceae	Ad	t					
Syzygium fastigiatum	Myrtaceae	Ad	t	Not	NE	No	No	No
Syzygium korthalsiana	Myrtaceae	Ad	t	Not	NE	No	No	No
Syzygium leucoxylon	Myrtaceae	Ad	t	Not	NE	No	No	No
Syzygium roseomarginatum	Myrtaceae	Ad	t	Not	NE	No	No	No
Syzygium sp 3	Myrtaceae	Ad	t					
Syzygium sp.	Myrtaceae	Ad	t					
Syzygium sp 1	Myrtaceae	Ad	t					
Syzygium sp 2	Myrtaceae	Ad	t					
Syzygium subdecusata	Myrtaceae	Ad	t	?	?	No	No	No
Syzygium valdevenosum	Myrtaceae	Ad	t	Not	NE	No	No	No
Tristaniopsis bilocularis	Myrtaceae	Ad	t	Not	NE	No	No	No
Tristaniopsis cf. *whiteana*	Myrtaceae	Ad	t	Not	NE	No	No	No
Ochanostachys amentacea	Olacaceae	Ad	t	Not	DD	No	No	No
Chionanthus pluriflorus	Oleaceae	Ad	t	Not	NE	No	No	No
Oleandra pistillaris	Oleandraceae	F	f	Not	NE	No	No	No
Bulbophyllum sp.	Orchidaceae	Am	h	?	?	No	Yes	Yes
Agrostophyllum stipulatum	Orchidaceae	Am	h	Not	NE	No	Yes	Yes
Liparis cf. *viridiflora*	Orchidaceae	Am	h			No	Yes	Yes
Freycinetia biloba	Pandanaceae	Am	sh	Borneo	NE	No	No	No
Freycinetia discoidea	Pandanaceae	Am	sh	Not	NE	No	No	No
Pandanus affinis	Pandanaceae	Am	sh	Not	NE	No	No	No
Pandanus discostigma	Pandanaceae	Am	sh	Borneo	NE	No	No	No
Ternstroemia aneura	Pentaphylacaceae	Ad	t	Not	NE	No	No	No
Ternstroemia beccarii	Pentaphylacaceae	Ad	t	Borneo	NE	No	No	No

Ternstroemia sp.	Pentaphylacaceae	Ad	t					
Antidesma neurocarpum	Phyllanthaceae	Ad	t	Not	NE	No	No	No
Aporosa acuminatissima	Phyllanthaceae	Ad	t	Not	NE	No	No	No
Aporosa confusa	Phyllanthaceae	Ad	t	Not	NE	No	No	No
Aporosa frutescens	Phyllanthaceae	Ad	t	Not	NE	No	No	No
Aporosa sp.	Phyllanthaceae	Ad	t					
Baccaurea lanceolata	Phyllanthaceae	Ad	t	Not	NE	Yes	No	No
Baccaurea membranacea	Phyllanthaceae	Ad	t	Not	VU	Yes	No	No
Baccaurea minor	Phyllanthaceae	Ad	t	Not	NE	Yes	No	No
Baccaurea sumatrana	Phyllanthaceae	Ad	t	Not	NE	Yes	No	No
Baccaurea tetrandra	Phyllanthaceae	Ad	t	Not	NE	Yes	No	No
Cleistanthus myrianthus var. *concinnus*	Phyllanthaceae	Ad	t	Not	NE	No	No	No
Cleistanthus podopyxis	Phyllanthaceae	Ad	t	Borneo	NE	No	No	No
Cleistanthus sp.	Phyllanthaceae	Ad	t					
Piper blumei	Piperaceae	Ad	c	Not	NE	No	No	No
Dinochloa sublaevigata	Poaceae	Am	c	Borneo	NE	No	No	No
Scrotochloa urceolata	Poaceae	Am	g	Not	NE	No	No	No
Falcatifolium falciforme	Podocarpaceae	G	t	Not	LC	No	No	No
Nageia wallichiana	Podocarpaceae	G	t	Not	LC	No	No	No
Phyllocladus hypophyllus	Podocarpaceae	G	t	Not	LC	No	No	No
Podocarpus cf. *neriifolius*	Podocarpaceae	G	t			No	Yes	No
Xanthophyllum adenotus var. *adenotus*	Polygalaceae	Ad	t	Not	NE	No	No	No
Xanthophyllum flavescens	Polygalaceae	Ad	t	Not	NE	No	No	No
Xanthophyllum griffithii var. *angustifolium*	Polygalaceae	Ad	t	Not	NE	No	No	No
Xanthophyllum montanum	Polygalaceae	Ad	t	Sabah	NE	No	No	No
Xanthophyllum schizoarpon	Polygalaceae	Ad	t	Borneo	NE	No	No	No
Xanthophyllum trichocladum	Polygalaceae	Ad	t	Borneo	NE	No	No	No
Calymmodon gracilis	Polypodiaceae	F	f	Not	NE	No	No	No
Selliguea stenophylla	Polypodiaceae	F	f	Not	NE	No	No	No
Ardisia forbesii	Primulaceae	Ad	t	Not	NE	No	No	No
Ardisia pachysandra	Primulaceae	Ad	t	Not	NE	No	No	No
Ardisia sp.	Primulaceae	Ad	t					
Embelia philippinensis	Primulaceae	Ad	c	Not	NE	No	No	No
Labisia pumila	Primulaceae	Ad	h	Not	NE	No	No	No
Myrsine porteriana	Primulaceae	Ad	t	Not	NE	No	No	No
Helicia attenuata	Proteaceae	Ad	t	Not	NE	No	No	No
Helicia petiolaris	Proteaceae	Ad	t	Not	NE	No	No	No

Helicia sp.	Proteaceae	Ad	t					
Heliciopsis artocarpioides	Proteaceae	Ad	t	Not	NE	No	No	No
Antrophyum callifolium	Pteridaceae	F	f	Not	NE	No	No	No
Antrophyum sessilifolium	Pteridaceae	F	f	Not	NE	No	No	No
Taenitis blechnoides	Pteridaceae	F	f	Not	NE	No	No	No
Drypetes sp.	Putranjivaceae	Ad	t					
Rafflesia tengku-adlinii	Rafflesiaceae	Ad	sa	Sabah	NE	No	Yes	No
Ziziphus angustifolia	Rhamnaceae	Ad	t	Not	NE	No	No	No
Ziziphus borneensis	Rhamnaceae	Ad	t	Borneo	NE	No	No	No
Prunus javanica	Rosaceae	Ad	t	Not	NE	No	No	No
Acranthera velutinervia	Rubiaceae	Ad	sh	Sabah	NE	No	No	No
Argostemma borragineum	Rubiaceae	Ad	sh	Not	NE	No	No	No
Cyanoneuron pubescens	Rubiaceae	Ad	t	Borneo	NE	No	No	No
Diplospora malaccensis	Rubiaceae	Ad	t	Not	NE	No	No	No
Hedyotis pulchella	Rubiaceae	Ad	t	Borneo	NE	No	No	No
Hymenodictyon orixense	Rubiaceae	Ad	t	Not	NE	No	No	No
Ixora brachyantha	Rubiaceae	Ad	t	Borneo	NE	No	No	No
Ixora linggensis	Rubiaceae	Ad	t	?	?	No	No	No
Ixora miliensis	Rubiaceae	Ad	t	Borneo	NE	No	No	No
Ludekia borneensis	Rubiaceae	Ad	t	Borneo	NE	No	No	No
Neonauclea artocarpoides	Rubiaceae	Ad	t	Borneo	NE	No	No	No
Pleiocarpidia enneandra	Rubiaceae	Ad	t	Not	NE	No	No	No
Praravinia suberosa	Rubiaceae	Ad	t	Borneo	NE	No	No	No
Urophyllum glabrum	Rubiaceae	Ad	t	Not	NE	No	No	No
Urophyllum griffithianum	Rubiaceae	Ad	t	Not	NE	No	No	No
Maclurodendron pubescens	Rutaceae	Ad	t	Sabah	NE	No	No	No
Maclurodendron sp.	Rutaceae	Ad	t			No	No	No
Meliosma simplicifolia	Sabiaceae	Ad	t	Not	NE	No	No	No
Caseria grewiaefolia var. *gelonioides*	Salicaceae	Ad	t	?	NE	No	No	No
Caseria grewiaefolia var. *grewiaefolia*	Salicaceae	Ad	t	?	NE	No	No	No
Dimocarpus longan	Sapindaceae	Ad	t	Not	NT	No	No	No
Nephelium ramboutan-ake	Sapindaceae	Ad	t	Not	NE	Yes	No	No
Madhuca mindanaensis	Sapotaceae	Ad	t	Not	NE	No	No	No
Madhuca multinervia	Sapotaceae	Ad	t	Sabah	NE	No	No	No
Madhuca sp.	Sapotaceae	Ad	t			No	No	No

Palaquium dasyphyllum	Sapotaceae	Ad	t	Not	NE	No	No	No
Palaquium leiocarpum	Sapotaceae	Ad	t	Not	NE	No	No	No
Palaquium rostratum	Sapotaceae	Ad	t	Not	NE	No	No	No
Palaquium sericeum	Sapotaceae	Ad	t	Borneo	NE	No	No	No
Selaginella brevipes	Selaginellaceae	L	l	Borneo	NE	No	No	No
Selaginella intermedia	Selaginellaceae	L	l	?	NE	No	No	No
Eurycoma longifolia	Simaroubaceae	Ad	t	Not	NE	No	No	No
Gomphandra cumingiana	Stemonuraceae	Ad	t	Not	NE	No	No	No
Stemonurus grandifolius	Stemonuraceae	Ad	t	Borneo	NE	No	No	No
Symplocos fasciculata	Symplocaceae	Ad	t	Not	NE	No	No	No
***Symplocos* sp.**	Symplocaceae	Ad	t			No	No	No
Symplocos tricocata	Symplocaceae	Ad	t	Not	NE	No	No	No
Pleocnemia irregularis	Tectariaceae	F	f	Not	NE	No	No	No
Tectaria pleiosora	Tectariaceae	F	f	Not	NE	No	No	No
Octomeles sumatrana	Tetramelaceae	Ad	t	Not	LC	No	No	No
Schima wallichii	Theaceae	Ad	t	Not	NE	No	No	No
Elatostema kabayense	Urticaceae	Ad	h	Not	NE	No	No	No
Tetrastigma* cf. *diepenhorstii	Vitaceae	Ad	c			No	Yes	No
Tetrastigma* cf. *lanceolarium	Vitaceae	Ad	c			No	Yes	No
Alpinia capitellata	Zingiberaceae	Am	h	Not	NE	No	Yes	No
Amomum testaceum	Zingiberaceae	Am	h	Not	NE	No	Yes	No
Boesenbergia gracilipes	Zingiberaceae	Am	h	Borneo	NE	No	Yes	No
Boesenbergia pulchella	Zingiberaceae	Am	h	Borneo	NE	No	Yes	No
Burbidgea schizocheila	Zingiberaceae	Am	h	Borneo	NE	No	Yes	No
Elettaria longituba	Zingiberaceae	Am	h	Not	NE	No	Yes	No
Plagiostachys parva	Zingiberaceae	Am	h	Borneo	NE	No	Yes	No
Zingiber griffithii	Zingiberaceae	Am	h	Not	NE	No	Yes	No
***Zingiber* sp.**	Zingiberaceae	Am	h			No	Yes	No

Notes:
IUCN/Malaysia Red List: CR=Critically endangered; EN=Endangered; VU=Vulnerable; NT=Near threatened; LC=Least concern; NE=Not Evaluated
G=Plant Group: Ad=Angiosperm (Dicotyledon); Am=Angiosperm (Monocotyledon); G=Gymnosperm; F=Fern; L=Lycophyte
H=habit: t=tree; c=climber; sh=shrub; h=herb; g=grass; sd=sedge; f=fern; ep=epiphyte; l=lycophyte; pt=palm tree; sa= saprophytic; str=strangler
SFDpro=Sabah Forestry Department prohibited species under Sabah Forest Enactment 1968
SWCE=Sabah Wildlife Conservation Enactment 1997
CITES=Convention on International Trade in Endangered Species of Wild Fauna and Flora
IdLoc= Location: BT=Batu Timbang

Journal of Tropical Biology and Conservation 16: 101–106, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

Short Notes on Saproxylic Arthropods of Batu Timbang Research Station, Imbak Canyon Conservation Area

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Abstract

A sampling of saproxylic arthropods was conducted from a fallen log located close to an existing trail and was situated under shaded area. All arthropods (larvae, pupae and adults) found in the fallen log were collected and kept in vials containing 75 % ethanol solution for identification. A total of 7 insect orders and 1 class of arthropod (Diplopoda) were collected. 15 species of ants were collected but only 1 species observed residing in the fallen log. Other ants were collected foraging on the deadwood. Only one species of termites (Blattodea: Havilanditermes atripennis) was recorded. Other orders found include Coleoptera (beetles), Orthoptera (mole cricket), Dermaptera (earwig), Diptera (fly) and Lepidoptera (moth).

Keywords: saproxylic, arthropods, fallen log, Imbak Canyon Conservation Area

Introduction

In general, Arthropoda is a phylum in the Animal Kingdom where the members of this phylum have bilaterally symmetrical body, jointed chitonous exoskeleton with sclerotized plates and jointed segmental appendages (Anderson, 2001). Examples of Arthropods are insects, arachnids and crustaceans.

In the present study, saproxylic arthropods were collected in Imbak Canyon Conservation Area. Saproxylic arthropods are defined as arthropods that ‘to be dependent, during some part of the life cycle, upon dead or dying wood, or wood inhabiting fungi, or upon the presence of other saproxylic species’ was coined by Speight in 1989 (Grove, 2002). Many of these saproxylic faunas are arthropods which use dead wood for food, shelter, foraging or reproductive activities (Hammond, 1997; Grove, 2002). Their roles as initial dead wood decomposers thus involved directly in the nutrient cycle has made them important components in the forest ecosystem (Lachat et al., 2006).

Received 01 March 2019
Reviewed 06 May 2019
Accepted 02 July 2019
Published 15 October 2019

Arthropods play vital roles in various ecosystem functions and respond acutely to habitat manipulation. As saproxylic arthropods depend on deadwood for their survival, any alteration of vegetation and habitat disturbance will greatly affect their presence in a particular area. It has been shown that arthropods showed a fragmentation effect with point diversity and abundance negatively correlated with fragment age (Bolger et al., 2000). Despite their important role as decomposers of organic materials, very few studies have been conducted on saproxylic arthropods in tropical regions (Mahadimenakbar et al., 2008).

Material and Method

Study site

The study area is located at Batu Timbang Research Station, ICCA. Collection of Saproxylic insects was carried out from August 18th to19th, 2017.

Collections and observations

Due to limited time available (only 2 days), only one fallen log was selected as the sampling station. This fallen log was located close to an existing trail and was situated under a shaded area. The length was measured from the top to bottom of the fallen log. Diameter was measured at the top, middle and bottom sections of the log by using a measuring tape (Araya, 1992; Araya, 1993).

In the field, all arthropods (larvae, pupae and adults) found in the fallen log were collected and kept in vials containing 75 % ethanol solution for identification. Whenever possible, the fallen log was broken into smaller pieces to extract as many arthropods as possible and was examined carefully to make sure no arthropods will be left unnoticed. Extra effort was taken if the examined sections contained high number of arthropods such as ants or termites. Specimens collected were identified in laboratory. All insects were identified up to Order level, except ants and termites identified to species level. Beetles were identified to family level. Other arthropods were identified up to Class level. Excluding ants and termites, the numbers of individuals sampled in each study site were calculated. Individual numbers of ants and termites were not calculated as there were too many of them, and not all individuals were sampled. Ants that came to the rotten log foraging for food were also sampled as additional samples.

Results and Discussion

A total of 7 insect orders and 1 class of arthropod (Diplopoda) were collected, as shown in Table 1.

Table 1: Individual numbers of arthropod orders collected.

Order/ Class	No. of taxa
ymenoptera (Ants)	1. *Dinomyrmex gigas* 2. *Myrmicaria* sp. 3. *Dacetinops* sp. 4. *Pheidole* sp. 5. *Lordomyrma* sp. 6. *Camponotus* sp. 7. *Meranoplus* sp. 8. *Aenictus* sp. 9. *Leptogenys* sp. 10. *Polyrhachis* sp. 1 11. *Polyrhachis* sp. 2 12. *Diacamma* sp. 13. *Echinopla* sp. 14. *Myopias* sp. 1 15. *Myopias* sp. 2
Coleoptera (Beetles)	1. Passalidae (2 morphospecies) 2. Staphylinidae (2 morphospecies) 3. Scarabaeidae, Aphodiinae 4. Carabidae 5. Elateridae (2 larvae) 6. Cerambycidae (1 larva) 7. Curculionidae (2 larvae)
Orthoptera (Mole Cricket)	1 morphospecies
Dermaptera (Earwig)	1 morphospecies
Diptera (Fly)	1 morphospecies; 2 larvae
Lepidoptera (Moth)	1 mophospecies; 2 larvae
Diplopoda (Pill Milipede)	1 morphospecies
Isoptera (Blattodea)	1 species *Havilanditermes atripennis*

Since the collection was carried out in a short period and only a small number of specimens were sampled, we have decided not to analyze the data in detail since this will not show the true assemblage of saproxylic beetles in this area. Instead, we will only discuss generally about habitat types and beetles associated with wood. A more reliable information would have been gained if the sampling was carried out in an appropriate period of time. Further intensive studies are needed in order to get reliable data on the diversity and composition of these beetles.

Ants use dead wood particularly as their nest, not for food. Only *Pheidole* sp. was seen making nests in the fallen log. They are not directly involved in decomposing dead wood like termites. Other ants were collected foraging on the deadwood. They might be attracted to the broken deadwood to scavenge for other invertebrates found living in the log. Termites, on the other hand, are wood decomposers. In this study, only one species of termites was recorded, *Havilanditermes atripennis*. Termites play the most important role in the process of decomposition of dead wood in Imbak Canyon. One of the characteristics of the Oriental biogeographical region is that soil-feeding termites are less prominent compared to wood-feeding species (Eggleton, 2000 in Homathevi et al., 2002).

Saproxylic beetles' association with wood can be categorized into several types of habitats as follows; (1) on bark and wood surface, (2) under bark (subcortical habitats) and (3) inside wood (wood borers) (Lawrence & Britton, 1994). In this study, the highest individual numbers were Passalidae, with 2 morphospecies, altogether 24 imagos, 8 larvae and 2 pupae. For Staphylinidae, there were 2 morphospecies collected where morphospecies 1 had 4 imagos while morphospecies 2 had only 1 imago. Other specimens in this order were in larval form.

These are all common saproxylic beetles. Beetles play a major role in decomposing dead wood since most of forest dwelling beetles use dead wood as habitat for their larvae. Passalidae and Cerambycidae larvae are xylophages which feed on wood. Staphylinidae, Carabidae (both imagos and larvae for both families) and Elateridae (larva only) are predators and they live in dead wood for predation and tend to concentrate in logs with high number of other beetle larvae.

Pill millipedes occur in moist environment, such as under logs and in leaf litter. Little is known concerning the feeding habits of these millipedes, but they most likely feed on rotting plant matters. As their name suggests, pill millipede are capable of rolling into a tight ball, presumably as a defensive mechanism (Harvey & Yen, 1989). Larvae of Diptera and Lepidoptera were also found in the log.

Diptera larvae could be fungus feeders while Lepidoptera larvae could be wood borers.

Conclusion

Deadwood play a vital role in conserving biological diversity because many organisms depend on it for their survival, or require at least one part of their life cycle on deadwood. Without deadwood, food chain of many species will be interrupted. Deadwood are also important in the nutrient cycling process through the decomposition of deadwood by microorganisms and also saproxylic arthropods. We can conclude that a fallen log can be an important microhabitat of various species of saproxylic arthropods.

Acknowledgements

We would like to thank Yayasan Sabah for organizing a scientific expedition in Batu Timbang, Imbak Canyon, especially to Dr. Yap Sau Wai, Dr. Hamzah Tangki and Dr. Jadda Gilong Kanju. We would like also to thank to the rangers from Yayasan Sabah for their assistance during this expedition.

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Journal of Tropical Biology and Conservation 16: 107–117, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Macrofungi of Imbak Canyon – Batu Timbang Area, Sabah

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Abstract
Macrofungi survey was carried out from 21st to 26th of August, 2017 during the Imbak Canyon Conservation Area (ICCA) Scientific Expedition at Batu Timbang Area, Imbak Canyon, Sabah. The purpose of the study was to survey the diversity of mushroom forming-fungi or macrofungi because such study and information is poorly documented and limited in Malaysia. In this scientific expedition, we obtained a total of 106 species from 13 different families within Basidiomycota and Ascomycota. The most dominant family found was Polyporaceae with 25 species were collected, 23% of the total samples collected. We identified four different species of edible mushrooms and two deadly poisonous mushrooms. About 47% unidentified group will be subjected for DNA analysis. Apart from that, we characterized some of the polypore till genus or species level. The most interesting species from this study area were *Earliella scabrosa* and *Panus similis* that were recorded to have a medicinal properties. A glowing mushroom, *Mycena illuminans* is a new record for the Imbak Canyon region. Diversity of the ectomycorrhizal mushrooms which are specific to dipterocarp trees in Batu Timbang should be explored. Endemic and IUCN red listed species like *Buglossoporus* sp. found in the study area should be preserved for DNA. Future studies are needed in order to conserve the hidden knowledge of undescribed groups of mushroom from this region.

Keywords: macrofungi diversity, gill mushrooms, polypores, conservation

Received 26 July 2019
Reviewed 10 September 2019
Accepted 08 October 2019
Published 15 October 2019

Introduction

Mushroom-forming fungi or macrofungi are a group of mushrooms that are visible to our naked eye. They play an important role in our ecosystem as part of the terrestrial ecosystems, forming a large share of their species diversity, and are key players in ecosystem processes (Chang & Miles, 2004; Senn-Irlet et al., 2007). They are good decayers, pathogens, parasites, and mutualistic symbionts of both plants and animals. The main macrofungi includes some of the ascomycetes and basidiomycetes with large, easily observed spore-bearing structures. This includes mushrooms, bracket fungi (polypores), puffballs, truffles, cup fungi, resupinate or corticioids and etc. Global distribution of macrofungi have been compiled and it is estimated that tropical Asia contains about 400 described species (Mueller et al., 2007). The number seems to be very low for a high biodiversity hotspot area such as Malaysia. This is because the study of macrofungi and their species diversity in this region, especially in Borneo, is still lacking (Chang & Lee, 2011; Hyde, 2003). Thus, the main objective of this study was to survey macrofungi (different forms) distributed within the Batu Timbang area of Imbak Canyon. In this scientific expedition, we compiled data from different group of mushrooms based on their nutritional modes and segregated them according to their closest families, genus and species level. All of the collections that were made from this expedition were described and identified in order to produce a checklist for the Batu Timbang Area. This study will serve as baseline information for future researchers who are interested to study the specific genus or species. In addition, research findings or information from this study especially endemic mushroom species known to this area, will be incorporated to enhance this area as a Class I Forest Reserve.

Methods and materials

Study Area

Imbak Canyon, approximately 30,000 hectares in size and encompassing two ridge top Virgin Jungle Reserves is probably Sabah’s last frontier of pristine tropical rainforest and is located between longitude 117°2'0"E and latitude 5°5'30"N. It was gazetted as a Class I (Protection) Forest Reserve by the Sabah State Government in 2009. Unlike Danum Valley and Maliau Basin Conservation areas, Imbak Canyon Conservation Area ICCA is located near 48 villages in the district of Tongod, with a combined population of 30,000. ICCA is reachable by road from Kota Kinabalu to Tongod on a journey of approximately 10 hours, and a four-wheel drive is essential as the latter part of the journey is on logging roads. The scientific expedition was conducted from the 16th to 26th of August, 2017 and was divided into two sessions, and we joined the second session that was conducted from 21st to 26th of August, 2017. The Imbak Canyon Conservation Area (ICCA) Scientific Expedition was organized by Yayasan Sabah in collaboration with Sabah Forestry Department and sponsored by PETRONAS. The expedition was participated by numerous agencies and academic institutions such as Universiti Malaysia Sabah (UMS), Sabah Parks, Sabah Museum, Universiti Kebangsaan Malaysia (UKM), Universiti Malaya (UM), Universiti Sains Malaysia (USM), Sabah Foundation and Ryukyus University, Japan. ICCA is located almost right in the heart of Sabah, just north of the famous Maliau Basin with unique heterogenous biodiversity in terms of the geological and geomorphic characteristic and studded with many stunning waterfalls.

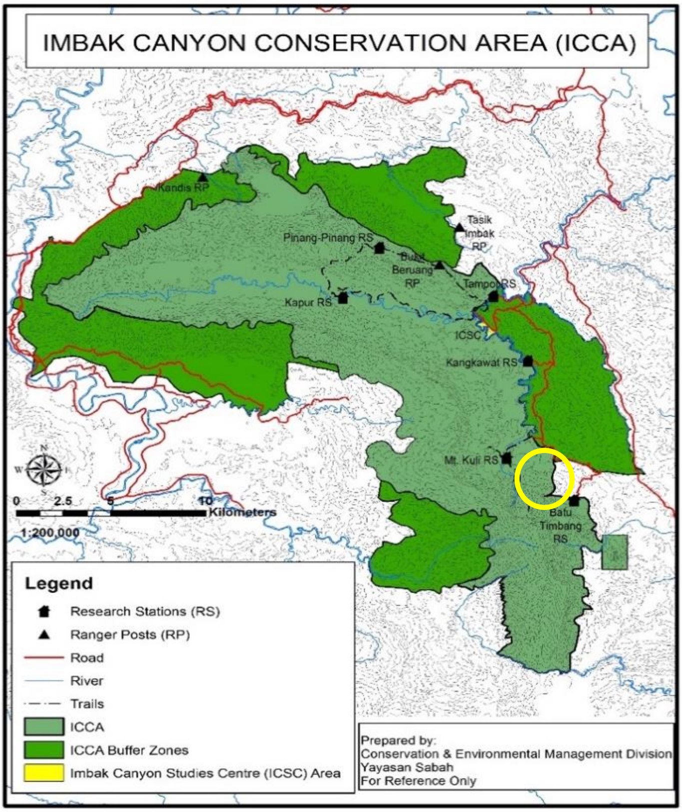

Figure 1. Sampling location of Batu Timbang Scientific Expedition Site

Sample Collections
Fresh macrofungi samples were collected based on their fruiting body occurrence on the substrates, mostly on fallen rotting branches, twigs, and dead trees. During the time of this trip, it was the rainy season. Photographs were taken for each specimen, including the top, side, and bottom view of the fungi. The specimens were then cut or carefully dug out using a knife or a trowel to any avoid damages. All specimens were properly tagged and wrapped appropriately in a paper bag to avoid drying. The habitat, substrate and their fresh morphological characteristics of the fungi were recorded. For each habitat, Global Positioning System (GPS) were recorded. Photographs were taken with DSLR Nikon D3200.

Macrofungi Identification
Spore prints were prepared from fresh samples and chemical reaction tests were done using potassium hydroxide (KOH). A small piece of the mushrooms tissues were kept in KOH for further study. The identification based on the morphology and some microscopic characteristic of the macrofungi was accomplished with the aid of current keys, descriptions and references (Evans & Kibby, 2004; Pegler, 1994; Zainuddin et al., 2010). When specimens could not be matched to known species descriptions, they were assigned to a genus and given a species number, for example, *Hyphodontia* sp. 1. The taxonomic status and description of these species will be examined and identified later using more advance method like molecular identification for further use. The specimens were brought back and dried in an incubator or oven at 45°C for 24-48 hours. The dried specimens were deposited in the BORNEENSIS Herbarium (BORH) at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah for further study.

Results and Discussion

In this study, we recorded macrofungi taxa that belongs to two major division of the fungi kingdom namely (i) Basidiomycota and (ii) Ascomycota. Most of the collections that were collected from Batu Timbang, were Basidiomycetes and one species of *Cookeina* (Ascomycota, Sarcoscyphaceae). The total families and genus of macrofungi are presented in Table 1. The total number of 56 species of macrofungi found during the survey on Batu Timbang, Imbak Canyon were from 13 families within Basidiomycotina (Figure 2). The highest distribution of macrofungi for Batu Timbang were Polyporaceae (23%), followed by Fomitopsidae (5%), Ganodermataceae (5%) and Marasmiaceae (5%).

About 47% of the collected fungi were unidentified as they lacked morphological references and need further identification using molecular analysis which is costly and time consuming.
In this survey, we discuss some of the interesting collections from Batu Timbang.

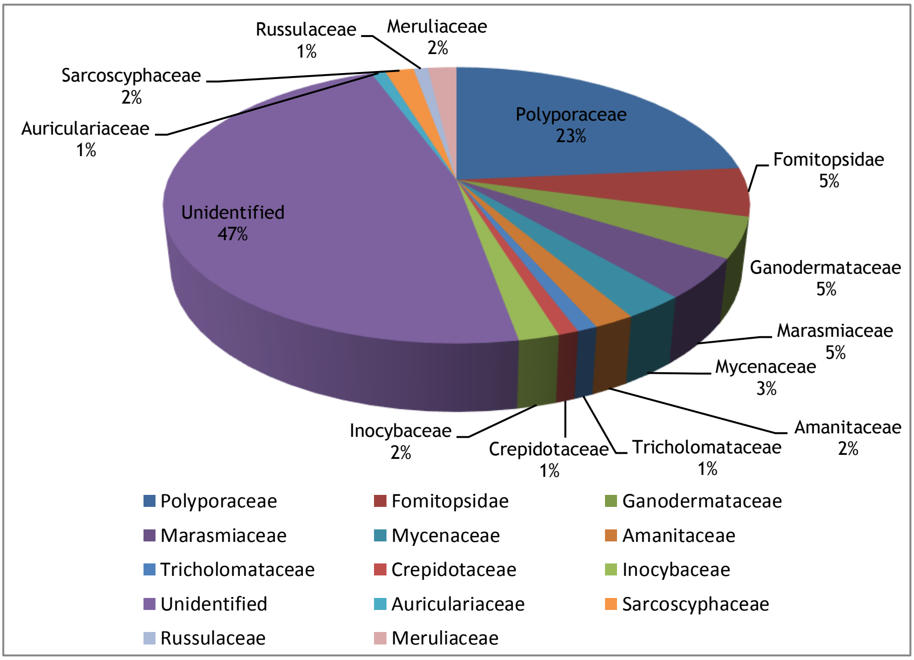

Figure 2. Percentage of macrofungi occurrence by families at Batu Timbang, Imbak Canyon

Polyporaceae, the wood decay fungi, was the dominant group around this area (Figure 3). We recorded 25 species within Polyporaceae family. Batu Timbang is an old growth forest area covered with dead logs and dead woods. This area may be accompanied with humidity and the high moisture content for the abundant polypore's to grow on the cellulose-rich substrates (Svrcek, 1997). Most of the polypores are white rot and favour dead wood and dead trees as their main substrate. We recorded two edible polypores, *Polyporus badius* and *Hexagonia tenuiculus*. Apart from that, an interesting finding of *Buglossoporus sp*. from Batu Timbang is considered as a new record for this area since this species only occurs at higher elevations of more than 2500m in montane regions like Mount Tambuyukon, Crocker Range and Sayap National Park, Kundasang. (Figure 5).

Figure 3. List of some interesting polypore mushrooms. A. *Polyporus badius*; B. *Amouroderma rugosum*; C. *Microporus xanthopus*; D. *Microporellus sp*.; E. *Favolus sp*.; F. *Fomitopsis dochmia*; G. *Microporus affinis*; H. *Piptoporus sp*.; I. *Favolus emericii*; J. *Ganoderma australae*; K. *Rhodofomitopsis feei*; L. *Tyroformes sp*.; M. *Coriolopsis sp*.; N. *Microporus vernicipes*; O. *Earliella scabrosa*; P. *Microporus sp*.

Agaricales are fleshy gill mushrooms. In this study, we were able to record at least 14 species from different families such as Mycenaceae, Russulaceae, Marasmiaceae, Amanitaceae, Thelophoraceae Meruliceae and Crepidotaceae. Among the Agaricales, Amanitaceae, Russulaceae, Thelophoraceae were ectomycorrhizal species which were associated with tree species like Dipterocarpaceae, Fagaceae, *Castanotiopsis* and etc. Ectomycorrhizal species are usually dependent on their host plants and in this study, we did not record any of the symbiotic relationship information due to time constraints. In addition, we recorded two deadly poisonous mushrooms, *Amanita similis* and *Trogia venenata* (Figure 4 - J & M). *A. similis* is a common tropical species that is widely distributed along the Javan and Bornean islands. Morphologically, the cap of *Amanita similis* ranges between 80 - 90 mm wide, convex then expanded and somewhat umbonate, viscid at first, innately streaked, bronze to honey yellow, darkest in the centre, with a sulcate-striate margin (30 - 50% of the radius). The flesh is pale yellow, bright yellow underneath the cap skin especially under the umbo. The Javan and the Bornean species are almost the same in terms of their spore structures. The spores size is 7.5 - 10.5 × 5.5 - 7.5

µm with broadly ellipsoid and inamyloid shape. On the other hand *Trogia venenata* is one of thetropical species that was recorded poisonous and this species is lethal to human due to their toxic amino acids.

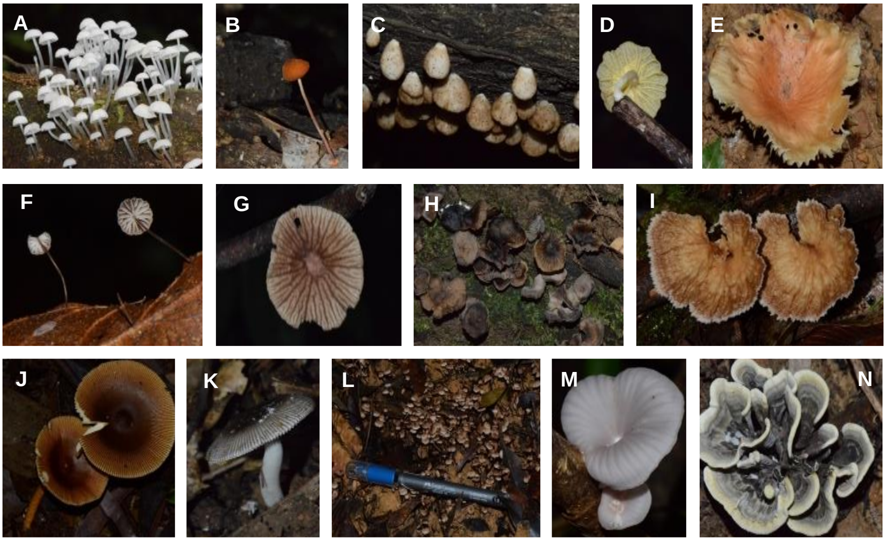

Figure 4. List of some interesting Agaricales mushrooms. A. *Mycena illuminans*; B. *Mycena sp.*; C. *Crepidotus sp.*; D. *Gerronema sp.*; E. *Russula sp.*; F. *Marasmius rotalis*; G. *Marasmius* sp.; H. *Inocybe sp.*; I. *Cymatoderma elegans*; J. *Amanita similis*; K. *Russula nigricans*; L. *Inocybe* sp. *(pink)*; M. *Trogia vennenata.*; N. *Thelephora sp.*

The distribution of Polypores were more abundant in this area than the Agaricales (Table 1). This might be due to the climate and seasonal factors that needs to be observed throughout the year (Svrcek, 1997). The fruiting of mushrooms sometimes needs to be monitored yearly. During the expedition, it was rainy and most of the gill mushrooms or fleshy mushrooms were damaged because of the wet environment and could not be dried well for storing. Spore prints for some of the fleshy mushrooms were restricted because some of the fruiting bodies were too moistured and damaged. However, we were able to identify the edible ones based on the records from previous studies done by Seelan et al. (2015).

Table 1. List of Macrofungi collections by family at Batu Timbang, Imbak Canyon (Sabah).

Family / Species	Number of samples	Habitat/Substrate	Remarks
Order: Polyporales			
Family: Polyporaceae			
Polyporus badius	1	Dead log/Saprophytic	Edible
Trametes versicolor	1	Dead wood/Saprophytic	Medicinal
Microporus xanthopus	6	Dead wood/Saprophytic	Medicinal

Microporus vernicepes	1	Dead wood/Saprophytic	
Microporus affinis	4	Dead log/Saprophytic	
***Microporus* sp.**	2	Dead log/Saprophytic	
***Trametes* spp.**	4	Dead wood/Saprophytic	
Hexagonia tenuiculus	1	Dead log/Saprophytic	Edible
***Skeletocutis* sp.**	1	Dead log/Saprophytic	
Panus similis	1	Dead wood/Saprophytic	Edible
Earliella scabrosa	1	Dead tree	
Favolus emericii	1	Dead log/Saprophytic	
***Favolus* sp.**	1	Dead log/Saprophytic	
Family: Fomitopsidae			
***Fomitopsis* spp.**	3	Dead log/Saprophytic	
***Piptoporus* sp.**	1	Dead log/Saprophytic	
***Coriolopsis* sp.**	1	Dead log/Saprophytic	
***Buglossoporus* sp.**	1	Dead log/Saprophytic	
Family: Ganodermataceae			
Ganoderma australae	2	Dead log/Parasitic	Pathogenic
Ganoderma lucidum	1	Dead log/Saprophytic	Medicinal
Amouroderma rugosum	1	Soil/Saprophytic	Medicinal
***Ganoderma* sp.**	1	Dead log/Saprophytic	
Family: Meruliaceae			
***Podoscypha* sp.**	1	Dead wood/Saprophytic	
Cymatoderma elegans	1	Dead wood/Saprophytic	
Order: Agaricales			
Family: Mycenaceae			
***Mycena* spp.**	3	Dead wood/Saprophytic	Glowing
Family: Marasmiaceae			
***Marasmius* spp.**	4	Dead wood/Saprophytic	
***Gerronema* sp.**	1	Dead branch	
Family: Tricholomataceae			
Trogia venenata	1	Dead wood/Saprophytic	Toxic
Family: Amanitaceae			
Amanita similis	2	Soil/Ectomycorrhizal	
Family Crepidotaceae			
***Crepidotus* sp.**	1	Dead wood/Saprophytic	
Family Inocybaceae			
Inocybe sp.	2	Soil/Ectomycorrhizal	
Order: Auriculariales			Toxic
Family Auriculariaceae			
***Auricularia* sp.**	1	Dead wood/Saprophytic	
			Edible
Order: Pezizales			
Family Sarcoscyphaceae			
Cookeina sulcipes	1	Dead log/Saprophytic	
Cookeina tricholoma	1	Dead log/Saprophytic	

Order: Russulales		
Family: Russulaceae		
***Russula* spp.**	1	Soil/Ectomycorrhizal
Unidentified	50	
TOTAL	106	

The ten days study in pristine forest of Imbak Canyon also recorded three interesting species of macrofungi which are the *Earliella scabrosa*, *Panus similis* and *Buglossoporus* sp. (Figure 5). *Earliella scabrosa* is a monotypic genus which comprises a single species and was described by Murril (1905) from Cuba. This species provides a conducive habitat for many beetle groups. Their fleshy hymenophores provide food sources and habitat for many arthropods especially beetles and are also recorded pathogenic to humans (Hong et al., 2018). The others species is *Panus similis*, which is the lentinoid fungi and is a saprophytic fungi. It was distributed only in Southeast Asia, Latin Americas and Australasia (Gunasekaran, 2015). The species was described by Corner in 1964 and rediscovered in Batu Timbang, Imbak Canyon in 2017. *Buglossoporus sp.* is recorded as one of the vulnerable species by the IUCN Red List and recorded as very rare distribution as it is only found in Tambuyukon so far. The species is annually grown, usually on lowland and montane forest distribution (Hattori, 2017).

Conclusion

The preliminary work will undoubtedly be valuable to those interested in studying fungi in the lowland and upland mixed dipterocarp forests of Imbak Canyon – Batu Timbang Area, Sabah. It is hoped that this work will act as a catalyst to increase and promote more studies on fungi, especially in Sabah.

Figure 5. List of some interesting mushroom from Batu Timbang. A. *Earliella scabrosa;* B. *Panus similis;* C. *Buglossoporus* sp.

Acknowledgements

We are grateful to Yayasan Sabah under the coordination of Dr Yap Sau Wai, who heads the Conservation and Environmental Management Division, Rita S. Galid, Dr Hamzah Tangki, Jadda Suhaimi and their team from Yayasan Sabah for their support throughout the expedition. We also wish to thank the then Chief Conservator of Forests, Datuk Sam Mannan, who is also the Chairman of Imbak Canyon Management Committee, Deputy Chief Conservators, Frederick Kugan and Dr Lee Ying Fah, for their support in this expedition. Sincere gratitude also goes to the Dipterocarp's team from Sabah Forestry Department headed by Mr. Richard Majapun and his team Martin Tuyok, Sandy Tsen and Lee Yew Leung for their invaluable help during the field work. We also wish to thank Dr Arthur Chung Yaw Chyang for his comments and suggestion on the manuscript.

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Journal of Tropical Biology and Conservation 16: 119–123, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

A preliminary survey of Araceae of Batu Timbang, Imbak Canyon Conservation Area (ICCA), Sabah, Malaysia Borneo.

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Abstract

During a scientific expedition to Batu Timbang, Imbak Canyon Conservation Area (ICCA), Sandakan, Sabah between 17 and 20 August 2017, 14 species in seven genera of Araceae were collected. The genera are *Pothos* (*Pothos* [Allopothos] sp.); *Rhaphidophora* (*R. korthalsii*, *R. sylvestris*, *R. latevaginata*); *Scindapusus* (*S. pictus*, *S. longistipitatus*, *S. kinabaluensis*, and *Scindapsus* sp. nov.); *Schismatoglottis* (*S. wongii*); *Aglaonema* sp.; *Ooia* sp. and *Alocasia* (*A. robusta*, *A. sarawakensis,* and *A. wongii*).

Keywords: Aroids, Imbak Canyon Conservation Area ICCA, Malaysian Borneo.

Introduction

The Araceae, comprising seven subfamilies, 144 genera and about 4,000 described species, is a subcosmopolitan family in distribution but most abundant and diverse in the ever wet or humid tropics (Boyce & Croat, 2011; Cusimano et al., 2011). The family is defined by having minute sessile flowers on spadix and covered by a spathe. The spadix may bear either unisexual or bisexual flowers. Most of the climbers have bisexual type flowers while others have unisexual flowers. Ecologically, aroids can be found in streams, ponds and canals, terrestrial habitats, tidal mud, swamps and wasteland, forest floor, climbers, epiphytes and rheophytes (Mashhor et al., 2012).

The aroids of Borneo currently stand at 575 species, of which 433 are formally described (Wong, 2016). Most Bornean terrestrial aroids show marked local endemism, often to a very high degree, and arefrequently associated with geological obligation. Fieldtrips in Sabah have resulted in numerous undeterminable species of aroids which on subsequent flowering in cultivation have proved to be taxonomic novelties (Kartini et al., 2017).

Received 06 May 2019
Reviewed 21 June 2019
Accepted 02 October 2019
Published 15 October 2019

Method

A preliminary survey of the aroids of Batu Timbang, ICCA was conducted between 17 to 20 August 2017. Several habitats including forested areas and riverside ecosystems were visited, specifically the Rafflesia Trail, Lanap Trail and Lanap riverbank adjacent to the base camp.

All aroids found were recorded. As so often is the case, many were encountered only as sterile plants. Although with experience assignment of sterile plants to genus is not overly difficult, identification to species, especially in an area never before studied, is often impossible since in many instances the species encountered will be undescribed taxonomic novelties. On such occasions, living specimens are collected and maintained at the Nursery, Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan campus awaiting flowering for further identification. Identifications are based on Boyce (2004), Boyce et al. (2001), Hay (1998), Wong (2016), and Wong & Boyce (2016).

Results

Three subfamilies and seven genera comprising 14 species were found in the sites surveyed: Rafflesia Trail, Lanap Train and Lanap riverbank (Table 1).

Discussion

The initial survey of Batu Timbang revealed a distinctly meager aroid flora comprised largely of rather widespread species associated with disturbed habitats. Of the three *Alocasia* encountered, *Alocasia robusta* is a fast-growing

Table 1. Aroids species in Batu Timbang, ICCA

Subfamily	Genus and Species	Rafflesia Trail	Lanap Trail	Lanap riverbank
Pothoideae	*Pothos* sp. [Allopothos]	+	+	-
Monsteroideae	*Rhaphidophora foraminifera* (Engl.)	-	-	+
	Engl	+	+	-
	Rhaphidophora korthalsii Schott	+	+	-
	Rhaphidophora latevaginata M. Hotta	+	+	-
	Scindapsus pictus Hassk.	+	+	-
	Scindapsus longistipitatus Merr.	+	+	-
	Scindapsus sp. 1	+	+	-
	Scindapsus sp. 2			
Aroideae	*Aglaonema* sp.	+	+	-
	Alocasia sarawakensis M. Hotta	+	-	+
	Alocasia robusta M. Hotta	-	-	+
	Alocasia wongii A. Hay	+	+	+
	Ooia sp.	-	-	+
	Schismatoglottis wongii A. Hay	-	-	+

but short-lived species frequenting forest gap-phases while *Alocasia sarawakensis* (Plate 1) is a semi-colonial (via rhizome bulbils) species adapted to open swampy sites where it often becomes almost weedy, for example at Danum Valley. Among the climbers, *Rhaphidophora korthalsii* is one of the most widespread species of the genus, occurring from the Andaman Islands to the tropical islands of the western Pacific. *Rhaphidophora foraminifera* is a large plant and a *Rhaphidophora* in the Hongkongensis clade. *Rhaphidophora latevaginata* (Plate 2) is locally common for North East Peninsular Malaysia (Sofiman & Boyce, 2010) and throughout northern and central Borneo. *Scindapsus,* was represented by four species, of which three are widespread and often associated with disturbed forest: *S. pictus* (Plate 3) occurs throughout the Sunda and the Philippines and into Sulawesi; *S. longistipitatus* is widespread in Sabah and East Kalimantan and extends out to the Philippines. The third of the widespread species (*Scindapsus* sp. nov. 1) (Plate 4) occurs throughout northern Borneo and as far South as the Kapuaus valley in the North West and Tarakan in the North East of Kalimantan, but has yet to be formally described.

Of slightly more interest was the central and eastern Sabah endemics *Alocasia wongii* and *Schismatoglottis wongii* (Plate 5), and an *Aglaonema* which did not appear to concur well with the three species of the genus currently recorded for Borneo (*A. nebulosum* N.E.Br., a species restricted to kerangas, *A. nitidum* (Jack) Kunth, and *A. simplex* (Blume) Blume. Unfortunately, owing to an oversight no images exist of this plant.

Three aroids encountered were deemed of special interest. A *Pothos* (Plate 6) in the Allopothos clade, a *Scindapsus (Scindapsus* sp. nov. 2 in Table 1) (Plate 7) and an *Ooia* (Plate 8). Unfortunately, all except the *Scindapsus* were encountered sterile. The *Ooia* is unquestionably undescribed (Wong & Boyce 2016), and furthermore the second species for Sabah – the other being *O. kinabaluensis* (Bogner) S. Y. Wong & P. C. Boyce.

The *Pothos* is very likely *P. miarabilis* Merr., and would represent the second ever collection for the species.

Scindapsus sp. 2 is a member of the taxonomically difficult Coriaceus complex and closely similar to the montane-kerangas *Scindapsus kinabaluensis* (Furtado) Kartini & P. C. (Kartini et. al., 2015), but differs in the spathe shape.

Conclusions

Batu Timbang proved to be disappointing as a site for Araceae, but given the limited duration of the visit it is highly probable that more awaits discovery. In particular, a concerted effort should be made to find the *Pothos* in flower, likewise the *Ooia*, and a search made for adult plants of the *Aglaonema*.

Acknowledgements

We are grateful to Universiti Malaysia Sabah (UMS) for providing grant No. GKP00010-ST-2016. We also thank Peter Boyce (LMU) and Wong Sin Yeng (UNIMAS) for species confirmation and the organisers of Batu Timbang, ICCA scientific expedition 2017 on their hard work in making the expedition a success.

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List of Plates


Plate 1-*Alocasia sarawakensis* M.


Plate 2-*Rhaphidophora latevaginata* M. Hotta


Plate 3-*Scindapsus pictus* Hassk.


Plate 4-*Scindapsus* sp. 1


Plate 5-*Schismatoglottis wongii* A. Hay


Plate 6-*Pothos* sp.


Plate 7-*Scindapsus* sp. 2


Plate 8. *Ooia* sp.

Journal of Tropical Biology and Conservation 16: 125–140, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Species Composition and Distribution of Zingiberaceae in Mt. Hamiguitan Expansion Site, Davao Oriental, Philippines

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Abstract

This study was conducted to assess the composition and distribution of gingers (Zingiberaceae) in Mt. Hamiguitan expansion site, Tumalite, San Isidro, Davao Oriental, Philippines. Transect walk and opportunistic sampling were carried out along established forest trails, rivers, creeks and streams. Fourteen (14) ginger species were found, of which 10 species are endemic to the Philippines, two species are introduced, and two species are unidentified to the species level. The species belong to two subfamilies (Alpinioideae and Zingiberoideae) and three tribes (Alpinieae, Globbeae, and Zingibereae). The species recorded include *Alpinia haenkei* C.Presl, *Alpinia* cf. *vulcanica* Elmer, *Alpinia rufa* C.Presl, *Alpinia* sp., *Curcuma zedoaria* (Christm.) Rosc., *Curcuma longa* L., *Etlingera dalican* (Elmer) A.D.Poulsen, *Etlingera hamiguitanensis* Naive, *Etlingera* sp., *Geocharis fusiformis* (Ridl.) R.M.Sm., *Globba campsophylla* K.Schum., *Hornstedtia conoidea* Ridl., *Hornstedtia microcheila* Ridl., and *Meistera muricarpa* (Elmer) Škorničk. & M.F.Newman. These species represent 47% of the total genera and 14% of the total species of Zingiberaceae in the Philippines. Cluster analysis (numerical analysis) using morphological descriptions supported present taxonomic placements of the species. The data indicated that *G. fusiformis* is the most abundant ginger in the area.

Keywords: *Etlingera hamiguitanensis*, *Geocharis fusiformis*, Gingers, *Meistera muricarpa*, Philippine endemic

Received 31 October 2018
Reviewed 19 March 2019
Accepted 10 April 2019
Published 15 October 2019

Introduction

Zingiberaceae or the ginger family has at least 1,500 species distributed in 53 genera (Kress et al., 2002; Lamb et al., 2013). Many of the species are commercially cultivated in several regions of the world, such as in Africa, India, China, Japan, Indonesia, Australia, Nigeria and the West Indies. In the Philippines, this family has 15 genera and more than 100 species (Pelser et al., 2011 onwards). The species are mostly characterized as small to large perennial plants with creeping horizontal or tuberous rhizomes and are usually aromatic in all plant parts. The inflorescence can be branched or lax in some species, with reduced pedicels, or even forming a spike, or with congested rachis (Leong-Škorničkova & Newman, 2015).

Knowledge about Zingiberaceae in many parts of the world is still insufficient (Larsen & Larsen, 2006). This could be due to inadequate collection of herbarium specimens from the respective areas (Larsen, 1980), which makes the identification of gingers in those areas inaccurate and hence insufficiently known in terms of taxonomy (Larsen & Larsen, 2006). Although the works of Merril (1924) and Steiner (1959) on gingers are accessible, the information reported by them is inadequate and needs updating. Madulid (1996) stated that a revision of the family is needed to understand its taxonomy, biology and ecology.

Mt. Hamiguitan Range Wildlife Sanctuary (MHRWS) is one of the wildlife sanctuaries in the Philippines that has unique biodiversity resources (Amoroso & Aspiras, 2010). It was designated as a World Heritage Site in June 2014 and an ASEAN Heritage Park in 2016. It is also a Mindanao Long Term Ecological Research (LTER) Site. Mt. Hamiguitan is a home to 152 species of plants belonging to 27 families and 72 genera (Amoroso et al., 2016), and hosts quite a number of endemic and endangered flora species in the Philippines especially the *Nepenthes* (Gronemeyer et al. 2016). Recently, Amoroso et al. (2019) recorded 228 taxa of plants in Mt. Hamiguitan, which consist of 74 species of ferns and lycophytes, six species of gymnosperms, 30 species of herbs and vines, and 118 species of trees and shrubs. Different plant species which have been particularly studied in MHRWS were pteridophytes, *Nepenthes*, and pandans. However, the wild gingers of Mt. Hamiguitan are still little understood, and so far only studies by Acma (2010) and Acma & Mendez (2018) are available literature on Zingiberaceae of this mountain. Thus, this study was carried out to determine the species composition and distribution of Zingiberaceae in Mt. Hamiguitan expansion site. This study covered only the expansion site of the mountain which is found at the periphery of the protected zone of MHRWS. It is predominantly a dipterocarp forest in which trees grow very tall and large, and form a large proportion of the forest canopy.

Materials and Methods

Entry Protocol

Permission to carry out the study was obtained from the respective government authorities and units (e.g., the Barangay Council, Municipal Mayor), Protected Area Superintendent (PASu), and Department of Environment and Natural Resources (DENR) of MHRWS. The field sampling was conducted in March 2017.

Sampling, Collection and Preservation of Specimens

Transect walk was carried out along established forest trails, covering the selected study area including rivers, creeks and streams (Fig. 1). Likewise, opportunistic sampling was done whenever there was the presence of ginger species and an opportunity to collect the said species at the area. One to three live specimens were collected and placed inside labeled plastic bags. The specimens were brought to the research cabin for measurement of parts, photo documentation, and preparation of voucher specimens. Global Positioning System (GPS) was used to determine the exact elevation and coordinates of the collected specimens.

Representative vegetative plant parts from the terminal part, middle, and basal portions of the plant body were prepared. Collected specimens were numbered and documented as to the collector, date of collection, locality, common name, habitat, height and inflorescence. Specimens were then laid out between folded sheets of newspapers with leaves flattened for pressing. The plant materials were pressed using a wood presser, tightened by ropes and dried in a mechanical dryer. Those dried specimens were mounted in herbarium sheets measuring 11 ½ × 16 ½ in. Corresponding labels were affixed and specimens were deposited at the Central Mindanao University Herbarium (CMUH).

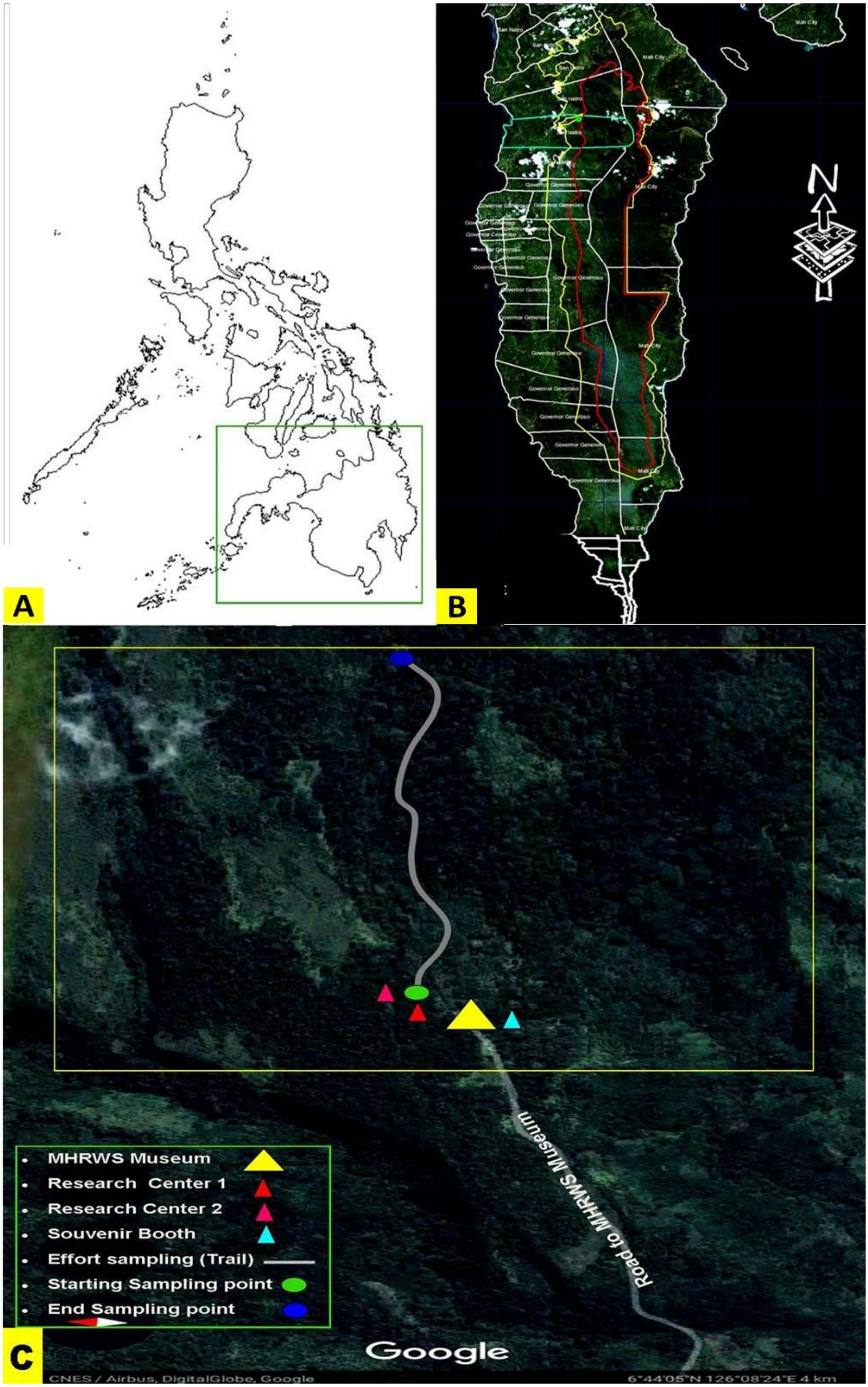

Figure 1. Study site. **A)** Map of the Philippines, **B)** Map of Davao Oriental province, **C)** Sampling trail in Mt. Hamiguitan expansion site.

Likewise, pickled collections of the floral parts were placed inside small plastic containers and preserved using 70% ethanol and pure glycerine which comprised about 10% of the total preservation mixture. The specimens were also deposited at the CMUH.

Species Identification, Distribution and Ecological Status Assessment
The morphological and taxonomic descriptions and numerical analyses were carried out at the College of Arts and Sciences of Central Mindanao University (CMU), Musuan, Bukidnon, Philippines. The specimens were first identified to genera and species by comparing with pre-identified specimens on gingers in the herbarium of CMU. Affinities of the species were determined through the Bray-Curtis cluster analysis. The elevations and coordinates for each species obtained using GPS were used in preparing the distribution map. A line graph indicating the species along with number of elevations in which those species occurred were utilized to determine whether they were abundant, rare or endemic to the area.

Results and Discussion

A. Species Identification and Composition of Zingiberaceae
The study revealed 14 species of Zingiberaceae in Mt. Hamiguitan expansion site, namely, *Alpinia haenkei* C. Presl, *Alpinia* cf. *vulcanica* Elmer, *Alpinia rufa* C.Presl, *Alpinia* sp., *Curcuma zedoaria* (Christm.) Rosc., *Curcuma longa* L., *Etlingera dalican* (Elmer) A.D.Poulsen, *Etlingera hamiguitanensis* Naive, *Etlingera* sp., *Geocharis fusiformis* (Ridl.) R.M.Sm., *Globba campsophylla* K.Schum., *Hornstedtia conoidea* Ridl., *Hornstedtia microcheila* Ridl., and *Meistera muricarpa* (Elmer) Škorničk. & M.F.Newman (Fig. 2).

The species mostly belong to the subfamily Alpinioideae and tribe Alpineae, except for the species of *G. campsophylla*, *C. zedoaria*, and *C. longa*, belonging to the subfamily Zingiberoideae under tribe Globbeae and Zingibereae, respectively. This study documented 47% of the total genera and 14% of the total species of Zingiberaceae in the Philippines.

Figure 2. Zingiberaceae species of Mt. Hamiguitan expansion site. **A)** *Alpinia haenkei*, **B)** *Alpinia* cf. *vulcanica*, **C)** *Alpinia rufa*, **D)** *Curcuma zedoaria*, **E)** *Curcuma longa*, **F)** *Etlingera dalican*, **G)** *Etlingera hamiguitanensis*, **H).** *Geocharis fusiformis*, **I)** *Globba campsophylla*, **J)** *Hornstedtia conoidea*, **K)** *Hornstedtia microcheila*, and **L)** *Meistera muricarpa*.

1. *Alpinia haenkei* C.Presl, Symb. 1 (1832) 66

Terrestrial herb, 1-2 m tall. Rhizome creeping, 8–10 mm in diameter, cream brown. Ligule bifid, 0.8–1 cm long and petiole 4–6 cm long. Leaf blade broad-lanceolate, margin entire with fine hairs, 33–45 x 9–10 cm. Leaf base oblique, apex broadly acuminate, leaf texture adaxially pubescent, glaucous-glossy abaxially. Inflorescence terminal, originates on the leafy shoots, raceme. Bracts, bracteoles, calyx, corolla white, labellum red inside at centre with striate marks and white in margin. Fruit globose, pubescent with fine hairs, red when mature. The specimen of this species was recorded at an elevation of 250 m a.s.l. at a shady area associated with grasses and trees (CMUH 00010981).

2. *Alpinia* cf. *vulcanica* Elmer, LPB 8 (1915) 2971
Terrestrial herb, 3–5 m tall. Rhizome and roots raised and exposed. Ligule notched with fine hairs, 1–1.5 mm in diameter. Petiole 4–5 cm long. Leaf blade broad, lanceolate, margin entire, base oblique, apex acuminate, glabrous abaxially, 45–65 x 10–12 cm. Inflorescence terminal, raceme. Bracts and bracteoles spatulate, white to pale green. Fruits globose, yellowish, glabrous to glaucous. Seeds black. The specimens of this species were collected near *Pandanus* sp. and recorded at elevations of 536, 548, 550 and 565 m a.s.l. (CMUH 00010982).

3. *Alpinia rufa* C.Presl, Rel. Haenk. 1 (1827) 114
Terrestrial herb, 1–2 m tall. Rhizome creeping, brown, 10–15 mm in diameter. Ligule entire, 0.8–1 cm long. Leaf blade lanceolate, sessile, margin entire to ciliate, base attenuate, apex acuminate, pubescent abaxially and adaxially, 18–27 x 6–7 cm. Inflorescence terminal in the leafy shoots, umbel. Fruit globose, pubescent, green when young, red when mature. Seeds numerous. Specimens of this species were recorded at an elevation of 436 m a.s.l. near forest trail. It has been reported to be associated with shrubs, vines and rattans (see Acma & Mendez, 2018).

4. *Alpinia* sp.
Terrestrial herb, 1–2 m tall. Rhizome creeping, fleshy to pinkish, 11–15 mm in diameter. Ligule notched, yellowish to maroon, 0.2–4 cm long. Leaves sessile, lanceolate, margin entire to sinuate, base oblique, apex acuminate to cuspidate, glabrous abaxially, 16–17 x 4–5 cm. Inflorescence terminal. The specimen of this species was recorded at an elevation of 604 m a.s.l. and associated with falcata trees, grasses and shrubs.

5. *Curcuma zedoaria* (Christm.) Rosc., Monandr. Pl. (1828) t. 109
Terrestrial herb, 1–1.5 m tall. Rhizome creeping, branched, fleshy, often with tuber-bearing roots, 10–15 mm in diameter. Petiole 10–16 cm long. Leaf blade lanceolate, base cuneate, apex acuminate, glabrous rarely narrowly linear, with purple band along the center, 20–36 x 5-8 cm. Inflorescence arising on a

separate side shoots in conic-spike cylindric form. Coma bracts pink to white. The specimens of this species were recorded at elevations of 124 and 463 m a.s.l. Introduced or cultivated mainly by local people and were associated with *Cocos nucifera* L. (coconut), trees and grasses (CMUH 00010988).

6. *Curcuma longa* L., Sp. Pl. (1753) 2
Terrestrial herb, 1 m tall. Rhizomes creeping, branched, fleshy roots bearing tubers, 9–13 mm in diameter. Petiole 9–14 cm long. Leaf blade lanceolate, margin entire, base cuneate, apex acuminate, glabrous, 12–23 x 7–10 cm. Inflorescence terminally arising on pseudostems in a conic-spike cylindric form. Coma bracts white and green. The specimen of this species was recorded at an elevation of 115 m a.s.l. Introduced or cultivated in the Philippines by local people (CMUH 00010992).

7. *Etlingera dalican* (Elmer) A.D.Poulsen, Blumea 48 (2003) 524
Terrestrial herb, 1–1.5 m tall. Rhizome creeping, fleshy pinkish to reddish, 5–7 mm in diameter. Ligule entire, 0.8–1 cm long. Leaf blade sessile, margin entire to sinuate, base cuneate, apex acuminate, glabrous on surface, broadly lanceolate, 16–35 x 6–10 cm. Inflorescence arises from the rhizome, obconic/truncate at the top. Bracts yellowish towards the top, pale green towards the base, oblong. Bracteoles tubular, cream brown, sepals 3-tipped, pinkish to whitish, yellow tip and whitish base, oblanceolate. Labellum faint yellow, lip and filament fused into a tube. Stigma bright red. The specimen of this species was recorded at an elevation of 364 m a.s.l. It has been reported to be associated with vines and grasses (see Acma & Mendez, 2018) (CMUH 00010991).

8. *Etlingera hamiguitanensis* Naive, Taiwania 62 (2017) 341
Terrestrial herb, 2 m tall. Rhizome creeping covered with fleshy purple or red bracts, 17–20 mm in diameter. Ligule entire, apex dark purple, 0.8–1 cm long. Petiole 1–2 cm long. Leaf blade broad lanceolate, margin entire to sinuate, base round, apex acuminate, glabrous abaxially and adaxially, 36–38 x 7–10 cm. Inflorescence emerges on rhizome, surrounded by several sterile coloured bracts appearing at soil level. Bracts and bracteoles red towards the top, white towards the base. Calyx pinkish, light red corolla. Labellum bilobed, curved which made it different from *E. philippinensis* having a distal part of the lip in curved. Specimens of this species were recorded at elevations of 486, 489 and 491 m a.s.l. and associated with *Bambusa* sp. and grasses (CMUH 00010987).

9. *Etlingera* sp.

Terrestrial herb, 1–2 m tall. Rhizome creeping, 12–15 mm in diameter, reddish to pinkish. Ligule entire, 0.5–0.8 cm long. Leaves sessile, broad lanceolate, margin entire to sinuate, base cuneate, apex acuminate, glabrous. 20–27 x 6–8 cm. Inflorescence arising on rhizomes, obconic. Bracts and bracteoles red towards the top, pinkish towards the base. Fruit globose, creamy pink. Specimens of this species were recorded at elevations of 386, 389 and 395 m a.s.l. in shady areas near creeks (CMUH 00010984).

10. *Geocharis fusiformis* (Ridl.) R.M.Sm., Notes Roy. Bot. Gard. Edinburgh 43 (1986) 458

Terrestrial herb, 2–3 m tall. Rhizome creeping, brownish, 18–25 mm in diameter. Ligule entire, 0 .7–1 cm. Petiole 1–2 cm long. Leaf blade broad to ovate lanceolate, margin entire, base rounded, apex acuminate to cuspidate, glabrous, upper surface green, whitish on lower, 29–43 x 5–10 cm. Whitish linear streak laterally present in the leaf sheath. Inflorescence arises on rhizomes in lax spike, prostate or erect on ground. Bracts and bracteoles light red, calyx light pink, corolla lobe long yellow with edges. Labellum bilobed, yellow. Stigma and anther bright red. Specimens of this species were recorded at elevations of 401–600 m a.s.l. in shady areas relatively open with few shrubs, falcata and mahogany trees (CMUH 00010983).

11. *Globba campsophylla* K.Schum. in Engl., Pflanzenreich 20 (1904) 145

Terrestrial herb, relatively short, 0.65–0.77 m tall. Rhizome creeping, short, white with fleshy roots, 1–1.5 mm in diameter. Ligule entire, 0.8–1cm long. Leaf blade sessile, lanceolate, base oblique, apex acuminate, glabrous, 33–36 x 5–7 cm. Inflorescence terminal, lax principally, white in colour. Long curved filament present and the reflexed, yellow streaked white labellum partially fused to floral tube. Lateral staminodes are attached below. Specimens of this species were recorded at an elevation of 263 m a.s.l. It has been reported in the shady dipterocarp forest associated with Araceae species and grasses (see Acma & Mendez, 2018) (CMUH 00010985).

12. *Hornstedtia conoidea* Ridl. in Elmer, LPB 2 (1909) 605; PJS 4 c (1909) Bot. 175

Terrestrial herb, 1–2 meters tall. Rhizome creeping, 8–12 mm in diameter, reddish. Ligule entire, 0.3–0.9 cm long with purple to black margin. Petiole 1–2 cm long. Leaf blade broadly lanceolate, margin entire to sinuate, base cuneate, apex acuminate to cuspidate, glabrous with reddish purple colour along its edge, 32–47 x 7–8 cm. Inflorescence arises on rhizome, spindle. Bracts red with white base, broadly ovate, sharp apex. Specimens of this species were recorded at elevations of 396 and 404 m a.s.l. in shady and stony substrate area (CMUH 00010990).

13. ***Hornstedtia microcheila*** Ridl., LPB 2 (1909) 606; PJS 4 c (1909) Bot. 176
Terrestrial herb, 2–3 m tall. Rhizome creeping, reddish to faint pink, 13–15 mm in diameter. Ligule entire, 0.8–1 cm long, blacken apex, light green base with fine hairs. Petiole 1–2 cm long. Leaf blade lanceolate, margin entire, base cuneate, apex acuminate to cuspidate, glabrous abaxially and adaxially, 29–36 x 6–7 cm. Inflorescence arises on rhizome. Bracts and bracteoles pinkish. Calyx and corolla transparent, white to yellowish. Labellum broad curved spatulate with straight marks at the centre in faint yellow. The specimen of this species was recorded near a creek area at an elevation of 511 m a.s.l. (CMUH 00010986).

14. ***Meistera muricarpa*** (Elmer) Škorničk. & M.F.Newman, Taxon 67 (2018) 26
Terrestrial herb, 1–2 m tall. Rhizome creeping, robust, 8–10 mm in diameter. Ligule entire, 0.4–0.8 cm long. Petiole 1–1.5 cm long. Leaf blade lanceolate, margin entire to sinuate, base cuneate, apex acuminate to cuspidate, glabrous abaxially and adaxially, 29–30 x 5–7 cm wide. Purplish lines along pseudostems in adjacent area. Inflorescence arises from rhizome, obconic. Bracts and bracteoles pinkish. Calyx light green to pinkish. Corolla yellowish towards base. Labellum brownish at centre with straight marks, yellow at sides. Infructescence have branched spines, rambutan-like. Capsule red, globbose or ellipsoid. The specimen of this species was recorded at an elevation of 371 m a.s.l. in sandy soil substrate (CMUH 00010989).

This study revealed that *C. zedoaria* and *C. longa* are the only introduced species and are cultivated by a specific community known as “Rizalian group” in the area, and they process these into a food drink. According to Smith et al. (1998), the classification of Zingiberaceae had been recognized widely based on morphological characters and molecular phylogeny. As for this study, morphological characters were used for plant identification. The members of Zingiberaceae have distichous phyllotaxy with simple leaves (Sirirugsa, 1999), and the attachments of the leaves vary from petiolate to sessile. *Alpinia rufa*, *Alpinia* sp., *E. dalican*, and *Etlingera* sp. are sessile while the rest are petiolate.

Leaf base varies from cuneate, oblique, attenuate and rounded. Most of the species exhibit oblique base, such as *G. campsophylla*, *A. haenkei*, *A.* cf. *vulcanica*, and *Alpinia* sp. *Alpinia rufa* possess attenuate base, while cuneate base in the case of *M. muricarpa*. However, *C. longa*, *C. zedoaria*, *E. hamiguitanensis*, *E. dalican*, *Etlingera* sp., *H. conoidea*, *H. microcheila*, and *G. fusiformis* have a rounded base.

Shapes of ligules among the species varied from entire, notched and bifid. *Alpinia haenkei* and *A. rufa* had bifid ligule with fine hairs, while *A.* cf. *vulcanica* and *Alpinia* sp. possess a notched ligule. *Alpinia* sp. had yellowish to maroon ligule colour, while *H. microcheila* had also ligule which are blacken at edge or tip and green towards the base having entire shape. The rest of the species, namely, *E. hamiguitanensis*, *E. dalican*, *Etlingera* sp., *G. campsophylla*, *G. fusiformis*, *M. muricarpa*, *H. conoidea*, and *H. microcheila* had entire ligule shape.

Variations on inflorescence and infructescence of the species were also observed. The inflorescence of each species is important since it is the main characteristic in distinguishing which genera they belong to. Based on their reproductive parts, inflorescence and infructescence emerge terminally on leafy shoots or on pseudostems or arise from the rhizome. Noteworthy in this paper was *G. fusiformis,* which is supposed to bear inflorescence arising from the rhizome; however, the inflorescence was also observed at the terminal of the leafy shoot. Thus, this study supported Larsen et al. (1999) which reported that terminal inflorescence on the leafy shoots of this species is rare. Labellum shapes of the Zingiberaceae species were also differentiated such as bilobe on *E. hamiguitanensis* and broad spatulate on *H. microcheila*. Other species exhibit a curved labellum with straight marks along the centre and colour varies from species. In addition, floral characters which comprise stamen, corolla, calyx, and bracts are also significant for their taxonomic delineation. However, using these characters as key in identifying the species level is quite tough since flowers are ephemeral, which means it would last for only a short period of time, blooming for a day, or even just for a few hours (Larsen et al., 1999).

B. Cluster Analysis

Bray-Curtis Cluster analysis revealed affinities of the species (Fig. 3). The different clusters generated revealed that the same genus belong to the same cluster which implies that the morphological descriptions support the present taxonomic placements of the species. The Bray-Curtis Cluster analysis revealed seven clusters, which are cluster I with *G. fusiformis*, cluster II with *H. conoidea* and *H. microcheila*, cluster III with *M. muricarpa*, cluster IV with *Etlingera* sp., *E. dalican* and *E. hamiguitanensis*, cluster V with *G. campsophylla*, cluster VI with *C. longa* and *C. zedoaria*, and cluster VII with *A. rufa*, *Alpinia* cf. *vulcanica*, *A. haenkei*, and *Alpinia* sp. The dendrogram generated from the cluster analysis implies that the identification based on the morphological descriptions is supported by numerical analysis, implying that species identification in this study is reliable.

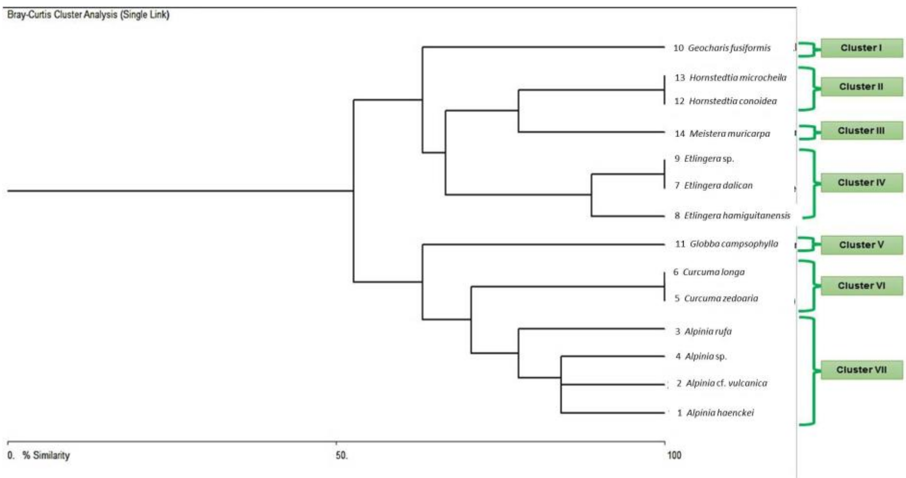

Figure 3. Dendrogram generated from Bray-Curtis Cluster Analysis.

C. Altitudinal Distribution

The distribution map revealed that the species were observed in different elevations (Fig. 4). For *C. longa,* it was recorded at an elevation of 115 m a.s.l., while *C. zedoaria* at an elevation of 124 m a.s.l. Both *Curcuma* species were abundant in their specific elevations. *Globba campsophylla* was the only recorded species at an elevation of 263 m a.s.l. *E. dalican*, on the other hand, was the only observed species recorded at an elevation of 364 m a.s.l., and nearer to it was *M. muricarpa* at an elevation of 371 m a.s.l. *Etlingera* sp.

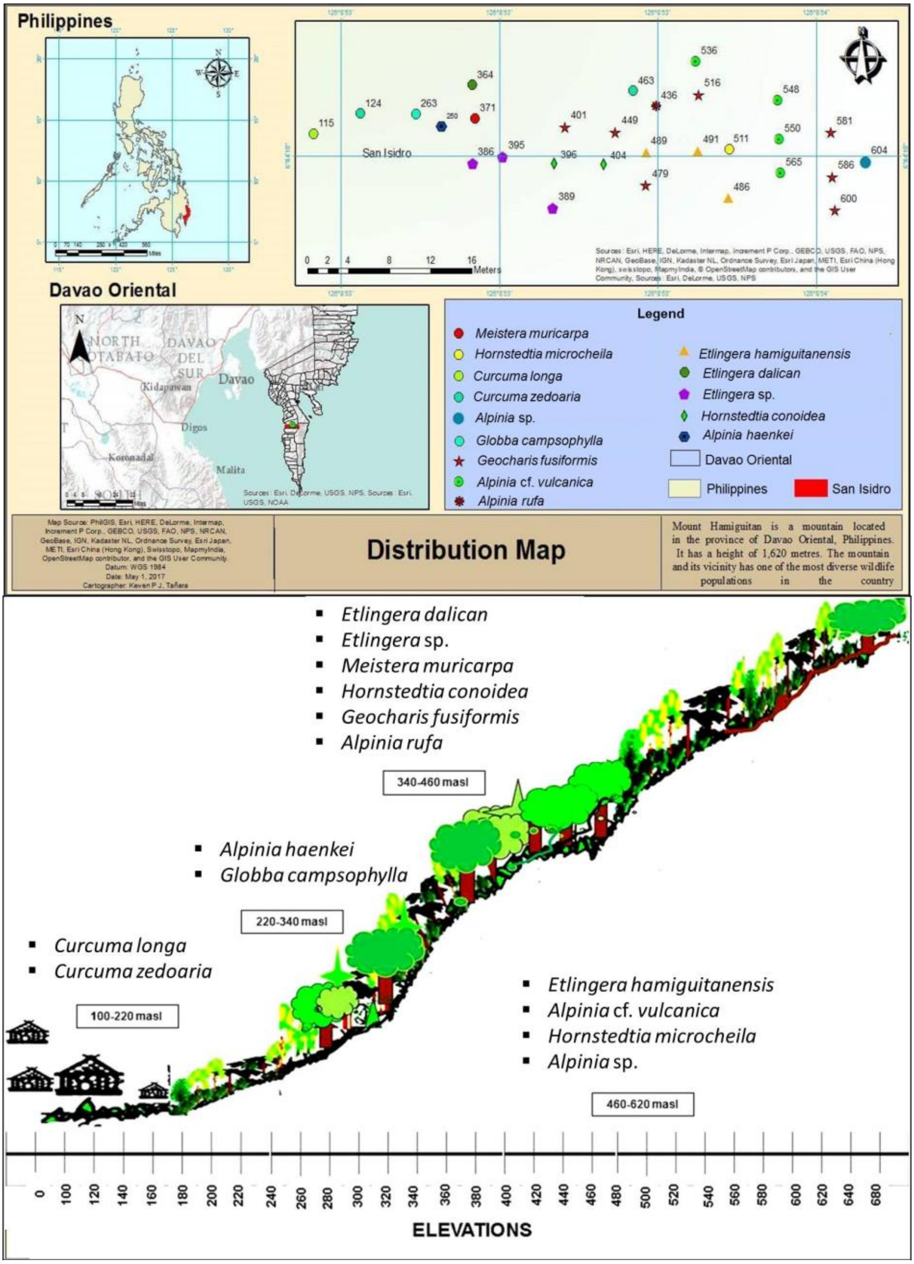

Figure 4. Map of Mt. Hamiguitan expansion site showing the distribution and elevation of Zingiberaceae species.

which is considered to be a possible new species is abundant at elevations of 386, 389, and 395 m a.s.l., while *H. conoidea* was abundant at 396 and 404 m a.s.l., and *G. fusiformis* at elevations of 401, 449, 479, 516, 581, 586 and 600 m a.s.l. With an elevation of 436 m a.s.l., *A. rufa* was the only recorded species, while from elevations of 486, 489 and 491 m a.s.l., *E. hamiguitanensis* was well observed, and in 511 m a.s.l. was *H. microcheila*. *Alpinia* cf. *vulcanica* was also diverse at elevations of 536, 548, 550 and 565 m a.s.l. Further, *Alpinia* sp. was only located at an elevation of 604 m a.s.l. which was the highest elevation recorded among the species.

D. Endemicity and Abundance of Species

Among the collected species, 10 are endemic to the Philippines, namely, *A. haenkei*, *A.* cf. *vulcanica*, *A. rufa*, *E. dalican*, *E. hamiguitanensis*, *G. fusiformis*, *G. campsophylla*, *H. conoidea*, *H. microcheila*, and *M. muricarpa*. Of these, *G. fusiformis* was the most abundant species in the area. Noteworthy also in this study is the *Alpinia* sp. which is the only recorded species found in the highest elevation which may be new to science together with *Etlingera* sp.

Conclusions and Recommendations

This study concluded that there are 14 species of Zingiberaceae in the expansion site of Mt. Hamiguitan. Of these, 10 species are endemic to the Philippines, two species are introduced and cultivated by local people, and two are still unidentified to the species level. The distribution of the Zingiberaceae species revealed that the species are generally scattered and dwell at elevations between 115–604 m a.s.l. *Geocharis fusiformis* was the most abundant species recorded in the area. Floral morphology including the inflorescence shape and origin/emergence and also their infructescence were found to be of taxonomic significant characters for identification of the species. Moreover, this study revealed that *Alpinia* sp. and *Etlingera* sp. are possibly new to science. The present data represents 47% of the total genera and 14% of the total species of Zingiberaceae in the Philippines. Further, the high endemicity of ginger species in this area is also noteworthy and adds a feature to the MHRWS as a UNESCO World Heritage Site and ASEAN Heritage Park.

Acknowledgments

The authors would like to thank the following: Central Mindanao University for the logistics support, Mt. Hamiguitan research funded by the DENR from

October 2016 to March 2017; Hon. Romero Gamoza (Brgy. Captain of La Union), Hon. Justina MB Yu (Municipal Mayor of San Isidro), and Ruel D. Colong (PASu of Mt. Hamiguitan) for allowing the authors to conduct this study at the Mt. Hamiguitan expansion site; Jun Limbaga, Edgar, and the staff of the DENR Region XI of the MHRWS for the warm welcome; and Keven Tanara for generating the distribution map.

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Journal of Tropical Biology and Conservation 16: 141–153, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Effects of the total solar eclipse of March 9, 2016 on the animal behaviour

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Abstract
Studies on animal behaviour associated with natural phenomenon such as a solar eclipse, provides valuable contribution to the ecology of the studied animal. An observation on the effect of the total solar eclipse on the environment and animal behaviour was done in Central Sulawesi, Indonesia on March 8-9, 2016. Four recorded environmental factors changed dramatically during the solar eclipse. Air temperature, light intensity, and wind-speed dropped and reached the peak around the maximum eclipse, whereas humidity increased at the same time. The observed animals, i.e. Heck's macaque, flying fox, maleo, amphibians, and several insects showed unusual behaviour as a response to the environmental changes. Meanwhile, tarsier showed no response to the solar eclipse. This observation revealed the effect of the total solar eclipse on the environment and animal behaviour.

Keywords: behaviour, eclipse, response, Sulawesi

Received 27 May 2018
Reviewed 04 December 2018
Accepted 21 January 2019
Published 15 October 2019

Introduction

A total solar eclipse is a rare natural phenomenon with only 68 occurrences during the 21st century, and has an effect on animals, plants and environmental factors (Pasachoff, 2009). Previous studies report the dramatic reduction of incoming global radiation, pronounced changes in surface temperature and humidity and also decrease in wind-speed associated with the eclipse event (Anderson et al., 1972; Founda et al., 2007). Environmental factor changes, especially decreasing light intensity and temperature effect animal behaviour.

The unusual behaviour of animals during the solar eclipse have been recorded in previous studies. The earliest records were of birds ceasing to sing and falling to the ground during total eclipses in 1544 and 1560 respectively. Thereafter, Newport (1837) reported bees returned to their hives during an eclipse. The scattered observations on mammals, some insects and plants have been done by astronomers who were primarily occupied with observing the eclipse, only when they had the good fortune to be a situation to observe the animals (Wheeler et al., 1935). Comprehensive records were presented by Wheeler at al. in 1935 by compiling reports from society during the total solar eclipse in New England states on August 31, 1932. After some decades, more scientists realized and gained information from their observations on animal behaviours. Beginning in 1955, Kullenberg reported bird and insect behaviour during the solar eclipse, and this was followed by other reports such as on bats (Krzanowski, 1959), freshwater invertebrates (Cadwallader & Eden 1977), chimpanzee in captivity (Branch & Gust 1986), and spiders (Uets et al., 1994). During the 2000s there were reports on birds by Tramer and ground squirrel by Kavanue and Rischer, in 2000 and 2009 respectively.

Although there are a number of reports, this phenomenon is always unique since it happens at different times of day, locations and conditions (Founda et al., 2007). On March 9, 2016, a total solar eclipse passed over the Indonesian archipelago. Almost the whole Indonesian territory was covered by the eclipse, with 65% of the minimum eclipse totality at the furthest area from the total eclipse's path. Thus, in order to study the effect of the total solar eclipse of March 9, 2016, we conducted observations and a field experimental study. This paper presents our result on recorded animal behaviour and environmental factors before and during the total solar eclipse.

Materials and Methods

Observations were conducted in Central Sulawesi i.e. Lore Lindu National Park (LLNP), Pangi-Binangga Nature Reserve (PBNR) and Pulau Kelelawar (**Figure 1**). LLNP and PBNR were located in the central axis of the eclipse with 100% sun obscuration, while Pulau Kelelawar was about 60 km away with 99% of sun obscuration.

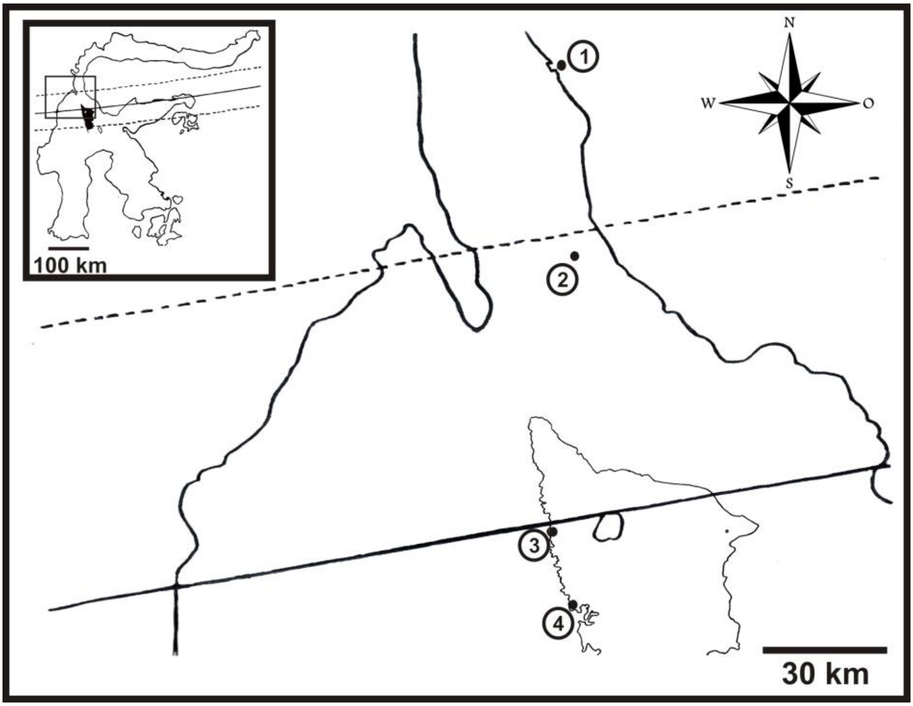

Figure 1. Observation sites: 1, Kelelawar Island; 2, Pangi-Binangga Nature Reserve; 3, Saluki (Lore Lindu National Park); and 4, Mata'uwe (Lore Lindu National Park). Black line is the center of the total solar eclipse, while the dotted line is the border of the total solar eclipse path with 100% obscuration.

The duration of the eclipse was 2 hours 32 minutes (details in Table 1 and Figure 2). The first partial eclipse started at 07.27 hours (T1, the first contact between the moon and the sun) until 08.37 hours (T2, the shadow of the moon fully covered the sun), while the second partial eclipse began at 08.40 hours (T3, the end of the total eclipse) until the end of the eclipse at 10.00 hours (T4, the sun was fully visible). The solar eclipse started when the height degree of the sun was 15-30°, which means that the eclipse started when the sky was already bright.

Table 1. Total Solar Eclipse on March 9, 2016: Time and sun position Lore Lindu National Park (Lat.: 1.4364°S Long.:120.1317°E) (generated and modified from https://eclipse.gsfc.nasa.gov/SEgoogle/SEgoogle2001/SE2016Mar09Tgoogle.html)

Event	UTC	Central Indonesian Time (WITA)	Altitude	Azimuthal
Start of partial eclipse (T1)	23:27:40.1	07:27:40.1	19.5°	094.2°
Start of total eclipse (T2)	00:37:21.9	08:37:21.9	36.8°	094.4°
Maximum eclipse (M)	00:38:43.8	08:38:43.8	37.2°	094.4°
End of total eclipse (T3)	00:40:06.4	08:40:06.4	37.5°	094.4°
End of partial eclipse (T4)	02:00:33.6	10:00:33.6	57.6°	095.9°

Therefore, these study sites were the best places for carrying out animal observation. In addition to the high animal diversity and endemism in Sulawesi, the duration of the total eclipse in these sites was longer. Moreover, there was a gap between dawn (sunrise) to the beginning of the eclipse.

The instantaneous scan-sampling technique was applied by observing one or a group of animals, recording what these animals did at predetermined time intervals, beginning one day before the eclipse and continuing through the day during the eclipse (Altmann, 1974; Branch & Gust, 1986). Several groups of animals which represent nocturnal and diurnal animals were observed in their natural habitats, i.e. Black flying fox (*Pteropus alecto*) in Pulau Kelelawar, Heck's macaque (*Macaca hecki*) in PBNR, Tarsier (*Tarsius dentatus*) in Mataue-LLNP, four species of amphibians: Sulawesian toad (*Ingerophrynus celebensis*), Common Asian toad (*Duttaphrynus melanostictus*), Crab eater frog (*Fejervarya cancrivora*), Chorus frog (*Microhyla* sp.) in paddy fields around Saluki, and also insects in Saluki-LLNP. Maleo (*Macrocephalon maleo*) was observed in the semi-natural captivity Saluki-LLNP. A group of maleo comprising adult males, adult females and chicks were kept in a cage which was built in their natural habitat at LLNP. Behaviour of the other animals were also recorded opportunistically.

Camera recording and direct observations were conducted to record the behaviour of birds and mammals. Camera traps (Bushnell NatureView HD) equipped with night vision devices were set up in the roosting site of the flying fox, nest tree of the tarsier, and the semi-natural captivity area of maleo. All the animals were observed directly. In the visual observation of the amphibian, an advertisement call recording was also be done using asound recorder (Olympus LS 11 with built-in microphone). The call recording provides information on the activity and identity of the observed species. An experimental field study was conducted to observe the behaviour of ball-rolling dung beetles (*Paragymnopleurus planus*) (Dacke et al., 2014). A circular dirt arena (3 metres in diameter) was prepared, and then one dung ball and a beetle were placed in the centre of the arena, letting the beetles roll the ball out of the circular line. Behaviour of the dung beetles were observed in two replications starting 30 minutes before the beginning of the eclipse until 30 minutes after the end of the eclipse. A light trapping was conducted to observe the response of diurnal-nocturnal insects, such as Coleoptera, Lepidoptera, Orthoptera, and Hemiptera. Light trap was constructed using two mercury lamps (*Phillips ML L* 160W 220V each) with electricity source provided from 1.000W *Honda EU 10i* portable generator. Lamps were mounted in the square white cloth, 3 x 2 metres in dimension. Light trappings were done from 07.00 to 10.00 hours at the Indonesian Central Time (WITA) the day before eclipse (March 8) and during the day when the eclipse happened. Additionally, four environmental factors were recorded from 06.00 to 11.00 hours beginning a day before the eclipse and ending after the eclipse. Air temperature, humidity, and wind speed were measured using a Kestrel 3000 pocket wind meter, while the light intensity was recorded by using the lux-meter.

Results and Discussion

Recorded environmental factors

All the measured environmental factors changed dramatically during the eclipse (**Figure 3**).

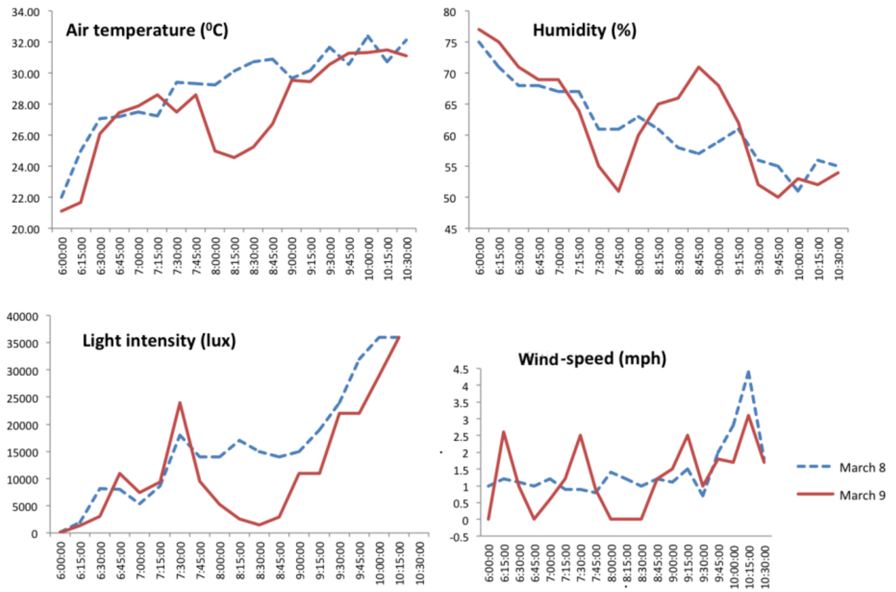

Figure 3. Recorded environmental factors. A day before eclipse, March 8 (dotted-blue line) and during the solar eclipse, March 9 (red line).

Air temperature, light intensity, and wind speed dropped and reached their peak around the time of the maximum eclipse, whereas humidity increased through the eclipse. The air temperature decreased significantly in all sites, from 28.61°C to 24.56°C, starting about 20 minutes after the first contact (T1), as a response to the sun radiation blocking (Anderson, 1999; Szalowski, 2002). Relative humidity increased for several minutes after T1, from 51% to 71%, as a consequence of the decreasing temperature (Founda et al., 2006). The light intensity dropped completely at T1, from 25.000 lux to near 0 lux during the maximum eclipse. Wind speed fluctuated during the day, however, it decreased at T1 and was stable around the maximum eclipse. Significant environment changes during the solar eclipse were expected to affect animal behaviour. Animals showed unusual behaviour, differing from their behaviour in normal conditions.

Animal responses

Black Flying Fox (*Pteropus alecto*) showed different behaviour during the solar eclipse. A day before the solar eclipse, almost all individuals produced a calling-sound and were noisy in their roost, from around 07.00 hours until noon. At 08.00 hours, some individuals flew around the tree-roosts, interacted with other individuals and flapped their wings more often. On March 9, they showed a similar behaviour in the morning but from 08.15 hours, the roost was very quiet, all individuals hung stably, and covered their bodies with wings **(Figure 4)**. This situation continued until 09.15 hours, about half of the second partial eclipse. At 09.30 hours some individuals flew around the tree-roosts, similar to the day before. It was predicted this unusual behaviour was a response to the environmental factor changes which suddenly happened during the total solar eclipse, especially the decrease in temperature and darkness (Wheeler et al., 1935). Additionally, flying fox did not emerge from their roost during the eclipse. It was argued that bats fly out from the roost during the eclipse because of the darker sky, creating a similar situation as evening when they emerge daily (Krzanowski, 1959).

Dian's Tarsier (*Tarsius dentatus*) showed no response to the solar eclipse. In daily normal conditions, this species produces an audible calling-sound which can be heard directly, and comes out from the nest to forage for prey in the evening (Řeháková-Petrů et al., 2012). Our observation and camera recording analysis show that the tarsier stayed inside the nest and did not produce a calling during the solar eclipse **(Figure 4)**. Although the tarsier is a nocturnal animal, we predicted that there is no effect on the tarsier's activity due to darkness caused by the eclipse.

Figure 4. Several observed animals. Black flying fox hang stably in their tree-roost (a), Tarsier stay inside the nest (b), Maleo in their semi-natural captivity (c), Amphibian (*Michrohyla* sp.) produce advertisement call during the eclipse (d), Dung beetles roll the dung ball (e), and moths in the light trap (f).

Heck's macaque (*Macaca hecki*) is one of the diurnal primates that was observed intensively. On March 8, they moved down from the resting tree and spent their time foraging, feeding, moving, and socializing from 05.43 to 10.30 hours, then took a rest on the canopy. At 11.23 to 17.36 hours they went deep into a forest with steep topography and could not be observed. However, they came back to their resting tree before the sky went dark as this is their usual daily activity (Reichard, 1998). On March 9, they showed similar activities in the morning. However, at 08.34 hours, they ceased all activities. Three males emitted a loud sound like '*wa-wa-wa*', as a command for other individuals to gather around the alpha male. The sound is supposed to be a call or a sign of a dangerous situation. Afterward, the alpha male moved down to the ground, followed by juveniles, females and some males and formed a circle with an alpha male at the centre. Thierry (1983) also reported the same pattern of emitted sound and circle formation of the Tonkean macaque (*M. tonkeana*) in LLNP when they were under threat or attack. When the total eclipse began, they did not move from their initial position, except for the alpha male and one other sub-adult male. After the end of the total eclipse, the adult male made some noises and interacted with the other individuals, then moved deep into the forest, and came back to the resting tree in the afternoon. A similar unusual behaviour of other primates during the solar eclipse has also been recorded by Branch & Gust (1986). According to their observation, the rare and uncommon events such as solar eclipse can influence and modulate the behaviour of chimpanzees.

Domesticated pig (*Sus* sp.) was observed opportunistically. Several minutes after the partial eclipse began (T1), they ceased their activities including eating, and took a rest position. These activities are appropriate to the dusk condition, which has been recorded in other mammals, such as squirrels (Wheeler et al., 1935). The normal activities began afterwards when the sky became brighter, before the end of the partial eclipse (T4).

The behaviour of the maleo (*Macrocephalon maleo*) has been observed visually and recorded using night vision camera in the semi-natural captivity. A day before the solar eclipse, all individuals showed normal activities such as feeding on the ground, moving around, and interacting among individuals. On March 9, from 08.00 hours (T1), unusual activities were recorded. Maleo showed anxious behaviour which was expressed by irregular movement in the cage. The male flew to the artificial nest, which was formed using a tree branch in a higher position inside the cage, and behaved like it was taking a rests prior to nightfall. This unusual behaviour is rarely seen during a normal day. Female maleo ceased the activities and stayed on the ground. Around 09.15 hours (T3), the female showed normal activity, while the male flew down to the ground and interacted normally with the females and chicks.

The other avian fauna, a colony of birds, foraged in the tree (*Ficus* sp.) and produced noisy calling in the morning until noon on March 8. However, on March

9, these birds foraged only in the morning until the first partial solar eclipse (T1). Close to the total solar eclipse, all of them flew away, probably back to their nests. Our observation showed a similar pattern with previous studies (i.e. Maccarone, 1997; Tramer, 2000), and agreed that although the solar eclipse was short, reduced light levels sufficiently interrupted normal avian diurnal behaviour patterns.

Four nocturnal species of amphibians were observed in the paddy field. The night before the eclipse, all of the observed species produced an advertisement call. On March 9, at the beginning of the first partial eclipse (T1), two species, *F. cancrivora* and *D. melanostictus* were actively feeding while *I. celebensis* and *Microhyla* sp. were inactive and stayed in the hollow embankment. When the sky started to become darker and the air temperature decreased (after T1), *F. cancrivora* changed its position, while *D. melanostictus* jumped to the upper embankment and hid under the grass. At the maximum eclipse, when the air temperature dropped significantly by 4°C, *I. celebensis, D. melanostictus* and *F. cancrivora* kept inactive at the same position, while *Microhyla* sp. produced their advertisement call. We predicted that the significant temperature change affected the activities of these amphibians making them less active. The decrease of the light intensity during the maximum eclipse reflects the situation at night. This situation triggered some amphibian species to produce the advertisement call (Wheeler et al., 1935).

An experimental field study was carried out to know the effect of the total solar eclipse to the roller-type dung beetles, *Paragymnopleurus planus*. The rolling speed was slower during the first and second partial eclipses, compared to normal conditions. Interestingly, during the maximum eclipse, *P. planus* stopped rolling the dung ball and buried itself inside the ground in the cower-sleep position. The dung beetles reappeared to the ground level when the sun began to become brighter and continued rolling the dung ball. According to Dacke et al. (2014), dung beetles utilize celestial compass cues such as the sun, moon, the pattern of polarized light of the sky and the milky-way to navigate a straight line while rolling the dung ball. Therefore, the lack of navigation signals which were caused by the total solar eclipse affected the dung beetles' behaviour. They stopped rolling the dung ball and could not navigate accurately.

Turning to the light trap results, no insects were trapped during 07.00 to 10.30 hours the day before eclipse, as we had expected. However, after the partial eclipse began (T1), two species of diurnal insects from the families Coccinellidae and Chrysomelidae were recorded coming to the trap. Meanwhile, there was no record of nocturnal insects very common to be found in the light trap i.e. Lucanidae, Scarabaeidae, and Cerambycidae. Interestingly one of the nocturnal insects, such as the moth, was recorded coming to the trap during the solar eclipse **(Figure 4).** Additionally, several unusual insect behaviour were also recorded opportunistically, i.e. chirping and shrilling of crickets and cicadas during the eclipse. Crickets became very noisy during the total solar eclipse, louder than usual (Wheeler et al., 1935). Chirping or shrilling of crickets and cicadas is common at night or in the evening when the sky gets darker, however, it is unusual to hear this during the day.

Our study recorded the environmental factor changes associated with the solar eclipse affecting the observed animal behaviour (**Table 2**). Diurnal animals ceased their activities, went back to their nests, or took the rest position, whereas nocturnal animals became active during the solar eclipse. All of these unusual behaviours are assumed as the exogenous rhythm expressions, and they revert to normal behaviour when conditions are stable (Hardy, 1970). A question related to this phenomenon can be addressed for future research, i.e. what kind of adaptation will be expressed if the influenced phenomenon, such as solar eclipse's similar condition situation occurs frequently?

Recorded data and information from this observation can be utilized to conduct future studies on animal behaviour associated with environmental changes. As we know, the environment is changing continuously due to many factors, such as increasing human population and habitat degradation. Therefore, a study on animal behaviour would be important to know their ecology and to develop the appropriate conservation management plan.

Table 2. Activities of the observed animal at normal conditions and during the solar eclipse

Animal	Usual activities	Activities during solar eclipse
Nocturnal animal		
Flying fox (*P. alecto*)	produce call-sound, flap the wing, interact each other, noisy, some individuals fly around the roost	quiet, cover their body with the wing, roost stably, do not emerge from the roost tree.
Dian's Tarsier (*T. dentatus*)	stay inside the nest, do not produce calling during the day	do not response the solar eclipse, stay inside the nest and do not produce calling
Sulawesian Toad (*I. celebensis*)	active and produce advertisement call at night	inactive
Common Asian Toad (*D. melanostictus*)	active and produce advertisement call at night	inactive
Crab-eater frog (*F. cancrivora*)	active and produce advertisement call at night	Inactive
Chorus frog (*Microhyla* sp.)	active and produce advertisement call at night	produce advertisement call
Moth	attracted with light trap during the night	attracted with light trap
Diurnal animal		
Heck's macaque (*M. hecki*)	forage, play/socialize	silent, cease activities, except males which emitting sound at partial eclipse. All individual cease activities at their position at total eclipse
Maleo (*M. maleo*)	feed in the ground, move around, interact each other	a male fly to the resting nest, female cease activities and stay in the ground
Domestic pig (*Sus* sp.)	eat the provided food	cease activities and take a rest position
Ball-rolling dung beetles (*P. planus*)	roll the dung ball normally	roll speed slower at first and second partial eclipse, stop rolling and burry itself inside the ground

Acknowledgements

We would like to thank the Head of RCB-LIPI, Head of Museum Zoologicum Bogoriense, Prof. Ibnu Maryanto, and Dr. Cahyo Rahmadi for their massive support during this study. We would also thank the LLNP for facilitating all team members during the observation. Our thanks to Mr. Arief Supriatna for his efforts in documenting during our observation. Great appreciation is addressed to Mr.

Fahri's students for their help. Last but not least, we would like to acknowledge the two reviewers who gave very constructive comments to improve our manuscript.

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Journal of Tropical Biology and Conservation 16: 155–163, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

A new record of *Euphorbia atoto* (Euphorbiaceae) in Bangka Belitung and notes of *Coptosapelta hammii* (Rubiaceae) for Borneo

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Abstract

Euphorbia atoto (Euphorbiaceae) and *Coptosapelta hammii* (Rubiaceae) have been recently collected from Belitung Islands, east of Sumatra, west Malesia. The discovery of *Euphorbia atoto*, combined with specimens kept in BO, is a new record for Bangka and Belitung Island, while *C. hammii* is considered to be an important rediscovery of a species thought to be endemic. Further examination of previously collected materials of *C. hammii* shows that this species has also been collected from Borneo, which means this species is no longer endemic to Belitung. Descriptions, photographs, notes on uses and a brief discussion are given.

Keywords: heath forest, plant diversity, Sumatra, west Malesia

Introduction

Bangka Belitung is one of the largest island complexes that lies outside the mainland of the Sumatran phytogeographical region. It takes its name from the two largest islands situated southeast of Sumatra, *viz* in the Karimata Strait. Both islands are situated between Sumatra and Borneo and Belitung Island is more or less located between Sumatra and Borneo.

Received 22 July 2019
Reviewed 07 August 2019
Accepted 19 August 2 019
Published 15 October 2019

In these islands, there are plenty of heath forests. It is one of the most unique ecosystems in Indonesia. The main constituent of the substrate in the heath forest is quartz and sand. The uniqueness of vegetation in the heath vegetation especially the one in Belitung was observed a long time ago by Valeton (1908a). He thought this vegetation is unique because most species have not been found in Java, one of the nearest island with good knowledge on the diversity of plants. Due to its peculiar stature, similar to the disturbed forest in appearance, heath vegetation sometimes was not considered as an important forest type in Indonesia (see Sulistyaningsih et al., 2019). Such kind of physiognomy, such as the small size of trees compared to other types found in the rainforest, e.g. dipterocarp forest, may be caused by the properties of the substrate which affect the availability of resources needed by plants, such as water (Becker, 2006).

After the 2000s, there was an increase in interest towards the diversity in these islands, particularly for plants. In Belitung, the now already established Belitong Geopark is perhaps one of the most important factors. The existence of widespread heath vegetation on this island also played quite a significant role in attracting scientists.

Some studies in plant diversity, ecology or even ethnobotany have been carried out (Oktavia, 2015; Oktavia et al., 2015; Rizqiani et al., 2018; Sulistyaningsih et al., 2019). Some of them yielded important discoveries that contribute to our knowledge of plant diversity either for Bangka Belitung, Sumatra or even Malesia. A good example is the discovery of *Archidendron borneense* (Benth.) Nielsen and *Syzygium claviflorum* (Roxb.) Wall. ex Steud. on Belitung Island (Sulistyaningsih et al., 2019).

During our study, we found that two noteworthy plant species had recently been collected from the islands. The first is *Euphorbia atoto,* which is the first record for Bangka Belitung Islands. The second is *Coptosapelta hammii*, a species previously reported as a Belitung endemic, which based on a further examination either from literature or herbarium, is no longer a Belitung endemic because it has been collected from Borneo. Its distribution is discussed.

Materials and Methods

Materials used in this study were collected in 2019 during a plant exploration to Belitung Islands carried out by the Ecology and Plant Resources Division, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB

University, Bogor, Indonesia. Plants were collected and preserved as dried herbarium specimens. The morphological description are based on dried materials supplemented by field notes for living characters. A further examination of specimens was also made in the Herbarium Bogoriense (BO), supplemented by digital images and database available in L (bioportal.naturalis.nl), K (apps.kew.org/herbcat), P (science.mnhn.fr/institution/mnhn/collection/p/list) (acronyms follow Thiers, 2019-continuously updated), and University of Brunei Darussalam (ubdherbarium. fos.ubd.edu.bn/).

Results and Discussion

A New Record of Euphorbia atoto in Bangka Belitung Islands

The Euphorbiaceae of Sumatra were revised by Airy Shaw (1981), who reported six species of *Euphorbia* of which three are native including *Euphorbia atoto*. The previous records of *Euphorbia atoto* in Sumatra included those from the southern mainland and islands in the west (Batu and Enggano). In BO, we found four specimens collected from Bangka that seems overlooked by Airy Shaw during the revision of Sumatran *Euphorbiaceae* (Airy Shaw 1981). Added with our recent collection from Belitung, therefore, these represents the first for Bangka Belitung Islands.

Euphorbia atoto Forst. f., Fl. Inst. Austr.: 36 (1786); Backer & Bakhuizen f. Fl. Java 2 (1963) 503; A.R. Smith in Airy Shaw, Euph. Born.: 111 (1975); Airy Shaw, Kew Bull. 36(2): 295 (1981); Ma & Gilbert, Fl. China 11: 292 (2008). - Type: Society Islands. Tahiti, *Forster s.n*. (Lecto K! designated by Smith (1981), rejected by Florence (1996)). - Figure 1.

Distribution: Widespread from east India to Ryukyu Island (Japan) and southeast to Queensland (Australia) and Polynesia in the Pacific Islands. In Sumatra, this species was reported to occur in the south and islands in the west (Batu and Enggano) (Airy Shaw, 1981). It is here reported from several locations in Bangka and the northwestern part of Belitung Island.

Habitat and Ecology: Coralline sandy beach and coral rock, including coconut plantation. In Belitung, this species grows in sand on granitic rock surfaces near the beach, below 5 m elevation. The large granitic rocks were quite frequent on the beach where this species grows.

Notes. *Euphorbia atoto* can be distinguished from other Sumatran species by the combination of the following characters: perennial herb or subshrub and by its non-succulent habit (Airy Shaw, 1981).

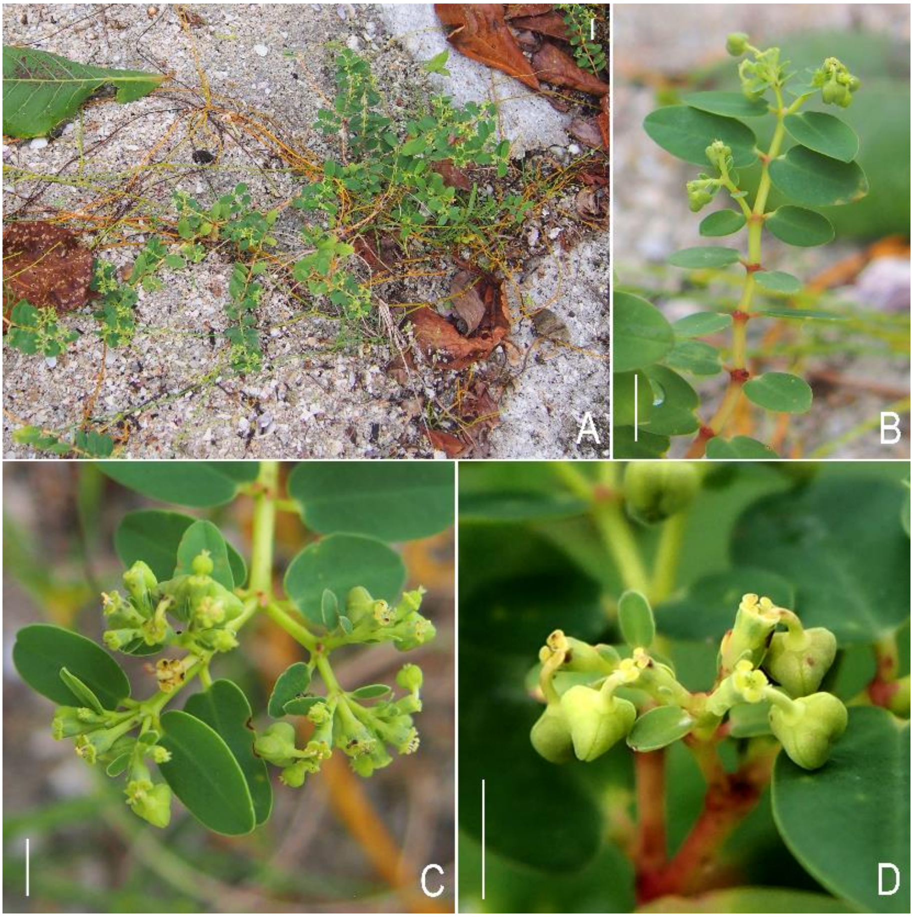

Figure 1. Morphology of *Euphorbia atoto*: A. Living plant. B. Branch. C. Inflorescence. D. Fruit (immature). Scale: A = 2 cm; B = 1 cm; C = 2 mm; and D = 5 mm. All from *WA Mustaqim et al*. ESDT 1.

The new records of this species in Bangka and Belitung possibly illustrate the lack or poor collecting efforts in both islands. This is probably because this is a coastal species which usually has a widespread distribution. The habitat recorded in Belitung Island is typical for the species, i.e. in the sandy areas near coasts (Ma & Gilbert, 2008). However, the sand where this species grows is derived from large granitic rocks, which seems to have never been mentioned in other literature. The elevation which is at c. 5 m is also typical for the species.

Specimens examined: Indonesia. Bangka Island, Soengai Liat, 10 m, 10 Nov 1917, *Bünnemeijer 1944* (BO!); *ibid.* Pulau Lepas, 50 m, 13 Dec. 1917, *Bünnemeijer 2455* (BO!); *ibid.* Tanjung Kalian, 11 Oct 1917, *Bünnemeijer 1557* (BO!); Bangka Archip. Ind., 25 Aug 1886, *Bokhorst 500* (BO!); Belitung Island, Tanjung Kelayang Beach, S2.56° E107.65°, *WA Mustaqim et al. ESDT 1* (BO!, FIPIA!).

Update and Bornean record of Coptosapelta hammii

The genus *Coptosapelta* Korth. consists of 16 accepted species. This genus is distributed in Myanmar, China including Taiwan, Indochina and many parts of Malesia. In Malesia, this genus has been recorded from Sumatra, Peninsular Malaysia, Java, Borneo, Philippines and New Guinea (Plants of the World Online, 2019).

On a few recent trips to Belitung Island, *Coptosapelta hammii*, a species hitherto known only from Belitung (Valeton, 1922; 1923; Govaerts et al., 2016; Plants of the World Online, 2019) was found. The species was described in 1908 by Theodore Valeton (1908b) based on Ham's collection gathered from Manggar and Budong (=Boedong) in 1907.

Accessing specimens both directly in BO or digital herbaria (K, L, P; acronym follow Thiers 2019, continuously updated), we noted that this species had also been recorded outside Belitung. Several collections had been gathered from Borneo, the first in 1937 but this was not reported (Govaerts et al., 2016; Plants of the World Online, 2019), except as listed material for Borneo *Tukirin 641* (L), which we did not find in BO in our last examination, in a palynological study by Verellen et al. (2004). It is formally reported here.

Coptosapelta hammii Valeton, Proc. Roy. Acad. Amsterdam 11: 129 (1908); *ibid.* 19: 281 (1922); *ibid.* 26: 373 (1923). - Type: Sumatra, Archip. Ind. Billiton W, around 1-31 Jan 1907, *Ham* SP 167 (lecto L, isolecto BO) (mentioned in Valeton 1922: 281).

Suberect *shrubs* or climbers, to c. 3 m length. *Stem* many angled, distinct in older stages, c. 2 mm diam., hirsute, hairs whitish, glabrescent with age. *Stipule* narrowly triangular lanceolate, 3–10 × c. 1 mm, hirsute, tapering toward the apex. *Leaves* elliptic, often subobovate, rarely elliptic-oblong or subovate, (2.3–) 5–9.3 × (1.1–) 2.9–4.5 cm, sparsely hirsute above, hairs dense on the midrib, glabrescent, hirsute beneath, especially along midrib and lateral veins, lately glabrescent; base cuneate, margin entire, apex acute to obtuse with short

Figure 2. Morphology of *Coptosapelta hammii*: A. Leafy branches. B. Flowers. C. Early fruiting stage. D. Submature fruit. Scale: A–B and D= 1 cm; and C = 0.5 cm. All from *WA Mustaqim et al*. ESDT 2.

acumen, or shortly acuminate; midrib immersed above, raised beneath; lateral veins 5–9 on each side of the midrib, arcuate especially near the margin. *Inflorescence* terminal, 4–6-flowered, lower flowers subtended by leaves, inflorescence axis to c. 2.6 cm long. *Flowers* 5-merous. *Calyx* velvety, hairs white; ovary portion subglobose, 3.5–4 mm long and wide; limb deeply lobed, lobes linear, 6–9 × 0.5–2 mm, acute, hirsute outside. *Corolla* hirsutulous throughout the tube, cylindrical, slender, 5.4–6 cm long, limb obovate-lanceolate, 1.4–1.7 × 0.4–0.5 cm, apex obtuse or rounded. *Stamens* alternatipetalous, filaments short, c. 0.5 mm long, anthers linear, c. 5.5 × 0.6 mm, apex acute, filaments and connective hairy, hairs as the corolla tube, the upper half of the connective glabrous. *Style* very slender, c. 5.8 mm long, glabrous, stigma clavate, c. 0.4 mm long, blackish when dry, exserted for the majority of the length. *Capsule* ellipsoid, 1.7–2.2 × 1.1–1.5 cm, calyx limb persistent, erect. - Figure 2.

Distribution: Sumatra (Belitung Island) and Borneo (Kalimantan).
Habitat and ecology: Valeton (1923) recorded the habitat as sandy barren soil. From the recent field trip, it grows in a wider range of habitats, including padang vegetation and also forest or shrub vegetation on podzolic soil.
Vernacular names and Uses: *Akar bunga padang* (Kalimantan); *Akar segendai* (Sumatra: Belitong).
Notes: This species is recognized by the combination of the following characters: corolla tube more than twice the length of the lobes, anthers without hairs, and secondary veins ranging from 5 to 7 on each side of the midrib (Valeton, 1923).

Valeton described *Coptosapelta hammii* as a shrub, but it is actually quite variable in habit, i.e. from shrub to liana-like. The shrub-like appearance seems to be the result of adaptation to the padang habitat, where no host plants occur and where its growth is limited. Valeton categorized it as xerophilic. Our observations in Belitung Island confirmed this for plants growing in heath vegetation, possibly the result is an adaptation to the quite dry environment in the heath forest (Turner et al., 2000).

Additional specimens examined: Indonesia. Sumatra: Belitung Island, Cendil Village, *WA Mustaqim et al*. ESDT 2 (BO!, FIPIA!). Borneo: West Kalimantan, Singkawang, Pasir Pandjang, 4 Oct 1937, *Dunselman 67* (BO! P!-image seen [P03984061]); *ibid*. 1 July 1949, *Polak s.n.* (BO!); *ibid*. 15 Feb 1937, *Dunselman* s.n. (BO!); *ibid*. Muara Kendawangan Nature Reserve, S02°34'19" E110°22'07", 6 May 2001, *Tahan Uji 4337* (*field no. 4239 and 4337*) (BO!). East Kalimantan, Bukit Tengkiling off 30 km NE of Palangkaraya, Central Kalimantan, 60 m, 30 Nov 1995, *Ambriansyah & Arifin* A.A.996 (L!-image seen [L.290573], P!-image seen [P04951575], WAN); East Kalimantan, W. Kutei, Kelinjau River, near Nelan, 15 June 1954, *Kostermans 9598* (BO!). Indonesia: location not recorded, *s.coll.*, *s.n.* (field number no 58) (K!-image seen [K001325114]); location not recorded, *s.coll.*, *s.n.* (field number no 40) (P!-image seen [P03984059]).

Acknowledgements

The authors thank the Indonesian Ministry of Tourism, Government of Belitung Regency, Government of Belitung Timur Regency, Bureau of Environmental Belitung Timur Regency and Belitong Geopark for funding, support, and permission during exploration where all the specimens used in this study were collected from Belitung Island.

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Journal of Tropical Biology and Conservation 16: 165–183, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

The Daily Activity Budgets of Long-tailed Macaque (*Macaca fascicularis)* at Padang Teratak Wildlife Sanctuary, Beaufort, Sabah, Malaysia

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Abstract

At present, the conversion of natural forests into urbanized and agricultural plantation areas are rising at an accelerated rate. Due to the loss of suitable habitats, wildlife i.e. macaques are forced to move into or nearby areas close to humans. The increased encounters between humans and macaques have led to conflicts between both when macaques utilize human resources. A preliminary study was conducted to explore the daily activity pattern of macaques based on age-sex and time in human-dominated areas (e.g. human settlements, oil palm plantation and fruit orchards). . The study was conducted in Padang Teratak Wildlife Sanctuary (PTWS) located in the district of Beaufort, eastern part of Sabah, Malaysia. Opportunistic observations were conducted along the roads encompassing seven villages in four days per month from December 2015 to January 2016. The survey was conducted in four time frames for 11 hours per day (06:00-09:00, 09:00-12:00, 13:00-15:00 and 15:00-18:00) to document behaviour of macaques with regards to the time of the day. Overall, a total of 1,462 individuals from 221 groups with total direct contact of 96 hours, 53 minutes out of 132 hours, consisting of multi-males multi-females (173 encounters), multi males (18), solitary male (28) and solitary female (2). There were 13 behavioural activities recorded and the study revealed that macaques spent most of their time for moving (28.4%), foraging (25.2%), resting (19.1%) and grooming (12.3%). Results from ANOVA showed that the daily activity patterns of each categories within age-gender varied showing there was a relation between daily activity budget and age-gender of macaques. In addition, Chi-square test indicated there were interactions between age-gender and time on daily activity. In contrast, time did not have an effect on their activity pattern. Good wildlife management practices by understanding the behaviour of macaques in human settlements near degraded forests is crucial since habitat sharing by humans and macaques raise the concern of safety and health issues.

Received 26 April 2019
Reviewed 11 June 2019
Accepted 10 September 2019
Published 15 October 2019

The frequent encounter between this species increases transmission of some zoonotic diseases to humans such as the malaria parasite (*Plasmodium knowlesi)*.

Keywords: *Macaca fascicularis*, long-tailed macaque, daily activity budget, altered habitats, human-macaque conflict.

Introduction

Forested areas are being converted into urbanized areas and agricultural plantations, mainly oil palm at an accelerated rate throughout Southeast Asia. The loss of habitat has forced macaques to exploit human settlements and its surrounding forested areas (Fuentes et al., 2005; Gumert et al., 2011; Sha et al., 2009; Hambali et al., 2012b). Due to the exploitation of areas close to settlements, this has frequently caused conflicts between humans and macaques. They are described as pests in many areas, which include fields, roadsides, temples, tourist lodges, reserves and towns (Lee & Priston, 2005). Species of primates with home ranges and resource utilization that overlap with humans, i.e macaques and baboons, are a predicament in Asia and Africa (Hill, 2000; Priston, 2005).

The habituation of macaques to both humans and human foods stem from being fed often by people, which have led to the foraging in human dominated areas such as garbage bins around the city (Hambali et al., 2012b). In addition, increased food resources such as oil palm fruit and human food wastes are clearly affecting macaque behaviours including their preference in choosing sleeping tree nearest to human settlements (Kurland, 1973). This strategy mirrors the natural ability of macaques in exploiting available opportunistic resources (Kurland, 1973), albeit the environment is disturbed (Wheatley, 1999). The expansion of human population has led to rapid and extensive invasion into the forested habitat of macaques (Hambali et al., 2012b). The accumulation of both human food rubbish and food crops plantation in remaining home ranges caused the macaques to become habituated and dependant on humans and human food which in turn results in conflicts among humans and primates. These environmental changes urged the macaques to adapt allowing them to survive in urban areas.

Macaca fascicularis (Primates: Cercopithecidae), or commonly known as the long-tailed macaque or crab-eating macaque, has a widespread and diverse geographical range in the mainland and Southeast Asian region (Fooden, 1995). They are a native within their home ranges occurring in Malaysia, lower north of Thailand, southern Laos, Cambodia, Myanmar, Vietnam, Java, Sumatra and the

Philippines (Marsh & Wilson, 1981; San & Hamada, 2009). Long-tailed macaques are the most common monkey of both disturbed and secondary forest in the lowlands up to around 1,300 m in the mountains (Phillipps & Phillipps, 2016). They are also inhabitants to many small islands in Borneo which includes Gaya Island (Kota Kinabalu), Pulau Tiga (West Sabah) and Maratua (Northeast Kalimantan). In Sabah, they are distributed throughout the state in almost all vegetation except in montane forests (Yasuma & Andau, 2000).

Macaques are one of the primate species closely associated with humans in terms of social behaviour. Research has shown that the social behaviour of primates is nearly similar to humans such as eating, playing, fighting and caring for their young (Rod, 1992). Krebs & Davies (1993) showed that behavioural activities of macaques are different between disturbed areas and those in their natural habitat (Krebs & Davies, 1993). Several studies have shown that activity budgets are affected by a variety of demographic and environmental factors: food sources, sex, social rank, reproductive condition, and the degree of human disturbance in the region (Peres, 1993; Passamani, 1998; Di Fiore & Rodman, 2001). According to van Schaik et al. (1983), as the group size increases, the activity budget of macaques is less encouraging. Macaques in bigger groups must expend more energy to get food sources and conflicts such as fighting within the group rises, whereas social interaction to reduce tension such as grooming decreases.

In true wild macaques, they have reduced home ranges with shorter day ranges and spend less time travelling and foraging and more time resting (Altmann & Muruthi, 1988; Kogenezawa & Imaki, 1999; Saj et al., 1999). Macaques that rely on anthropogenic foods in or surrounding urbanized areas spent less time consuming in wild fruits and flowers, reduced resting time and more time moving (Sha & Hanya, 2013). Forested habitats were used less often and they had a greater overall home range and mean monthly home range. Although there were contrasting results where food-motivated capuchins sustained a larger territory compared to their wild counterparts with comparable activity budget (McKinney, 2011), orangutans raiding on crops moved further on raiding days compared to days where they forage mainly on wild fruits (Campbell-Smith et al., 2011) and, in chimpanzees where they travelled further during crop-raiding days (Hockings et al. (2012). The diverse patterns emphasize on the complexity in ecological reactions of primate, depending on species and individual group and site condition (McKinney, 2011; Hockings et al., 2012; Sha & Hanya, 2013). Primates dependent on anthropogenic foods present opportunities to observe possible adaptation flexibility in their ecology and behaviour, which is predicted to differ depending on how anthropogenic food resources are utilized (Sha & Hanya, 2013).

Padang Teratak Wildlife Sanctuary (PTWS), gazetted as a wildlife sanctuary reserve in 1978, is located in the district of Beaufort. It covers 2,270 hectares of wetland with its surrounding area consisting of fragmented forests, mangrove, oil palm plantation, rumbia plantation and villages. According to Berenstain (1986), macaques are more concentrated and widespread in disturbed forests compared to undisturbed forests and open areas. The loss of habitat and food resources in their habitat of origin pushed them to invade agricultural areas in addition to human settlements causing losses. Therefore, there is a need for comprehensive research and monitoring of daily activity budgets and behaviour of macaques covering various habitats such as PTWS.

Macaques have a multimales - multifemales social system where alpha males usually mate with large numbers of females throughout the year which lead to a population increase in a particular area. The increase in number causes a conflict between humans and macaques when they intrude and raid plantations, and the homes of villagers. Several cases of human-macaque conflict in the study area include house break-ins, raiding of orchards, obstructing roads and the chasing of women and children. Thus, the present study was conducted to explore the activity budget of macaques surrounding PTWS in terms of time of the day and age-gender groups. It is crucial to understand the behaviour of macaques inhabiting various habitats such as settlements, disturbed forests and agricultural areas. It is expected that macaques in altered habitats would rely highly on anthropogenic foods which will affect their behaviour (e.g. moving and foraging). The findings of this study are expected to assist in controlling and reducing conflict between humans–macaques, by providing necessary information to the Sabah Wildlife Department so that they are able to devise suitable management plans for example group relocation. It is vital to know which areas and what time macaques go out to forage for food for the placement of cages during translocation.

Methodology

Study Area

Padang Teratak Wildlife Sanctuary (PTWS), Beaufort (N 05 ° 19'39.4, E 115 ° 31'06.58) is located between the Padas Damit Forest Reserve and the Menumbok Forest Reserve Class V (Extension II), which covers 2,270 hectares of grassy marshland with scattered clumps of low bushes, surrounded on one side by agricultural land and a small village as well as peat swamp forest on the other side (Sabah Forest Enactment, 1984; Malaysian Wetland Working Group, 1987). The agricultural land consists of oil palm and rumbia plantations.

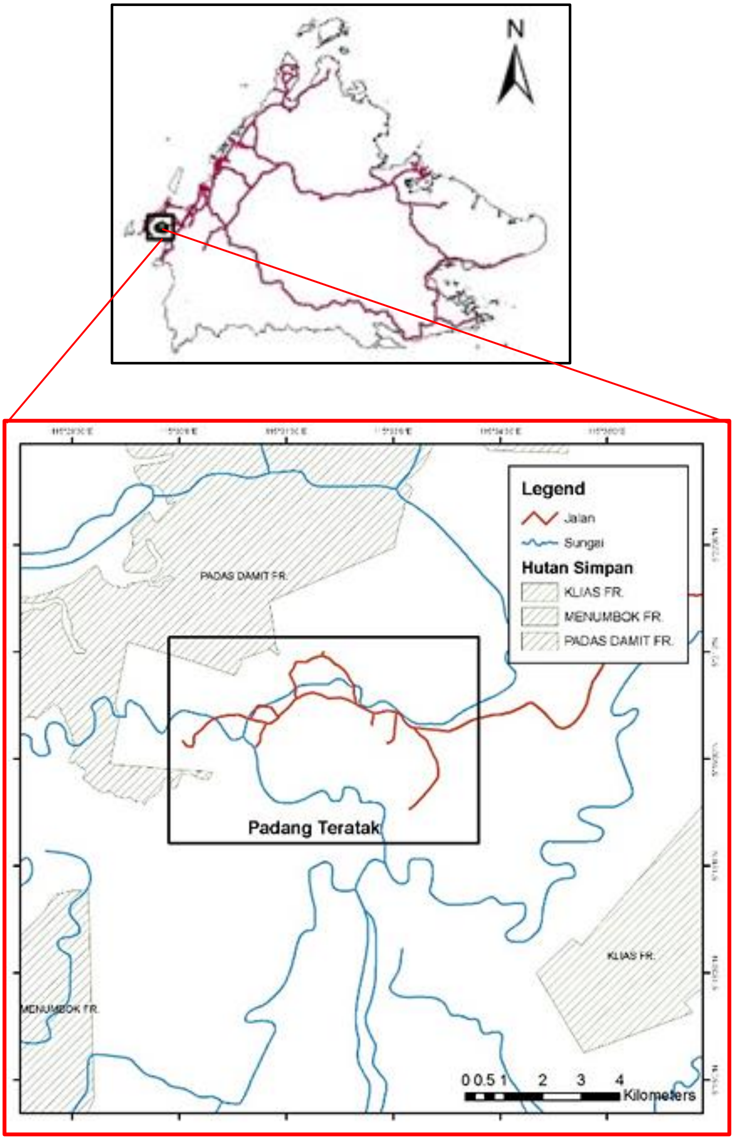

Figure 1. Map of the study area: Padang Teratak, Beaufort, western Sabah. (Source: GIS Laboratory, ITBC, Universiti Malaysia Sabah).

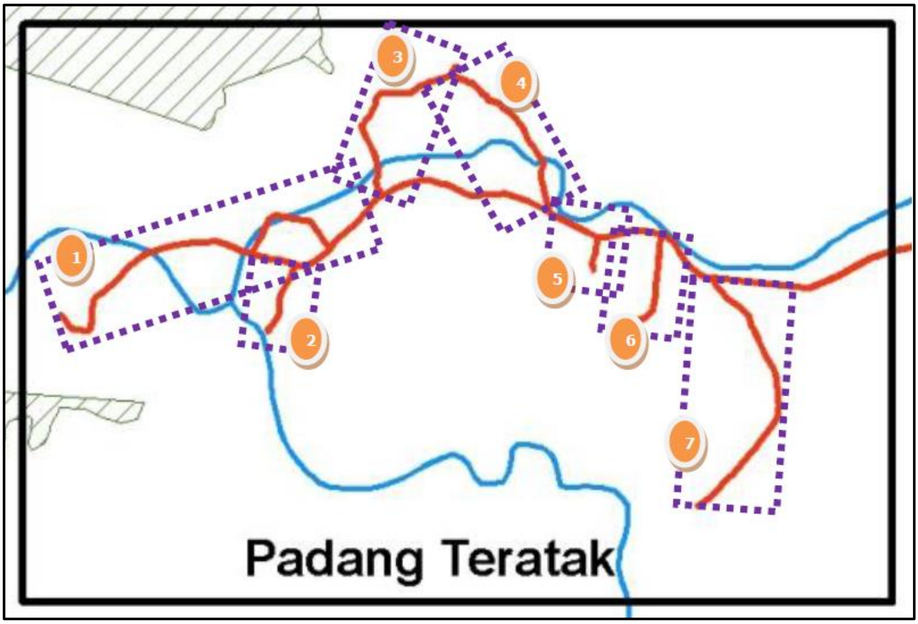

Figure 2. Map of PTWS showing the seven villages encompassing the main road (red line) used as observation transect: 1) Bentuka; 2) Teratak; 3) Shah Bandar; 4) Kukut; 5) Padas Damit; 6) Mentulud and 7) Bengkalalak. (Source: Mahad, 2016).

It is located about 120 km away from the state capital, Kota Kinabalu. It was gazzeted in 1978 as a wildlife sanctuary reserve as it is a suitable environment for various wildlife in Sabah (Russell, 2006). The reserve is known as an important site for resident migratory birds such as ducks and egrets, which they use as a wintering site from November to January. Meanwhile, other parts of the reserve offers habitat to the Estuarine crocodile and Proboscis monkey (Malaysian Wetland Working Group, 1987; Payne & Vaz, 1998).

Data collection

The present study was conducted between the months November 2015 to January 2016 for four days per month. Ad libitum sampling method was applied since macaques in this area were not fully habituated to humans. Opportunistic observations were carried out every three hours for a total of four times per day (06:00-09:00, 09:00-12:00, 13:00-15:00 and 15:00-18:00), where the total direct contact hours with macaque groups was 11 hours. The survey was conducted along roads encompassing seven villages: Bentuka, Teratak, Shah Bandar, Kukut, Padas Damit, Mentulud and Bengkalalak. The starting point of the sampling were chosen at random every three hours at which village, road or junction surveyed. Surveys were discontinued during rain to reduce bias as the macaques would usually seek shelter or hide to avoid getting wet. The behavioural activities were recorded at random where groups of macaque were observed until the group disappeared from the observer's sight. Apart from that, this method was also used to construct an activity catalogue (ethogram). The data recorded during sampling are: date, time, coordinate type of area, age-gender and behavioural activity. Macaques were distinguished by age by referring to characteristics in Table 1 and activities definitions in Table 2.

Table 1. Demography categories of macaques used to differentiate individuals in this study.

Age-Gender	Approximate Age	Characteristics
Adult Male (AM)	At least 5 years old	Taller, tail is longer and thicker compared to other age classes, limbs are relatively longer than AF, facial hair is short, have a moustache, penis is more visible, scrotum is bigger and pink in colour.
Sub-adult Male (SM)	4 ½ - 5 ½ years	Height is similar to younger AM, thinner, less muscular, penis can be seen clearly but scrotum is smaller than M.
Adult Female (AF)	At least 4 years old	Body hair colour is greyish and faded, nipples are longer, facial hair is always present.
Sub-adult Female (SF)	4 – 5 years	Thinner and smaller, nipples are shorter than F, hair is relatively shorter on the face.
Juvenile (JV)	1 – 4 years	Thinner, gender is normally difficult to distinguish.
Infant (IN)	0 – 1 year	Clinging to mother's body or carried by its mother.

Source: Fittinghoff, 1978.

Table 2. Definition of activity budget category used in this research.

Activity	Definition
Moving (MOV)	Any movement between 2 locations. Divided into: 1. Moving on the same tree; 2. Moving between different trees; 3. Moving on the ground; walking; jumping; climbing
Foraging (FOR)	Subject inserting food item into the mouth or chewing on food.
Resting (RES)	Subject is sitting or lying down and is not involved in any other activities.
Grooming (GRO)	Any activity of scratching or cleaning of the body with hands, feet or mouth. This activity is divided into: 1. Subject is grooming itself; 2. Subject is grooming other individual; 3. Subject is groomed by other individual.

Drinking (DRI)	Subject is drinking or licking any fluid.
Playing (PLA)	Chasing, wrestling, exploring and other movements with no particular purpose. Subject playing by itself or involving 2 or more individuals.
Agonistic (AGO)	Subject giving or receiving offensive action and threatening. This activity is divided into: 1. Without physical touch such as face expression; 2. With physical touch such as biting or grasping.
Copulation (COP)	Subject having sex by taking positions behind or above other individuals in ventral-dorsal position.
Vocalization (VOC)	Any sound produced by the subject.
Cling (CLI)	Subject (infant) hugging or hugging the ventral of another subject.
Urination (URI)	Subject discharging urine.
Defecation (DEF)	Subject discharging faeces.

Source: Bennett, 1983.

Data Analysis

Analysis of Variance (ANOVA) was conducted using SPSS statistical analysis version 10 to observe the differences between the daily activities in terms of gender and time in addition to the effect of interaction between age-gender and time factors on the daily activity of macaques. Chi-square test (x^2) was conducted to observe the relation between daily activities of macaques with age-gender and time, whether the activities were dependent with both independent variables. The relation between daily activities with age-gender and time were considered as significant when the statistic value of Chi-square test is high whereas the critical value (K) is less than 0.05. The result of the test is shown in a cross-table to compare percentage of daily activities for each category within the independent variables, age-gender and time.

Results

Overall, a total of 1,462 individuals from 221 different groups of macaques were observed during the study. The age structure of macaques sighted were males (n=398), females (n=311), sub-males (n=236), sub-females (n=105), juveniles (n=335) and infants (n=77). The total of direct observation time with the macaques encountered was 96 hours, 53 minutes out of 132 hours. Because of the shyness of the macaques, we did not manage to follow the animals throughout the daylight hours. Most of our observations (63%, the total number of observations was 915 = 42hours, 29 minutes) were conducted only during the morning (06:00-9:00 h) and evening (15:00-18:00). Even so, we managed to record a total of 14 behavioural activities (based on pooled data across all individual monkeys observed). The major behavioural activities were moving (28.4%), foraging (25.2%), resting (19.1%) and grooming (12.3%). Nine types of behavioural activities form the minor activities which were playing (3.6%), agonistic (3.1%), clinging (2.6%), swimming (1.6%), vocalization (0.5%), pest behaviour (0.4%), drinking (0.3%), breastfeeding (0.2%), and defecate (0.1%). The types of groups found throughout the survey period were multi-males multi-females (173 encounters), multi males (18), solitary male (28) and solitary female (2) (Mahad, 2016).

Table 3. The overall percentage and frequency of daily activity budgets (diurnal) of *M. fascicularis* at PTWS.

Type of Daily Activities	Activities	Frequency	Percentage (%)
Major	Moving	414	28.4
	Foraging	368	25.2
	Resting	280	19.1
	Grooming	180	12.3
Minor	Playing	52	3.6
	Agonistic	46	3.1
	Cling	38	2.6
	Copulation	38	2.6
	Swimming	24	1.6
	Vocalization	7	0.5
	Pest Behaviour	6	0.4
	Drinking	5	0.3
	Breastfeeding	3	0.2
	Defecate	1	0.1
	Total:	**1,462**	**100.0**

The result from the two way ANOVA showed the main effect of daily activities were influenced by age-gender factor ($F_{5,\ 1437} = 17.491$, k <0 .05). Overall, it was found that the effect of interaction between both dependent variable (age-gender*time) on dependent variable exist significantly ($F_{5,\ 1437} = 2.253$, k < 0.05). As much as 14.4% of the variation in daily activities is contributed by gender and the combination between age and time.

Table 4. Two way ANOVA test to observe the effect of gender, time and the interaction between age-gender and time on daily activity budget of macaques.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1487.849[a]	23	64.689	10.524	0.000
Intercept	9942.317	1	9942.317	1617.420	0.000
Time	15.359	3	5.120	0.833	0.476
Age-Gender	537.586	5	107.517	17.491	0.000
Time * Age-Gender	207.710	15	13.847	2.253	0.004
Error	8833.273	1437	6.147		
Total	27160.000	1461			
Corrected Error	10321.123	1460			

a. R Squared = 0.144 (Adjusted R Squared = 0.130)

The Chi-square test (x^2) showed that there was a significant relationship between gender and daily activities of macaque (X^2= 1000.653, df = 65, K < 0.05). In addition, there was a significant relationship between time and overall daily activities of macaques (X^2= 80.231, df = 39, K < 0.05). In contrast, there was no significant relationship between time and daily major activities of macaques (X^2= 13.418, df = 9, K > 0.05).

Table 5. Daily activities percentage (diurnal) of macaques for each age-gender category based on cross-table of age-gender and daily activities. The highest percentage of daily activities by each age group in major and minor activities were bolded.

Daily Activities	Time-Gender	Adult Male	Adult Female	Sub-male	Sub-female	Juvenile	Infant
Major (%)	Moving	**30.4**	**25.1**	**26.6**	**21.9**	**37.6**	5.2
	Foraging	**26.1**	**25.4**	**29.5**	**35.2**	**19.1**	**18.2**
	Resting	22.4	21.5	17.7	19.0	16.7	7.8
	Grooming	10.1	17.7	14.3	13.3	7.2	**16.9**
Minor (%)	Antagonistic	**4.5**	1.0	**8.0**	0.0	1.8	0.0
	Copulation	**4.3**	**6.1**	0.4	1.0	0.0	0.0
	Playing	0.8	0.3	**1.3**	1.0	**13.1**	0.0
	Clinging	0.0	0.0	0.0	0.0	0.0	**49.4**
	Swimming	0.3	**2.6**	0.4	**3.8**	**3.0**	0.0
	Vocalization	0.8	0.3	0.4	1.0	0.3	0.0
	Pest Behaviour	0.0	0.0	0.8	**2.9**	0.3	0.0
	Drinking	0.5	0.0	0.4	1.0	0.3	0.0
	Breastfeeding	0.0	0.0	0.0	0.0	0.3	**2.6**
	Defecation	0.0	0.0	0.0	0.0	0.3	0.0

Table 6. Daily activities percentage of macaques for each time period based on cross-table of time and daily activities.

Daily Activities	Time	06:00-09:00	09:00-12:00	12:00-15.00	15:00-18:00
Major (%)	Moving	29.3	27.0	29.3	28.4
	Foraging	23.6	27.2	29.3	23.6
	Resting	19.8	14.6	19.3	22.1
	Grooming	14.5	11.1	6.7	13.1
Minor (%)	Antagonistic	2.7	4.0	2.0	3.2
	Copulation	3.2	1.5	5.3	2.1
	Playing	2.7	5.8	1.3	3.2
	Clinging	2.7	2.8	0.7	2.9
	Swimming	0.5	4.0	4.0	0.0
	Vocalization	0.2	0.8	0.7	0.4
	Pest Behaviour	0.5	0.3	0.7	0.4
	Drinking	0.0	1.0	0.0	0.2
	Breastfeeding	0.2	0.0	0.7	0.2
	Defecation	0.0	0.0	0.0	0.2

Discussion

The Chi-square test shows that the daily activity patterns in each category age-gender vary indicating that there is a strong relationship between the daily activities of long-tailed macaques. Daily activities of macaques were divided into main and minor activities. Based on the observation (Table 3), macaques spend most of their time moving (28.4%), followed by foraging (25.2%), resting (19.1%) and grooming (12.3%). Vocalisation, antagonistic, drinking, breastfeeding and defecating were less visible which led to fewer records made resulting in low percentage in those activities.

The highest activity observed in this study was moving. This is supported by Hambali et al. (2012b) and Md-Zain et al. (2010) where macaques travel to forage for food during the day. Moreover, the presence of researchers indirectly affected the percentage of moving activities since they were not fully habituated to human presence. Since macaques are diurnal, they spend most of their time travelling from one area to another in search of food resources during daylight. The chart shows that juvenile monkeys were more active in moving compared to adult males. In contrast, infants recorded the lowest percentage as they cling to adult females to move. This behaviour allows the movements of the group to go smoothly.

PTWS provides the macaques with diverse and high availability of food resources, with abundant foraging sites such as oil palm plantations, human settlements, mangrove, fruit orchards and areas with areca nut and rubber trees. Thus, foraging was observed the second highest. They have been observed to forage on grasses; young tree leaves (fig, bamboo, oil palm, rubber, coconut, bushes and tree sapling) and fruits (coconut, guava, areca nut and banana). This incidence verified the abundance and continued availability of resources in PWTS. Oil palm was widespread in the study site, where it was planted by several plantation companies and the villagers of the surrounding areas on their private land. Mature oil palms are able to produce 1,000 to 4,000 fruit seeds per bunch depending on the age and durability of the trees. The increase in quality resources from anthropogenic sources is clearly influencing primate behaviour (Fuentes et al., 2005; Hadi et al., 2007). Anthropogenic foods provide higher energy compared to natural food as they are more palatable, rich in calories, easier to digest, abundant and in cluster (Forthman-Quick, 1986; Forthman-Quick & Demment, 1988; Saj et al., 1999). Due to the ease in obtaining food in human-dominated areas, one of the effects that is frequently observed are reduction in territory range and daily distance travelled, where more time spent to rest and forage (Malik & Southwick, 1988; Wheatley et al., 1996; Saj et al., 1999; Strum, 2010).

The observation revealed that males (n=104), females (n=79), sub-males (n=70) and sub-females (n=37) were more frequent in foraging for food. This behaviour allows males to maximize the absorption of nutrients to gain energy and for muscle building. Large and muscular body size benefits the males to compete for a mating partner (Cowlishaw & Dunbar, 1991). Meanwhile, female macaques need to extract more food sources to meet their reproductive needs. The findings of this study were supported by the study of Trivers (1972) and Wrangham (1979) where the quality and number of offspring produced by the females were determined by the quality of the resources obtained.

Resting is the third highest observed behaviour during the survey period. For sub-adults, the percentages of these two activities were of inverse proportion to one another. This finding was similar to Hambali et al. (2012), where when foraging behaviour increase, macaques spend less time resting. Since macaques only forage during daylight, they exert more effort in eating to be able to last through the night. Moreover, this study shows that males rested more than females, similar to the observation by Fuentes et al. (2007) in Padangtegal, Bali Indonesia. Since macaques are generally in a multi-male multi-female group with an alpha male, the number of females is usually higher than males within the group. Females are less likely to rest as they are often interacting with other members of the group such as grooming infants.

Grooming is one of the main activities that show the hierarchical structure within their group. Adult females (n=55) recorded the highest frequency of grooming activity compared to the other gender groups: adult males (n=40), sub-males (n=34), sub-females (n=14), juveniles (n=24), and infants (n=13). This finding is supported by Gumert (2007) where grooming is considered as an exchange to gain access to infants as females were seen to have an interest in caring for young other than their own. Meanwhile, adults of both genders were normally seen grooming each other after mating. As a result from Table 5, copulation activity was highest for adults compare to sub-adults. The study of Gumert (2007) stated that grooming can be regarded as a reward for couples after copulation. Lower-ranked females tend to groom higher-ranked females. The purpose of this behaviour is to obtain cooperation during aggression, to reduce interference and to gain access to food during resources shortages (Hambali, 2012). Macaques are polygamous species where males were observed to copulate with more than one female. Engelhardt (2005) found that dominant males are able to detect fertile females. Females typically prefer to mate with dominant males (Md-Zain et al., 2010).

Males obtain a high position in the hierarchy through successful antagonistic interactions such as fighting (Arlet et al., 2009). These studies (Sussman et al., 2003; Md-Zain et al., 2010; Hambali et al., 2012) agree that antagonistic behaviour is driven by competition for food and mating partners. Agonistic behaviour and mating were influenced by the number of fertile females within the group (Girard-Buttoz et al., 2009). This means that the more fertile females are available within the group, the less competition there are among males. Thus, leading to higher copulation activities within the group. There were a group of multi-males during the observation period that exhibited agonistic behaviour by staring aggressively, making squeaky sounds and shaking tree branches after seeing researchers. This group did not escape but threatened researchers by moving towards them.

Playing is a social interaction involving more than one individual (Kipper & Todt, 2002). Opposite to Hambali et al., (2012), this study did not record any playing activity by infants. This occurred due to infants constantly clinging to adult females. Sub-adults were often seen playing with juveniles. An example of their playing activity observed includes jumping from one branch to another, pulling of tails and chasing each other. They had been observed swimming several times in the nearby small river and drainage. Fooden (1995) that this species are good swimmers among the primate species and often enter the water for fun. Swimming may be one of the mechanisms for cooling down since the weather is usually warm between 12:00-15:00. Furthermore, this behaviour is a survival strategy when their feel threatened. A group was swimming before they spotted the researcher and fled to another location through drainage.

Despite the low pest behaviour recorded, it was significant enough to trigger the anger and fear of villagers around Padang Teratak. Some 0.4% of the interrupting activities around Padang Teratak were done by adults and juveniles. Their small body sizes allowed them to enter houses through holes on floor and walls, windows and roofs. The same group of macaque often enter houses between 12:00 to 15:00 when the residents were not at home. They do not only rummage dustbins and properties inside the house, but have been seen stealing food such as rice, eggs and snacks. In the fruiting season, this species invades the orchards to eat fruits and other crops. Victims of monkey disruptions at this area claimed that macaques block roads and chase out women and children. However, there was no report on any injury cases received by the Wildlife Sanctuary, Padang Teratak.

Conclusion

Padang Teratak Wildlife Sanctuary and its surrounding areas provide easy access of varied anthropogenic foods and protection against natural predators to macaques. These have led to the increase in the number of macaques over the years. Their presence in this area poses a conflict between humans and macaques. Based on observation from the study, the most common daily activity budgets of macaques were moving, feeding, resting and grooming. Side activities such as mating and pest behaviour also need to be investigated in the future. The understanding of the behaviour of macaques is vital in order to manage the increase in population, avoid conflict with humans and for health reasons as they are known to be a vector for zoonotic disease (i.e. malaria, herpes B virus, pox virus, measles, rabies and bacterial infections).

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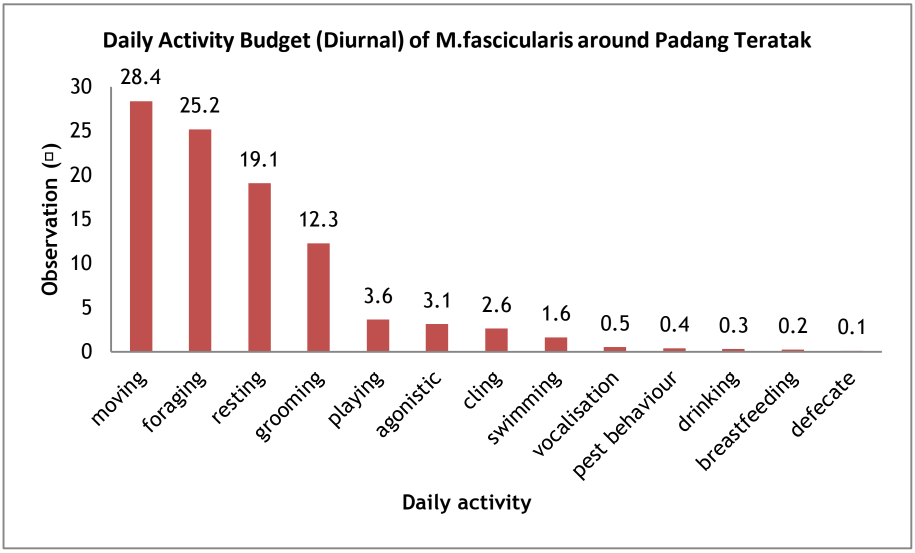
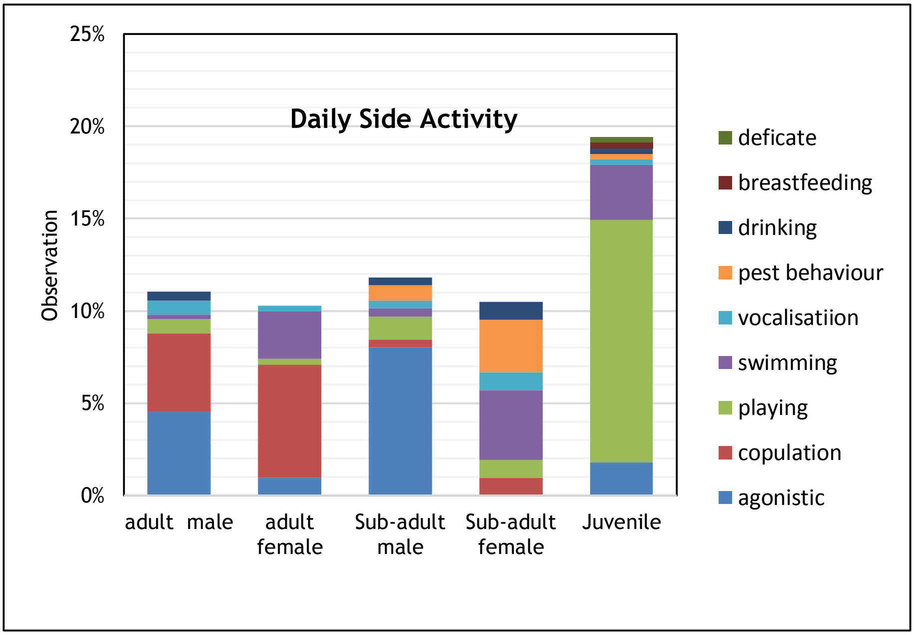
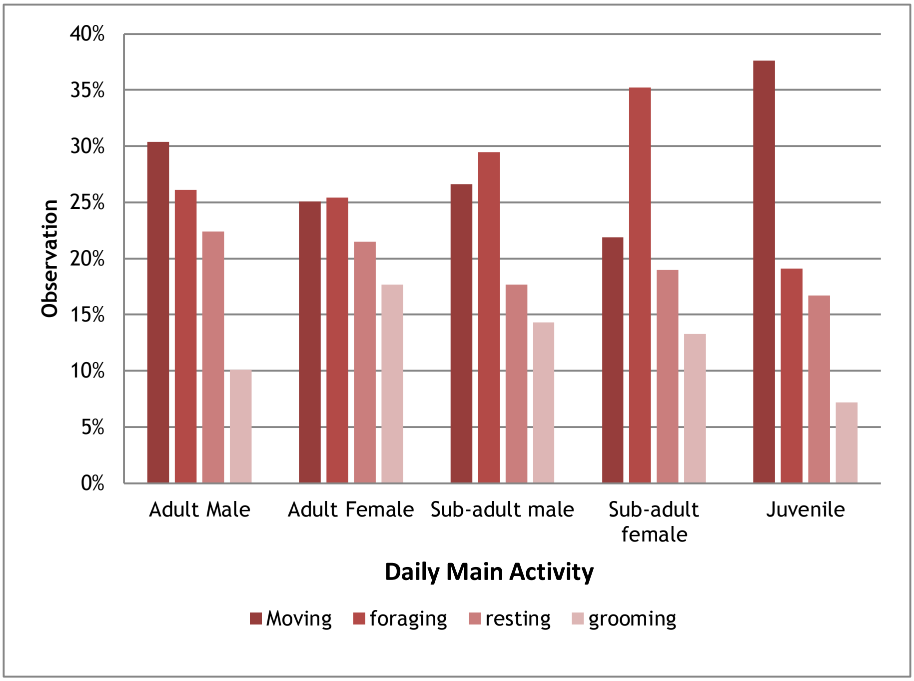
Appendix



Appendix 1. Percentage of Daily Activity Budgets (Diurnal) of macaques at around Padang Teratak.



Appendix 2. Percentage of daily minor activities for adult, sub-adult and juvenile.



Appendix 3. Percentage of daily main activity of macaques by age group: adult, sub-adult and juvenile.

Journal of Tropical Biology and Conservation 16: 185–196, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Diversity of praying mantises (Insecta: Mantodea) in Bukit Piton Forest Reserve, Lahad Datu, Sabah

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Abstract
This study was the first attempt to investigate the praying mantis diversity and composition on a long term basis in a regenerating forest of Bukit Piton Forest Reserve. The study area was a disturbed forest which had been affected by logging activities and forest fires, and undergone a restoration programme. Twelve sampling sessions were carried out over a period of one year, from August 2016 to July 2017, totalling 144 sampling days and nights. Methods included light traps, baited traps, and manual searching. A total of 187 individuals belonging to 19 species were successfully collected. The species sampled represent approximately 16% of the species that occur in Borneo. Mantidae was the dominant family, accounting for about 53% of total species and 36% of total individuals. The dominant species was *Tropidomantis tenera* which belongs to the family Iridopterygidae and made up 27% of the total individuals. The diversity indices showed that the praying mantis cenosis in the area was moderately diverse. This study highlighted the importance of a regenerating forest for the preservation of a significant portion of the biodiversity. We hope that the information obtained from this study will contribute towards a better understanding of the diversity of this fascinating group of insects, as well as the importance of a regenerating forest as a habitat worthy of conservation efforts.

Keywords: praying mantis, Sabah, composition, diversity, disturbed habitat, regenerating forest

Introduction
Praying mantises (order Mantodea) are a group of invertebrates in the class Insecta and the phylum Arthropoda (Sureshan, 2009). In the arthropod community, mantises are known to be top predators (Balakrishnan 2012) and play important roles in the ecosystem. Approximately 2,400 species have been described worldwide (Rivera & Svenson, 2014). Praying mantises are widely distributed in many ecoregions (Ehrmann 2002; Battiston & Fontana, 2010), but most species inhabit the tropics and subtropics (Rivera & Svenson, 2014). Many species can be found in habitats with high structural complexity (Hill et al.,

Received 04 December 2018
Reviewed 02 May 2019
Accepted 10 September 2019
Published 15 October 2019

2004). To date, Borneo is recognized as the island with the highest praying mantis diversity, with a total of 118 species having been recorded (Schwarz & Konopik, 2014).

These fascinating insects are well known for their peculiar habits of prey capture, camouflage, and cannibalistic sexual behaviour. Most species camouflage as part of vegetation such as flowers, leaves, twigs, grasses and tree bark (Mukherjee et al., 1995; Ehrmann 2002; Ghate et al., 2012; Rivera & Svenson, 2014; Schwarz & Konopik, 2014). The camouflage enables them to blend remarkably well with their surroundings (Sureshan, 2009), making them less detectable to their preys and tpredators (Gemeno et al., 2005). However, these characteristics also make praying mantises difficult to observe and sample in the field.

Based on the studies conducted in Sabah, the primary forest harbour a higher number of praying mantis species compared to disturbed habitats (Ling, 2011; Musi, 2017). On the other hand, a short term study conducted in Bukit Piton Forest Reserve showed that the area is a potential habitat for praying mantises (Norman, 2015). Therefore, this study aimed to gather more comprehensive information regarding the species diversity and composition of praying mantises in the area. The information would be useful to understand how regenerating forests could help to sustain biodiversity particularly praying mantises, which are highly dependent on their surroundings for their persistence.

Materials and Methods

a. Background of the study site

Bukit Piton Forest Reserve (N 05°09’, E 118°00’) is located in Lahad Datu, Sabah, and covers an area of 11,612 hectares (Figure 1). It is a lowland mixed dipterocarp rainforest and known to be inhabited by a large population of Orangutans (Simon, 2012; Chung & Majapun, 2013; WWF, 2015). Despite this, the area was severely logged from 1980-2007, and experienced two major fires that completely destroyed the forest in 1983 and 1997-1998 (WWF, 2015). Starting in 2008, a comprehensive restoration programme was introduced to recover the area from the ravages of uncontrolled logging, forest fires and the encroachments of the past.

Previously, this forest reserve was known as Northern Ulu Segama (NUS) before it was renamed Bukit Piton Forest Reserve which forms part of the Ulu Segama-Malua Forest Reserve (Chung & Majapun 2013; WWF, 2015). In 2012, Bukit Piton

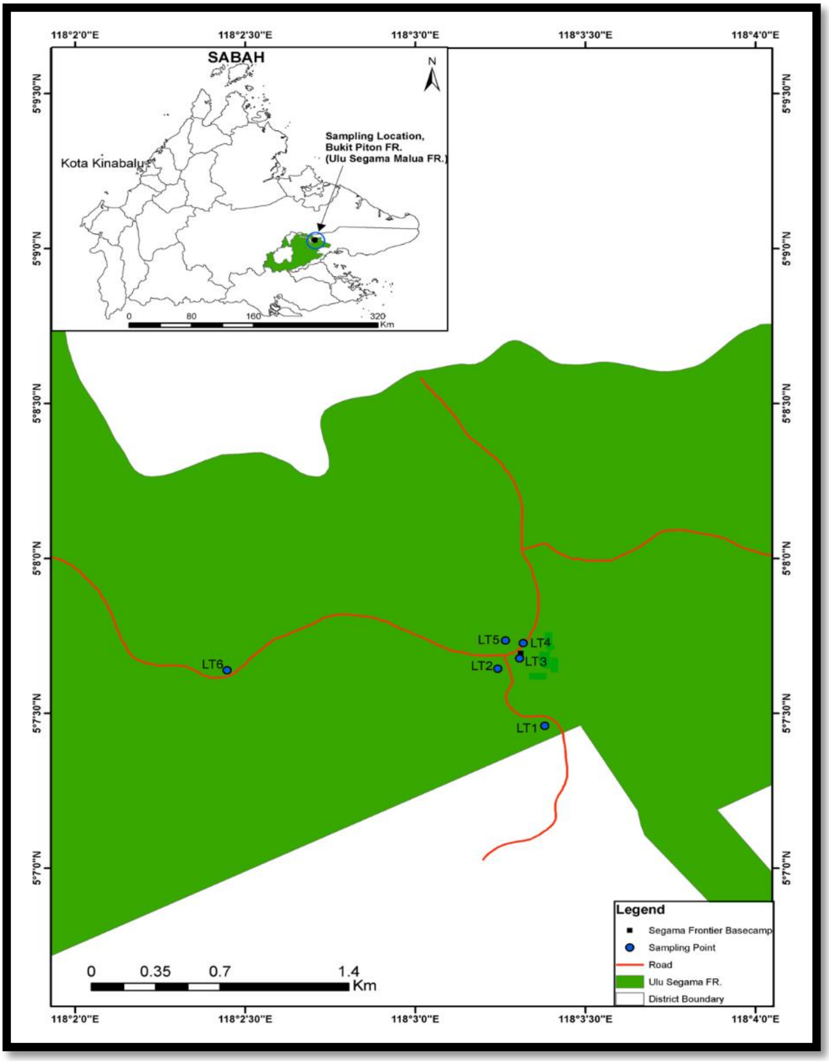

Figure 1. Map of Bukit Piton Forest Reserve (LT=Light trap)

Forest Reserve was upgraded to a Class I Protected Forest from a Class II Commercial Forest Reserve by the State Government of Sabah (Chung & Majapun, 2013).

b. Sampling methods

Praying mantis samplings were conducted over a one year period (August 2016 – July 2017) which included twelve sampling sessions. Three sampling methods were applied, i.e. light traps, baited traps and manual search. The samplings were conducted at six different sites within the study area (Figure 1). Every site was visited twice to reduce seasonal bias. In each month, sampling was carried out for 12 consecutive days and nights. Overall, there were 144 sampling days and nights.

i. *Light Trap*

This method is widely used for praying mantis sampling and has proven to be the most efficient way to collect praying mantises (e.g. Helmkampf et al., 2007; Ling, 2011; Musi, 2017). The trap was set up by using a vertical white sheet which was illuminated by a light source (in this study a lamp powered by a portable generator) (Figure 2). The trap was placed in a suitable area within the study site and operated from 7.00 p.m. to 00.00 a.m. The trap was checked every half an hour and all mantises that came to the trap were collected. To increase the sample size, the area within a 5 m radius around the light trap was also searched for praying mantises by taking into account that females and nymphs have less ability or unable to fly when compared to males, limiting their mobility to the trap.

ii. *Direct Searching*

Mantises were manually searched for along a line transect of 500 m. Every 50 m was marked as a sampling station and mantises were searched for 20 minutes within a 5 m radius of each station. Intense searching and good observation skills are necessary to locate a praying mantis among the surrounding vegetation.

iii. *Baited trap*

Figure 2. The light trap used in this study

Figure 3. A baited trap used in this study

Ten baited traps were set up at 50 m intervals along a 500 m transect. Five traps were baited with bananas while the remaining five traps were baited with tuna.

Each of the traps was hung on a tree branch at breast height (Figure 3). This method was conducted simultaneously while manually searching for mantises. This method was used as an indirect way to catch mantises while they went after prey lured at the baited traps.

c. Mantis preservation

All the specimens were dry preserved and mounted following standard entomological procedures (Figure 4).

The mantises were identified following the available references including Oliveira (1996) (Key to praying mantids), Bragg (2008), Bragg (2010), Helmkampf et al., (2007), Ling et al., (2013), Schwarz & Konopik (2014), and Musi (2017). The specimens were also examined with the mantis collections in BORNEENSIS (collection centre at Institute for Tropical Biological and Conservation, UMS). Most of the specimens were identified to species level, however, several individuals could only be identified to genus level due to ambiguous characteristics. All specimens were deposited in BORNEENSIS.

Figure 4. A praying mantis specimen (*Hierodula* sp.)

d. Data analyses

i. Diversity indices

The diversity of praying mantises at the study site was analysed using the Shannon-Wiener's Diversity Index (H'), Simpson's Reciprocal Index (1/D) and Margalef's Index (D_{mg}) (Magurran, 2004). The analyses were performed by using the Software 'Species diversity and richness' version 2 (Henderson & Seaby, 1998).

ii. <u>*Species rank abundance curve*</u>

A species rank abundance curve was used to analyse the diversity pattern in terms of species abundance at the study area. This method ranks the species from the most abundant to the least (McCabe, 2011). The calculations were done using Microsoft Excel 2010.

Results

a. Praying mantis composition

A total of 187 praying mantis individuals were sampled, comprising 19 species. They belong to five families and 10 subfamilies (Table 1). The species sampled represent 16% of the species that occur in Borneo. Mantidae was the dominant family with 9 species accounting for 53% of the total species and 36% of the individuals.

Tropidomantis tenera was recorded as the dominant species with 51 individuals, i.e. approximately 27% of the individuals collected (Table 1). This species is a member of the family Iridopterygidae.

All individuals were collected from the light trap and only one individual by manual searching, while the baited traps failed to sample any praying mantis.

Table 1. The list of praying mantis species and number of individuals sampled in BPFR

Family	Subfamily	Species	Individual
Hymenopodidae	Acromantinae	***Acromantis* sp.** Saussure, 1870	12
		Citharomantis falcata Rehn, 1909	1
		Psychomantis borneensis De Haan, 1842	5
	Phyllothelyinae	***Phyllothelys* sp.** Wood-Mason, 1876	1
		Ceratocrania macra Westwood, 1889	6
	Hymenopodinae	***Creobroter granulicollis*** Saussure, 1870	2
Iridopterygidae	Tropidomantinae	***Tropidomantis tenera*** Stål, 1860	51
Liturgusidae	Liturgusinae	***Humbertiella ocularis*** Saussure, 1872	14
Mantidae	Angelinae	***Euchomenella matilei*** Roy, 2001	39
	Amelinae	***Gonypeta punctata*** De Haan, 1842	5

		Myrcinus tuberosus Stål, 1877	3
	Mantinae	***Hierodula dyaka*** Westwood, 1889	1
		***Hierodula* sp.** Burmeister, 1838	1
		Hierodula venosa Olivier, 1792	2
		Tenodera blanchardi Giglio-Tos, 1912	1
		Statilia maculata Thunberg, 1784	11
	Deroplatyinae	***Deroplatys desiccata*** Westwood, 1839	1
		Deroplatys truncata Guérin-Méneville, 1843	3
Tarachodidae	Caliridinae	***Leptomantella* sp.** Uvarov, 1940	27
5	**10**	**19**	**187**

b. Species diversity

The overall findings showed that the praying mantises in the study area were moderately diverse, as indicated by the diversity indices (Shannon-Wiener's Diversity Index (H') - 2.20; Simpson's Reciprocal Index (1/D) - 6.54; Margalef's index (D_{mg}) - 3.44). The result could be influenced by a few abundant species such as *Tropidomantis tenera.*, *Euchomenella matilei* and *Leptomantella* sp. Several species were sampled as singletons.

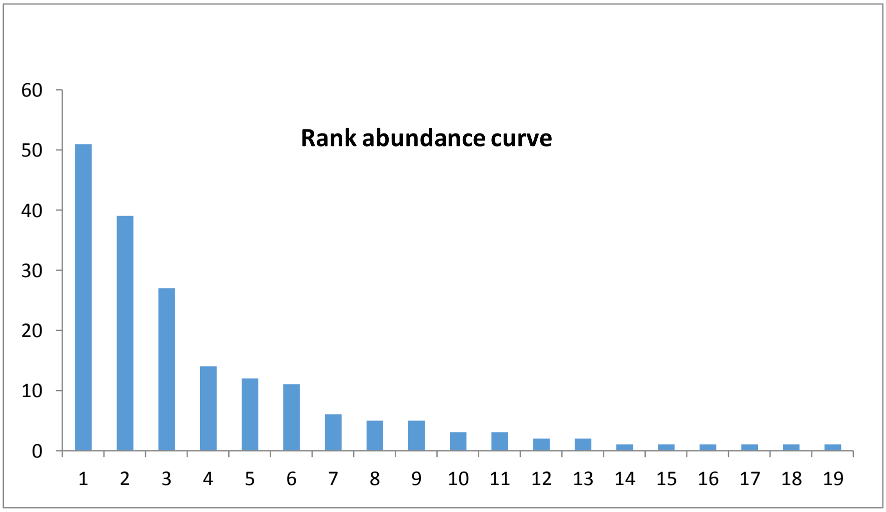

Figure 5. Species rank abundance curve

Discussion

Habitat disturbance is one of the major threats to biodiversity (Montoya, 2008). Forest fires and logging have been recognized as disturbances that commonly occur in forest habitats. However, few studies have been carried out to document species diversity in disturbed forest habitats which have undergone regeneration. Some studies show that habitat structure is important for species survival, mainly for those species that depend on highly cluttered surroundings, such as praying mantises (Hill et al., 2004; Mustaffa et al., 2015). The importance of habitat selection varies across species. For example, heterogenous surrounding vegetation increases camouflaging ability in praying mantises, thus enabling them to hunt for prey or hide from predators efficiently.

This study showed that even a highly disturbed habitat such as Bukit Piton FR can still harbour a moderate diversity of mantis species. Several species that have been recorded in primary forests were also found in the study area, such as *Psychromantis borneensis, Humbertiella ocularis, Euchomenella matilei* and *Deroplatys desiccata* (Musi, 2017). The species richness and abundance of praying mantises in Bukit Piton FR may be related to the regenerating vegetation within the area. Nine species were recorded as singletons. Ulu Segama-Malua FR currently boosts the largest extent of restored and rehabilitated Dipterocarp forest in the world (Sabah Forestry Department, 2017). The forest restoration activities that have been implemented in the area might have improved the forest habitat quality, therefore allowing more species to inhabit and exploit the available resources. Regenerating tropical forest can sustain about 57% of the primary forest biodiversity and has high conservation value (Whitworth et al., 2016). Furthermore, a study by Edwards et al. (2017) suggested that secondary forest can play a vital role in conserving phylogenetic diversity of birds.

In this study, *Tropidomantis tenera* was found to be dominant in the study area. This species was reported to be a generalist species and common in both primary and disturbed habitats, since the females are capable of flying and are attracted to light (Schwarz & Konopik, 2014). This could explain its high abundance in the study area. In comparison, most studies conducted in the primary forests in Sabah have recorded several species of Mantidae as dominant species (e.g Ling, 2011; Musi, 2017; Helmkampf et al., 2007).

In conclusion, a regenerating forest could provide sustainable resources for particular species to occur despite the damage that the forest had experienced in the past. This aspect has been widely neglected and the focus for species conservation has mostly been on primary forest or an undisturbed habitat. We hope that more initiatives will be put forward to study species persistence and biodiversity in regenerating forest habitats.

Acknowledgements

First of all, we would like to thank Universiti Malaysia Sabah for the research grant (GUG0044-ST-M-1/2016) and the Institute for Tropical Biology and Conservation (ITBC) for the technical support. We also thank Sabah Forestry Department for the permit to conduct the fieldwork at Bukit Piton Forest Reserve (Ulu Segama-Malua Forest Reserve) Lahad Datu, Sabah. We would also like to express our highest gratitude and appreciation to the Director of Segama Frontier, Mr. Norman Burhan and his staffs for their assistance in providing facilities, logistic and field support. Finally, we are grateful to Madam Nurain binti Musi for her assistance in the specimen identifications.

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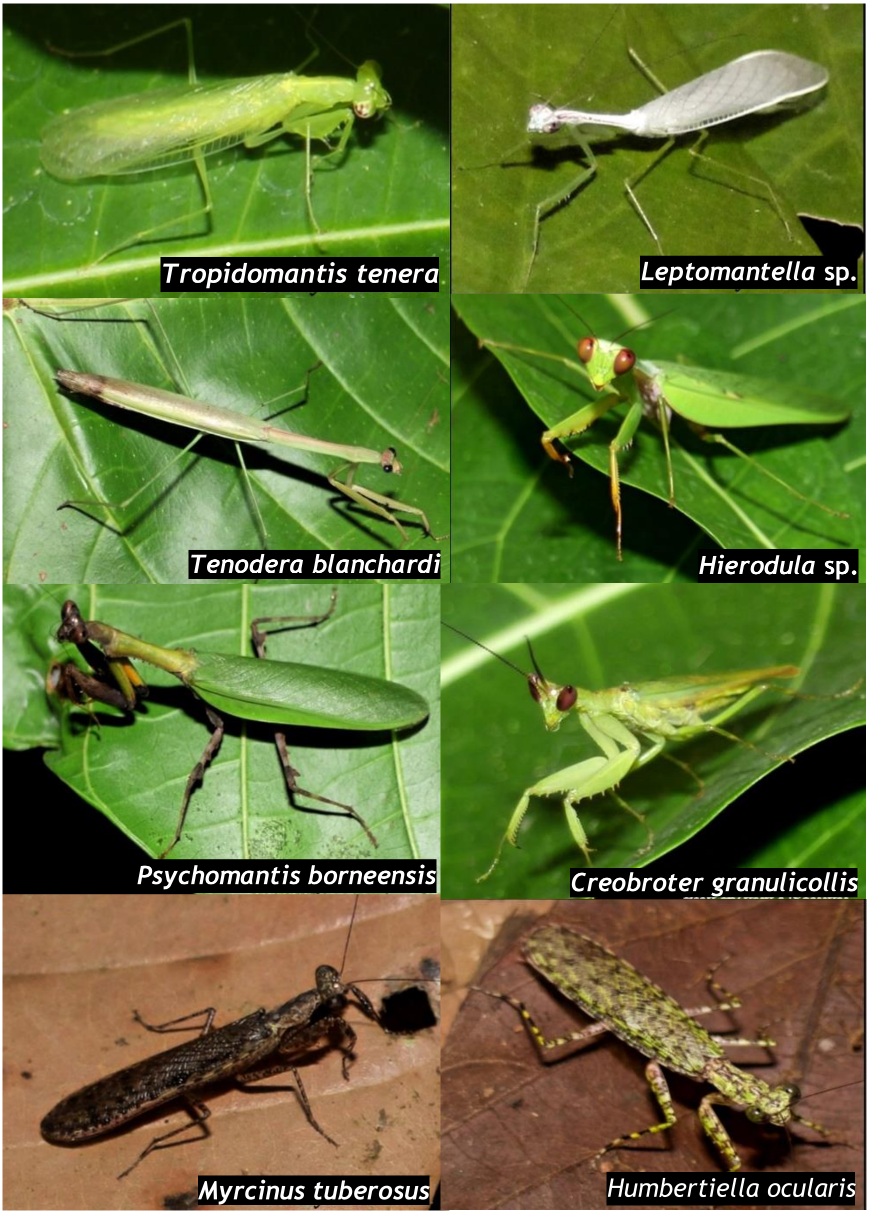
Appendix

Examples of praying mantis species collected in Bukit Piton Forest Reserve, Sabah. Photo by Effa Liyana Norman (2019).




Deroplatys desiccata
Ceratocrania macra
Gonypeta punctata
Statilia maculata
Deroplatys truncata
Euchomenella matilei

Journal of Tropical Biology and Conservation 16: 197–211, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

Assessing the Relatedness of *Abelmoschus* Accessions using Morphological Characters

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Abstract
Character analysis of Okra (*Abelmoschus* [Medik.] species, Malvaceae) accessions was carried out using morphological data to evaluate their genetic distinction and relatedness. Seeds of five *Abelmoschus* accessions (NG/MR/01/10/002, A.E 3, NG/MR/MAY/09/009, NGAE-96-0065 and NG/OA/05/12/160) were obtained from the Gene Bank of National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria. Based on the International Board for Plant Genetic Resources standard descriptors for Okra, 16 qualitative morphological characters were selected based on their relevance to *Abelmoschus* breeding, crop distinction, utilization and conservation. The five accessions present significant differences with two of the accessions (NG/MR/MAY/09/009 and NG/OA/05/12/160) closely related and other three (NG/MR/01/10/002, A.E 3 and NGAE-96-0065) closely related too. Accessions NG/MR/01/10/002, A.E 3 and NGAE-96-0065 had medium or intermediate growth habit while accession NG/MR/MAY/09/009 and NG/OA/05/12/160 shows erect growth habit. General aspect of the stem, nature of branching, fruit pubescence, fruit shape, position of the fruit on the main stem, leaf shape, fruit colour, and fruit length at maturity had the most effect on observed relationship between the accessions. Scatter plots derived from the principal component analysis suggest moderate tendency of grouping with the genus where two distinct clusters were obtained from the dendrogram. Together, these results suggest that the five okra accessions may be the descendants of the two commonly cultivated *Abelmoschus* species in Southern Nigeria (i.e. *A. esculentus* and *A. caillei*).

Keywords: Okra (*Abelmoschus* species), Genetic diversity, Plant Conservation, Character analysis, Morphological data

Received 16 April 2019
Reviewed 08 July 2019
Accepted 22 July 2019
Published 15 October 2019

Introduction

Okra (*Abelmoschus* [Medik.] species) belongs to the family Malvaceae and order Malvales. Angiosperm Phylogeny Group (2009) recognized it as a monophyletic group. The group contains economically important crops used for food, fibre vegetable oil, horticulture, timber, medicine, etc. Common examples include jute (*Corchorus*) and cotton (*Gossypium*). Members of this group are cultivated throughout the tropics and subtropics in home gardens as well as on large commercial farms (Osawaru et al., 2014). Their cultivation is influenced by environmental conditions. For instance, Shahid et al. (2011) reported that increasing salinity causes a decrease of germination percentage, shoot and root length, plant height, pod weight, pod length, photosynthesis rate, and stomatal conductance. *Abelmoschus* species is a multipurpose and functional food crop with increasing importance especially with regard to its nutritional, medicinal, and industrial value (Reddy et al., 2013; Ogwu et al., 2016a). Osawaru et al. (2011) reported that although about 60% of okra grown is for subsistence as fresh vegetable, okra parts and products remain the subject of major economic activities in West Africa. Okra production plays a significant role in the economy, hence more attention should be given to the selection of high yielding cultivars to maximize its socio-economic potential (Adeoluwa & Kehinde, 2011; Kumar et al., 2010).

Abelmoschus species have diverse morphological characteristics in Africa and Asia. Some are annual herbs, perennial shrubs or trees and produce a characteristic mucilaginous substance (Edwin et al., 2006). In spite of its high acceptability among growers, consumers and their wide genetic pool variability, optimum productivity is still lagging (Das et al., 2012). Eshiet and Brisibe (2011) opined that cultivated okra rarely reach their maximum yield potential due to several constraints including poor investments in breeding programmes aimed at enhancing their yield in the field. Nonetheless, okra has huge potential for enhancing livelihoods in urban and rural areas as it offers a possible route to prosperity for small-scale and large-scale producers as well as to all those involved in the okra value chain, including women producers and traders (Kumar et al., 2010). The first step towards this goal is to address their taxonomy. Taxonomy of *Abelmoschus* has a complex history with uncertainty in the generic and specific status because of the inconsistent treatment for some *Abelmoschus* species (Patil et al., 2015). Characterization studies will help highlight distinct descriptive features that may be explored in plant breeding. The success of a breeding programme depends on the variability of the initial material as well as available knowledge base of the genetic system, which is important for devising an efficient selection programme through the use of a suitable mating design

(Reddy et al., 2013). More so, characterization of genetic resources is an essential first step in any crop improvement (Das et al., 2012) and conservation programme (Ogwu et al., 2014; Osawaru and Ogwu, 2014). Characterization and quantification of genetic diversity within and among closely related crop varieties are essential for their rational use (Adeoluwa and Kehinde, 2011). Diversity based on phenotypic and morphological characters usually varies with environments; hence the evaluation of traits may require their cultivation to maturity prior to identification and characterization (Adeoluwa and Kehinde, 2011). Through characterization, the nature of the relationship between and among plant species can be highlighted with the help of description keys (Osawaru et al., 2015a). This is necessary since the selection of superior genotypes may be based on outward appearance (phenotype), which is subject to variation due to fluctuating environmental factors (Adeoluwa and Kehinde, 2011).

Reproductive and vegetative morphology of *Abelmoschus* species is known to be variable but the patterns of this variation and their relevance in the crop distinction, breeding, germplasm utilization and conservation have not been sufficiently addressed (Patil et al., 2015, Osawaru et al., 2015b; Ogwu et al., 2016). This study aims to characterize *Abelmoschus* accessions based on morphological data with a view to assess their genetic relationship, highlight the key descriptive character and to promote the conservation and sustainable utilization of the germplasm.

Materials and Methods

Material collection and cultivation: Seeds of five accessions of *Abelmoschus* species (NG/MR/01/10/002, A.E 3, NG/MR/MAY/09/009, NGAE-96-0065 and NG/OA/05/12/160) were obtained from the Gene Bank of the National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria.

The seeds were grown on an experimental field beside the Botanical Garden, University of Benin, Nigeria using randomized complete block design with three replicates per accession. Fifty pre-soaked (for 24 h) seeds (10 from each accession) were sown directly in the soil (3 – 5 cm deep) per plot with a spacing of 40 × 30 cm (row to row and plant to plant) and covered with top soil. Seedlings were thinned to three plants per stand two weeks after germination. The physicochemical and microbial characteristics of the plot have been reported by Osawaru et al. (2013a; 2013b); Osawaru and Ogwu (2014b); Ogwu and Osawaru

(2015b). Standard agronomic practices such as weeding and watering were adopted from Remison (2005).

Morphological characterization: Data were collected on morphological characters using standardized descriptors for o (IBPGR, 1991). Based on this descriptor, 16 qualitative characters were measured.

Table 1. Code used in morphological characterization.

S/N	Parameter measured	Parameter key	Character state
1	General aspect of stem	GAS	3=erect, 5=medium, 7=procumbent
2	Stem colour	StC	1=green, 2=green with red patches, 3=purple
3	Stem pubescence	SPu	3=glabrous, 5=slight, 7=conspicuous
4	Nature of Branching	Brc	3=orthotropic stem only, 5=medium, 7=strong
5	Leaf shape	LSh	From types 1 to 11
6	Leaf colour	LCl	1=green, 2=green with red veins, 3= red
7	Petiole colour	PtC	1=green, 2=green with red veins, 3=purple
8	Flowering span	FSp	1=single flowering, 2=grouped flowering
9	Petal colour	PCl	1=cream, 2=yellow, 3=golden
10	Fruit colour	FCl	1=yellowish green, 2=green, 3=green with red patches, 4=red
11	Position of fruit on main stem	PFS	3=erect, 5=horizontal, 7=pendulous
12	Fruit shape	FSh	From types 1 to 15
13	Fruit pubescence	FPu	3=downy, 5= slightly rough, 7=prickly
14	Length of peduncle	LPe	1=from 1 to 3cm, 2=more than 3cm
15	Fruit length at maturity	FLM	1=less than 7cm, 2= from 8 to 15cm, 3= more than 15cm
16	Seed shape	SSh	1=round, 2=reniform

Statistical analysis: Multivariate statistical analyses were employed to assess the genetic relationship among accessions as suggested by the morphological data. Data collected were analyzed using SPSS (version 20.0) and PAST (paleontological statistics, version 1.34). Principal Component Analysis (PCA), Scatter Plot and Single Linkage Cluster Analysis (SLCA) were used to determine the extent of genetic variation and percentage similarities within and between accessions. Eigen-values, factor scores and scatter diagram obtained from PCA were used to determine the relative discriminative power of axes and their associated characters. The dendrogram was generated from the SCLA to display position of accessions and their distance similarity.

Result

The results are shown in Tables 2 - 4 and Figures 1 and 2.
The results of morphological trait assessment of the five accessions of *Abelmoschus* species is presented in Table 3.

The principal component analysis was conducted for 16 qualitative morphological characters among the five accessions of *Abelmoschus* spp. This shows that only one of the 16 principal component axes had eigenvalues greater than 2.43 as Joliffe cut-off standard value according to Joliffe (2002).

The eigenvalue tells the importance of each principal component axes and its contribution in explaining the variability in characters of five accessions of *Abelmoschus* spp. The PCA eigenvalue includes: PC 1 (10.54), PC 2 (2.36), PC 3 (0.58) and PC 4 (0.43); 0.40 was taken as the standard.

Table 2. Qualitative traits that varied among the five accessions of *Abelmoschus* species

S/N	Accession Parameter Measured	NG/MR/01/10/002	A.E 3	NG/MR/MAY/09/009	NGAE-96-0065	NG/OA/05/12/160
1	GAS	5	5	3	5	3
2	StC	2	2	2	2	2
3	SPu	3	3	3	3	3
4	Brc	5	7	3	7	3
5	LSh	4	4	4	4	4
6	LCl	2	2	2	2	2
7	PtC	2	2	2	2	2
8	FSp	1	1	1	1	1
9	PCl	3	3	2	2	2
10	FCl	2	3	2	2	3
11	PFS	7	7	3	7	3
12	FSh	4	4	4	4	1
13	FPu	5	7	3	5	3
14	LPe	1	2	1	2	1
15	FLm	2	2	1	2	2
16	SSh	2	2	2	2	2

Key:
GAS- general aspect of stem, **SP**- stem pubescence, **Br**-branching **StC**-stem colour **LC**-leaf colour, **PC**- petal colour, **PFMS**-position of fruit on main stem, **FC**- fruit colour, **FLm**- fruit length at maturity, **LP**-length of peduncle, **FSh**- fruit shape, **FP**- fruit pubescence, **SSh**- seed shape, **FSp**- flowering span **LSh**- leaf shape, **PtC**-petiole colour

Table 3. Principal Component Analysis (PCA) of 16 qualitative morphological characters among five accessions of *Abelmoschus* species

Characters code	PC 1	PC 2
GAS	1.04	0.08
SPu	2.26e-17	3.15e-17
Brc	0.59	0.27
StC	-8.42e-20	8.39e-18
LCl	-3.12e-22	1.74e-19
PCl	0.11	0.03
PFS	0.32	0.08
FCl	-0.02	0.29
FLM	0.06	0.23
LPe	0.13	0.10
FSh	0.30	-0.59
FPu	0.48	0.26
SSh	0	0
FSp	0	0
LSh	-0.30	0.59
PtC	0	0
Eigenvalue	10.54	2.36
% Variance	75.80	16.97

Key:
GAS- general aspect of stem, **SPu**- stem pubescence, **Brc**-branching **StC**-stem colour **LCl**-leaf colour, **PCl**-petal colour, **PFS**-position of fruit on main stem, **FCl**- fruit colour, **FLM**- fruit length at maturity, **LPe**-length of peduncle, **FSh**- fruit shape, **FPu**- fruit pubescence, **SSh**- seed shape, **FSp**-flowering span **LSh**-leaf shape, **PtC**-petiole colour **PC**- Principal component.

The loading of each character on the different principal component axes is used to assess their relative contribution in showing variation. The following characters was heavily loaded along PC axis 1; GAS (1.04), Brc (0.59), FPu (0.48), FSh (0.30) and PFS (0.32) while LSh (0.59), FCl (0.29) and FLM (0.23) were loaded along PC axis 2.

Table 4. The Standardized Principal Component scores

Accession	PC 1	PC 2	PC 3	PC 4
NG/MR/01/10/002	**1.077**	-0.576	0.588	0.966
A.E 3	**3.326**	0.883	0.649	-0.711
NG/MR/MAY/09/009	-2.450	-2.201	0.058	-0.428
NGAE-96-0065	**2.284**	0.032	-1.245	0.063
NG/OA/05/12/160	-4.237	**1.862**	-0.044	0.110

Key:
PC - Principal component.

The standardized principal component scores of the various accessions suggest that the first two principal component axes account for 92.77 % of the variance from the 16 qualitative morphological parameters (axes). PCA 1 loaded characters are possessed by accession A.E 3, NGAE-96-0065 and NG/MR/01/10/002 respectively. PCA 2 loaded characters are best possessed by accession NG/OA/05/12/160 only.

Accession A.E 3, having seen to have a high PCA score in PCA 1 could be well distinguished by characters in PCA 1. For instance, the general aspect of stem, branching and fruit pubescence are seen to be high in accession A.E 3 and NGAE-96-0065 respectively and highly loaded in PCA 1. These characters are outlined in Table 3.

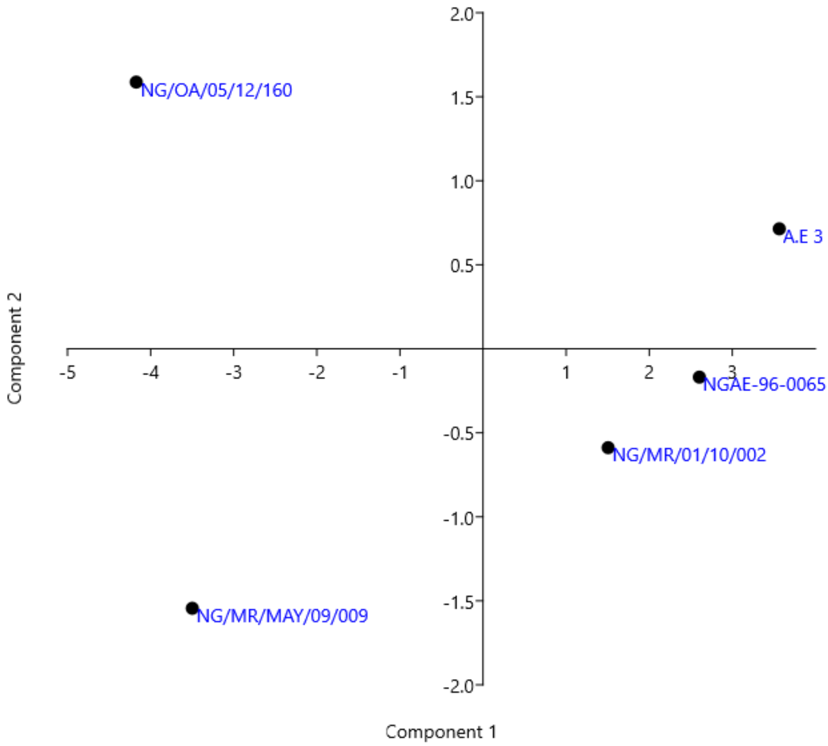

Figure 1: PCA scatter diagram produced by plotting the first PC against the second PC for 16 qualitative morphological characters of five accessions of *Abelmoschus* species

The scatter plots derived from the PCA also show the grouping tendency of the accession studied by grouping accessions NG/MR/MAY/09/009 and NG/OA/05/12/160 together and accessions NG/MR/01/10/002, A.E 3 and NGAE-96-0065 in the other component.

The clustering pattern of the dendrogram also suggests the existence of two groups like *A. caillei and A. esculentus*, which are the two common *Abelmoschus* species in Southern Nigeria and West Africa.

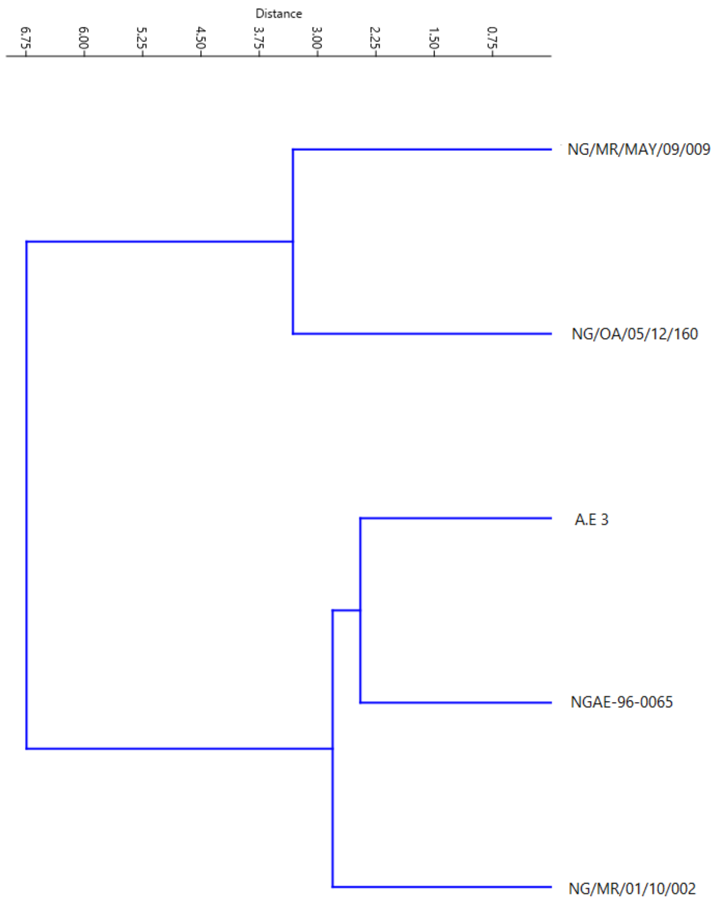

Figure 2: Dendrogram based on 16 qualitative morphological characters of five accessions of *Abelmoschus* species

Discussion

Variation is an important attribute in breeding programmes (Hazra and Basu, 2000; Omonhinmin and Osawaru, 2005). *Abelmoschus* species characterized in this study showed variation for eight traits including general aspect of the stem, nature of branching, fruit pubescence, fruit shape, position of the fruit on the main stem, leaf shape, fruit colour, and fruit length at maturity. These characters may allow for the identification, distinction, breeding and conservation of okra accessions in Nigeria and all over the world. This supports the findings reported in Yildiz et al. (2015); Ogwu et al. (2016) that fruit, leaf, and stem morphologies are the primary characteristics exploited to distinguish the okra accessions. The variation in general aspects of the stem, branching, leaf shape, petal colour, fruit colour, fruit shape, position of fruit on the main stem, fruit pubescence and length of peduncle are easily recognizable with visual approach by the use of colour chart and IBPGR, 1991 descriptor list of *Abelmoschus* species.

The observed variations in the growth habit and other characters may have evolved over time to suit environmental conditions. For instance, the erect stems allow for maximum and uniform exposure or distribution of all leaves and other vegetative parts for better interception of sunlight, and would also result in an increase in dry matter production and subsequent increase in yield (Oppong-sekyere et al., 2011). The intermediate nature of *Abelmoschus* species allows for larger and continuous fruit harvest. This is an advantage when the price of vegetables fluctuate (Oppong-sekyere et al., 2011). Variations in fruit characteristics may be indicative of differences in the genetic make-up of the plant (Ogwu et al., 2016). In addition, *Abelmoschus* species with determinate growth pattern and pronounced branching is likely *A. caillei* while those with indeterminate growth pattern and orthotropic branching as *A. esculentus* (Omonhinmin & Osawaru, 2005).

Moreover, Omonhinmin and Osawaru, (2005) reported that profuse branching in *Abelmoschus* may indicate high yield potential, as branches are production sites and hence the higher their number the greater the potential for yield. Profuse branching that was observed in NG/MR/01/10/002, A.E 3 and NGAE-96-0065 may be incorporated into NG/MR/MAY/09/009 and NG/OA/05/12/160 in order to produce *Abelmoschus* species that are less lodging with higher yield potentials for commercial production. Fruit with characteristics such as smooth, spineless, slender with green skin are desirable characteristics in Nigeria markets. Accession NG/MR/MAY/09/009 and NG/OA/05/12/160 display such characters. These can, therefore, be selected for breeding by crossing them with accession

NG/MR/01/10/002, A.E 3 and NGAE-96-0065, which are high yielding with longer harvest duration.

Results obtained in this study revealed variation in fruit colour among the five accessions. Accession NG/MR/01/10/002, NGAE-96-0065 and NG/MR/MAY/09/009 were green in colour while accession A.E 3 and NG/OA/05/12/160 were green with red patches. This was in accordance with the result of Oppong-sekyere et al., (2011). The results also suggest that the five accessions of *Abelmoschus* species exhibited varying degree of fruit pubescence including downy, slightly rough and prickly. Accessions NG/MR/MAY/09/009 and NG/OA/05/12/160 had downy fruit pubescence while accessions NG/MR/01/10/002 and NGAE-96-0065 had slightly rough fruit pubescence and accession A.E 3 showed prickly fruit pubescence. This was in accordance with the result of Thomas, (1991) and Bish et al., (1995). These features serve protective functions for the fruit.

The position of fruit on the main stem was pendulous in NG/MR/01/10/002, A.E 3 and NGAE-96-0065 and erect in NG/MR/MAY/09/009 and NG/OA/05/12/160. Omonhinmin and Osawaru (2005) reported that *Abelmoschus* spp with the pendulous position of fruit in relation to the stem are *A.* caillei while that one with erect to horizontal position was reported as *A. esculentus*. Several researchers Bish et al. (1995); Akinyele and Oseikita (2006); Oppong-sekyere et al. (2011); Omonhinmin and Osawaru (2005); Osawaru *et al.* (2013); Osawaru et al. (2014); Osawaru et al. (2015b); Osawaru *et al.* (2016) have investigated the diversity of different morphological traits within *Abelmoschus* species. Put together, their results suggest that a large number of *Abelmoschus* characters such as pigment colour and spines on fruit surface are inheritable suggesting that they are controlled by relatively few genes.

The multivariate analysis applied in this study was useful in tracing the possible relationship between the five accessions. Using multivariate techniques Osawaru et al., (2012) and other researchers showed its importance in numerical taxonomy. Twenty-one morphological characters were standardized, analyzed and subjected to principal component analysis and single linkage clusters analysis. The PC loading of each character on the different principal component axes is shown in Table 4 and was used to assess their relative contribution in showing variation. PC 1 was affected by characters such as general aspect of stem, branching, fruit pubescence, fruit shape, position of fruit on main stem and leaf shape. PC 2 was significantly loaded with characters such as fruit colour and fruit length at maturity. These characters account for the high variability observed in the accessions studied. However, principal component analysis alone would not give an adequate character representation in terms of relative importance when numerous characters are considered simultaneously (Shalini et al., 2003). To complement such result; scatter diagram was employed with idea of tracing the grouping tendency within the genus *Abelmoschus* as used by Wickremasinghe and Herat, (2006) and single linkage cluster analysis was also employed to classify the variation and show relationship pattern among the five accessions as used by Ariyo and Odulaja (1991). The scatter plots of PC 2 versus PC 1 (Figure 1) indicates a good tendency of grouping of the five accessions. Accession A.E 3 was grouped with NGAE-96-0065, this shows that the above accessions are to a certain extent, related morphologically. The five accessions were grouped into two distinct cluster groups with accession NG/MR/01/10/002, A.E 3 and NGAE-96-0065 all clustering in one and NG/MR/MAY/09/009 and NG/OA/05/12/160 160 (Figure 2). This could be used to identify them as having a common ancestral origin with a common gene. These variations within the genus *Abelmoschus* could be used for breeding that incorporates required traits and for proper classification. Hence, we recommend crosses to be done between accessions in the different cluster groups. From a phylogenetic perspective, all the accessions are likely descendants of the commonly cultivated *Abelmoschus* in Southern Nigeria and parts of West Africa.

Conclusion

This study highlighted the relevance of key descriptive *Abelmoschus* characters including general aspect of stem, branching, fruit pubescence, fruit shape, position of fruit on main stem, leaf shape, fruit colour and fruit length at maturity. These characters may be used to distinguish different *Abelmoschus* accession and can be exploited in the selection of okra germplasm for cultivation. The 16 morphological characters suggest that the five accessions probably belong to two *Abelmoschus* species. However, further studies using molecular characterization is required to confirm the findings reported in this study.

Acknowledgement

The authors appreciate the help of the National Center for Genetic Resources and Biotechnology, Ibadan, Nigeria.

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Journal of Tropical Biology and Conservation 16: 213–227, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Effects of mechanical and acid scarification on germination performance of *Schizolobium parahyba* (Fabaceae – Caesalpinioideae) seeds

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Abstract

Improving seed germination of native species is fundamental for assisting restoration practices, particular in highly degraded ecosystems such as tropical moist forests. Tropical moist forests of Central and South America continue to decrease as a result of fragmentation and conversion of forested land to agriculture. *Schizolobium parahyba* is a pioneer legume tree species widely used in restoration practices due to its fast growth rate, nitrogen-fixing capacity, and wood properties. Seeds of this species exhibit low germination as a result of physical dormancy, which highly limits its propagation on a large scale. We evaluated the effects of mechanical and acid scarification treatments with solutions of sulfuric (10%, 20%) and chloridric (25%, 50%, 75%) on *Schizolobium parahyba* seed germination. Mechanically scarified seeds had higher germination percentage (92.5%) than seeds treated with chloridric acid (50%), sulfuric acid (33.13±.2.11%) or intact seeds (17.5%). Seeds soaked in 10% sulfuric acid for 1 and 5 minutes exhibited higher germination values than seeds soaked in 20% for 10 minutes. Seeds soaked in 75% and 50% chloridric acid solutions for 5 and 10 minutes had an overall higher and faster germination than seeds soaked in 25% for 1 minute. This study indicates that mechanical scarification and acid scarification with solutions of chloridric acid solutions of 50% and 75% can highly improve large-scale propagation of *S.parahyba* and thus assist habitat restoration and conservation practices in degraded moist tropical forests.

Keywords: endangered ecosystems, physical dormancy, seed conservation, seed germination, seed vigour, tropical trees.

Introduction

Improving seed germination rates of native species is fundamental for assisting restoration practices, particular in highly degraded ecosystems. Tropical forests play important roles in biodiversity conservation, carbon storage and climate

Received 21 January 2019
Reviewed 09 July 2019
Accepted 07 August 2019
Published 15 October 2019

regulation (Spracklen et al., 2015). However, about 1.1 million km^2 of tropical forest has been lost over the period 2000 to 2012 (Hansen et al., 2013). From 2010 to 2015, the natural forest area has decreased by a net 6.5 million ha per year (FAO, 2016). Tropical forest cover in South America continues to decrease at an annual rate of 0.40 million ha (FAO, 2016) as a result of habitat fragmentation, logging, fire, and conversion to agriculture and other land uses (Celis & Jose 2011; Gustafsson et al., 2016). Ongoing tropical deforestation threatens global biodiversity and ecosystem services significantly (Spracklen et al., 2015) and global climate change is largely altering species distribution, composition and forest structure (Deb et al., 2018). Therefore, restoration efforts are highly needed to recover at least some of the functions and diversity of heavily degraded tropical forests (Chadzon, 2003).

Forest restoration practices of degraded habitats often involve the establishment of single-species tree plantations that increase site fertility and thus facilitate native forest succession (Chadzon, 2003). Pioneer plant species are particularly important because they can ameliorate above- and below-ground environmental conditions and thus facilitate the establishment of late-successional native species (Rodrigues & Rodrigues, 2014). Rapidly-growing tree species, particularly nitrogen-fixing legumes, can increase organic matter in the soil, prevent erosion, and enhance nutrient cycling (Chadzon, 2003). Direct seeding, a technique in which seeds are introduced directly on regeneration sites, has been shown to improve disturbed habitats in the tropics (Bonilla-Mohello & Holl, 2010; De Souza & Scariot, 2014; Hossain et al., 2014; Muñoz-Rojas et al., 2016). However, before starting a forest restoration programme with native tree species, it is essential to know the germination requirements of the species to be used (Ferreira & Santos, 2012). The knowledge of seed germination requirements is thus fundamental to assist habitat restoration practices and the reestablishment of native plants (Blakesley et al., 2002).

Schizolobium parahyba (Vell. Conc.) S. F. Blake (Fabaceae–Caesalpinioideae) is a pioneer deciduous tree species found mostly in gaps and along forest borders from southern Mexico to Brazil (Maldonado & Escobar, 1999). Mature trees typically have a straight trunk, up to 40 meters tall and 80 cm wide, that branches out only near the top. This tree species is widely used in silvicultural, agroforesty, and restoration practices due to its fast growth rate (it can reach up to 8 -10 m tall after 2 years) and nitrogen-fixing capacity (Lorenzi, 2002). The ability of fixing nitrogen allows this species to survive in poor-quality soils and hence facilitates the establishment of native species by improving soil fertility (Orwa et al., 2009). Trees also provide shade in coffee plantations and are tolerant to a wide range of rainfall levels, temperatures and soil conditions (http://www.worldagroforestry.org). Its lightweight wood (density 0.32 g/ cm^3) is used to make boxes, liners, boards, matches, toys, model aircrafts (Freire et al., 2015) light construction, furniture, and handicrafts (Oliveira Silva et al., 2018). Trees are also highly valued as ornamentals for roadsides and urban planting.

Despite its ecological importance, low and erratic germination due to physical seed dormancy highly limits its large-scale propagation (Carvalho, 2005). Physical dormancy is caused by one or more water-impermeable layers of palisade cells in the seed or fruit coat (Baskin & Baskin, 2014). Physical seed dormancy can be overcome with mechanical and chemical scarification methods that disrupt the seed coat allowing water movement to the embryo (Baskin & Baskin, 2014). Pre-sowing treatments such as cold stratification, mechanical disruption, or immersion in acid and hot water are widely used because they can improve germination within a relatively short period (Tadros et al., 2012). Although several chemical and mechanical methods have been used to break the physical dormancy of *S. parahyba* seeds (Alves de Azeredo et al., 2003; Freire et al., 2007; Yubero, 2011; Castro Nina, 2016), large-scale propagation programmes and restoration practices of degraded habitats still require rapid and uniform seed germination of *S.parahyba* (Mateus Alves et al., 2017). Testing scarification treatments is thus necessary to further improve seed germination rates of *S.parahyba*. In this study we evaluated the effects of several pre-sowing treatments such as mechanical scarification (sandpaper) and acid scarification with solutions of sulfuric acid (10% and 20%) and chloridric acid (25,50 and 75%) for 1,5 and 10 minutes on the germination of *S. parahyba* seeds. The effects of these acid concentrations and times of immersion have not been tested previously in the seeds of this species.

Materials and Methods

Mature seeds were randomly collected from about 50 trees located in a five ha forest plantation located 35 km south of the town of San Luis, Antioquia, Colombia (05° 37’ 54’’N, 75º02’02’’W, 450 m.a.s.l). This forest plantation was established using seeds from outstanding phenotypes of different forest populations in Colombia. Approximately 100 seeds were collected from each tree. Seeds were collected from selected trees that exhibited straight trunks, high quality wood, high seed production, and represent about 70% of the population of the best trees in the plantation (Rodriguez and Nieto, 1999).

Brown, flat ovate freshly-harvested seeds were about 25 mm in length, had a moisture content of 8% (fresh weight), with approximately 2,200 seeds per kilogramme. In the laboratory, seeds were cleaned with Tween® 20 for 1 minute and rinsed with distilled and deionised water. After cleaning, air-dried seeds were stored in paper envelopes under ambient laboratory conditions (21-23°C, 50% RH) for approximately 90 days until the beginning of the germination trials. Imbibitions curves for intact and mechanically scarified seeds were determined. Two samples of 50 seeds at each condition were tested. Initial weight and fresh weight of each seed sample were measured each hour with an analytic balance until the seed weight had stabilised. Seed viability was determined using 1% tetrazolium solution (2, 3, 5-triphenyl tetrazolium chloride) following ISTA (2012). Two samples of 50 seeds were used. Seeds were soaked in the Tetrazolium solution in flasks totally wrapped with aluminium foil, which were placed in an oven at 37ºC for 22 h. We previously determined that embryo staining was completed within 22h. After this period of time, embryo coloration patterns were evaluated under a dissecting scope. Seeds were recorded as viable when embryos where homogeneously stained (i.e., both radicle and cotyledons).

Germination trials
We conducted germination trials to test the effects of mechanical and acid scarification on *S. parahyba* seeds using a factorial experimental design. Seeds were mechanically scarified by laterally rubbing them against sand paper sheets to make a small hole in the seed coat. Seeds were immersed in solutions of sulfuric acid at 10 and 20% and in solutions of chloridric acid at 25%, 50% and 75% for 1, 5 and 10 minutes to test the effect of acid scarification on germination. Seeds were then placed inside 400 ml flasks. Flasks were placed on a shaker plate so seeds were equally exposed to the acids. After being immersed seeds were then rinsed with distilled and deionised water for 10 minutes. Eight replicates of 10 seeds were used in each of the 16 treatments (including the control treatment) for a total of 1,280 seeds. Seeds were sown in 90 mm-diameter glass Petri dishes on filter paper wetted with 10 ml distilled, deionised water. The dishes were placed in a growth chamber at 24°C, 50% RH and 12-/12-h photoperiod. Germination (radicle protusion was recorded two to three times per week up to 20 d (when no more germination was observed). Seeds were watered as needed.

Data analysis
For each replicate, final germination percentage (GC) and the following germination rate indices were calculated: Germination Rate Index (GRI) = G1 / T1 + G2/T2 + G3/T3 +...Gn/Tn, where, G1=number of germinated seeds on T1;

T1=days of first counting and Gn=number of germinated seeds between Tn-1 and Tn; Tn=days at the final counting; time (d) to reach 50% of maximum recorded germination (R50'); peak value (PV), a measure of seed vigor, calculated as the maximum cumulative percentage germination on any day divided by the number of days to reach that percentage (Czabator, 1962); mean daily germination (MDG), the accumulated percentage of germinated seeds at the end of the test period; and germination value (GV), which expresses the germinative energy and the speed of germination and calculated as GV = MDG x PV.

Differences in each of the germination indices (GC, GRI, PV, R50', MDG and GV) among all treatments were examined by 1-way ANOVA. Tukey's HSD post-hoc tests for multiple comparisons of means were used. Two-way ANOVA tests were conducted to examine the proportion of variation explained by acid concentration and time of seed immersion as fixed factors. Finally, a principal components analysis (PCA) was performed on all multivariate trait data (germination indices) to examine patterns of co-varying traits. Data normality and homogeneity of variances were examined with Kolmogorov-Smirnov tests and Levene's tests (Sokal & Rohlf, 1995). All analyses were performed in JMP 14 (SAS Institute, Cary, NC, USA).

Results

Patterns of seed water uptake differed significantly between intact and mechanically scarified seeds (t = 4.79, P <0.0001). While seed water uptake of intact seeds was 0%, seed water uptake of mechanically scarified seeds was 34.09%. Water uptake of scarified seeds was slow during the first 4 h but increased fast over the next 6-13 h of imbibition, and reached a plateau after 14-16 h. Based on tetrazolium testing, the viability (homogeneously stained embryos) of *S. parahybum* seeds was 90%.

Table 1. Treatment effects of acid scarification (concentration, time and its interaction) on *S. parahybum* percentage seed germination (GC), germination rate index (GRI), time to 50% of maximum recorded germination (R50'), peak value (PV), mean daily germination (MDG), and germination value (GV). Values are percentage of total variation (%) explained by each effect (Type I sum of squares for each effect as percentage of total sum of squares).

Acid	Source of variation	GC	GRI	PV	R50	MDG	GV
Sulfuric	Concentration (C)	25.69*	16.94*	2.04	17.31	25.69*	2.35
	Time (T)	73.01**	78.35**	68.34**	35.60	72.01**	81.26**
	C x T	2.30	4.71	29.61*	44.55	2.30	16.40
Chloridric	Concentration (C)	73.78*	67.79**	30.51*	27.08	73.78*	53.76*
	Time (T)	20.92	23.98*	3.46	12.60	20.92	6.33
	C x T	5.31	8.24	66.03*	60.32	5.31	39.91

*,**Significant at p ≤ 0.01 and p ≤ 0.001, respectively.

Germination percentage, rate and vigour differed significantly among seeds subjected to different treatments according to the germination indices ($F_{GC,}$ = 16.40; P <0.0001; F_{GRI} = 20.86; P<0.0001; F_{PV} =10.19, P <0.0001; F_{MDG} =16.40, P <0.0001; F_{GV} =12.58, P <0.0001, Figure 1). Overall, mechanically scarified seeds had on average germination index values (except for R50') significantly higher than seeds soaked in chloridric, sulfuric acid or intact seeds, respectively (Figure 1). Mechanically scarified seeds had on average significantly higher final germination percentages (92.5±2.5%) than seeds soaked in chloridric acid (50.03±2.09%), in sulfuric acid (33.13±.2.11%) or intact seeds (17.5±1.64%).

Acid concentration and time of immersion significantly affected seed germination (Table 1). However, the magnitude of the effect of concentration and time on seed germination differed between sulfuric and chloridric treatments. For seeds soaked in chloridric acid, concentration had a larger effect on germination than time. In contrast, for seeds soaked in sulfuric acid, time of immersion had a larger effect on germination than concentration (Table 1). For both acids, concentration and time interacted significantly only for PV (Table 1). Overall, seeds soaked in 10% sulfuric acid for 1 and 5 minutes exhibited higher germination values than seeds soaked in 20% sulfuric acid for 10 minutes (Figure 1). Seeds soaked in 75% and 50% chloridric acid solutions for 5 and 10 minutes had higher mean germination than seeds soaked in 25% chloridric acid solution for 1 minute (Figure 1). The first two axes of the PCA explained 72.9% and 20% of the variation in the six germination indices (Figure 2). Mechanically scarified seeds and seeds treated with 75% chloridric acid exhibited the highest values for most germination indices (except for R50'). In contrast, control seeds and seeds treated with 20% sulfuric acid exhibited the lowest values. Seeds treated with 10% sulphuric acid and with 25%, and 50% exhibited intermediate values (Figure 2).

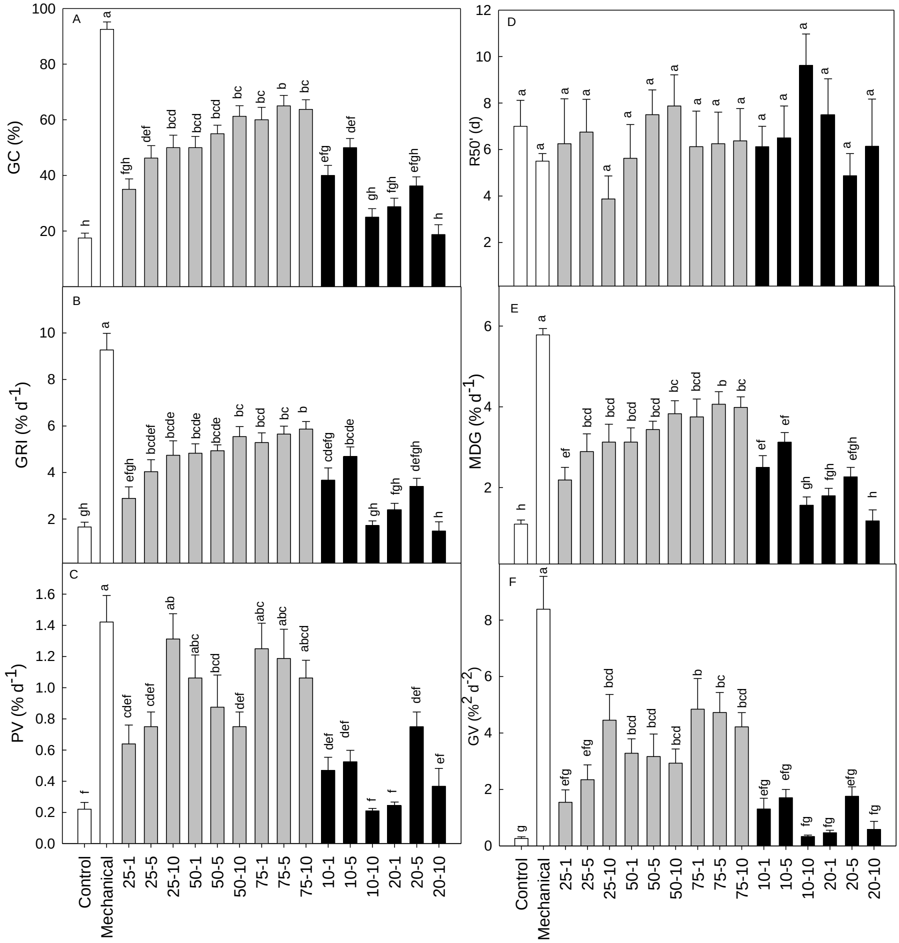

Figure 1. Percentage germination (GC) and germination indices of *Schizolobium parahyba* seeds following either mechanical or acid scarification with chloridric acid (gray bars) or sulfuric (black bars) compared to intact (control) seeds. GC: percentage germination; PV: peak value; GRI: germination rate index; MDG: mean daily germination; R50': time to 50% of maximum germination; GV: germination value. Bars are means ± SE (n=8). Bars topped with different letters differ significantly ($P < 0.05$) according to Tukey's HSD post-hoc tests.

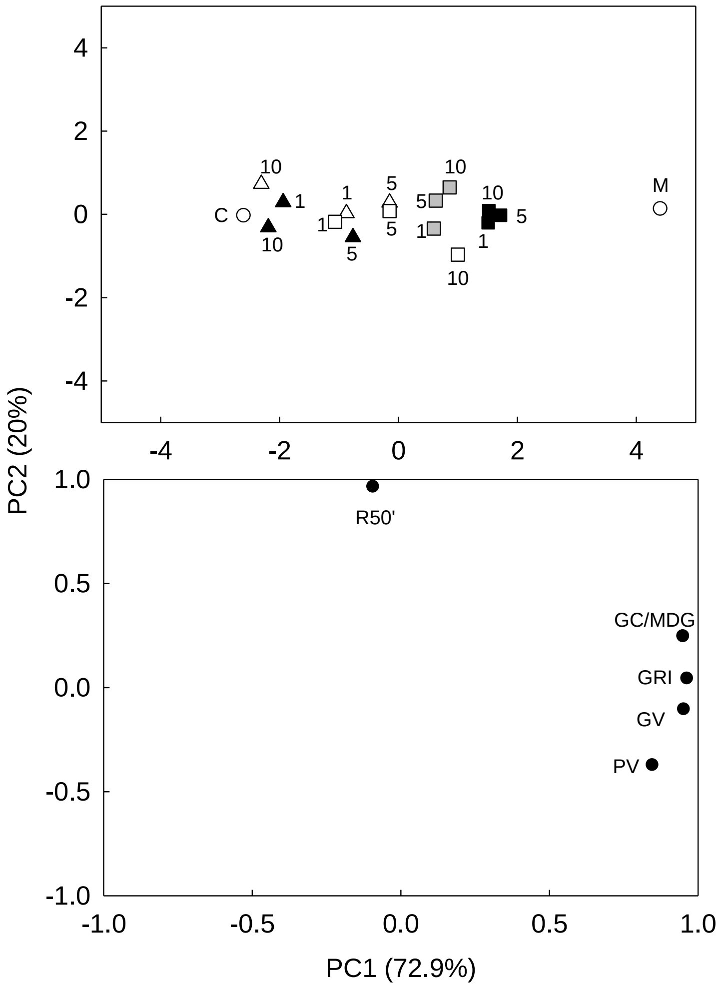

Figure 2. (a) PCA ordination for *S. parahybum* intact seeds (C) and seeds subjected to mechanical (M) or acid scarification treatments with sulfuric acid at 10% (white triangles), 20% (black triangles) and chloridric acid at 25% (white squares), 50% (gray squares) and 75% (black squares) for 1,5, and 10 minutes. (b) Position of germination indices on the first two axes of the principal components analysis for all the treatments included in this study.

Discussion

Mechanical scarification with sand paper and soaking seeds in acid solutions improved germination of *S.parahyba* seeds. Mechanically scarified seeds, however, had higher germination than acid scarified seeds. Our results agree with those of Azeredo et al., (2003), Yubero (2011), Castro Nina (2016) who found that *S. Parahyba* scarified seeds with sandpaper reached 95%, 93% and 97% of final germination, respectively. Oliveira Silva et al., (2018) also found that mechanical scarification and seed immersion in hot water enhance germination rates in the studied species. Mechanical scarification enhances germination in several tropical hard-seeded species because cracks or cuts facilitate water entry and gas exchange which promotes seed germination by activating enzymatic hydrolysis (Missanjo et al., 2013). We observed that coats of freshly harvested seeds of *S. parahyba* were impermeable to water as intact seeds did not imbibe water but mechanically scarified seeds did. In addition, we observed that embryos were fully developed according to the tetrazolium viability tests. Thus, we confirmed that covering layers restrain water uptake by the embryo in this tree species. In seeds with physical dormancy, prevention of water uptake causes the seed to remain dormant until some factor (s) render the covering layer (s) permeable to water (De Souza et al., 2012). In nature, these factors include high temperatures, widely fluctuating temperatures, fire, drying, freezing/thawing and passage through the digestive tracts of animals (Baskin et al., 2000). The use of scarification methods is well known in overcoming dormancy of seeds with hard impermeable coats as it disrupts seed coats and allows water uptake and further radicle emergence (Baskin & Baskin, 2004). Acid scarification methods are often used to overcome physical seed dormancy (Baskin & Baskin, 2014). For example, sulfuric acid was effective to overcome dormancy in several Fabaceae species such as *Schizolobium amazonicum* seeds (Cruz et al., 2007), *Senna obtusifolia*, *Crotalaria senegalensis* and *Crotalaria verrucosa* (Okonwu & Eboh, 2017). However, the efficiency of the treatment varies with the concentration of the acid, plant species and treatment duration (Cruz et al., 2007; Okonwu & Eboh, 2017).

In our study, the type of acid, concentration and time of seed exposure affected germination. Seeds treated with 10% sulfuric for 1 and 5 minutes acid had higher germination than seeds treated with 20% for 10 minutes. Thus, exposure of seeds to sulfuric acid solutions of concentrations higher than 10% for more than 5 minutes may have affected negatively seed coat integrity and/or the plugged natural openings in the studied species. Our results agree with those of Oliveira Silva et al., (2018) who found that *S. parahyba* seeds immersed in concentrated sulfuric acid for 10 and 20 min had lower seed vigour than mechanically scarified seeds or seeds immersed in hot water. Oliveira Pereira et al., (2011) and Mateus Alves et al., (2017) also found that mechanical scarification was a more effective method to overcome seed dormancy than acid scarification.

In our study, seeds treated with 50-75% chloridric acid for 5-10 minutes exhibited higher germination than seeds treated with 25% for 1 minute. Our results are consistent with Okonwu & Eboh, 2017 who found that chloridric acid at 75% and 50% enhanced seed germination of *Crotalaria verrucosa* (Okonwu & Eboh, 2017) and *Parkia biglobosa* (Abubakar et al., 2013), respectively. Further studies are necessary to test other concentrations of chloridric acid along with different soaking times in the seed germination of *S. parahyba*. In addition, studies have found that seed origin may affect seed germination and seedling vigour in *S. parahyba* (Canchignia-Martínez, 2007; Freire et al., 2015). Thus, further studies testing the effect of seed origin on the germination of this species are necessary.

Our results indicate that mechanical scarification and soaking in chloridric acid at 75% or 50% for 10 minutes enhance seed germination of *S. parahyba*. Therefore these pre-germinative treatments can be used to increase and synchronize germination particularly during nursery stages of restoration projects (Basto & Ramírez, 2015) or to support direct field seed sowing and seedling planting in habitat restoration practices (Heeleman et al., 2012; Muñoz-rojas et al., 2016). In natural conditions, exposure to high constant temperatures or fluctuating temperatures is the most likely cause of release from physical dormancy of *S. parahyba* seeds (De Souza et al., 2012). Seed responses to temperature changes in the habitat usually increase seed coat permeability to water. Studies with species from tropical forests have shown that alternating temperatures on a wet surface effectively broke physical dormancy of seeds (De Paula et al., 2012). Therefore, restoration projects involving seedling establishment of *S. parahyba* in tropical degraded-habitats would highly benefit if seeds are previously scarified and sown during the rainy season to increase the likelihood of seedling survival and growth because the transition from germinated seed to emerged seedling has been identified as the life-stage transition most limiting the success of direct seeding (Muñoz-Rojas et al., 2016). Direct seeding studies indicate that *S. parahyba* is a successful species for restoring tropical moist forests (Engel & Parrotta 2001). In abandoned agricultural lands in Southeastern Brazil, adequate seed germination and survival as well as rapid growth during the first two years after sowing, yielded developing *S. parahyba* forests that facilitated natural regeneration by native woody shrub species in their understories (Engel & Parrotta 2001). Further studies however, are necessary to continue improving restoration techniques, select appropriate microsites for direct seeding and seedling planting to increase population sizes of *S.parahyba* in the field (Hüller et al., 2017).

Conclusion

Our study shows that mechanical scarification and seed immersion in acid solutions improve germination of *Schizolobium parahyba* seeds. Mechanically scarified seeds had higher germination percentage (92.5%) than seeds treated with chloridric acid (50%), sulfuric acid (33.13±.2.11%) or intact seeds (17.5%). Acid concentration and time of immersion significantly affected seed germination but the magnitudes of the effects of concentration and time on seed germination differed between sulfuric and chloridric treatments. Seeds soaked in 10% sulfuric acid for 1 and 5 minutes exhibited higher germination values than seeds soaked in 20% for 10 minutes. Seeds soaked in 75% and 50% chloridric acid solutions for 5 and 10 minutes had an overall higher and faster germination than seeds soaked in 25% for 1 minute. Therefore, mechanical scarification and seed immersion in chloridric acid solutions of 50% and 75% for 5 and 10 minutes could facilitate large-scale propagation of this pioneer species and thus assist habitat restoration and conservation practices in highly degraded ecosystems such as tropical moist forests.

Acknowledgments

This work was supported by funds from the Pontificia Universidad Javeriana (PUJ) Biology Department. We thank The Biología de Plantas y Sistemas Productivos group at PUJ for logistical support. We are grateful to Corporación Nacional de Investigación y Fomento Forestal (CONIF) for providing seed material. We also thank S. Carrizosa and an anonymous reviewer for providing valuable comments on previous versions of this manuscript.

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Journal of Tropical Biology and Conservation 16: 229-251, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Diversity of Frogs in Tawau Hills Park, Sabah, Malaysia

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Abstract
A study on the diversity of anurans was carried out via Visual Encounter Survey (VES) method in Tawau Hills Park (THP), from June 2009 to September 2010. Twenty-eight line transects were established and surveyed, resulting in 925 individuals from 51 species, representing six families. Eighteen species were new locality records for THP, bringing the total number of identified frogs to 68 species. These frogs occupy elevations from lowland area (200 m a.s.l.) at the headquarters of THP, Balong substation and Merotai substation to submontane area (>900 m a.s.l.) at Mount Maria, Mount Lucia and Mount Magdalena. All 68 species recorded represent 62% of the total frog species found in Sabah, with 15% of them endemic to Borneo. Frog species in THP were dominated by Ranidae (39.4%) and Dicroglossidae (24.4%). *Limnonectes leporinus* was the most abundant species (10.2%), followed by *Meristogenys orphnocnemis* (10.1%). The high species diversity and richness in THP could be due to the rich topography of this park, which provides ample feeding, breeding and shelter for frogs.

Keywords: Anurans, Lowlands, Submontane, Visual Encountered Survey, Endemic, Borneo

Introduction
Borneo is one of the most important biodiversity hotspots for amphibians where inventory studies have been systematically carried out in Sabah and Sarawak since the late 1850s (Inger & Stuebing, 2005; Matsui 2006, Das and Haas, 2010). There were only 138 frog species recorded in Borneo in 1996, but the figure increased to 150 species in 2005 (Malkmus et al., 2002; Inger and Stuebing, 2005), and 167 species in 2012 (Inger & Tan, 2010; Frost, 2012).

Received 26 April 2019
Reviewed 21 Jun 2019
Accepted 03 July 2019
Published 15 October 2019

According to Frost (2012), there are seven families comprising 46 genera and 234 species of frogs recorded in Malaysia (Peninsula Malaysia, Sabah and Sarawak). In Borneo, frogs consist of seven families of 37 genera and 160 species. There are 108 species of frogs from over seven families and 30 genera in Sabah. Approximately 57% or 96 species of frogs recorded are endemic to Borneo (Malkmus et al., 2002; Inger & Stuebing, 2005; Inger & Tan, 2010, Frost, 2012).

There are only three published data on the frog species found in Tawau Hills Park. The earliest study was done by Stuebing and Nor (1995) who recorded 31 species of frogs in three habitats - on the boundary of, within the lowland areas of Tawau Hills Park, and the area adjacent to cocoa plantations during a scientific expedition organized by Sabah Parks and Universiti Kebangsaan Malaysia Sabah Campus (UKMS) in 1989. Inger et al. (2000) conducted a comprehensive study from 1991 to 1993 and they recorded 42 frog species in the lowlands of Tawau Hills Park. The latest study was done by Kueh and Sudin (2008) with the discovery of *Gastrophrynoides borneensis* in Tawau Hills Park. These studies have documented a total of 50 species of amphibians.

Frogs are functionally vital to many ecosystems they live in, as they play significant roles in the earth's biota (Heyer et al., 1994; Werner & McPeek, 1994). They act as a biological control and beneficial environment bio-indicator. They are predators, consuming a great variety of prey, such as insects and invertebrates, and they are also prey to snakes, birds and reptiles. Even as tadpoles, they are eaten by large fishes and other animals. In addition, frogs act as a keystone species in many communities (Beebee, 1996; Stewart & Woolbright, 1996; Inger & Tan, 2010). Anuran surveys do not only provide information on distribution, abundance and density, but also aid in understanding the habitat requirements of the species and environmental variables controlling its diversity. Such information is needed for effective conservation planning and management of forests, including monitoring of anuran populations in a period of apparent global decline and management of effective nature conservation (Parris, 1999).

Methodology

Study site: Tawau Hills Park

This study was conducted in Tawau Hills National Parks or more commonly known as Tawau Hills Park (THP), Sabah (Figure 1). The park is surrounded by Ulu Kalumpang Forest Reserve on its northern side and Mt. Andrassy Forest Reserve on its south, whilst its western and southern sides have boundaries with cocoa

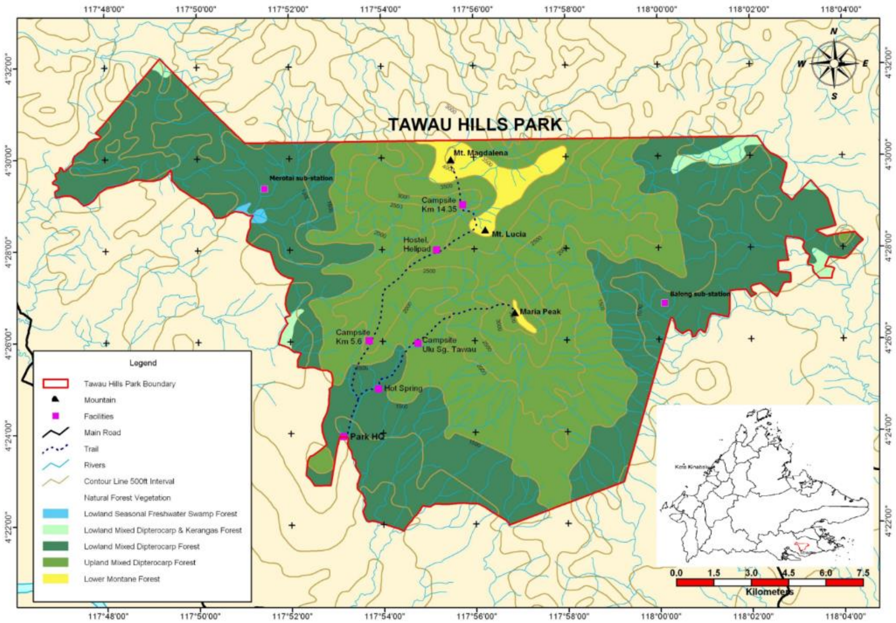

Figure 1. The physiographic of Tawau Hills Park. (Source: GIS laboratory of Institute for Tropical Biology)

and oil palm plantations. The park occupies a land area of 280 km^2, in which 60% of the park is virgin lowland dipterocarp forest, while the remaining area, particularly the lower elevations of the eastern and western areas, were logged before they were gazetted (Omar & Nais, 1995; Mohamed et al., 1999).

There are at least seven rivers in Sabah originated from Tawau Hills Park: Sg. Tawau, Sg. Kinabutan, Sg. Mantri, Sg. Balung, Sg. Merotai Kanan, Sg. Merotai Kecil and Sg. Junap. The main river system in THP is Sg. Tawau which flows through the middle of the 280 km^2 of the park forming many natural deep-water pools and waterfalls, including the Table Waterfall. There is also a hot spring feeding into one of its tributaries (Omar & Nais, 1995).

The terrain within THP is relatively rugged and hilly, with altitudes ranging from 30 m to 1,310 m. The three main peaks: Mt. Magdalena (1,310m), Mt. Lucia (1,189m) and Mt. Maria (1,067m) consist of inactive volcanoes, which were last active about 27,000 years ago. Only about 8% of the park area lies at an altitude of above 900 m, while more than 50% of the park is below an elevation of 457 m. Generally, the study site can be categorized into three different habitats, which are sub-montane forest habitat (Mt. Magdalena, Mt. Lucia and Mt. Maria), lowland mixed dipterocarp forest habitat (lowland area around THP headquarters) and logged forest habitat (Balong sub-station and Merotai sub-station).

Sampling of frogs

Frog surveys were carried out randomly at the study area to minimise sampling bias. Visual Encounter Survey (VES) was used as the sampling method in this study (Crump & Scott, 1994; Heyer et al., 1994). Samplings were done once every six weeks with an average sampling effort of seven days for each visit from June 2009 to September 2010, covering dry and wet seasons. Sampling and observations were performed day and night for at least 3 hours at each site. Frogs were sampled using the hand-grabbing technique (Heyer, 1994; Matsui, 2006) and placed in separate plastic bags. Details, such as date and time of capture, microhabitats, and weather data were recorded on data sheets.

A total of 28 transects consisting 17 stream transects and 11 forest-floor transects were established in Tawau Hills Park: nine at the lowland area of the park, four at Balong substation, five at Merotai substation, four at Mt. Lucia, five at Mt. Maria and one at Mt. Magdalena. Each line transect was divided into stations of 10-m intervals. All stream transects were 500 m in length, except for one in Merotai substation, which was 300 m, as it was located in a very small creek. The width of each river was measured and recorded (Table 1). The length of forest floor transects were varied and established to the greatest accessibility (Table 2).

Table 1. River transects established at different localities in Tawau Hills Park

Transect	Name	Transect Length	Locality	River Width
RT1	Sg. Tawau 1	500 m	Lowland	7.5 m
RT2	Sg. Tawau 2	500 m	Lowland	7.5 m
RT3	Sg. Gelas	500 m	Lowland	8 m
RT4	Sg. belakang rest house 10km	500 m	Lucia (10km)	3.5 m
RT5	Sg. Balong	500 m	Balong	10.5 m
RT6	Sg. Papaya	500 m	Balong	4.5 m
RT7	Sg. Api Kecil	300 m	Merotai	2 m
RT8	Sg. Merotai Kanan	500 m	Merotai	11 m
RT9	Sg. Limau	500 m	Merotai	3.5 m
RT10	Lucia Waterfall 1	500 m	Lucia	3.5 m
RT11	Lucia Waterfall 2	500 m	Lucia	3.5 m
RT12	Gelas Waterfall	500 m	Lowland	2.5 m
RT13	Hotspring	500 m	Lowland	5.5 m
RT14	Sg. Tawau 1	500 m	Maria	8 m
RT15	Sg. Tawau 2	500 m	Maria	8 m

RT16	Sg. Maria 1	500 m	Maria	8 m
RT17	Sg. Maria 2	500 m	Maria	8 m

Table 2. Forest floor transects established at different localities in Tawau

Transect	Forest Floor Transects	Length	Locality
FT1	Trail along Sg. Tawau	2000m	Lowland
FT2	Lowland Botanical Garden	3000m	Lowland
FT3	Trail Lucia (km-3 to 5)	3000m	Lowland
FT4	Trail Lucia (km-6 to 9)	4000m	Lucia
FT5	Trail Sg. Kecil	1000m	Balong
FT6	Trail Sg. Papaya	1000m	Balong
FT7	Trail Canopy	2000m	Lowland
FT8	Trail Sg. Api Kecil	500m	Merotai
FT9	Trail Sg. Limau	1000m	Merotai
FT10	Trail Magdalena (km-14 to 17)	4000m	Magdalena
FT11	Trail Maria	3000m	Maria

Specimen Identification and Preservation

All specimens were taxonomically identified to species based on morphological characteristics keys by Malkmus et al. (2002); and Inger and Stuebing (2005). Only selected frog specimens were euthanized using chlorobutanol and fixed in 10% formalin. Specimens are kept in 90% ethyl alcohol for long-term storage (Heyer, 1994; Matsui, 2006) in BORNEENSIS, the wet specimen collection centre at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

Species Diversity Indices

A biological community has an attribute termed as species diversity, and various approaches have been suggested to measure this parameter (Colwell, 2005; Baumgärtner, 2006; Lamb et al., 2009). Four diversity indices were used in this study: Shannon Index, Simpsons Diversity Index, Margalef Diversity Index and Brillouin Index. In addition, species rarefaction curves and species accumulation curves were produced to estimate the possible total number of species occurring in THP and evaluate sampling effort (Krebs, 1999; Thompson et al., 2003; Colwell et al., 2004).

Results

Frogs recorded in Tawau Hills Park

Overall, 51 species of frogs comprising six families were recorded throughout this study. Of these species, 18 species are new records to Tawau Hills Park (Table 3).

Table 3: Frog species recorded in year 1995, 2000 and current study

	Species name	1995	2000	Current study
Megophryidae				
1	*Leptobrachella gracilis*	+		
2	*Leptobrachium abbotti**	+	+	+
3	*Leptobrachium hendricksoni*			+
4	*Leptobrachium montanum**			+
5	*Leptolalax dringi**		+	+
6	*Leptolalax pictus**			+
7	*Megophrys nasuta*	+	+	+
Bufonidae				
8	*Ansonia fuliginea**			+
9	*Ansonia leptopus*	+	+	
10	*Ansonia longidigita**			+
11	*Ansonia spinulifer**		+	+
12	*Ingerophrynus divergen**	+	+	+
13	*Phrynoidis juxtaspera*	+	+	+
14	*Rentapia hosii*	+	+	+
Microhylidae				
15	*Chaperina fusca*		+	+
16	*Kalophrynus heterochirus**		+	+
17	*Kalophrynus pleurostigma*		+	
18	*Kalophrynus subterrestris**			+
19	*Kalophrynus punctatus*	+		
20	*Kaloula baleata*	+	+	
21	*Metaphrynella sundana*	+	+	
22	*Microhyla borneensis*		+	+
23	*Microhyla perparva**		+	
Ranidae				
24	*Amninara nicobariensis*	+		
25	*Huia cavitympanum**		+	+
26	*Meristogenys orphnocnemis**	+	+	+
27	*Meristogenys phaeomerus**			+
28	*Meristogenys whiteheadi**			+
29	*Meristogenys sp.*	+		
30	*Hylarana chalconota*	+	+	+

31	*Hylarana erythraea*			+
32	*Hylarana luctuosa*			+
33	*Hylarana picturata*		+	+
34	*Hylarana signata*	+		
35	*Odorrana hosii*	+	+	+
36	*Staurois latopalmatus**	+	+	+
37	*Staurois natator*	+	+	+
38	*Staurois tuberilinguis**			+
39	*Staurois parvus*			+
Dicroglossidae				
40	*Fejervarya limnocharis*		+	+
41	*Limnonectes finchi**	+	+	+
42	*Limnonectes kuhlii*	+	+	+
43	*Limnonectes leporinus**	+	+	+
44	*Limnonectes maleisianus*			+
45	*Limnonectes palavanensis*		+	+
46	*Limnonectes paramacrodon*	+	+	
47	*Occidozyga baluensis*	+	+	+
48	*Occidozyga laevis*			+
Rhacophoridae				
49	*Nyctixalus pictus*	+	+	
50	*Philautus aurantium**	+		+
51	*Philautus hosii**		+	+
52	*Philautus petersi*			+
53	*Polypedates colletti*			+
54	*Polypedates leucomystax*	+	+	+
55	*Polypedates macrotis*	+	+	+
56	*Polypedates otilophus*		+	+
57	*Rhacophorus appendiculatus*	+	+	+
58	*Rhacophorus bimaculatus*		+	
59	*Rhacophorus dulitensis*	+	+	
60	*Rhacophorus everetti*			+
61	*Rhacophorus gauni**	+	+	+
62	*Rhacophorus harrissoni**		+	+
63	*Rhacophorus kajau**		+	
64	*Rhacophorus nigropalmatus*		+	
65	*Rhacophorus borneensis*			+
66	*Rhacophorus pardalis*	+		+
67	*Rhacophorus rufipes**		+	+
	Subtotal	**31**	**42**	**51**

+ Presence of frog.
*Species endemic to the island of Borneo
Source: Stuebing and Nor, 1995; Inger et al., 2000
Note: *Gastrophrynoides borneensis* (Anura: Microhylidae) was discovered in 2008 (Kueh & Sudin, 2008), hence, the total recorded frog species in THP is Now 68 species.

This study adds 18 species to the existing herpetofaunal checklist of THP. The newly documented species are: Leptobrachium hendricksoni, L. montanum, Lepotolalax pictus, Ansonia fuliginea, A. longidigita, Kalophrynus subterrestris, Meristogenys phaeomerus, M. whiteheadi, Hylarana erythraea, H. luctuosa, Staurois tuberilinguis, S. parvus, Limnonectes maleisianus, Occidozyga laevis, Philautus petersi, Polypedates colletti, Rhacophorus everetti and Rh. borneensis. Overall, 25 species of frogs from the checklist are endemic to the island of Borneo and among these 23 were encountered during this study.

Frog Abundance

A total of 925 individuals of frogs were encountered throughout the 16 months of sampling, from June 2009 until September 2010. The number of individuals encountered for each species at six different localities are indicated in Table 4.

Lowland area at THP Headquarters had the highest abundance with 467 individuals encountered, followed by Mt. Maria (143 individuals), Balong Substation (123 individuals), Mt. Lucia (90 individuals), Merotai Substation (73 individuals) and the lowest abundance was at Mt. Magdelena (29 individuals) as shown in Table 4.

Table 4. Number of individuals encountered for each frog species at six different localities in THP

Species name	HQ	Balong Substation	Merotai Substation	Mt. Magdalena	Mt. Lucia	Mt. Maria	Total
Megophryidae							
*Leptobrachium abbotti**	15	0	2	1	0	0	18
Leptobrachium hendricksoni	27	3	0	0	0	0	30
*Leptobrachium montanum**, LU	0	0	0	0	2	0	2
*Leptolalax dringi**	22	15	0	0	0	9	46
*Leptolalax pictus**, LU	0	0	0	0	7	0	7
Megophrys nasuta	4	0	0	2	1	0	7
Subtotal	68	18	2	3	10	9	110
Bufonidae							
*Ansonia fuliginea**	0	0	0	2	8	0	10
*Ansonia longidigita**	31	0	0	0	1	0	32

*Ansonia spinulifer**, HQ	2	0	0	0	0	0	2
*Ingerophrynus divergen**	6	1	0	0	0	0	7
Phrynoidis juxtaspera	6	2	6	0	0	2	16
Rentapia hosii	9	1	0	0	0	0	10
Subtotal	54	4	6	2	9	2	77
Microhylidae							
Chaperina fusca	6	1	0	0	0	0	7
*Kalophrynus heterochirus**, MG	0	0	0	1	0	0	1
*Kalophrynus subterrestris**	1	0	0	2	0	0	3
Microhyla borneensis	6	6	0	0	0	0	12
Subtotal	13	7	0	3	0	0	23
Ranidae							
*Huia cavitympanum**	4	0	0	0	0	4	8
*Meristogenys orphnocnemis**	42	16	2	0	16	17	93
*Meristogenys phaeomerus**	6	1	1	2	4	5	19
*Meristogenys whiteheadi**, MG	0	0	0	1	0	0	1
Hylarana chalconata	38	2	12	0	0	8	60
Hylarana erythraea	15	0	0	0	0	1	16
Odorrana hosii	30	3	0	0	0	20	53
Hylarana luctuosa HQ	1	0	0	0	0	0	1
Hylarana picturata	15	19	4	0	0	5	43
*Staurois latopalmatus**	2	0	0	0	0	16	18
Staurois natator	18	0	0	0	8	13	39
Staurois parvus MG	0	0	0	1	0	0	1
*Staurois tuberilinguis**	8	0	0	0	3	1	12
Subtotal	179	41	19	4	31	90	364
Dicroglossidae							
Fejervarya	1	2	0	0	0	0	3

limnocharis							
*Limnonectes finchi**	13	0	0	0	1	0	14
Limnonectes kuhlii	22	7	15	0	16	12	72
*Limnonectes leporinus**	40	19	20	0	0	15	94
Limnonectes malesianus HQ	2	0	0	0	0	0	2
Limnonectes palavanensis	2	0	0	2	7	0	11
Occidozyga baluensis	12	1	1	0	2	0	16
Occidozyga laevis BA	0	14	0	0	0	0	14
Subtotal	92	43	36	2	26	27	226
Rhacophoridae							
*Philautus aurantium**, LU	0	0	0	0	2	0	2
*Philautus hosii**	13	0	0	0	12	0	25
Philautus petersi MG	0	0	0	12	0	0	12
Polypedates colletti HQ	1	0	0	0	0	0	1
Polypedates leucomystax HQ	15	0	0	0	0	0	15
Polypedates macrotis HQ	8	0	0	0	0	0	8
Polypedates otilophus	3	0	1	0	0	0	4
Rhacophorus appendiculatus	3	0	7	0	0	0	10
Rhacophorus everetti MG	0	0	0	3	0	0	3
*Rhacophorus gauni**, ME	0	0	2	0	0	0	2
*Rhacophorus harrissoni**	2	0	0	0	0	2	4
Rhacophorus pardalis	14	10	0	0	0	7	31
Rhacophorus borneensis HQ	1	0	0	0	0	0	1
*Rhacophorus rufipes**	1	0	0	0	0	6	7
Subtotal	61	10	10	15	14	15	125
Total	467	123	73	29	90	143	925

*Species endemic to the island of Borneo.

HQ Species found only at lowland headquarters of THP.
BA Species found only at Balong Substation.
ME Species found only at Merotai Substation.
LU Species found only at highland Mt Lucia.
MG Species found only at highland Mt Magdelena.

Dominancy of Frog Family

Based on the number of individuals in each family (Figure 2), frog species in THP was dominated by Ranidae, with 364 individuals encountered (39.4%), followed by Dicroglossidae (226 individuals; 24.4%), Rhacophoridae (125 individuals; 13.5%), Megophryidae (110 individuals; 11.9%), Bufonidae (77 individuals; 8.3%) and Microhylidae (23 individuals; 2.5%).

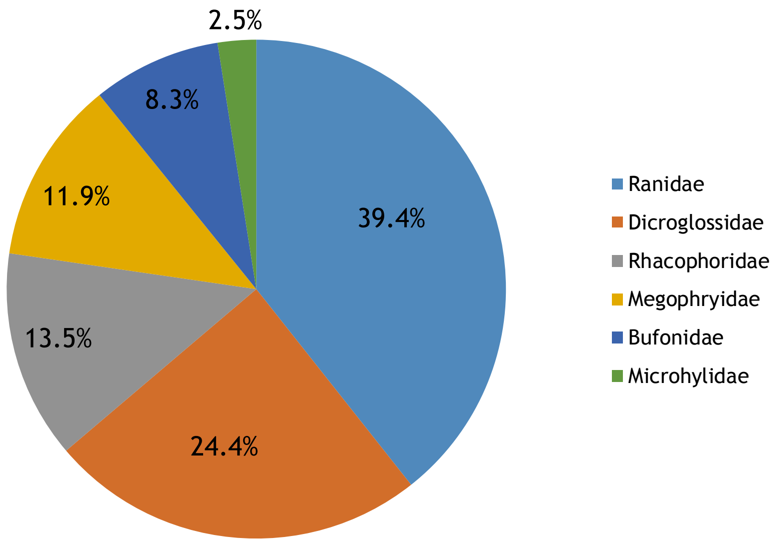

Figure 2. Dominance of frogs encountered in THP.

Abundance Rank

Limnonectes leporinus from the family Dicroglossidae was the most abundant species (94 individuals or 10.2%) in Tawau Hills Park; followed by *Meristogenys orphnocnemis* from Ranidae (93 individuals or 10%) and *Limnonectes kuhlii* from Dicroglossidae (72 individuals or 7.8%). The abundance of these three species (28%) comprises more than a quarter of the total number of individuals encountered in THP. In contrast, *Kalophrynus heterochirus*, *Hylarana luctuosa*,

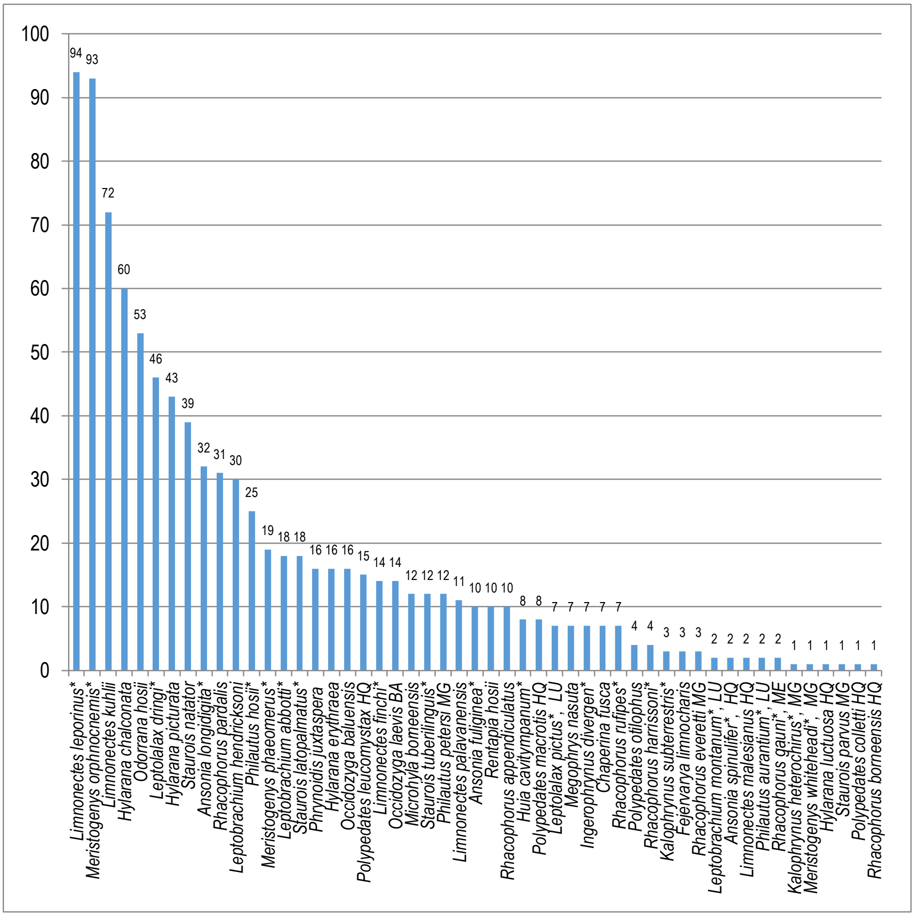

Figure 3. Abundance rank of frog species found in Tawau Hills Park.

Meristogenys whiteheadi, *Staurois parvus*, *Polypedates colletti*, and *Rhacophorus borneensis* were rare species, which were only found once (Figure 3). *Meristogenys orphnocnemis* was the most abundant species (42 individuals or 8.99%) found at headquarters of THP{, followed by *Limnonectes leporinus* (40 individuals or 8.56%), and *Hylarana chalconota* (38 individuals or 8.14). On the other hand, *Kalophrynus subterrestris*, *Hylarana luctosa*, *Fejervarya limnocharis*, *Polypedates colletti*, *Rhacophorus borneensis*, and *Rh. rufipes* were species found only once.

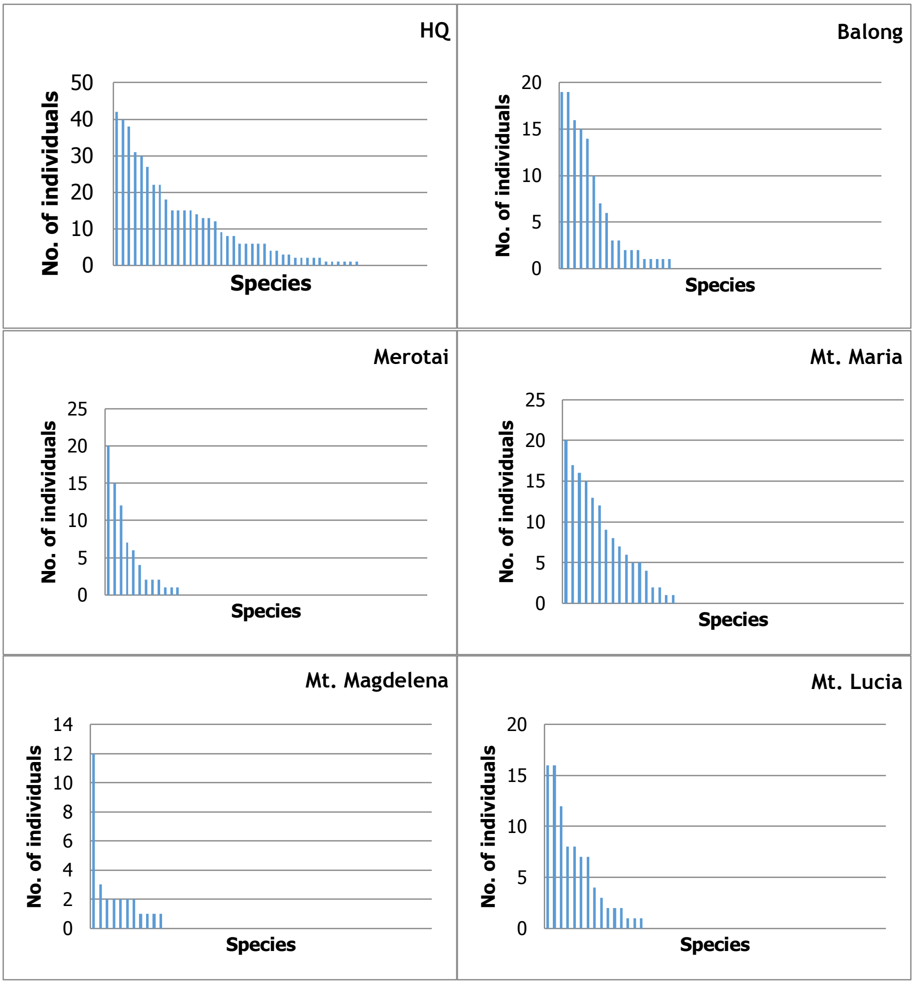

Figure 4. The abundance rank of frogs at each locality.

The frog species with the highest abundance found at Balong substation were *Hylarana picturata* and *Limnonectes leporinus* (19 individuals or 15.45%), followed by *Meristogenys orphnocnemis* (16 individuals or 13%). There were five singleton frog species found which were *Ingerophrynus divergens, Rentapia hosii, Chaperina fusca, M. phaeomerus and Occidozyga baluensis* (Figure 4).

Limnonectes leporinus (20 individuals or 27.4%) was the most abundant species found at Merotai substation, followed by *L. kuhlii* (15 individuals or 20.55%) and *Hylarana chalconota* (12 individuals or 16.44%). On the contrary, *Meristogenys* *phaeomerus, Occidozyga baluensis* and *Polypedates otilophus* were species found only once at Merotai substation (Figure 4).

The total frog individuals found at Mt. Magdalena was 29 individuals with *Philautus petersi* (12 individuals or 41.38%) as the most abundant species found, followed by *Rhacophorus everetti* (3 individuals or 13%). There were four species with only one individual found; these were *Leptobrachium abbotti, Kalophrynus heterochirus, Meristogenys whiteheadi* and *Staurois parvus* (Figure 4).

The most abundant frog species found at Mt. Lucia were *Meristogenys orphnocnemis* and *Limnonectes kuhlii* (16 individuals or 17.78%), followed by *Philautus hosii* (12 individuals or 13.33). There were three singleton species found, namely *Megophrys nasuta, Ansonia longidigita and Limnonectes finchii* (Figure 4).

Odorrana hosii (20 individuals or 13.99%) recorded the highest abundance at Mt. Maria, followed by *Meristogenys orphnocnemis* (17 individuals or 11.89%), and *Staurois latopalmatus* (12 individuals or 11.19%). *Hylarana erythraea* and *Staurois tuberilinguis* were found only once (Figure 4).

Species diversity Indices

The results of the four diversity indices for frog sampling at six localities in THP are summarized in Table 5 and Figure 5. Four diversity indices applied in this study show no significant difference overall (One sample T-test, df = 3, P > 0.05).

Table 5. Four diversity indices accounted for frog sampling in Tawau Hills Park

Sample	Shannon Index	Simpsons Index	Margalef Index	Brillouin Index
Overall	3.357	20.938	7.329	3.248
THP Headquarters	3.244	21.198	6.345	3.091
Balong Substation	2.439	10.031	3.533	2.227
Merotai Substation	2.038	6.473	2.564	1.818
Mount Lucia	2.366	9.627	3.111	2.128
Mount Maria	2.575	12.337	3.224	2.381
Mount Magdelena	1.986	5.487	2.970	1.587

Based on pooling data from all 28 transects surveyed and 16 months of sampling period, the species accumulation curve almost levelled off and reached asymptote starting from the 41st survey (Figure 6). This suggests that most frog species within THP have now been encountered.

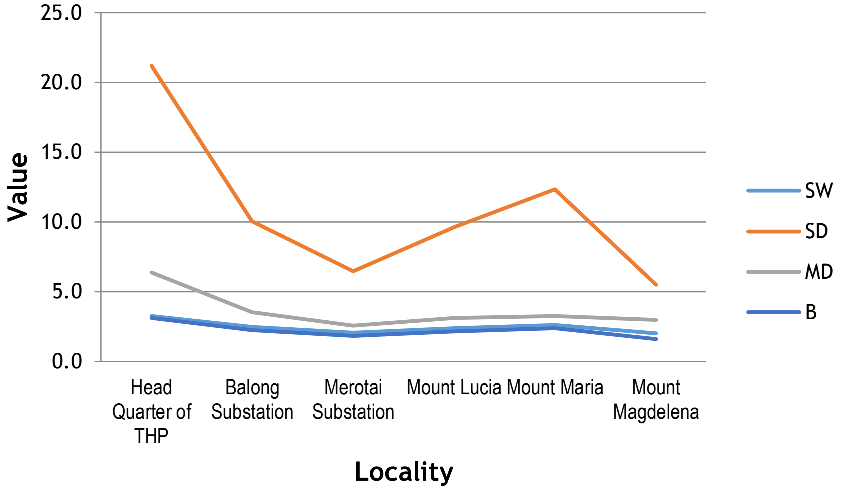

Figure 5. The diversity indices at each locality.

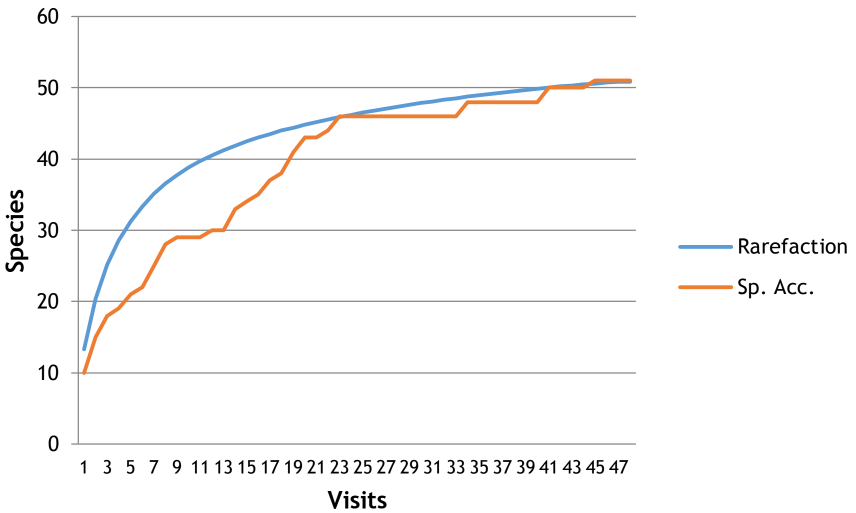

Figure 6. Species accumulation curve fitted on rarefaction curve.

Discussion

Throughout this study, a total of 51 frog species were identified in Tawau Hills Park, Sabah, with 33 frog species recorded previously (Stuebing & Nor, 1995; Inger et al., 2000). Inger and his group (2000) added 18 frog species to the amphibian checklist of THP in 2000, while this study, similarly supplemented 18 frog species to the list, bringing the total number of species identified in Tawau Hills Park to 68 species. Of the 68 species encountered in THP, they represent approximately 42% of the total frog species found in Borneo, with 24% of the species endemic to the island. The result correspondingly represents 62% of the total frog species found in Sabah.

Results of pooling data from 28 transects surveyed in this study show the species accumulation curve almost levelled off and reached asymptote starting from the 41st survey (Figure 6) in this study that was conducted up to 48 visits. This suggests that most frog species within THP had been encountered. Nevertheless, the increment in number of frog species in THP also suggests there may be more frog species to be found in future.

There were 16 frog species listed previously and not encountered during this study (Table 3). Among them, *Leptobrachella gracilis, Kalophrynus punctatus, Meristogenys sp., Amnirana nicobariensis,* and *Hylarana signata* were species encountered during the first survey in 1995 but not found in the subsequent study (Stuebing & Nor, 1995). Their absence during the survey could be due to their secretive habit or they were well hidden. Habitat loss and human disturbance may possibly contribute to this as well as the park is now open partly as a recreation area for both local and international tourists.

As shown in the results (Table 4), the frog individuals obtained were different at each locality. The lowland areas at the park headquarters had high abundance compared with the other five sampling sites. The abundance of frogs can be reflected by the availability of prey in habitats (Duellman & Trueb, 1994). The THP headquarters is home to a variety of microhabitats that promote the growth of green algae and aquatic organisms. This in turn enriches the availability of prey for frogs (Duellman & Trueb, 1994), leading to higher frog abundance around the headquarters.

More than half of the park lies below 457 m a.s.l, allowing easy access to most rivers, while approximately 8% of the park areas lies above 900 m a.s.l, which hampers the establishment of transects due to the difficult terrain.

Mt. Magdalena had the least abundance among the sampling sites (Table 4). High altitude is one of the rationales for this. The abundance of frogs is related to the availability of breeding sites and frogs distribute correspondingly to appropriate nursery sites for reproduction. As there are not many suitable breeding sites or water sources with the increasing altitude (Wong, 1994), only specialist frogs exhibiting breeding behaviour independent of water bodies will likely be present at higher altitudes (Wong, 1994; Rahbek, 1995). In addition, physical changes such as lower temperature, reduced rainfall and reduced aquatic microhabitats also influence the distribution of frogs in higher altitudes. Steep slopes and limited access in that area were the main reasons only one forest floor transect but no river transect was established.

Limnonectes leporinus, *Meristogenys orphnocnemis* and *Limnonectes kuhlii* were the three most abundant species encountered in THP (Table 4). This is consistent with the results recorded in most amphibian studies in Borneo (Kitayama, 1992; Inger & Voris, 1993; Stuebing & Nor, 1995; Voris & Inger, 1995; Maklarin et al., 1999; Inger et al., 2000; Traeholt et al., 2007; Grafe & Keller, 2009; Keller et al., 2009; Grafe et al., 2010; Inger & Tan, 2010). These are the common frogs found along rivers in both primary and old secondary forests.

In this study, *L. leporinus* was present at all lowland sampling sites and on Mt. Maria at 907 m a.s.l. The fact that this species was present at Mt. Maria was surprising as it is a lowland specialist (Inger & Stuebing, 2005; IUCN, 2018) There were 15 individuals of *L. leporinus* at Mt. Maria, suggesting its occurrence was not a coincidence. This finding indicates this species may travel from the downstream of Sg. Tawau to the upstream at Mt. Maria, as this species is also present at the same river at a lower elevation.

In addition, *M. orphnocnemis* and *L. kuhlii* were present at all sampling sites, except at Mt. Magdalena (Table 4). Both species are riparian species (Inger & Stuebing, 2005; Inger & Tan, 2010), thus, it is not likely to be found elsewhere. At all sampling sites where both *L. leporinus* and *L. kuhlii* co-existed, the abundance of *L. leporinus* was approximately double that of *L. kuhlii*. This incident is reliable, as according to Inger and Stuebing (2005), *L. kuhlii* is very common along medium sized streams but their existence is rare where *L. leporinus* is abundant.

There were six singleton species found in this study, of which *Kalophrynus heterochirus*, *Meristogenys whiteheadi* and *Staurois parvus* were found at Mt. Magdalena sampling sites, and *Hylarana luctuosa*, *Polypedates colletti* and

Rhacophorus borneensis in the lowland area around the headquarters of THP (Figure 4). All, except for *H. luctuosa* and *Rh. borneensis,* were not considered as rare species elsewhere (Inger & Stuebing, 2005), yet it seems that they are dwindling in the park and its surrounding habitat. As for the situation of *H. luctuosa* and *Rh. borneensis*, it appears to be the general trend in most amphibian studies as not enough data is available to make an assessment among fossorial (*H. luctuosa*) and arboreal (*Rh. borneensis*) frogs. In fact, none of the arboreal frogs can be regarded as highly abundant in those studies (May, 1975). The lack of quantitative abundance data for arboreal frogs suggests there is a need to develop appropriate frog sampling methods to access the vegetation above three meters from ground (Grafe & Keller, 2009).

According to the frog descriptions by Inger and Stuebing (2005), out of the 148 species of frogs listed in Borneo, there are only 17 montane species found above 900 m a.s.l., and of these four are new records at THP (Table 3). *Philautus petersi* and *Rhacophorus everetti* were only found on Mt. Magdalena at the elevation of 1,216 m a.s.l. and 1,198 m a.s.l., respectively, *while Leptobrachium montanum* was only found on Mt. Lucia sampling site at 1,088 m a.s.l. (Table 4). These montane species have narrow habitat tolerance as they confine themselves only in high elevation microhabitats and microclimates (Inger & Stuebing, 2005). They are well adapted to low temperature and high humidity. These frog species may be incompetent at a slightly higher temperature occurring in lowlands or they may require more moisture, usually occurring in the highlands due to thick fog (Inger, 1999; 2005). As the altitude increases, the drainage basin gets smaller and lesser and these frog species have little to no dependence on water bodies for breeding. *Philautus petersi* has a life cycle that is completely independent of water bodies, as their larvae undergoes a process of development within the egg as a tiny froglet, not as a tadpole (Malkmus et al., 2002; Inger & Stuebing, 2005).

As for *Ansonia fuliginea* which was expected to only occur at a higher elevation above 2,000m a.s.l (Inger et al., 2000), it was found in both the Mt. Magdalena (1,304m a.s.l) and Mt. Lucia (1,007m a.s.l) sampling sites. As no amphibian survey was carried out in the highland areas of THP before, it was not surprising that *A. fuliginea* was only found in this study. Navas (2006) had suggested that the plasticity of frog thermal physiology will determine the ability of frogs to adapt along different altitudinal gradients and that plasticity might be related to the evolutionary history of a taxon. Hence, the study result suggests that *A. fuliginea* has exhibited great agility in its thermal physiology, allowing this species to distribute in different altitudinal gradients rather than its own gradient range as stated in a previous study. In addition, suitable microclimate and microhabitat availability could be a determining factor for the distribution of this species as well.

There were only 17 frog species present at the lowland area with an elevation of near sea level to 700m a.s.l. (headquarters of THP, Balong substation and Merotai substation). These frog species are confined to the lowland tropical forest, that is to say, they are not adapted to montane areas (Inger & Stuebing, 2005). These frog species are adapted to flat terrain, in turn, eliminating clear streams and moderate to strong currents which these frogs require for breeding. This suggests that these species are well adapted and are associated to disturbed areas, as well as to human activities (Duellman, 1999; Inger, 1999; Inger, 2005). However, this could not be applied to *H. luctuosa*, *P. colletti* and *R. borneensis*, as these species are rare, only encountered once and also new records to THP, simultaneously.

Occidozyga laevis was only found in Balong substation, whereas *Rhacophorus gauni* was only found in Merotai substation. These two frog species adapted themselves to the forest edge and cultivated plantations as Balong and Merotai substations border oil palm and cocoa plantations. Frog species that live in relatively disturbed, modified and open environment have adaptations either physiologically or behaviourally. These adaptations enable them to withstand or avoid unfavourable extremes of heat or humidity (Inger, 2005). However, there was no significant evidence to prove these occurrences, as more time is needed to observe their behaviour and collect data. Yet, this result can serve as the baseline for a future study.

Throughout the study, *Meristogenys phaeomerus* was the only species found in all six sampling sites, indicating high tolerance for broad habitat types, ranging from low to high elevation, from forest to plantation areas and from riparian to forest areas far from water source.

Results of this study imply that THP has considerably high species diversity and richness of frogs (Table 5) as compared to other areas in Borneo; for instance, Ulu Temburung National Park in Brunei with 66 frog species, Nanga Tekalit in Sarawak with 60 frog species, Crocker Range National Park with 59 frog species and Segaham in Sarawak with 47 frog species (Inger, 2003; Das, 2006; Grafe & Keller, 2009).

The high species diversity and species richness found in THP may be due to the various habitat types in this park. Sampling sites at various elevations is one of the reasons many species were found. The three peaks, Mt. Magdelena, Mt. Lucia, and Mt. Maria provide primary montane and submontane microhabitats for specialist frog species, such as *Rhacophorus everetti* (mossy tree frog) at Mt. Magdelena, since mossy forest only occurs on this mountain (Stuebing and Nor, 1995; Inger et al., 1996; Richards, 1996; Malkmus et al., 2002; Inger & Stuebing, 2005).

Lowland forests and hill forests are also present in THP, apart from montane forests. These two types of forests provide different types of microhabitats to lowland frog species, particularly those needing swift, torrential streams and rivers to breed but occurring below 900m a.s.l. (Grafe & Keller, 2009). Apart from that, tree canopy in lowland primary rainforests provides shelter for arboreal species, such as *Rhacophorus borneensis* (Borneo flying frog). This species remains within the canopies and only descends to breed around semi-permanent pools on forest floor (Richards, 1996; Inger & Stuebing, 2005; Inger & Tan, 2010).

Conclusion

A total of 28 transects were established and surveyed in six sampling sites - headquarters of THP, Balong substation, Merotai substation, Mount Magdalena, Mt Lucia and Mount Maria. A total of 925 individuals of frogs were found, from 51 species comprising six families. This study has successfully added 18 new frog species to the checklist of frogs in Tawau Hills Park. The park headquarters is located in the lowland area and had the highest abundance with 467 individuals from 40 frog species, while Mount Magdalena is the least abundant with only 29 individuals from 11 species. The Shannon Diversity Index of frogs in this study was 3.357, indicating that THP has high frog biodiversity.

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Journal of Tropical Biology and Conservation 16: 253–266, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Establishing optimal conditions for nursery production and domestication of *Crassocephalum crepidioides* (Benth.) S. Moore.

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Abstract

Crassocephalum crepidioides (Benth.) S. Moore is a plant consumed as a green leaf vegetable in several regions of Benin. But the species is still not domesticated and is harvested from wild according to the seasons. The plant remains as an undervalued food plant in Benin. This study aims to find the optimal conditions for nursery production of this species for its better valorization and domestication in order to contribute towards reducing food insecurity. We tested seed germination capacity and seedling growth of *C. crepidioides* in a nursery. We used cow dung and poultry manure to fertilize the transplanting board before transplanting seedlings from the seedbed whereas control plots were kept without fertilization. In addition, we tested shade impact on seedlings considering two variants (under shade and out of shade). Seedlings were transplanted in the following three spacings (20x20 cm, 30x30 cm and 40x40 cm). We used two water doses (22 liters/day and 44 liters/day) to water each 3.30 m^2 seedbed. The freshly harvested seeds showed about 19 day's latency with a germination rate to 15.10 % while conserved seeds showed a latency time to 5 days with a germination rate to 12.70 %. Fertilization and shade influenced significantly the growth of *C. crepidioides*. Cow dung and poultry manure are suitable for soil fertilization for better plant development. The dose of water supplied to the seedbeds and transplanting spacings during the dry season also significantly influenced the growth of *C. crepidioides*. We found that that the 20x20 cm transplanting spacing reduces evaporation and transpiration, which is better for plant development.

Keywords: greens vegetable, ecological requirements, gbolo, *Crassocephalum crepidioides* Benin

Received 04 April 2018
Reviewed 29 April 2019
Accepted 22 May 2019
Published 15 October 2019

Introduction

Of the 3,000 plant species inventoried in Benin's forest ecosystems (MEHU, 2011), 162 species are consumed by local populations as food plants (Codjia et al., 2003). Of these, *C. crepidioides* occupies a prominent place given its nutritional and medicinal importance. It is an annual plant that grows spontaneously on newly cleared fields. The leaves of this plant are sold in local markets in almost all regions of Benin, and consumed as a green leaf vegetable during the period of the plant's occurrence (Akoegninou et al., 2006). Its leaves are nutritionally very rich in vitamin C and minerals (100 g of fresh leaves contain 9.17 mg of vitamin C, 308.45 Kcal and 1012 mg of calcium) (Adjatin et al., 2013). Vitamin C is prescribed in most of our hospitals, for the synthesis of collagen and inter-cellular; a substance that gives contraction to muscles, vascular tissue, bones, tendons and ligaments (Olayinka et al., 2012). Thus the amount of vitamin C contained in the fresh leaves of *C. crepidioides* helps with health problems. The hot extracts of the leaves also play an antibacterial role (Omotayo et al., 2015). *C. crepidioides* also contains a large amount of satisfactory protein and a large amount of essential amino acids that constitute an alternative source of energy when the carbohydrate metabolism is weakened via gluconeogenesis contributing to stunting in children and lack of development during pregnancy (Iheanacho et al., 2009).

Despite all these qualities, consumers do not plant the species. Its exploitation remains in the form of collection from the wild during the period of the occurrence on newly cleared farms. It thus urged to domesticate C. *crepidioides*. However, the search for horticultural conditions of production is an alternative that could contribute to the domestication of this species. In this study, we did a germination test of *C. crepidioides* seeds that were not preserved and those that were preserved. We also compared the growth and morphology of *C. crepidioides* according to two animal dung (cow dung and poultry manure) under the shade and without shade. Similarly, the growth and morphology of *C. crepidioides* is studied according to different spacings and doses of water. We discuss the implications of our results for the enhancement of *C. crepidioides* and also help the population to obtain information related to the production of the species.

Materials and Methods

Study area

The study was conducted in the village of Adakplamè, Kétou district which is located about 150 km from Cotonou, the economic capital of Benin. The district of Kétou is located at the northern end of Plateau department between 7°10 and 7°41'17" north latitude and between 2°24'24" and 2°47'40" East and covers an area of 1,775 square kilometers (INSAE/RGPH3, 2002). The village of Adakplamè which is the largest one in the district of Kétou is distanced from the town centre by about 12 km and is surrounded by two forest reserves which are Dogo classified forest and Kétou classified forest. The climate in this zone is tropical, Guinean type with bimodal rainfall with two rainy seasons and two dry seasons. The long rainy season covers the period of March to July and the short dry season covers the month of August. The short rainy season covers the period of September to October and the long dry season covers the period of November to February. The average annual rainfall varies from 1,200 to 1,300 mm. The annual average temperature varies from 25 °C to 29 °C and the average relative humidity varies from 68 % to 85 %. Annual evapotranspiration varies from 89.19 mm to 144.13 mm.

Plant material

C. crepidioides seedlings are obtained from germination of seeds in the nursery. The common name of the species is "Crassocephale" or *faux crépide* in French and vernacular names are Gbolo (Yoruba and Adja), Akogbolo or Hôwounhôgui (Mahi), or Terikiagborou (Bariba), Wankadjobrou (Ditamari), This herbaceous annual plant of up to 1 m height (Akoegninou et al., 2006) belongs to the Asteraceae family. The leaves are simple, lobed form with toothed edge, surrounded by fine hairs. The flower is attached on a peduncle with a set of long sepals with tube shape containing a hundred white stamens (setaceous) bearing each, a seed at the base and showing red colour at the maximum petals height (Figure 1). The seeds are particularly very small and their average weight is 0.15 mg/seed.

Figure 1. Seeds, flowers and C. crepidioides

a. Data collection methods

i. Seed germination tests

C. crepidioides seeds were collected from identified local individuals in the farms at Adakplamè (municipality of Kétou). Two germination tests have been performed. The first germination test focused on freshly harvested seeds, dried and then sown two days after harvest and the second germination test was carried out on dried seeds that were stored in a perforated box for ventilation for 7 months. In the first test 1,800 seeds of *C. crepidioides* were sown on a straw seedbed straw of which 96 plants were replicated. In the second test 3,000 seeds of *C. crepidioides* were conserved for 7 months then sowed in the same conditions of which 232 plants were replicated. Seeds were sowed to the flight on two traditional seedbeds (1 m x 1 m). The seedbeds were watered two times per day (2 litres in the morning and evening). For the tests, the number of seeds germinated were counted daily for two months and seed germination rate was determined as follows:

$$\mathrm{Tg} = \frac{\text{Number of germinated seeds}}{\text{Number of sowed seeds}} \text{ x } 100$$

ii. Transplanting seedlings

❖ **Effect of soil fertilization and shade on the growth of *C. crepidioides*.**

This trial was conducted during the rainy season from August to October 2016. Six weeks after sowing, seedlings obtained from the first seed germination test were transplanted at a spacing of 60x60 cm on 12 planting boards of 3 m x 1.10

m fertilized with cow dung and poultry manure. Two factors were studied; three level of fertilization (cow dung (**cd**), poultry manure (**pm**) and control (**c**)) and two level of shade (under shade and out of shade). The experimental design is a randomized block design with two replications (Figure 2). Each block is composed of six planting boards with two planting boards per treatment. Twelve (12) kilogrammes of fertilizer were provided to each fertilized board.

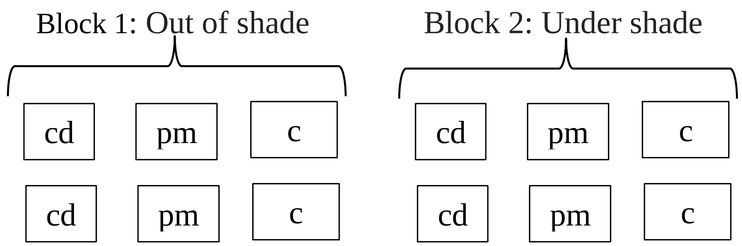

Figure 2. Experiment design of first trial of rainy season.

❖ **Effect of transplanting spacing and amount of water on growth of *C. crepidioides*.**

This trial was carried out during the dry season, from January to March 2017. Two factors were studied; spacing between plants and water doses. Six weeks after sowing, seedlings resulting from seed germination at the second germination test were transplanted on six boards of 3x1.10 m, regularly watered twice a day. The boards were fertilized with cow dung. The experimental design is a randomized bloc design with two replications (Figure 3). Two treatments were applied which are water dose: 2.68 L/m^2/day either 22 liters of water a day, with 11 liters in the morning and 11 liters in the evening; 5.36 L/m^2/day either 44 liters of water a day (22 liters morning and 22 liters evening) and transplanting spacing (20x20 cm (**S1**), 30x30 cm (**S2**) and 40x40 cm (**S3**)).

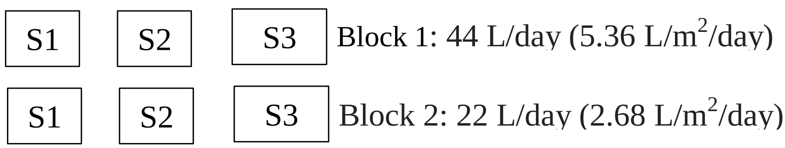

Figure 3. Experimental design of second trial of the dry season.

b. Measurement and data analysis

Quantitative variables such as plant size (Ht), number of leaves (Nf), length (Lf) and width (lf) of limb, number of internodes (Nen), Leaf weight (Pf) and number of flowers (Nfr) were measured on 5 plants per planting board. Data was collected weekly starting from the third week after transplanting. Roots weight (Pr), fresh leaves weight and dry leaves weight were measured on the seventh week after transplanting. We used Kruskal-Wallis test (non-parametric equivalent of ANOVA one-way) to test the effect of fertilization on Ht, Lf, lf, Pf, Pr and Ps. Generalized linear model (GLM) was also used to test the effect of fertilization on Nf, Nen, and Nfr and Wilcoxon test was used for comparison of means. We generated a correlation matrix using all measured variables. Analyses were performed using R.3.2.4 (R Core Team, 2016) software.

Results

Germination rate of C. crepidioides

Figure 4 shows germination rates of freshly harvested seeds and stored seeds for 9 months respectively. The analysis of this figure revealed that the freshly harvested seeds have a latency period of 19 days and a germination rate of 15.10 % in 47 days after sowing while stored seeds have a latency time of 5 days and a germination rate of 12.70 % in 19 days.

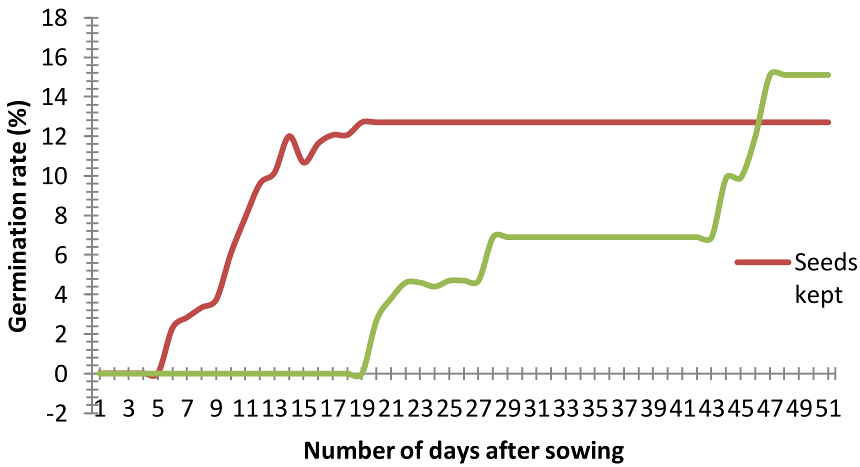

Figure 4. Germination rate of *C. crepidioides*.

Influence of soil fertilization on the growth of C. crepidioides

Table 1 shows the effect of soil fertilization on plant height, leaves size and roots weight. Soil fertilization statistically has a significant effect (Prob. <0.1 %) on plant height, leaves length and roots weight. However, means comparison test shows that *C. crepidioides* apparently has uniformed growth, whether the soil is fertilized with cow dung or poultry manure. In contrast, plants grown on soils without fertilization (controls) showed lower values of growth parameters (Table 1) with a highly significant difference.

Table 1. Effect of fertilization on the growth of *C. crepidioides*.

Variables	Fertilizers	chi-sq	Prob	Means
Plant height (Ht in cm)	Cow dung Poultry droppings Control	21.22	0.000***	22.85[a] 23.33[a] 11.50[b]
Length of leaves (Lf in cm)	Cow dung Poultry droppings Control	19.46	0.000***	19.90[a] 20.52[a] 13.18[b]
Width of Leaves (lf in cm)	Cow dung Poultry droppings Control	20.38	0.000***	7.19[a] 7.24[a] 4.85[b]
Weight of Roots (Pr in g)	Cow dung Poultry droppings Control	13.93	0.000***	0.09[a] 0.07[ab] 0.05[b]
Weight of Fresh leaves (Pf in g)	Cow dung Poultry droppings Control	18.39	0.000***	0.19[a] 0.15[a] 0.09[b]

There are strong positive correlations between plant height, leaves length (r = 0.87) and width (r = 0.87) leaves and between length and width of leaves (r = 0.95) (Table 2). Thus, *C. crepidioides* leaves sizes (length and width) are proportional to plant height. Table 3 shows the effect of soil fertilization on number of leaves and number of internodes. Soil fertilization has a highly positive statistically effect (prob. <1 %) on number of leaves and number of internodes of *C. crepidioides*. Fertilization of the soil with cow dung or poultry droppings increased the number of leaves produced by *C. crepidioides*. Mean number of leaves produced per plant varied from 21.80 to 26.70 on fertilized soils whereas number of leaves was 12.25 on the control.

Table 2. Matrix of correlations between growth parameters.

Variables	Ht	Nf	Lf	Lf	Nen	Pf	Pr
Ht	1						
Nf	0.54	1					
Lf	0.87	0.72	1				
lf	0.87	0.65	0.95	1			
Nen	0.28	0.83	0.54	0.43	1		
Pf	0.69	0.76	0.78	0.75	0.66	1	
Pr	0.71	0.49	0.74	0.71	0.33	0.74	1

Ht: plant height; Nf: number of leaves; Lf: length of leaves; lf: width of leaves; Nen: number of internodes. >0.70: strongly correlated; 0.30 to 0.70: moderately correlated; < 0.30: weakly correlated.

Table 3. Effect of fertilization on number of leaves and number of internodes.

Variables	Fertilizers	Coefficient	Standard error	t value	means	Prob
Number of leaves (Nf)	Cow dung	0.779	0.148	5.23	26.70	0.000***
	Poultry droppings	0.576	0.154	3.74	21.80	0.000***
Number of internodes (Nen)	Cow dung	1.115	0.341	3.26	9.15	0.001**
	Poultry droppings	0.993	0.347	2.861	8.10	0.005**

Influence of the shade on the growth of *C. crepidioides*

Table 4 shows the effect of the shade on the growth of *C. crepidioides*. Plant size, length and width of leaves, weight of fresh leaves and weight of roots are higher under the shade. In contrast, the number of leaves and number of internodes are higher when out of shade. A significant statistically effect of shade is noted on plant size (Prob. <0.1 %), length (prob. <5 %) and width (p <1%) of leaves, number of internodes (Prob. <0.1 %) and weight of roots (Prob. <1 %). Figure 5 (a and b) shows pictures of *C. crepidioides* plants under the shade and out of the shade, respectively.

Table 4. Effect of shade on growth of *C. crepidioides.*

Variables	V	Prob.	means
Size (Ht in cm)	370	0.001**	23.7 14.75
Number of leaves (Nf)	146	0.124	18 22.5
Length of leaves (Lf in cm)	341.5	0.025*	19.28 16.45
Width of leaves (lf in cm)	384	0.001**	7.04 5.82
Number of internodes (Nen)	38.8	0.000***	3.73 9.77
Weight of roots (Pr in g)	191	0.7	0.15 0.14
Weight of Fresh leaves (Pf in g)	205.5	0.001**	0.09 0.06

Figure 5. Under the shade plants (a) and out of the shade plants (b) on fertilized soil.

Influence of amount of water on growth of *C. crepidioides*.

The dose of water used to irrigate the seedbed has significant effect (Prob <5%) on the number of internodes, number of leaves and the dry weight of the leaves. The total height, width, length and wet weight of the leaves are not influenced by the dose of irrigating water. The seedbeds watered with 44 litres of water per day, produced highly grown plants (11.05 cm and 18.91 cm height; 35 and 48.44 leaves respectively for 22 litres and 44 litres). Table 5 presents the mean values of growth parameters of *C. crepidioides* according to the water doses used to irrigate the seed beds.

Table 5. Effect of water dose on the growth of *C. crepidioides*.

Variables	P value	22 liters	44 liters
Total height	0.49	11.05	18.91
Leaf width	0.49	2.97	4.37
Leaf length	0.84	8.58	12.53
Number of internode (Nen)	0.02	4.27	7.55
Number of leaves	0.02	35	48.44
Number of flowers	0.00***	1.77	11.38
Fresh leaf weight	0.06	25.5	48.94
Dry leaf weight	0.005	2.23	5.27

Influence of spacing on growth of *C. crepidioides*.

The average values of growth parameters of *C. crepidioides* are higher for 20x20 cm planting spacing (Table 6) compared to 30 x 30 cm and 40 x 40 cm. The variance analysis probabilities indicate that transplant space have significant effects (Prob <5 %) on number of internodes, number of leaves, and leaf dry weight, while the dose of water used to irrigate does not influence the total height, width, length and wet weight of the leaves (Table 5).

Table 6. Effect of transplanting spacings on the growth of *C. crepidioides*.

Variables	P value	20cm x 20cm	30cm x 30cm	40cm x 40cm
Total height	0.48	16.58±5.12	14.87±7.94	13.50±5.11
Leaf width	0.49	4.09±0.95	3.20±1.17	3.71±0.61
Leaf length	0.84	11.86±2.37	9.57±3.22	10.23±2.02
Number of internode (Nen)	0.02	6.83±2.83	4.41±5.40	5.50±2.64
Number of leaves	0.02	42.66±20.91	38.58±26.54	43.91±15.12
Number of flowers	0.00***	6.83±9.68	10.91±21.09	2.00±3.93
Fresh leaf weight	0.06	39.50±19.92	34.33±30.06	37.83±20.24
Dry leaf weight	0.005	4.16±2.08	3.61±3.33	3.47±2.26

Discussion

Germination of *C. crepidioides* seeds

The germination rates of *C. crepidioides* is low; this could be due to high density of seeds sowed within a small space as seeds were so small and were sprayed without counting. Indeed, according to (Sounon et al., 2009), the high density of seeds sowed within a restricted space could reduce the space available for germination of each seed and then induce competitive effects between seeds; which can slow down their germination. Much lower germination rates could also result from infertility of some seeds in fruits. There are 30 to 50 seeds in a fruit of *C. crepidioides*. The long periodof germination (from the 19th to 47th day) of freshly harvested seeds could be a result of embryonic dormancy of seeds, due to the presence of inhibiting substances in the pericarp; which slow down the metabolic reactions of seed germination (Wédjangnon et al., 2016). Compared to these freshly harvested seeds, those which have been dried for days then stored for months have a very short germination time which starts from the 5th to 19th day after sowing. The long dry period and the long duration of conservation would have allowed better drying of seeds and reduction of inhibiting substance that would have consequently reduced the duration of latency and germination time.

Effect of soil fertilization and shade on growth of *C. crepidioides*

Soil fertilization with cow dung and poultry manure improved the growth of *C. crepidioides* plants. This growth is apparently uniformed on soils fertilized with cow dung and poultry manure. However, a statistically significant difference is observed between all plant growth parameters on fertilized soils compared to controls (Table 1). These results could be explained by the physicochemical properties of both fertilizers. Cow dung and poultry manure are organic fertilizers rich in organic matter, nitrogen, potash and phosphorus, which allow for the best growth of seedlings (Charbonnier et al., 2012; Lehmann et al., 2012).

Figure 6. Plants produced with soil fertilization (a) and without soil fertilization (b).

Plants produced out of shade have more leaves than those produced under shade. This could be explained by significant photosynthesis out of shade, which causes large branching and consequently a large number of internodes. The height growth of plants leaves length and width under shade can be explained by the phototropism phenomenon under shade, where well channeled light has tends to attract the plants in their height growth, which is not the case in diffuse light (Djidji et al., 2010). This growth of plants under shade, answers the preference of green vegetable consumers, who when at the market choose green vegetables with long and wide leaves.

Figure 7. *C. crepidioides* plants produced under shade (a) and out of shade (b).

Effect of spacing and amount of water on growth of *C. crepidioides*

The comparison of plants produced during the various seasons indicates that the number of leaves produced during the dry season is higher than the in the rainy season whatever the spacing between plants. However, the leaves grow more in rainy season. This could be a result of insufficient water and high transpiration of plants produced in the dry season when plant evapotranspiration is the highest. The production of this green vegetable during the dry season requires the provision of sufficient quantity of water. It is also observed that there was leaf shrinkage on seedbeds irrigated with 22 liters per day (Figure 8). This shrinkage is even more accentuated on plants with large spacing (30x30 cm and 40x40 cm). Thus evaporation and transpiration are less important when plants are closer.

Figure 8. Morphology of leaves in dry season. (a) seedbeds irrigated with 22 L of water per day; (b) seedbeds irrigated with 44 L of water per day.

Conclusion

It appears clearly from the different experiments that fertilization of soil with cow dung or poultry droppings allows for the best growth of *C. crepidioides* plants to satisfy the preference of green vegetable consumers. Cultivation of this plant under shade induces the best growth of leaves. However, *C. crepidioides* can also be grown out of shade with good growth of leaves. Cultivation of this plant requires a lot of water. The transplanting of seedlings to close spacings limits evaporation and transpiration of the plant in the dry season, which improves its growth. Apart from very low germination rates of the seeds, which forthcoming investigations on production of this green vegetable must improve in terms of its nursery production, there is no difficulty in producing the green vegetable of *C. crepidioides* in regards to its abundant fruiting. It is necessary to valorize this green vegetable because of the nutritional composition of leaves and their importance in terms of health.

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Journal of Tropical Biology and Conservation 16: 267–283, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Assessment of Spatial Variability and Temporal Dynamics of Dissolved Organic Matter (DOM) at Lower Kinabatangan River Catchment, Sabah.

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Abstract

The spatial and temporal variability of dissolved organic matter (DOM) characteristics and surface water quality in the Lower Kinabatangan River Catchment were determined between October 2015 and May 2016. The objectives of this study were: (i) to distinguish the DOM absorption characteristics and physicochemical quality of surface water draining from different types of land use: oil palm plantation (OP), secondary forest (SF) and semi-natural vegetation (SV); and to examine its temporal variations during dry and wet periods. The collected physicochemical parameters data was analysed and classified based on the Malaysian National Water Quality Standard (NWQS). Findings indicated all the parameters fall into Class I, except for pH, total suspended solids (TSS) and concentration dissolved oxygen (DO). Linear discriminant analysis has been applied to distinguished the physico-chemical and absorption DOM properties data into mutually-exclusive spatial and temporal groups. Interestingly, the pH, DO and total nitrogen values were exhibited as dominant parameters at SV during both low and high rainfall periods. The dominance of these parameters suggested that the spatially and temporally varied water quality were influenced by both natural processes (e.g precipitation rate) and anthropogenic factor (e.g land use change). Whereas, both absorption coefficients (a_{340}) and spectral slope ($S_{275-295}$) were more dominant at SF and OP respectively. This might be due to increasing terrestrial DOM loadings as well as significant degradation of DOM via microbial and/or photochemical reaction.

Keywords: Dissolved organic matter (DOM), physico-chemical water quality, Lower Kinabatangan River Catchment.

Received 23 August 2018
Reviewed 27 May 2019
Accepted 02 July 2019
Published 15 October 2019

Introduction

Tropical river catchment is a complex ecosystem, linking various components with each other; biotic and abiotic, terrestrial and aquatic, plants and soils, atmosphere and vegetation, and soils and water (Giller & Malmqvist, 1998; Baxter et al., 2005). However, despite its well known ecological importance, significant areas of the tropical catchment are increasingly disappearing over the years as a result of development and economic gains. Changes in the catchment surface characteristics have been found to disrupt the components of hydrologic system in the region such as streamflow, surface runoff, groundwater recharge as well as water quality (Shukla et al., 2014). Conversion of forested areas to agricultural land also result in increased rate of sediment loads, surface runoff and nutrient influx (Jakobsen et al., 2007). As the tropical river system is characterized by a distinct annual cycle in precipitation, rain period and high solar radiation (Saigusa et al., 2008), temporal variant may also create a difference in the flow velocity, water chemistry and metabolic rates (Tan et al., 2017).

The spatial and temporal variability of water chemistry has been previously addressed in other studies. The findings by Tan et al. (2017) indicated water temperature, total suspended solids (TSS) and conductivity were varied spatially at different types of land use at Maliau Basin Conservation Area (MBCA), while the values of pH and concentrations of dissolved oxygen (DO) displayed variation during dry and wet period respectively. Harun et al. (2014) had reported seasonally variable contributions of TSS and chemical oxygen demand (COD) at the Lower Kinabatangan River Catchment, with higher values during the wet season. However, precipitation anomalies had been identified in the duration of the study period during 2005/2006, which explained by the occurrence of a weak La Nińa event. In another water quality study within the same catchment area, significant variations of dissolved organic matter (DOM) were also observed by Harun et al. (2016), with respect to types of land use and seasonal variability. The characterization of DOM quality and quantity on both spatial and temporal scales is important as it reflects the proper functioning of the aquatic ecosystem.

Riverine DOM is a heterogeneous mixture of various organic compounds in natural water. The aggregations of these molecules had increased the complexity of DOM structure which described its reactivity and bioavailability within the aquatic ecosystem (Aiken et al., 2011; Bejarano et al., 2015; Yates et al., 2016). Allochthonous DOM made up major aquatic DOM pool, along with *in-situ* heterothropic and anthropogenically derived DOM. As rivers at the lower catchment areas are usually wider, slower-flowing water and less coverage by forest canopy as compared to the upper catchment (Harun, 2013), the autochthonous DOM may play a more important role in this part of the river where higher penetration by sunlight may increase microbial activities and photochemical reaction in water bodies. Characterization of the quality and quantity of DOM by spectroscopic measurement has been widely applied, as it enables quick analysis and low operational cost. Numerous studies on the amount and composition of DOM had demonstrated its significant role in examining land use conversion and anthropogenic activities that influence the local surface water quality, for instance, findings by Limpens et al. (2008) showed that the DOM in oil palm plantations seems to have a significant signature.

This paper focuses on the characterization of the absorption bands of riverine DOM in the UV-Visible range and surface water quality in forested and agricultural catchments during both dry and wet periods. The quality of DOM and surface water draining from three different types of land use (oil palm plantation, semi-natural vegetation and secondary forest) at Lower Kinabatangan River catchment were explored in this study. The rapid expansion of agricultural land was known to have a profound effect on the aquatic ecosystems as well as altering the quantity and quality of DOM. Although the water quality study had been previously conducted within the same river catchment areas, both spectroscopic DOM properties in the UV-visible range and physico-chemical water quality have not been addressed in any publication. In addition, the study also aims to provide a better understanding on water quality trend analysis and its relationship to the water quality of the lower Kinabatangan river, which highlights the crucial requirement of consistency in water quality monitoring.

Methodology

Study site

The Lower Kinabatangan River Catchment is located on the east coast of the Malaysian state of Sabah. The river flows 560 km easternly from its headwaters in the Southwest region of Crocker Range to the Sulu Sea (Boonratana, 2013; Fletcher, 2009). It is the second longest river in Malaysia, draining a total of 16,800 square km of water catchment area or about 23% of the total land area of Sabah. Lower Kinabatangan land areas can be classified into natural rainforest wetland habitat, agriculture and village settlement (Fletcher, 2009). Currently, almost half of the Kinabatangan district is dominated by agricultural areas, mainly oil palm and a very small percentage of other crops. Oil palm is grown on cleared vast area of forest land or logged areas that were transformed into plantations. Malaysia had become the second biggest palm oil exporter in the world, with Sabah as the major contributor in total production of palm oil in the country.

Sample collection and in-situ *analysis*

A total of 12 sampling stations were selected along the lower reaches of Kinabatangan River (Table 1). Sampling was conducted based on its accessibility and characterized by three types of land use which included oil palm plantation (OP), semi-natural vegetation (SV) and secondary forests (SF) (Figure 1). A total of 60 surface water samples were collected during the fieldwork campaigns (October to November 2015 and March to May 2016). The collected samples were stored in 200 mL of high-density polyethylene (HDPE) bottles, which are pre-washed with 10% hydrochloric acid to avoid contamination. Samples filtration were immediately conducted using Whatman GF/F glass fibre filter. Samples collected were acidified using HCl to pH~2 for preservation until further DOC analysis at Universiti Malaysia Sabah (UMS). In-situ water quality parameters were determined by using a YSI Profesional Plus (ProPlus) (Model 6026 S/N Y5173) multiparameter.

Table 1. Sampling stations and its GPS coordinates

Selected rivers	Stations	GPS coordinates
Pin River (SV1)	P1	N 05° 23.901 E 117° 56.087
	P2	N 05° 23.788 E 117° 56.096
	P3	N 05° 23.682 E 117° 56.093
Takala River (SV2)	T1	N 05° 25.056 E 117° 58.814
	T2	N 05° 24.114 E 117° 58.781
	T3	N 05° 24.056 E 117° 58.743
Resang River (OP)	R1	N 05° 32.973 E 118° 20.159
	R2	N 05° 32.933 E 118° 20.209
	R3	N 05° 32.903 E 118° 20.255
Menanggol River (SF)	M1	N 05° 30.293 E 118° 16.389
	M2	N 05° 30.284 E 118° 16.328
	M3	N 05° 30.276 E 118° 16.260

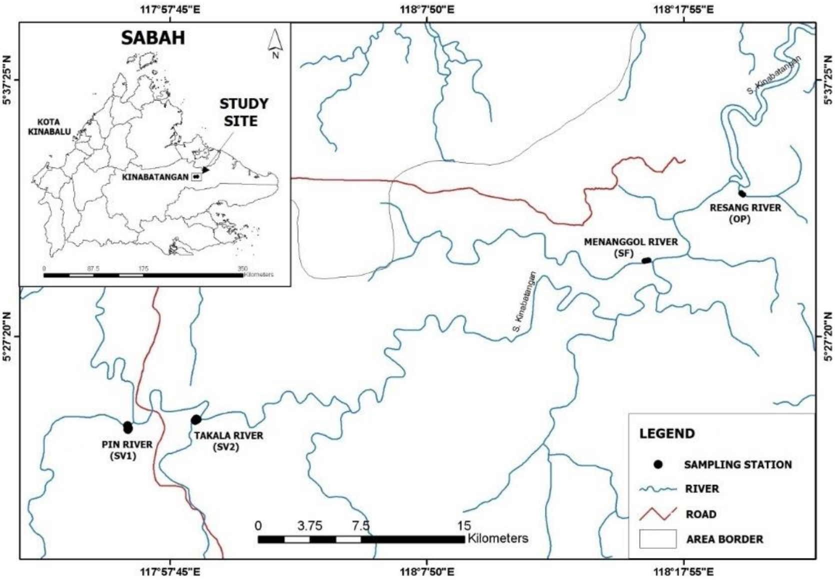

Figure 1. The locations of sampled rivers

Spectroscopic DOM analysis

In this study, Agilent Cary 60 UV-Visible Spectrophotometer were used to measure the UV-visible absorption spectra of DOM within all samples with deionized water used as a reference. The absorption spectra were obtained between a wavelength of 200 nm to 800 nm and each data were collected at 1 nm intervals. The wavelength of 200-400 nm was selected to represent the ultraviolet (UV) of a region of the light spectrum, while 400-800 nm represented the visible light portion. All the samples were allowed to reach room temperature before spectrophotometric analysis began. The reason was to avoid the condensation to form on the wall of the cuvette (Carter et al., 2012) and interfere with the spectrum of radiation from the spectrophotometer. The surface cuvette was also cleaned before measurement to keep the reflection and scatter losses to a minimum. Then, absorption spectra recorded by the spectrophotometer were converted to absorption coefficient using Beer-Lambert Law (1). Beer's Law states that the absorption of light is directly proportional to both the concentration of the absorbing medium and the thickness of the medium of the light path (Dhikale et al., 2015).

$$\alpha = 2.303*A/l \quad (1)$$

Where α is absorption coefficient, A is the absorbance of the sample by the spectrophotometer and l is the path length of the cuvette in meters. The absorption coefficient was obtained at wavelength 340 nm (a_{340}). This wavelength was used to characterize dissolved organic matter as organic molecules absorb and reacts with UV light in this wavelength (Baker et al., 2004). Besides that, the spectral slope from the wavelength interval of 275 to 295 nm ($S_{275-295}$) was also identified and calculated as a linear regression of the log transformed spectra. Absorption coefficient a_{254} and a_{340} were used to indicate the concentration of chromophoric DOM while spectral slope has been widely used to indicate the molecular weight and aromaticity of DOM (Helms et al., 2008).

Dissolved Organic Carbon analysis
Shimadzu TOC-L Analyzer with auto-sampler ASI-L was used to determine the concentrations of dissolved organic carbon (DOC) in the sample. Catalytic combustion oxidation method was used in this analysis, which involves complete oxidation of carbonaceous materials with the aid of a catalyst. Prior to sample analysis, all the samples were allowed to warm to room temperature (Loginova et al., 2016), which then acidified and sparged for 8 minutes at 75 or 100 mL/min with ultra-pure oxygen. These steps were carried out to remove the inorganic carbon compounds from the samples (Zigah et al., 2012). Apart from that, the concentration of dissolved nitrogen (DN) were also determined using this method.

Statistical analysis
Linear discriminant analysis (LDA) were applied to classifying physicochemical water quality and spectroscopic DOM properties into mutually exclusive spatial and temporal groups. Discrimination between groups and minimisation of misclassification error rates resulted in a linear combination of these parameters (Gazzaz et al., 2012). The LDA standardized coefficient out represents the partial contribution of the physicochemical and spectroscopic DOM properties and rank the importance of each parameter to the discriminant function (Tan et al., 2017). Statistical analysis of linear discriminant analysis (LDA) was run by using R statistical software 3.4.4.

Result and discussion

Rainfall data and environmental condition during sampling session

The Lower Kinabatangan River Catchment had received 2614.0 mm of the total annual rainfall for 2015, followed by approximately 2494.8 mm recorded in 2016 (Figure 2). The wet period (the total monthly rainfall is slightly above than the average annual rainfall each respective year) for the sampling occasions were recorded in November 2015 and May 2016. Meanwhile, the dry period was taken in October 2015, March and April 2016 (the total monthly rainfall is slightly lower than the average annual rainfall each respective year). The lowest rainfall was recorded during March 2016 (third sampling session) with significantly reduced river water levels, followed by April 2016 and October 2015. Rainfall data in December 2016 is unavailable.

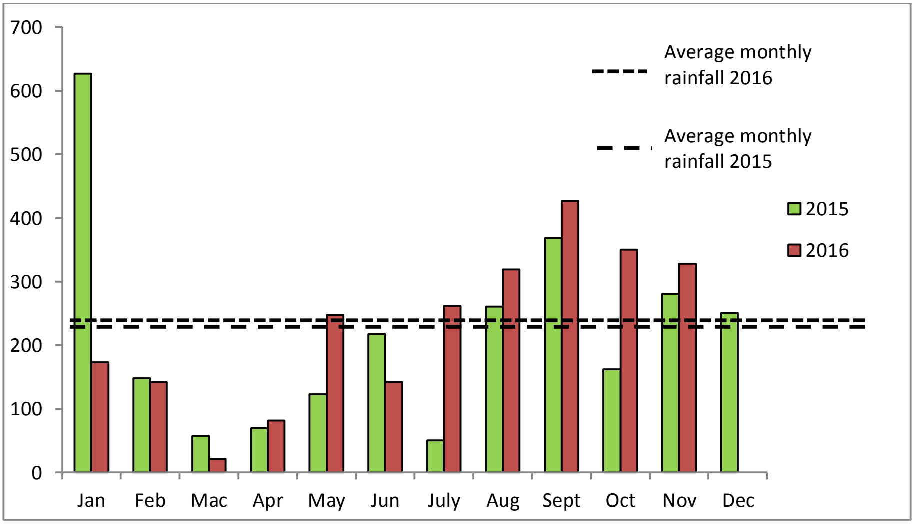

Figure 2. Monthly rainfall data recorded at Lower Kinabatangan River Catchment from January 2015 until November 2016. (Meteorological Department, Kota Kinabalu)

Surface water quality

Most of the water quality parameters in this study were classified under Class I, based on the Malaysian National Water Quality Standards (NWQS), except for dissolved oxygen (DO), total suspended solids (TSS) and pH values categorized as class IV. The water quality at Lower Kinabatangan River catchment was considered to be polluted and suitable to be used only for irrigation purposes. The low pH level and high TSS concentration were observed at agricultural-based land use (OP and SV) compared to SF (Table 2). Although loads of organic matter usually higher during land preparation and planting periods within young oil palm plantation, lack of land cover by vegetation as well as the unpaved road of harvesting path of mature plantations at OP may have caused significant soil erosion and washed into the river. As organic matter are naturally acidic, increase in its concentration

Table 2. Mean ± SE of physicochemical and spectroscopic parameters at Lower Kinabatangan River Catchment during different sampling months.

	pH	Temperature °C	Conductivity µS/cm	DO mg/L	TSS mg/L	DN mg/L	DOC mg/L	a_{254} /m	a_{340} /m	$S_{275-295}$ /nm
Semi-natural vegetation (SV)										
Oct	6.8 ±	28.1 ±	242.1 ±	5.1 ±	399.9 ±	1.4 ±	9.0 ±	144.0	74.1 ±	0.008 ±
2015	0.4	0.7	3.5	0.1	21.9	0.4	0.4	± 32.4	26.8	2.2x10^{-3}
Nov	7.1 ±	26.5 ±	174.0 ±	4.5 ±	320.8 ±	0.9 ±	6.1 ±	100.3	47.1 ±	0.009 ±
2015	0.3	0.3	38.9	0.4	96.8	0.4	0.7	± 4.2	1.8	2.0x10^{-4}
Mar	8.2 ±	32.8 ±	226.8 ±	5.8 ±	25.8 ±	N/A	7.2 ±	75.6 ±	34.3 ±	0.010 ±
2016	0.7	0.8	43.7	1.5	9.7		1.8	29.3	5.8	1.1x10^{-3}
Apr	6.6 ±	30.2 ±	265.8 ±	2.4 ±	199.6 ±	3.4 ±	9.1 ±	85.1 ±	63.6 ±	0.009 ±
2016	0.2	1.2	57.1	0.4	41.6	0.4	1.8	5.4	5.6	3.4x10^{-4}
May	7.5 ±	34.2 ±	152.4 ±	7.8 ±	54.8 ±	0.5 ±	7.8 ±	92.6 ±	70.3 ±	0.008 ±
2016	0.6	1.6	28.4	2.9	14.5	0.2	2.5	7.4	14.2	3.9x10^{-4}
Oil Palm Plantation (OP)										
Oct	6.5 ±	28.4 ±	411.8 ±	3.1 ±	56.3 ±	0.8 ±	8.8 ±	114.3	49.6 ±	0.010 ±
2015	0.2	0.7	71.7	0.4	19.3	0.2	1.4	± 18.3	5.4	3.5x10^{-4}
Nov	4.0 ±	27.2 ±	875 ±	2.6 ±	53.9 ±	0.9 ±	9.6 ±	116.0	45.9 ±	0.013 ±
2015	0.0	0.1	69.3	0.1	2.0	0.0	0.1	± `.3	1.2	3.5x10^{-4}
Mar	7.3 ±	30.5 ±	372.3 ±	4.1 ±	39.6 ±	N/A	9.3 ±	`07.2	60.4 ±	0.010 ±
2016	0.0	0.2	46.1	0.1	6.8		0.6	± 45.5	9.5	3.8x10^{-4}
Apr	6.4 ±	31.3 ±	166.6 ±	3.5 ±	349.3 ±	0.4 ±	8.6 ±	91.9 ±	54.2 ±	0.009 ±
2016	0.2	0.1	47.7	0.4	56.6	0.1	2.7	10.0	2.7	2.5x10^{-4}
May	4.2 ±	27.8 ±	516.3 ±	4.5 ±	75.8 ±	1.8 ±	6.8 ±	91.13	44.9 ±	0.008 ±
2016	0.1	0.3	19.0	0.3	8.1	0.4	0.2	± 8.7	0.5	2.1x10^{-4}
Secondary forest (SF)										
Oct	6.5 ±	28.4 ±	176.3 ±	3.0 ±	55.2 ±	0.8 ±	7.5	113.8	51.9 ±	0.010 ±
2015	0.1	0.2	44.7	0.9	5.9	0.2	±1.9	± 8.4	1.3	8.9x10^{-4}
Nov	6.4 ±	26.6 ±	188.6 ±	1.5 ±	14.7 ±	0.4 ±	16.0	300.9	122.0	0.012 ±
2015	0.1	0.0	0.9	0.0	4.5	0.0	± 0.4	± 2.4	± 1.5	1.5x10^{-4}
Mar	7.5 ±	30.5 ±	128.3 ±	4.1 ±	24.6 ±	N/A	12.0	89.8 ±	59.1 ±	0.009 ±
2016	0.1	0.1	1.3	0.2	0.6		± 2.2	31.5	5.1	2.1x10^{-4}
Apr	6.9 ±	31.0 ±	90.1 ±	3.6 ±	90.9 ±	0.2 ±	6.2 ±	89.4 ±	74.9 ±	0.009 ±
2016	0.1	0.3	3.5	0.6	10.7	0.2	3.6	5.8	8.7	3.5x10^{-4}
May	6.2 ±	29.4 ±	74.1 ±	4.0 ±	142.7 ±	0.4 ±	5.3 ±	88.7 ±	66.8 ±	0.008 ±
2016	0.0	0.1	0.1	0.2	6.1	0.0	0.9	2.6	2.2	5.7x10^{-5}

N/A – Data not available

may lower the pH level at OP. Apart from that, fertilizer usage such as ammonium-based nitrogen fertilizer may cause plantation soil to be more acidic and may lower the pH when washed into the river. In addition, accumulation of organic matter at SF (DOC: 16 mg/L; Table 2) could lead to low concentration of DO (1.5 mg/L; Table 2). High organic matter content may limit the fluxes of photosynthetically available radiation (PAR) into the water column, thus decrease the aquatic primary production and release lesser dissolved oxygen compounds (Kelble et al., 2005; Mostofa et al., 2012).

The relationship between Absorption Properties of DOM and DOC concentrations
Quantification of dissolved organic matter (DOM) to assess the total concentration of organic compounds in the aquatic environments, are often represented as dissolved organic carbon (DOC) (Tan, 2014; Spencer et al., 2012; Zhang et al., 2013; Hansen et al., 2016). Many studies investigating the utility of spectroscopic DOM measurements to determine the concentrations of DOC in the freshwater ecosystem have revealed the strong correlation between absorbance values and riverine DOC. Highly correlated UV-visible absorption coefficients at 254 nm (a_{254}) and 340 nm (a_{340}) with DOC concentrations (R^2 = 0.933 and R^2 = 0.915, $p < 0.05$, respectively) have been observed in a DOM study of surface water in Maliau Basin, Sabah (headwaters system for Kinabatangan River Catchment) (Tan et al., 2017).

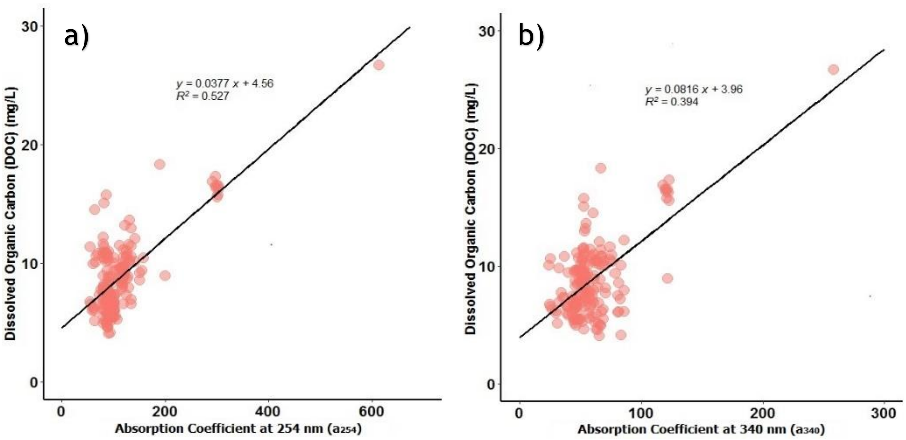

Figure 3. (a) – (b) UV-Visible absorption coefficients at 254 and 340 nm against DOC concentrations.

However, in this study, only weak to moderate relationships were obtained between DOM absorbance of a_{254} (R^2 = 0.527, p< 0.05, Figure 5.1) and a_{340} (R^2 = 0.394, p<0.05, Figure 5.2) and DOC concentrations. This suggested that the organic compounds fraction within the water column changes over time, that can either be dominated by organic compounds with poor UV-absorbing ability or/and light-absorbing chromophoric DOM fractions (Asmala *et al.*, 2014). As the Lower Kinabatangan River Catchment is comprised of various types of land use, exported DOM into the river may originate from a widerange of sources including forested areas as well as anthropogenically altered DOM from agricultural land and human settlements. These results thus suggest that the absorption coefficient at 254 and 340 nm were less suitable to be used as a proxy for DOC concentration in natural waters impacted by anthropogenic activities.

Spatial and temporal variation of water quality

Changes in water chemistry were greatly influenced by catchment characteristics as well as climatic conditions (Detail rainfall data of Lower Kinabatangan River Catchment is illustrated in Figure 2). In this study, the linear discriminant analysis (LDA) was applied to the dataset of physicochemical and spectroscopic DOM properties to investigate the spatial and temporal water quality patterns. As illustrated by the ordination plot of LDA (Figure 4 and Figure 5), water draining from different types of land use (SV, SF and OP) and sampling months showed significant variations with each other. Based on the LDA output (Figure 4 and Figure 5, Table 3), it has been found that pH, dissolved oxygen (DO), conductivity and total nitrogen (TN) were recognized as the dominant parameters in discriminating both spatial and temporal water quality pattern. Absorption coefficient at 340 nm (a_{340}) was able to only spatially discriminate water quality dataset, while the concentration of DOC and water temperature varied temporally.

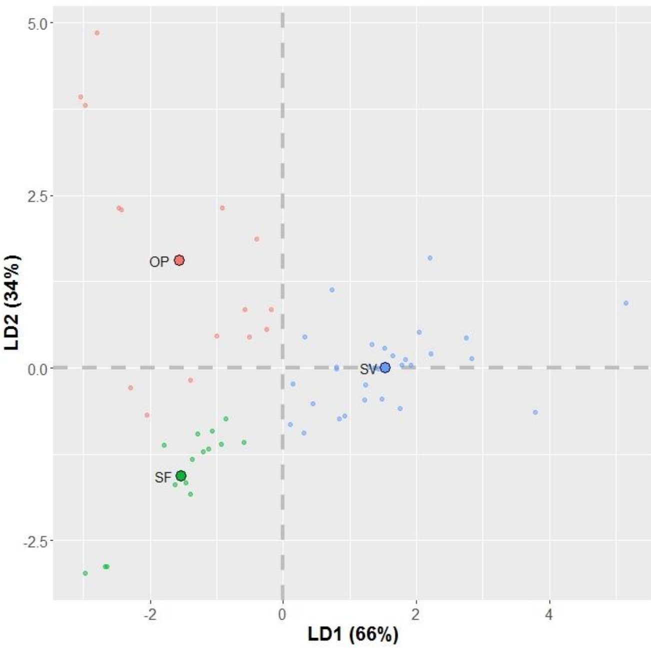

Figure 4. Linear discriminant analysis functions for each type of land use at Lower Kinabatangan River catchment (SV – Semi-natural vegetation, OP - Oil Palm Plantation, and SF - Secondary forest).

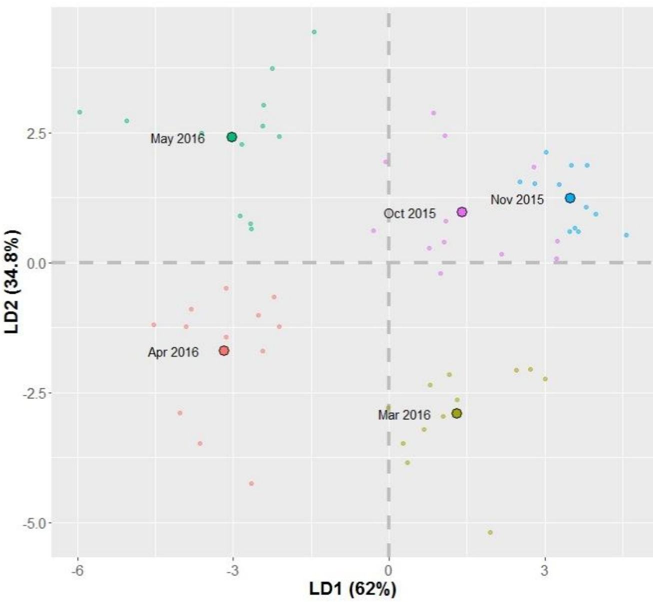

Figure 5. Linear discriminant analysis functions during different sampling months (October 2015, November 2015, March 2016, April 2016 and May 2016).

Table 2. Standardized linear discriminants coefficients and eigenvalue from the linear discriminant analysis that employed to examine the spatial and temporal variations of water quality at Lower Kinabatangan River catchment.

	Spatial Variation		Temporal Variation	
	LD1	LD2	LD1	LD2
pH	**1.32**	0.05	**1.88**	**-0.51**
Water temperature	-0.25	0.31	**-1.53**	**-0.50**
Conductivity	0.39	**1.11**	**1.33**	-0.10
Dissolved oxygen, DO	**0.84**	0.03	**0.60**	-0.02
Total suspended solids, TSS	0.40	0.20	-0.08	0.13
Dissolved nitrogen, DN	**1.14**	-0.08	**-0.50**	**0.75**
Dissolved organic carbon, DOC	-0.03	0.08	0.44	-0.37
a_{340}	0.15	**-0.52**	-0.09	0.34
$S_{275-295}$	0.02	**0.57**	0.48	-0.10
Eigenvalue	8.45	6.06	10.27	7.69

The pH level was observed to vary substantially across selected land use and tend to fluctuate inversely with precipitation events. Higher value of pH at SV was particularly determined during March 2016, while low pH reading was obtained in November 2016 (Table 2). Under a normal monsoon cycle, heaviest rainfall usually occurs between November to March (Northeast monsoon). However, the unexpected dry period of low rainfall was recorded during March 2016, with the apparent decline in water level of river observed within the vicinity of SV land use observed. High pH level obtained in this month was likely due to the low concentration of organic acid compounds in the river system (Pagano et al., 2014). Contrarily, heavy rainfall during November 2015 may increase DOM loadings into the fluvial system via leaching and surface run-off. As humic-rich DOM containing colloidal suspensions are generally acidic in nature, this leads to low pH during the wet period. (Fatema et al., 2014).

Results reveal that the mean DO concentrations recorded in this study ranges from 1.5 to 7.8 mg/L (Table 2). Since aquatic organisms are sensitive to DO levels, low concentrations of DO were highly harmful as at least 4 to 5 mg/L were required to sustain the normal functioning of a healthy ecosystem (Wang et al., 1978). Similar to pH level, high values of DO were also determined at SV. This may possibly occur due to the low quantity of organic acid present in the water column. In contrast, extremely low DO level was recorded during November 2015 (1.5 mg/L, Table 2), which may be due to the high concentration of DOC. Accumulation of organic matter was known to promotes the oxygen dependence reaction and limit the photosynthetically available radiation, thus lowered the DO level (Cory et al., 2015). Higher water temperature has been recorded during April 2016 (dry period), which reflect the significant exposure to solar radiation. The increase rate of light penetration may initiate the photosynthetic reaction in the water column, that resulted in a higher concentration of DO.

In regards to the comparison between different types of land use, concentrations of dissolved nitrogen (DN) were demonstrated to influence the spatial as well as temporal trends of water quality (Table 3). High concentrations of DN were recorded particularly at SV. Nutrient enrichment at SV had caused eutrophication where the extensive growth of algae was observed during the wet period (May 2016). The input of nitrogen compounds most probably originated from an oil palm plantation adjacent to the river channel. This finding was supported by Gharibreza et al. (2013) suggesting increased levels of nitrogen than natural forested content in Lake Bera catchment area that may result from the utilization of fertilizer by rubber and oil palm plantations. Over the years, nitrogen based fertilizer has been commonly used in the plantation to maximize productivity (Zin and Tramizi, 2007; Othman et al., 2014). The build up of nitrogen in agricultural soil was more easily leached away by rainwater into the aquatic system than most other essential elements (Vitousek et al., 2002; Khan et al., 2007).

Generally, conductive ions in the fluvial system are typically in accordance with the concentrations of dissolved solids and salts. Highest conductivity was exhibited by OP. Anthropogenic discharges and lack of riparian barrier in OP areas may applicable in explaining the spatial variability in conductivity values. In addition, high conductivity values obtained at OP may also be associated with the alluvial soils, which described by Acres and Folland (1975) to predominantly exist as silty clay loams in Kinabatangan areas. Clay soil were known to be readily ionized as it dissolved in water, raising the river conductivity levels. A spike in conductivity value occur during November 2015, as heavy rainfall during this wet period may enhance the rate of runoff from the terrestrial landscape.

Absorption coefficient at 340 nm (a_{340}) has been commonly used to quantitatively indicate the presence of chromophoric DOM (Baker and Spencer, 2004; Stedmon and Nelson, 2015). Chromophoric DOM was mainly represented by terrestrially derived DOM such as plant litter and soil organic matter. Thus, the predominance of a_{340} may suggest the accumulation of chromophoric DOM originated from the forested areas of SF. On the other hand, high calculated values of spectral slopes ($S_{275-295}$) were exhibited at OP. These spectroscopic properties of DOM generally used to qualitatively describes the shifts in the composition of DOM (Helms et al., 2008), higher values of $S_{275-295}$ indicates DOM with lower molecular weight and aromaticity as observed at OP land use. Low molecular weight and aromaticity of DOM might have resulted as a by-product of microbial processing and/or photochemical degradation.

Conclusion

Based on the evaluation of surface water quality properties, it is concluded that the sampling stations at Lower Kinabatangan River catchment were generally considered to be polluted as pH, DO and TSS parameters were classified under class IV, according to Malaysian National Water Quality Standard (NWQS). The surface physicochemical water quality properties were also spatially and temporally varied as illustrated by graphical LDA output, where findings showed that pH, DO and TN was significant at SV, while conductivity at OP. This suggests these parameters were influenced by agricultural activities as well as precipitation rate, where higher values were observed during both dry and wet periods. On the other hand, the spectroscopic data of DOM were only varied at different types of land use. High values of a_{340} at SF indicates accumulation of terrestrial DOM, while dominant $S_{275-295}$ at OP reflect the alteration of DOM either by the microbial or photochemical reaction. Further studies are is needed to characterise DOM composition in more detail as well as to compare DOM dynamics in headwaters and the lower part of the river to better understanding its functions within the tropical catchment.

Acknowledgement

The authors thank Universiti Malaysia Sabah for providing financial support under fundamental research grant scheme (FRGS/2/2014/STWN01/UMS/02/1). We also would like to thank the staff at KOPEL and Ms. Asnih Atin for their help with sampling.

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Journal of Tropical Biology and Conservation 16: 285–305, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Description of New *Pseudeustetha* species from Malaysia (Coleoptera: Chrysomelidae: Galerucinae s. str.)

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Absract
The small oriental genus of Chrysomelidae, *Pseudeustetha* Jacoby, 1899 from Malaysia was studied. A total of eight species of the genus are recorded from Malaysia, of which seven are described as new to science: *Pseudeustetha minima*, *P. nakasekoi*, *P. rufohirsuta*, *P. sabahcola*, *P. sarawacensis*, *P. sinarutensis* and *P. unicolor*, n. spp. The occurrence of *P. hirsuta* (Jacoby) in Borneo (Sabah, Sarawak) and Peninsular Malaysia was not confirmed in this study. A tentative key to the 11 known species of the genus is provided.

Keywords: *Pseudeustetha*, Galerucinae, Chrysomelidae, New species, Malaysia

Introduction
A small galerucine genus *Pseudeustetha* was established by Jacoby in 1899. It is characterized by the combination of the following: dorsal surfaces closely covered with fine erect hairs; eyes rather large; genae shallower than half the transverse diameter of an eye; antennal insertions separated, situated behind anterior margin of eyes; pronotum not very short, with a pair of lateral depressions, without longitudinal furrows, distinctly marginate on all margins; anterior coxal cavity closed behind, or partially opened; mesosternum rather vertical and free, not covered by a process of metasternum; male with last abdominal segment trilobed, with distinct median lobe; posterior tibiae with many spines at apex, with first tarsal segment shorter than remainder combined; tarsal claws appendiculate.

With a combination of these characters, this genus belongs to the section Antiphites Chapuis of the tribe Sermylini Wilcox. Its appearance may be

Received 04 December 2018
Reviewed 21 January 2019
Accepted 19 March 2019
Published 15 October 2019

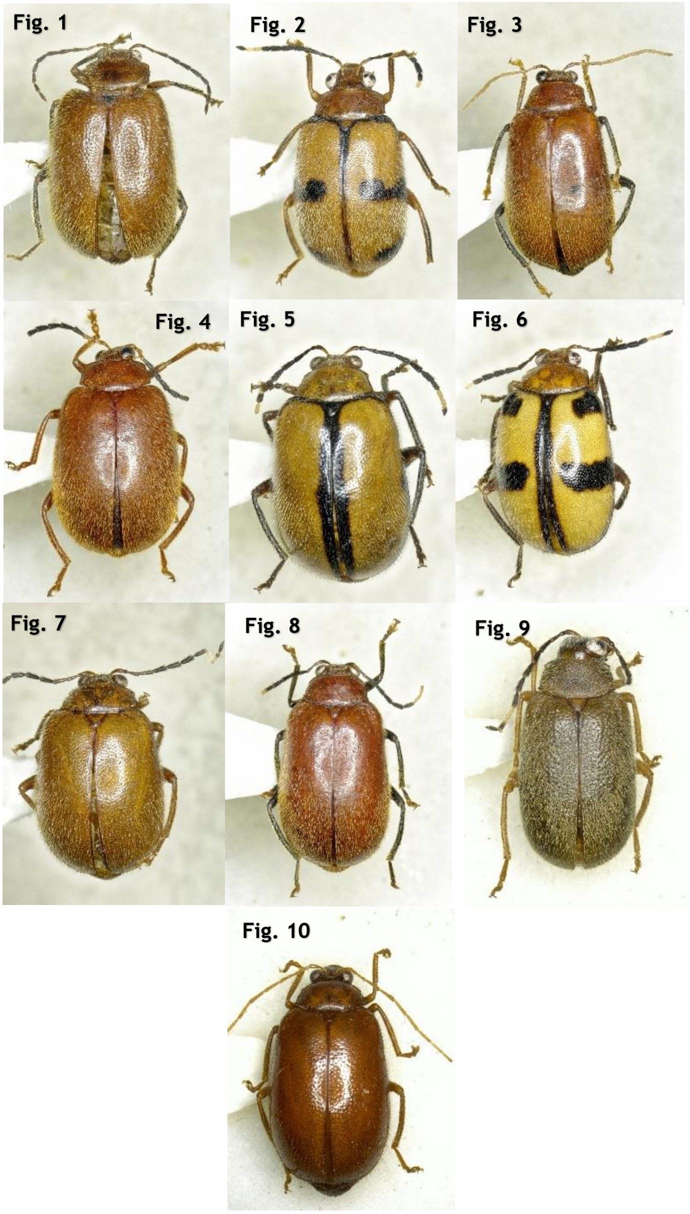
expressed as densely pubescent *Dercetina* or *Sermyloides* species.
It is, so far, composed of four species: *P. hirsuta* (Jacoby), *P. philippina* Medvedev, *P. quadriplagiata* Jacoby and *P. variabilis* (Jacoby) recorded from India, Indochina, Peninsular Malaysia, Sumatra, Borneo and the Philippines. Mohamedsaid (2004) cited *P. hirsuta* (Jacoby) and *P. variabilis* (Jacoby) from Malaysia. Almost nothing is known of the biology of *Pseudeustetha* species, except that Mohamedsaid (2004) cited *Ovaria* sp. (Annonaceae) as a host of *P. hirsuta*.

Species of this genus show rather wide ranges of colour variations as shown in Figures 21-25. Furthermore, the aedeagus, which is usually weakly chitinized, shows slight differences among species. These features together with variability in the punctuation of the pronotum make discrimination of species rather difficult.

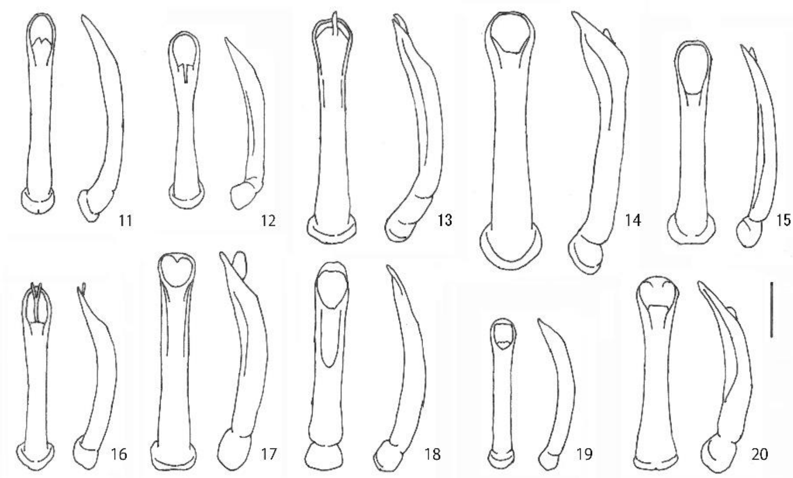
All the holotypes and a series of representative specimens will be deposited in the BORNEENSIS collection of the Institute of Tropical Biology and Conservation (IBTP), Universiti Malaysia Sabah, Kota Kinabalu, Sabah. "DM coll." refers to Dr. M. Daccodi's private collection in Verona, Italy.

Result and discussion

We collected in this study some 700 specimens of the genus from Malaysia and its neighbouring areas. After detailed examination of these specimens, we concluded that there are 9 species of the genus occurring in Sabah and Sarawak. Among these, 7 species are described in this paper as being new to science. At present, the genus is represented by 11 species, distributed widely in India (1 species), Indochina (1 species), Peninsular Malaysia (1 species), Borneo (8 species), Sumatra (2 species) and the Philippines (1 species). There are some questionable specimens that are undetermined, including one specimen from Al Dalan, Bario in Sarawak. Further extended research on the genus of the Great Sunda area is strongly recommended.



Figs. 1 – 10. Habitus of *Pseudeustetha* species.
Fig. 1, *Pseudeustetha hirsuta* (Jacoby) (from Lake Reh, Myanmar); Fig. 2, *P. minima* n. sp. (holotype); Fig. 3, *P. nakasekoi* n. sp. (holotype); Fig. 4, *P. rufohirsuta* n. sp. (holotype); Fig. 5, *P. sabahcola* n. sp. (holotype); Fig. 6, *Pseudeustetha* sp. (from Al Dalan, Bario, Sarawak); Fig. 7, *P. sarawacensis* n. sp. (holotype); Fig. 8, *P. sinarutensis* n. sp. (holotype); Fig. 9, *P. unicolor* n. sp. (holotype); Fig. 10, *P. variabillis* (Jacoby) (from Kg. Moyog, Penampang, Sabah).



Figs. 11 – 20. Aedeagus (left:dorsal view, right: lateral view) of:
Fig. 11, *Pseudeustetha hirsuta* (Jacoby) (from Lake Reh, Myanmar); Fig. 12, *P. minima* n. sp. (holotype); Fig. 13, *P. nakasekoi* n. sp. (holotype); Fig. 14, *P. rufohirsuta* n. sp. (holotype); Fig. 15, *P. sabahcola* n. sp. (holotype); Fig. 16, *Pseudeustheta* sp. (from Al Dalan, Bario, Sarawak); Fig. 17, *P. sarawacensis* n. sp. (holotype); Fig. 18, *P. sinarutensis* n. sp. (holotype); Fig. 19, *P. unicolor* n. sp. (holotype); Fig. 20, *P. variabilis* (Jacoby) (from Poring Park, Sabah).

Descriptions

Pseudeustetha hirsuta **(Jacoby, 1891)** （Figures 1, 11）

Antipha hirsuta Jacoby, 1891. Entomologist, 24 (suppl.): 32 (Assam: BMNH).

Antipha pubescens (Jacoby, 1892). Annali del Museo civico di storia naturale di Genova, 32: 971 (Burma: Carin, Cheba) – Kimoto, 1989. Esakia, 27: 216 (synonymized: India, Burma, Thailand, Cambodia, Laos).

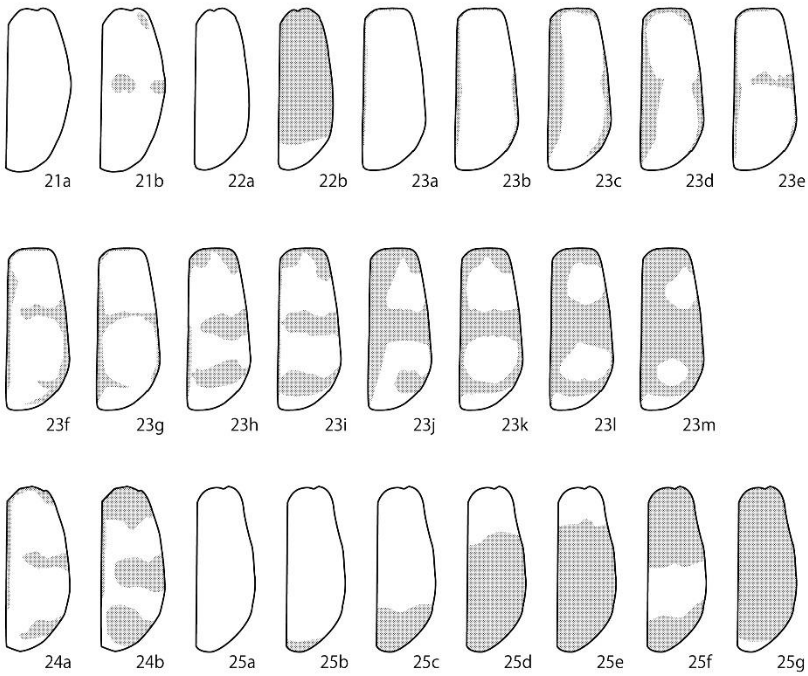
Pseudeustetha hirsuta: Aslam, 1972. Journal of Natural History, 6: 501 — Kimoto, 1989. Esakia, 27: 216—Mohamedsaid, 2004. Catalogue of the Malaysian Chrysomelidae (Insecta: Coleoptera): 78 (Peninsular Malaysia, Sarawak, India, Myanmar, Thailand, Cambodia, Laos).

Pseudeustetha pubescens: Aslam, 1972. ditto.

Avinasa pubescens: Maulik, 1936. Fauna British India, Galerucinae: 457 (Burma).

Male. Body parallel-sided, 6 mm; dark reddish brown, with antennae except for the three basal segments, tibiae and tarsi blackish; venter blackish brown; dorsum densely covered with yellowish pubescence.

Vertex with short median sulcus anteriorly; antennae 0.6 times as long as body,



Figs. 21—25. Variations of elytral patterns in:
Fig. 21, *Pseudeustetha minima* n. sp.; Fig. 22, *P. nakasekoi* n. sp.; Fig. 23, *P. sabahcola* n. sp.; Fig. 24, *P. sarawacensis* n. sp.; Fig. 25, *P. variabilis* (Jacoby)

weakly widened on 4th to 8th segments; 1st segment shorter than 4th, sub-equal to 5th and 11th; 7th longest; 8th 5.3 times and 10th 3.3 times as long as wide. Pronotum twice as wide as long, narrowed from base to anterior angles; disc broadly depressed obliquely behind the anterior angles, densely covered with large punctures, rugose on lateral areas. Elytra subparallel-sided, each 2.8 times as long as wide; disc densely covered with large punctures, of which diameter is as wide as their interspaces; interspaces densely covered with small punctures; 5th visible abdominal sternite with a small median lobe; aedeagus slender and subparallel-sided, weakly dilated ventrally before middle (Figure 11).

Material examined. 1♂, Lake Reh, Chin State, Myanmar, 7-9.VI.2013, A. & R. Abe leg.

Distribution. India, Myanmar, Thailand, Cambodia, Laos.
Host plants. *Ovaria* sp. (Annonaceae: after Mohamedsaid, 2004).
Remarks. This species is characterized by the pronotum rugosely punctate on lateral areas, antennae largely black without yellowish white segments, robust on intermediate segments, and by elytra densely covered with large and small punctures.
It was recorded from Peninsular Malaysia and Sarawak (Mohamedsaid, 2004). Since we could not trace the Malaysian specimens identified as *P. hirsuta* by the second author, and found no Malaysian specimens of this species, we tentatively exclude Peninsular Malaysia and Sarawak from its distribution.

Pseudeustetha minima **n. sp.** (Figures 2, 12, 21)
Male. Body weakly widened posteriorly, 4.8-5.0mm in length; yellowish brown to reddish brown; sometimes head and pronotum darker than elytra; elytra narrowly margined with black on basal half of suture, basal and lateral margins excepting for apex, with narrow transverse band near middle, a lateral patch near apex black; sometimes these bands and patches enlarged as in Figure 21; antennae black, with basal two segments reddish brown, with 9th and 10th yellowish white; dorsum covered with yellowish pubescence.
Head rather densely pubescent; frontal tubercles oblique, separated from each other by short upper process of frontal carina; antennae almost half as long as body, with 4th to 8th segments robust and densely pubescent; 1st longest, distinctly longer than 11th; 2nd sub-equal to 10th, slightly shorter than 9th ; 8th 1.8 times and 10th 1.5 times as long as wide. Pronotum transverse, 2.2 times as wide as long, gently narrowed from base to anterior angles, weakly rounded at middle on lateral margins, gently emarginated on anterior margin, broadly produced on basal margin; anterior angles robust, obliquely truncate; disc densely and distinctly punctate, sometimes almost rugose on lateral area, with a broad oblique depression behind anterior angle to middle. Elytra subparallel-sided, each 2.5 times as long as wide; disc matt, densely covered with punctures, of which diameter is as wide as interspaces; interspaces with smaller punctures. Fore and middle legs with 1st tarsal segments slender. Fifth visible abdominal sternite with a small median lobe. Aedeagus rather strongly narrowed near middle (Figure 12).
Female. Body 5.0-5.2mm in length; 5th visible abdominal sternite simply produced at apex; fore and middle legs with 1st tarsal segments slender.
Holotype. Male, Poring Park, Ranau, Sabah, Malaysia, 19.IV.2014, H. Takizawa leg. (IBTP). Paratypes. 1♀, Poring Park, Ranau, Sabah, 25.III.2015, H. Takizawa leg.; 1♀, ditto, 19.IV.2014, H. Takizawa leg.; 1♂2♀, ditto, 25-26.IX.2008, H. Takizawa leg.; 1♂, ditto, 21-22.X.2008, H. Takizawa leg. 1♂, Kg. Sinarut Baru,

Ranau, Sabah, 18.III.2012, H. Takizawa leg.
Distribution. Borneo (Sabah).
Host plants. Unknown.
Remarks. This new species is characterized by its smaller body size, antennae robust on intermediate segments, pronotum densely punctate, and by elytra with 2 or 3 transverse bands or patches. This is clearly distinguished from *P. sarawacensis*, n. sp. by densely punctate pronotum, and from *P. unicolor*, n. sp. by elytra with black pattern. This new species is collected by sweeping along forest trails at Poring substation, Ranau. Its specific name refers to its smaller body size.

Pseudeustetha nakasekoi **n. sp.** (Figures 3, 13, 22)
Male. Body weakly widened posteriorly, 6.5-7.0mm in length; colouration variable, yellowish brown to reddish (Figure 22); venter and legs except for fore tibiae and tarsi infuscate to blackish; pale individuals usually wholly yellowish brown, sometimes with fore and middle legs blackish; sometimes head and pronotum blackish; elytra sometimes black except for apical 1/4; dorsum covered with yellowish pubescence.
Vertex rather densely pubescent; antennae slender, 0.6 times as long as body; each segment rather short, 1st longer than each of 5th to 8th ; 8th 2.5 times and 10th 3 times as long as wide. Pronotum transversely trapezoid, almost twice as wide as long, gently narrowed from base to anterior angles; disc with a broad oblique depression behind anterior angles, densely covered with large punctures laterally; punctuation becoming smaller on median part. Elytra each 3 times as long as wide, widest before apical 1/3rd, thence gently narrowed to both ends; disc densely covered with hair bearing small punctures, of which diameter is narrower than their interspaces; 5th visible abdominal sternite weakly truncate at apex with a small median lobe. Legs with first tarsal segments slender. Aedeagus widely rounded at apex, gently widened from middle to both ends (Figure 13).
Female. Body slightly larger, 6.5-7.5 mm in length; 5th visible abdominal sternite simply produced at apex in an arched manner.
Holotype. Male. Kg. Naudu, Tambunan, Sabah, Malaysia, 8.XI.2015, H. Takizawa leg. (IBTP). Paratypes. *Sabah* —2♂1♀, Kg. Sinarut Baru, Ranau, 26-27.III.2016, H. Takizawa leg.; 1♂, ditto, 2.IV.2015, H. Takizawa leg.; 1♂, ditto, 3-4.IV.2016, H. Takizawa leg.; 1♂, ditto, 16.IV.2013, H. Takizawa leg.; 2♀, ditto, 20.IV.2013, H. Takizawa leg.; 1♂, ditto, 28.X.2012, H. Takizawa leg.; 1♂, ditto, 2.XI.2015, H. Takizawa leg. 1♀, Poring Park, Ranau, 28.III.2016, H. Takizawa leg. 1♂, Kg. Naudu, Tambunan, 23.III.2015, H. Takizawa leg.; 1♀, ditto, 30.III.2015, H.

Takizawa leg.; 1♀, ditto, 5.IV.2016, H. Takizawa leg.; 2♂1♀, ditto, 13.IV.2013, H. Takizawa leg.; 1♂, ditto, 25.VIII.2013, H. Takizawa leg.; 1♀, ditto, 30-31.X.2012, H. Takizawa leg.; 1♀, ditto, 9.XI.2015, H. Takizawa leg. 1♂, Keruak, Kg. Sukau, Sandakan, 15.XII.2009, H. Takizawa leg. 1♀, Tongod Forest Reserve, upper Kinabatangan, Sandakan, 22-23.II.2008, H. Takizawa leg. *Sarawak* —2♂2♀, Al Dalan, Bario, Kerambit Highland, 5-7.IX.2007, H. Takizawa leg. 1♂, Pa Ukal, Bario, Kerambit Highland, 4.IX.2007, H. Takizawa leg.

Distribution. Borneo (Sabah, Sarawak).

Host plants. Unknown.

Remarks. This new species is characterized by larger body with heavily punctate pronotum, and antennae with shorter segments. It is somewhat similar to *P. variabilis*, but clearly distinguished from the latter by the heavily punctate pronotum and antennae with shorter segments.

It is distributed on low mountains of 500 to 800 m alt. in Sabah, at 1000 m alt. in Sarawak, and was collected by sweeping along forest trails.

This species is dedicated to Mr. M. Nakaseko in Sabah, a butterfly photographer who accompanied me to various collecting localities in Sabah.

Pseudeustetha rufohirsuta **n. sp.** (Figures 4, 14)

Male. Body weakly widened posteriorly, and rather robust, 7.0-7.2mm in length; brownish red, densely covered with long golden red pubescence; antennae black, with basal 3 segments yellowish brown, with 9th yellowish white.

Head covered with fine pubescence; antennae 0.6 times as long as body, with 4th to 8th segments distinctly widened to apex, with apical 3 segments slender; 8th 2.2 times and 10th twice as wide as long. Pronotum transverse, slightly wider than twice the length, distinctly narrowed from base to anterior angles, arched on both anterior and posterior margins, weakly produced near middle on lateral margins; disc weakly and sparsely punctate medially, with a pair of distinct oblique impressions laterally, densely punctate outside the impressions; anterior angles robust and obliquely truncate. Scutellum acutely triangular. Elytra each 2.3 times as long as wide, widest behind middle, thence gently narrowed to both ends; disc densely covered with small punctures, with the interspaces smooth and shining; elytral epipleura pubescent, rather horizontal, reaching to apical 1/4th. Mesosternum almost vertical; 5th visible abdominal sternite truncate at apex, with a small median lobe; two anterior legs with the first tarsal segment weakly widened. Aedeagus rather robust, slender, and gradually narrowed from sub-basal constriction to apical 1/4th, thence gently widened to round apex, expanded ventrally at subapical area (Fig. 14).

Female. Body 6.0-7.0 mm in length; last abdominal sternite produced at apex

in an arched manner; first tarsal segments slender.
Holotype. Male, Poring Park, Ranau, Sabah, Malaysia, 5.III.2010, H. Takizawa leg. (IBTP). Paratypes. 1♀, Poring Park, Ranau, Sabah, 25-26.II.2008, H. Takizawa leg.; 1♂, ditto, 30.III.2016, H. Takizawa leg.; 1♀, 29-30.IX.2007, H. Takizawa leg. 1♀, Kg. Sinarut Baru, Ranau, Sabah, 3-4.IV.2016, H. Takizawa leg.; 1♀, ditto, 14.VI.2018, H. Takizawa leg. 1♂, Kg. Silau, Ranau, Sabah, 25.III.2015, H. Takizawa leg. 1♀, Mahua, B. C. Crocker Range Park, Sabah, 23.X.1999, K. Mizota leg. 1♀, Ulu Kimanis subst., Crocker Range Park, Papar, Sabah, 26-28.II.2010, H. Takizawa leg. 1♀, Kg. Podos, Kota Belud, Sabah, 13.XII.2014, H. Takizawa leg.
Distribution. Borneo (Sabah).
Host plants. Unknown.
Remarks. This new species is characterized by rather broad and red body, densely covered with golden red pubescence. Its antennae are robust on intermediate segments, black except 3 basal segments that are yellowish brown, and the 8th yellowish white. This unique combination of characters easily distinguishes this species from known congeners, as in the key.
This species is found at lowland forests to mountains (200-1,300m alt.). The host plant is unknown. The specific name refers to its golden red pubescence on the dorsum.

Pseudeustetha sabahcola n. sp. (Figures 5, 15, 23)
Male. Body subparallel-sided, 5.0-6.0 mm in length; pale yellowish brown; head and pronotum with more or less reddish brown tinge; venter yellowish to reddish brown, sometimes dark reddish brown to blackish; legs yellowish brown, tibiae apically and tarsi infuscate, sometimes with tibiae and tarsi black; antennae blackish, with basal two segments reddish brown, with 9th and 10th yellowish white; sometimes 11th basally yellowish white; dorsum covered with dense yellowish pubescence; colouration of elytra variable (Figure 23): a, elytra margined with black on all margins, except for apical area; b, elytra with one or two transverse bands; sometimes these stripes and bands enlarged in various degrees to leave three yellowish brown patches; sometimes sutural stripe almost disappeared; elytral epipleura always black.
Vertex sparsely covered with fine punctures and hairs; frontal tubercles subquadrate and raised, scarcely delimited laterally, distinctly separated from each other by acute process of frontal carina; frons weakly raised and finely granulate; antennae half as long as body, densely pubescent and robust on 4th to 8th; 3rd segment as long as 11th; 8th twice and 10th 1.3 times as long as wide. Pronotum transverse, twice as wide as long, distinctly narrowed from base to anterior angle, gently emarginated at anterior margin, produced at posterior

margin in a broad and arched manner; anterior angles widely and obliquely truncate; disc obliquely and weakly depressed behind the anterior angles to middle, rather finely punctate. Elytra each 1/3 as wide as long, widest behind apical 1/3rd, thence gently narrowed to base, strongly so to apex; disc densely covered with small hair-bearing punctures, with smooth interspaces; 5th visible abdominal sternite with small median lobe at apex; fore and middle legs with first tarsal segment slender. Aedeagus gently widened from middle to both ends (Figure 15).

Female. Body slightly larger, 5.5-6.5 mm in length; 5th visible abdominal sternite gently produced at apex.

Holotype. Male, Kinabalu Park, HQ, Ranau, Sabah, Malaysia, 5.XI.2015, H. Takizawa leg. (IBTP). Paratypes. 4 exs., Kinabalu Park, HQ, Ranau, Sabah, 19-20, 23.I.2008, H. Takizawa leg.; 3 exs., ditto, 20-24.II.2009, H. Takizawa leg.; 3 exs., ditto, 17-19.III.2008, H. Takizawa leg.; 3 exs., ditto, 3.IV.2015, H. Takizawa leg.; 4 exs., ditto, 13-14.V.2010, H. Takizawa leg.; 3 exs., ditto, 20.VII.2011, H. Takizawa leg.; 5 exs., ditto, 4.VIII.2011, H. Takizawa leg.; 1 ex., ditto, 4.IX.2013, H. Takizawa leg.; 3 exs., ditto, 17-19.X.2008, H. Takizawa leg.; 2 exs., ditto, 16-17.XI.2007, H. Takizawa leg.; 4 exs., ditto, 15.XII.2014, H. Takizawa leg. 4 exs., Mesilau, 1,500m, Ranau, Sabah, 23.III.2008, H. Takizawa leg. 4 exs., Upper Mesilau, Ranau, 19.IV.2013, H. Takizawa leg.; 3 exs., ditto, 28.V.2010, H. Takizawa leg. 1 ex., Mt. Kinabalu, Sabah, 28.V.1999, Z. Snrz leg. (DM Coll.). 2 exs., Mesilau Watergate (Water plant), Ranau, Sabah, 28.I.2011, H. Takizawa leg.; 8 exs., ditto, 24.II.2011, H. Takizawa leg.; 4 exs., ditto, 26.III.2015, H. Takizawa leg.; 3 exs., ditto, 28-29.V.2010, H. Takizawa leg.; 5 exs., ditto, 24.VII.2013, H. Takizawa leg.; 4 exs., ditto, 30.VIII.2013, H. Takizawa leg. 1 ex., Kundasang, Ranau, Sabah, 18.I.2009, H. Takizawa leg.; 1 ex., ditto, 20.II.2010, H. Takizawa leg.; 3 exs., ditto, 16.III.2008, H. Takizawa leg.; 4 exs., ditto, 4.IV.2015, H. Takizawa leg.; 2 exs., ditto, 31.VII.2013, H. Takizawa leg.; 1 ex., ditto, 16.VIII.2008, H. Takizawa leg.; 2 exs., ditto, 22.IX.2007, H. Takizawa leg.; 1 ex., ditto, 6.XI.2008, H. Takizawa leg.; 3 exs., ditto, 20.XII.2009, H. Takizawa leg. 2 exs., Poring Park, Ranau, Sabah, 8-9.I.2010, H. Takizawa leg.; 6 exs., ditto, 13-15.II.2009, H. Takizawa leg.; 1 ex., ditto, 19-20.III.2012, H. Takizawa leg.; 5 exs., ditto, 4-5.IV.2008, H. Takizawa leg.; 2 exs., ditto, 14.V.2017, H. Takizawa leg.; 1 ex., ditto, 10.VII.2008, H. Takizawa leg.; 2 exs., ditto, 2-3.VIII.2010, H. Takizawa leg.; 8 exs., ditto, 29-30.IX.2007, H. Takizawa leg.; 2 exs., ditto, 29.X.2012, H. Takizawa leg.; 5 exs., ditto, 3.XI.2015, H. Takizawa leg.; 9 exs., ditto, 19-20.XII.2007, H. Takizawa leg. 1 ex., Sayap substation, Kota Belud, Sabah, 28-29.IV.2014, H. Takizawa leg.; 1 ex., ditto, 21.VII.2010, H. Takizawa leg. 2 exs., Hot Spring Poring, Mt. Kinabalu, Sabah, II.2000, M. Snizek leg. (DM coll.). 1 ex., Kg. Kiau, Kota Belud, Sabah, 31.V-2.VI.2008, H. Takizawa leg. 1 ex., 26km peak, Jln. Kimanis, Papar, Sabah, 24.III.2012, H. Takizawa leg.; 1 ex., ditto, 17.V.2017, H. Takizawa leg.;1 ex., ditto, 24.VII.2013, H. Takizawa leg.; 1 ex., ditto, 24.VIII.2013, H. Takizawa leg.; 1 ex., ditto, 10.XI.2015, H. Takizawa leg. 1 ex., Gn. Alab, Crocker Range Park, Tambunan, Sabah, 29.VII.2011, H. Takizawa leg. 1 ex., Mahua waterfall, Tambunan, Sabah, 29.I.2011, H. Takizawa leg.; 1 ex., ditto, 21.III.2012, H. Takizawa leg.; 1 ex., ditto, 25.IV.2014, H. Takizawa leg.; 1 ex., ditto, 24.VII.2011, H. Takizawa leg. 1 ex., Malangan subst., Tambunan, Sabah, 31.III.2015, H. Takizawa leg. 1 ex., Kg. Naudu, Tambunan, Sabah, 30.III.2015, H. Takizawa leg.; 1 ex., ditto, 15.V.2017, H. Takizawa leg.; 1 ex., ditto, 25.VIII.2013, H. Takizawa leg.; 1 ex., ditto, 9.XI.2015, H. Takizawa leg. 3 exs., Kg. Ragkan, Tambunan, Sabah, 14.IV.2013, H. Takizawa leg.; 2 exs.,ditto, 28.X.2012, H. Takizawa leg. 3 exs., Pk. Tambunan, Tambunan, Sabah, 25.III.2012, H. Takizawa leg. 1 ex., W. of Apin V., Crocker Range E., V.1997, M. Snizek leg. (DM coll.). 1 ex., Crocker Range Park, HQ, Keningau, Sabah, 23.III.2012, H. Takizawa leg.: 1 ex., ditto, 6.XII.2014, H. Takizawa leg. 1 ex., Ulu Senagang substation, Keningau, Sabah, 10-12.III.2012, H. Takizawa leg.; 1 ex., ditto, 24-25.VII.2010, H. Takizawa leg. 1 ex., Kalang waterfall, Tenom, Sabah, 22.III.2016, H. Takizawa leg. 3 exs., Muaya waterfall, Kg. Muaya, Sipitang, 7-9.III.2009, H. Takizawa leg.

Additional material examined (all the specimens were collected by the first author). Kinabalu Park HQ: 2 exs., 22-23.I.2010; 2 exs., 20-24.II.2009; 1 ex., 24.II.2008; 3 exs., 24.II.2008; 2 exs., 28-29.II.2008; 3 exs., 5.III.2010; 1 ex., 13.III.2012; 2 exs., 14.III.2012; 3 exs., 17-19.III.2008; 2 exs., 23-25.III.2010; 1 ex., 29.III.2016; 1 ex., 29-30.III.2010; 1 ex., 31.III.2016; 3 exs., 1.IV.2016; 1 ex., 2.IV.2016; 1 ex., 3.IV.2015; 2 exs., 20.IV.2013; 3 exs., 12.V.2017; 3 exs., 13-14.V.2010; 2 exs., 14.V.2017; 3 exs., 20.V.2017; 9 exs., 22.V.2017; 5 exs., 27-28.V.2008; 1 ex., 8.VII.2010; 1 ex., 8.VII.2011; 3 exs., 20.VII.2011; 2 exs., 23-25.VII.2008; 1 ex., 4.VIII.2011; 1 ex., 19-20.VIII.2008; 2 exs., 4.IX.2103; 1 ex., 17-18.IX.2008; 1 ex., 22.X.2007; 2 exs., 5.XI.2015; 1 ex., 6.XI.2015; 1 ex., 7.XI.2015; 1 ex., 8.XI.2015; 2 exs., 15.XII.2014; 2 exs., 16.XII.2014; 2 exs., 21-22.XII.2007; 11 exs., 23-24.XII.2008. Upper Mesilau: 6 exs., 17.IV.2013; 2 exs., 19.IV.2013; 3 exs., 28.V.2010. Mesilau Watergate (water plant): 3 exs., 28.I.2011; 2 exs., 25.II.2008; 3 exs., 28.II.2009; 3 exs., 6.III.2010; 1 ex., 26.III.2015; 1 ex., 31.III.2016; 2 exs., 12.V.2017; 2 exs., 28-29.V.2010; 2 exs., 30.VIII.2013. Kundasang: 2 exs., 30.I.2010; 1 ex., 20.II.2010; 7 exs., 23.II.2009; 1 ex., 31.VIII.2013; 1 ex., 22.IX.2007; 2 exs., 20.X.2007; 3 exs., 6.XI.2008; 2 exs., 11.XI.2012; 2 exs., 17.XII.2007; 1 ex., 28.XII.2008. Poring Park: 3 exs., 9-10.I.2009; 1 ex., 21-22.I.2008; 3 exs., 11-12.III.2009; 1 ex., 12.III.2009; 1 ex., 16-17.III.2008; 1 ex., 19-20.III.2012; 3 exs., 24.III.2009; 1 ex., 19.IV.2014; 3 exs., 4-5.VII.2009; 7 exs., 10.VII.2008; 6 exs., 2-3.VIII.2010; 3 exs., 21-22.VIII.2008; 1

ex., 25-26.VIII.2007; 5 exs., 28-29.VIII.2013; 2 exs., 25-26.IX.2008; 5 exs., 29-30.XI.2007. Jln. Kimanis 26km peak: 1 ex., 29.III.2015;. Mahua waterfall: 2 exs., 24.VII.2011. Kg. Malangan: 3 exs., 12-14.III.2010. Kg. Naudu: 1 ex., 23.III.2015. Sayap substation: 1 ex., 21.VII.2012. Crocker Range Park, HQ, Keningau: 1 ex., 9.III.2012. Ulu Senagang substation: 1 ex., 24-25.VII.2010.

Distribution. Borneo (Sabah).

Host plants. *Desmodium* sp. (Fabaceae).

Remarks. This new species is characterized by its colouration that is yellowish to brownish dorsum with variable black stripes or patches, the pronotum distinctly narrowed anteriorly, and the antennae with robust median segments. The dorsal patterns in the patched type may be similar to those of *P. sarawacensis* , n. sp. However, the latter has a smaller body, and elytra densely covered with distinctly large punctures, and with the interspaces almost as wide as diameter of punctures.

One specimen (Figure 6) from Al Dalan, Bario highland in Sarawak seems closely related to this, but differs in the elytral pattern. This might belong to a different species. Since we saw only a single male specimen, we refrain from further identification at the moment.

This species is widely distributed in Sabah, and is found in a wide range of habitats from wet, shadowed floors in forests to sunny, open roadsides. It covers 300m alt. (Kalang waterfall in Tenom) to 1,800m alt. (Kinabalu Park, Headquarters area and Gunung Alab), but mainly occurs in montane area of 900 to 1,800m alt. The striped type generally occupies higher area, whereas the banded type tends to extend to lower areas. It feeds on a lot of plant species, including *Desmodium* sp.

Its specific name refers to its occurring area, Sabah State in Malaysia.

***Pseudeustetha sarawacensis* n. sp.** (Figures 7, 17, 24)

Male. Body small, weakly widened posteriorly, 4.5-5.0mm in length; yellowish brown to reddish brown, covered densely with yellowish pubescence; antennae black with basal 3 segments yellowish brown, with 9th and 10th yellowish white. One female specimen with three small black spots on elytron, one on humerus, two transversely arranged at middle.

Head sparsely punctate and hairy; frontal tubercles subquadrate and slightly oblique, distinctly separated by upper portion of frontal carina; antennae robust and densely pubescent on 4th to 8th segments, 0.7 times as long as body; 1st segment longest and sub-equal to 5th; 8th and 10th each 3 times as long as wide. Pronotum transverse, 2.2 times as wide as long, weakly narrowed to anterior angles, slightly rounded at middle on lateral margins, gently emarginated on anterior margin, broadly produced at posterior margin; anterior angles robust and obliquely truncate; disc broadly depressed obliquely behind anterior angles to middle, sparsely covered with small hair-bearing punctures, with interspaces smooth. Elytra subparallel-sided, each 2.5 times as long as wide; disc densely covered with larger punctures, of which diameter is distinctly narrower than interspaces; elytral epipleura concave and pubescent. Fifth visible abdominal sternite with a small median lobe at apex. Fore and middle legs with 1st tarsal segment weakly widened. Aedeagus weakly and gently widened from middle to both ends (Figure 17).

Female. Body 5.0-5.2mm in length; 5th visible abdominal sternite simply produced at apex; fore and middle legs with 1st tarsal segment slender.

Holotype. Male. Lanjak Entimau Wildlife Sanct., HQ, Sarawak, Malaysia, 18-28.VI.2008, H. Takizawa leg. (IBTP). Paratypes. 1♂2♀, data same as holotype; 1♂, Ridon, Lanjak Entimau Wildlife Sanct., HQ, Sarawak, 18, 20.VI.2008, H. Takizawa leg.

Distribution. Borneo (Sarawak).

Host plants. Unknown.

Remarks. This new species is characterized by its smaller body size, antennae with robust intermediate segments, and by the pronotum sparsely punctate. Similarly sized *P. minima* and *P. unicolor,* n. spp. are distinguished by the dense, almost rugose punctuation on the pronotum.

This species was collected by sweeping at a lowland riverside at Lanjak Entimau Wildlife Sanctuary in Sarawak.

Its specific name refers to its occurring area, Sarawak State in Malaysia.

***Pseudeustetha sinarutensis* n. sp.** (Figures 8, 18)

Male. Body 6.0 – 6.5 mm. in length, weakly widened posteriorly; reddish brown, with legs black except for apical segments of tarsi reddish brown; sometimes metathorax dark reddish brown; antennae black on 3rd or 4th to 8th segments, yellowish white on apical 3 segments; colouration variable: sometimes dorsum yellowish orange wholly, or dark reddish brown; antennae sometimes wholly pale yellowish brown; venter black, with pronotum and mesosternum reddish brown; fore legs yellowish brown; dorsum densely covered with yellowish red pubescence.

Antennae shorter, less than 0.6 times body length; robust on 4th – 8th segments; 8th and 10th each 3.2 times as long as wide. Pronotum transverse; disc rather densely covered with obscure punctures on antero-median area, where the diameter of punctures are slightly smaller than interspace. Elytra each 3 times as long as wide, rather straightly widened from base to apical 2/5th, thence roundly narrowed to apex, where both apices are separately rounded; disc distinctly impressed longitudinally inward to humerus, densely punctate with diameter of punctures smaller than interspaces; last visible abdominal sternite weakly tri-lobed, with median lobe produced posteriorly in an ached manner. Aedeagus subparallel-sided, rather truncate at apex, with a weak lateral ridge on apical half, weakly expanded ventrally at subapical area (Figure 18).

Female. Body slightly larger, 6.5—7.5 mm in length; 5th visible abdominal sternite roundly produced at apex.

Holotype. Male, Kg. Sinarut Baru, Ranau, Sabah, Malaysia, 15.III.2012, H. Takizawa leg. (ITBC). Paratypes. 1♂, same as the holotype, 2.IV.2015, H. Takizawa leg. 1♀, Poring park, Ranau, 19-20.III.2012, H. Takizawa leg.; 1♀, ditto, 7.IV.2013, H. Takizawa leg.; 1♂, ditto, 19.IV.2014, H. Takizawa leg.; 1♀, ditto, 14.V.2017, H. Takizawa leg. 2♀, Pk. Tambunan, Tambunan, 16.III.2012, H. Takizawa leg.; 1♀, ditto, 19.III.2012, H. Takizawa leg.; 1♀, ditto, 25.III.2012, H. Takizawa leg. 1♀, Kg. Naudu, Tambunan, 5.IV.2016, H. Takizawa leg.; 1♂, ditto, 15.IV.2013, H. Takizawa leg. 1♀, Tabin Wildlife Reserve, Lahad Datu, 25-26.XII.2008, H. Takizawa leg.

Distribution. Borneo (Sabah, Sarawak?).

Host plants. Unknown.

Remarks. This new species is characterized by the antennae robust on 4th to 8th segments, the pronotum rather densely punctate on antero-median area and by the dorsum wholly yellowish brown to reddish brown. *P. sarawacensis*, n. sp. is somewhat similar to it, having stout antennae and the dorsum wholly reddish brown, but the latter is easily distinguished from *P. sinarutensis*, n. sp. by almost impunctate pronotum and smaller body size. From similarly coloured *P. nakasekoi*, n. sp. and reddish brown type of *P. variabilis* (Jacoby), it is immediately distinguished by the robust antennae.

There are two female specimens collected at the Lanjak Entimau Wildlife Sanctuary, the headquarters area, Kapit, Sarawak (27-28.VI.2008). These probably belong to this new species, but the pronotum strongly punctates like in *P. nakasekoi*, n. sp. They are not included in the paratypes.

Its specific name is derived from the type locality, Kg. Sinarut Baru near Ranau.

***Pseudeustetha unicolor* n. sp.** (Figures 9, 19)

Male. Body parallel-sided and slender, 4.0-5.5mm in length; wholly yellowish brown; sometimes dorsum dark reddish brown to blackish brown; elytra sometimes reddish brown on basal 1/3, blackish on apical 2/3; dorsum covered with yellowish pubescence; antennae blackish, with 1st segment yellowish brown, with 9th and 10th basally yellowish white.

Vertex densely punctate, with a short median sulcus anteriorly; frontal tubercles subquadrate, delimited by a narrow furrow interiorly; antennae almost 0.6 times as long as body, densely pubescent and robust on 4th to 8th; 1st segment subequal to 4th, 5th, 6th and 11th in length; 8th and 10th each 3 times as long as wide. Pronotum transverse, twice as wide as long, gently and almost straightly narrowed from base to anterior angles, gently emarginated at anterior margin, broadly and somewhat sinuately produced on basal margin; anterior angles robust, obliquely truncate; disc densely and rugosely punctate, with a broad semilunar depression leaving raised area antero-medially. Elytra subparallel-sided, each three times as long as wide, densely covered with large punctures which have a tendency to arrange longitudinally; diameter of larger ones distinctly wider than their interspaces; interspace densely covered with small punctures; elytral epipleura rather oblique; 5th visible abdominal sternite with a small median lobe at apex. Fore legs with 1st tarsal segment slightly widened. Aedeagus rather short and subparallel-sided (Figure 19).

Female. Body slightly larger, 4.0-5.8 mm in length; 5th visible abdominal sternite simply produced at apex; fore and middle legs with 1st tarsal segment slender.

Holotype. Male. Poring Park, Ranau, Sabah, Malaysia, 13-15.II.2009, H. Takizawa leg. (IBTP). Paratypes. *Sabah*: 1♂, Poring Park,13-15.II.2009, H. Takizawa leg. 1♂, Kawang Forest Reserve, Papar, 4.XII.2014, H. Takizawa leg. 1♀, Ulu Kimanis subst., Crocker Range Park, Papar, 26-28.II.2010, H. Takizawa leg. 1♂2♀, Ulu Senagang subst., Crocker Range Park, Keningau, 10-12.III.2012, H. Takizawa leg.; 3♀, ditto, 31.I-1.II.2011, H. Takizawa leg. 2♂1♀, Microwave 197km, Bukit Tavitu Forest Reserve, Telupid, 8.III.2012, H. Takizawa leg. 1♂1♀, Kg. Singgahmata, Telupid, 27.VII.2013, H. Takizawa leg. 1♀, Tawai Forest Reserve, Telupid, 25-26.I.2011, H. Takizawa leg.; 1♀, ditto, 24.III.2015, H. Takizawa leg. 1♂3♀, Microwave, Tawai Forest Reserve, Telupid, 22-24.IV.2014, H. Takizawa leg.; 1♀, ditto, 10.XII.2014, H Takizawa leg. 2♂4♀, Muaya waterfall, Kg. Muaya, Sipitang, 7-9.III.2009, H. Takizawa leg. 3♀, Tibow Forest Reserve, HQ, 6km to Sapulut, Pensiangan, 17-19.XII.2008, *Sarawak*: 2♂2♀, HQ, Lanjak Entimau Wildlife Sanct., Kapit, 18-26.VI.2008, H. Takizawa leg. 1♂2♀, Bagua, HQ, Lanjak Entimau Wildlife Sanct., Kapit, 19.VI.2008, H. Takizawa leg. 1♂, Joh, HQ, Lanjak Entimau Wildlife Sanct., Kapit, 21.VI.2008, H. Takizawa leg. 3♀, Ridon, HQ, Lanjak Entimau Wildlife Sanct., 18-20.VI.2008, H. Takizawa leg. *Peninsular Malaysia*: 3♀, Awana Hotel, Genting Highlands, Pahang, 25.X.2012, H. Takizawa leg. 1♂2♀, Robinson waterfall, Cameron Highlands, Pahang, 12.III.2018, H. Takizawa leg.; 2♀, ditto, 15.III.2018, H. Takizawa leg.; 1♂, ditto, 16.III.2018, H. Takizawa leg.; 1♂4♀, ditto, 13-14.XII.2013, H. Takizawa leg. 1♀, Gn. Jasar, Cameron Highlands, Pahang, 13.III.2018, H. Takizawa leg.; 1♂1♀, ditto, 9.XII.2013, H. Takizawa leg.

Distribution. Borneo (Sabah, Sarawak), Peninsular Malaysia (Pahang).

Host plants. Undetermined tree (Figure 26).

Remarks. This small species is uniquely characterized by the pronotum and elytra densely pubescent, heavily and partially confluently punctate, antennae with robust intermediate segments, and by elytra lacking distinct patterns. It is similar to *P. hirsuta* (Jacoby) from Myanmar, but the latter species has the pronotum much densely punctate, with smooth and shining interspace, and the antennae without yellowish white segments.

This new species is distributed widely in Peninsular Malaysia, Sabah and Sarawak. It is usually collected by sweeping in secondary forests in lower altitude. At Tibow in Sabah it was found feeding on leaves of an undetermined tree in a garden. The specific name refers to its dorsal colouration, which usually uniformly yellowish brown to blackish.

Fig. 26. Undetermined host tree of *Pseudeustetha unicol*

Pseudeustetha variabilis (Jacoby, 1886) (Figures 10, 20, 25)

Antipha variabilis Jacoby, 1886. Annali del Museo civico storia naturale di Geneva, 24: 101 (Sarawak: Genova, MCZ).

Anthipa variabilis: Jacoby, 1896. Annali del Museo civico storia naturale di Geneva, 36: 476.

Dercetina variabilis: Wilcox, 1971, Coleopterorum Catalogue, 78: 179.

Pseudeustetha variabilis: Mohamedsaid, 1995. Wallaceana, 74: 5 (Sabah) – 2004, Catalogue of the Malaysian Chrysomelidae (Insecta, Coleoptera). p. 78 (Peninsular Malaysia, Sarawak, Sabah).

Body convex dorsally and weakly widened posteriorly, 6.0-8.0mm in length; colouration variable, generally yellowish to reddish brown; sometimes venter wholly or partially black; pronotum very often black; elytra often with variable black patches (Figure 25); legs sometimes black; dorsum covered with dark brown to black pubescence.

Antennae slender, 0.8 as long as body; 7th segment longest, distinctly longer than 1st; 4th sub-equal to 8th, 9th and 11th; 10th subequal to 1st, slightly shorter than 11th; 8th and 10th 3.3 and 3.6 times as long as wide; in female 4th to 7th, each sub-equal to 11th; 9th distinctly longer than 8th or 10th. Pronotum weakly narrowed from base to anterior angles; disc sparsely covered with small punctures, of which diameter is distinctly narrower than interspaces; interspaces smooth and shining; elytra each 2/3 as wide as long, widest near middle; 5th visible abdominal sternite with a small median lobe in male, simply produced in female; fore and middle legs with 1st tarsal segments slender; aedeagus slender, gently narrowed to middle, thence gently widened to subapical area, weakly truncate at apex (Figure 20).

Material examined. *Peninsular Malaysia*: 1 ex., Genting Highland, Awana Hotel, Pahang, 25.X.2012, H. Takizawa leg. 1 ex., Kg. Sungai Buloh, Kuala Lumpur, 30.III.2007, H. Takizawa leg. *Sabah*: 1 ex., Kg. Kiapad, Inanam, Kota Kinabalu, 13.I.2010, H. Takizawa leg.; 2 exs., ditto, 14.II.2010, H. Takizawa leg.; 1 ex., ditto, 13.III.2012, H. Takizawa leg.; 1 ex., ditto, 30.III.2008, H. Takizawa leg.; 1 ex., ditto, 6.IV.2010, H. Takizawa leg.; 2 exs., ditto, 1.V.2014, H. Takizawa leg.; 1 ex., ditto, 18.V.2008, H. Takizawa leg.; 2 exs., ditto, 26.VI.2010, H. Takizawa leg.; 1 ex., ditto, 5.VII.2008, H. Takizawa leg.; 1 ex., ditto, 12.VII.2009, H. Takizawa leg.; 1 ex., ditto, 8.IX.2013, H. Takizawa leg.; 2 exs., ditto, 10.XI.2012, H. Takizawa leg.; 1 ex., ditto, 11.XI.2015, H. Takizawa leg. 1 ex., Kg. Moyog, Jln. Tambunan, Penampang, 20.I.2011, H. Takizawa leg.; 1 ex., ditto, 13.II.2010, H. Takizawa leg.; 1 ex., ditto, 3.V.2014, H. Takizawa leg.; 2 exs., ditto, 15.XI.2012, H. Takizawa leg.; 1 ex., ditto, 12.XII.2009, H. Takizawa leg.; 1 ex., ditto, 17.XII.2014, H. Takizawa leg. 1 ex., Kg. Kipouvo, Jln. Tambunan, Penampang, 8.I.2008, H. Takizawa leg. 1 ex., Kawang Forest Reserve, Papar, 4.XII.2014, H. Takizawa leg. 2 exs., Kg. Tambatuon, Kota Belud, 9.IV.2013, H. Takizawa leg.; 3 exs., ditto, 17.IV.2014, H. Takizawa leg.; 1 ex., 22.IV.2013, H. Takizawa leg.; 1 ex., ditto, 3.IX.2013, H. Takizawa leg.; 2 exs., ditto, 12.XII.2014, H. Takizawa leg. 1 ex., Kg. Kiau, Kota Belud, 31.V-2.VI.2008, H. Takizawa leg. 1 ex., Sayap subst., Kota Belud, 20.VII.2010, H. Takizawa leg. 1 ex., Poring Park, Ranau, 8-9.I.2010, H. Takizawa leg.; 1 ex., ditto, 9-10.I.2009, H. Takizawa leg.; 1 ex., ditto, 11-12.III.2009, H. Takizawa leg.; 2 exs., 19-20.III.2012, H. Takizawa leg.; 1 ex., ditto, 30.III.2016, H. Takizawa leg.; 2 exs., ditto, 1.IV.2015, H. Takizawa leg.; 1 ex., ditto, 4-5.IV.2008, H. Takizawa leg.; 1 ex., ditto, 26.IV.2014, H. Takizawa leg.; 2 exs., ditto, 21.V.2017, H. Takizawa leg.; 1 ex., ditto, 4-5.VII.2009, H., Takizawa leg.; 1 ex., ditto, 3.XI.2015, H. Takizawa leg. 1 ex., Kinabalu nr. Ranau, 1,200m, 15.VII.1989, K. Maruyama leg. 1 ex., Kg. Sinarut Baru, Ranau, 26-27.III.2016, H. Takizawa leg.; 2 exs., ditto, 21.IV.2014, H. Takizawa leg.; 2 exs., ditto, 27.IV.2014, H. Takizawa leg. 1 ex., Mamut Mine, Ranau, 27.I.2011, H. Takizawa leg.; 1 ex., ditto, 21.VII.2011, H. Takizawa leg. 1 ex., Mamut, Lohan, Ranau, 23.VII.2011, H. Takizawa leg. 1 ex., Mahua waterfall, Tambunan, 25.IV.2014, H. Takizawa leg. 2 exs., Kg. Naudu, Tambunan, 30.III.2015, H. Takizawa leg. 4 exs., Kg. Singgahmata, Telupid, Sandakan, 27.VIII.2013, H. Takizawa leg. 4 exs., Kalang waterfall, Tenom, 22.III.2015, H. Takizawa leg. *Sarawak*: 1 ex., Mt. Serapi, Kuching, 21.V.1990, Y. Nishiyama leg. 4 exs., Lanjak Entimau Wildlife Sanct., HQ, Kapit, 18-28.VI.2008, H. Takizawa leg. 1 ex., Joh, Lanjak Entimau Wildlife Sanct., HQ, Kapit, 21-23.VI.2008, H. Takizawa leg. 2 exs., Menyaring, Lanjak Entimau Wildlife Sanct., HQ, Kapit, 24-25.VI.2008, H. Takizawa leg. 3 exs., Ridon, Lanjak Entimau Wildlife Sanct., HQ, Kapit, 18-20, 24-25.VI.2008, H. Takizawa leg.

N. Sumatra: 2 exs., Dolok Barus, Siborangit, 29.X.1999, H. Takizawa leg. 2 exs., Urung Tama, Sibolangit, 24.IV.1998, 13,18.IX.1998, H. Takizawa leg. 1 ex., Derek, Siborangit, 16.IX.1998, H. Takizawa leg. 1 ex., Passar Baru, Siborangit, 15.IX.1998, H. Takizawa leg. 1 ex., Susuk, 24.V.1994, K. Maruyama leg. 1 ex., Kota Cane, Aceh, 26-28.IV.1998, H. Takizawa leg. 1 ex., Banyak Is. 2,000m, 15-30.VI.1999, Awi leg.

Distribution. Peninsular Malaysia, Borneo (Sabah, Sarawak); Sumatra.

Host plants. unknown.

Remarks. This species is characterized by slender antennae and is distinguished from most species of the genus with this character. *P. nakasekoi,* n. sp. has similar slender antennae, but is distinguished from this by the heavily punctate pronotum.

There are considerable variations in dorsal colouration, pronotal punctuation and shape of aedeagus, and width/length ratios of antennal segments. This may suggest the heterogenous nature of the examined material. At present they are placed under *P. variabilis.*

This species is distributed generally in lowland, reaching 700-1,100m in Poring and Kg. Kiau in Sabah. It is collected by sweeping along trails in the forests.

Conclusion

In conclusion the known species of the genus are distinguished by the following keys.

Tentative key to species

1. Antennae slender, sometimes 3 apical segments shorter than the preceding ones ---------- 2

\- Antennae with middle segments robust and more or less widened, with 3 apical ones shorter than preceding ones ---------- 4

2. Head with deep groove above frontal tubercles; vertex finely punctate; pronotum finely and very densely punctate; elytra strongly and densely punctate; body 5.3-6.3 mm.; dark red to red fulvous or flavous, with elytra sometimes almost black (based on original description) ---------- *P. philippina* Mededev

\- Head without deep groove above frontal tubercles ---------- 3

3. Pronotum sparsely punctate, especially on lateral 1/3; pubescence yellowish brown ---------- *P. variabilis* (Jacoby)

\- Pronotum heavily and rugosely punctate at baso-lateral area ---------- *P. nakasekoi* n. sp.

4. Body small, less than 5 mm; yellowish to reddish brown, sometimes elytra with three small black spots; pronotum subquadrate, weakly rounded at lateral margins ---------- *P. minima* n. sp.

\- Body larger than 5 mm; yellowish brown to reddish brown, with/without black patch, bands or stripes on elytra ---------- 5

5. Elytra densely punctate, more or less rugose and matt on interspaces ---------- 6

\- Elytra sparsely/densely punctate, smooth and shining on interspaces ---------- 8

6. Pronotum weakly rounded on lateral margins; reddish brown with 3 patches/bands and sutural and lateral stripes black ---------- *P. sarawacensis* n. sp.

\- Pronotum almost straight on lateral margins; colouration variable, with/without black patches and stripes ---------- 7

7. Pronotum rugosely punctate; body slender, subparallel-sided; yellowish to reddish brown, or dark greyish brown; elytra densely covered with large punctures ---------- *P. unicolor* n. sp.

\- Body widened posteriorly; dark reddish brown; pronotum subquadrate, straight on lateral margins, strongly and densely punctate; elytra covered with large and small punctures; larger ones with their diameter as wide as interspaces ---------- *P. hirsuta* (Jacoby)

8. Pronotum subquadrate, weakly converging anteriorly, finely rugose; fulvous, elytra with large patch basally, and another one near apex black; elytra finely punctate, with indistinct rows of larger punctures (based on original description) ---------- *P. quadriplagiata* Jacoby

- Pronotum distinctly converging anteriorly ---------- 9

9. Pronotum much strongly narrowed anteriorly; elytra pale yellowish brown, with distinct black stripes or bands ---------- *P. sabahcola* n. sp.

- Pronotum distinctly narrowed anteriorly; elytra yellowish to reddish brown, without distinct black pattern ---------- 10

10. Body robust and ovate, covered with long golden pubescence; elytra each 2.3 times as long as wide; legs reddish brown ---------- *P. rufohirsuta* n. sp.

- Body narrower, widened posteriorly; elytra each 3 times as long as wide; yellowish to reddish brown; two posterior legs blackish in reddish brown individuals ---------- *P. sinarutensis* n. sp.

Acknowledgement

This study started during the first author's tenure at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah as a volunteer researcher appointed by Japan International Cooperation Agency (JICA). He wishes to acknowledge authorities of both the organizations, and also Sabah Parks for their cooperation and help in the study. We wish to thank Dr. M. Daccordi in Verona for the loan of interesting materials, and also to Ms Masami Yamada of Tokyo University of Agriculture for preparing photos.

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Journal of Tropical Biology and Conservation 16: 307–322, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Traditional Knowledge on Plants Utilization in Postpartum Care: An Ethnobotanical Study in Local Community of Cimande, Bogor, West Java, Indonesia

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Abstract

An ethnobotanical study was conducted in five villages in the vicinity of Cimande Resort, Bogor, West Java, predominantly occupied by the Sundanese people. Forty-eight species of plants were recorded in their application in postpartum care and four species of plants in the care of the newborns. “Jamu Godogan” or boiled herbs, taken by mothers on day 8-15 after birth, contained most diverse herbs of 11 plants species. Three species (Curcuma longa, Piper betle, and Plectranthus scutellarioides) have more than two utilization categories. Nine out of 50 species of utilized medicinal plants have not been registered in the Indonesian Medicinal Plants Index and Medicinal Plants Dictionary; thus, new records.

Keywords: Bogor, Cimande, ethnobotany, medicinal plant, postpartum care, Sundanese

Introduction

Human beings desire a healthy, long and prosperous life. Efforts have been made to accomplish this, not limited to medication but also to include prevention, rehabilitation and upgrading the quality of life (Nala, 2007). Long before modern treatments were invented, every society had their own knowledge of finding cures and maintaining health (Wiart, 2006; Balangcod & Balangcod, 2011). This ability comes from hundreds of years of experience which have been passed down from their ancestors. Along with the process, they have learned to utilize every material from their surroundings, including flora, fauna and minerals. Indonesia is well known for its diversity in medicinal plants and their utilization in accordance with local cultures and traditions. In this regard, the country is nicknamed “the living laboratory” (Aisyah et al., 2011). This option of medicinal plants is recognized as herbs or herbal medicine by Westerners, whereas in

Received 12 October 2018
Reviewed 02 May 2019
Accepted 21 Jun 2019
Published 15 October 2019

Indonesia it is known as “jamu”, generally *jamu* tastes bitter (Riswan & Sangat-Roemantyo, 2002; Yadav, 2007). Nowadays, *jamu* has gained popularity due to better hygiene, and is consumed by the wider society (Anonymous, 2010; Tilaar et al., 2010). Postpartum care is a health indicator that affects education and economic capacity improvement. Most Indonesian women receive their first postpartum care at home (Fort et al., 2006). Although most are treated by trained health personnel, some are cared for by local midwives. The local midwives play a significant role in increasing the chances of mother and newborn survival. Many people in West Java still tend to use “dukun beranak” or “paraji” (midwife) service to assist childbirth and postpartum care (Rajab, 2009), including Cimande. It is known that medicinal plants are also used for postpartum care in West Java (Sangat et al., 2000). Cimande is located in West Java and and is directly adjacent to Gunung Gede Pangrango National Park, West Java. This research is aimed at gaining knowledge on medicinal plants used in postpartum care in Cimande, West Java, providing a strong foundation for further studies on their pharmacological and phytochemical properties. This supports efforts to meet requirements for traditional medicine raw materials as requested by the Ministry of Health.

Methods and Location of Study

Figure 1. Location of the study: Cimande Resort, Caringin District, Bogor Regency, West Java Province.

The focus of the research was Cimande resort forest area which administratively is included in the Caringin District, Bogor Regency, and West Java Province. This area is located at an altitude of 500 to 800 m above sea level. The villages located around the forest area include Pancawati Village, Cimande, Cileungsi, Lemah Duhur, and Cibeureum. Cimande village itself has long been known to foreigners as an area with traditional experts for alternative treatment for broken bones (Notosiswoyo, 1992; Suwandono & Suhardi, 2003). Majority of the population in these villages are of Sundanese tribe, they are Muslim and their main livelihood is farming.

Figure 2. An interview with village midwives.

Ethnobotany research data collection was according to Vogl et al. (2004), Suminguit (2005), and Nolan & Turner (2011) using a modification by nonstructural and open- ended-interview and direct observation in the field, such as observation of postpartum care activities by interviewees, etc. The interviews were directed at two “dukun beranak” or village midwives known locally as "paraji" or "mak berang"; it was also done to six women/families whose labour process was with the help of a mak berang. They were selected based on the criteria mentioned above. The plant species used in postpartum and infant care were recorded to obtain data on location, local names, parts used, preparation and treatment methods, and their benefits.

Plants with of unknown scientific names were sampled in the form of herbarium specimens with reference to Nesbitt (2014). Herbarium specimens were then identified at the Herbarium Bogoriense, Research Center for Biology-LIPI. The data was then analyzed descriptively.

Results

Villagers in Cimande have access to modern health facilities such as Puskesmas/Primary Health Facility or hospitals; however, the role of dukun beranak to help the delivery process is still important. Some factors that influence this include strong local culture and tradition, the limited availability of modern facilities and medical personnel, and low income and education levels of local communities. Fifty plant species used in post-natal care were recorded; 4 of which are used for the care of a newborn (Table 1).

Some foods which are forbidden to be consumed during pregnancy include "cau" fruit or locally known as banana (*Musa* spp.), "Gedang" or papaya (*Carica papaya*), "waluh siyem" or siyem pumpkin (*Sechium edule*), "kulur" or kluwih (*Artocarpus altilis*), jackfruit (*A. heterophyllus*) and goat's meat. The use of medicinal plants in post-natal care can be single or a mixture of several species. During postnatal care, in addition to the use of different types of medicinal plants/jamu, women also receive a massage to all parts of the body on days 3, 7, 15, 30 and 40 after delivery. This massage aims to stimulate blood circulation to quickly restore body fitness.

Nine of the 50 plant species used in post-natal care -- *Amaranthus hybridus*, *Ficus sagittata*, *Lasianthus inodorus*, *Litsea robusta*, *Macaranga* cf. *triloba*, *Psophocarpus tetragonolobus*, *Selaginella plana*, *Vigna unguiculata* and *Vitis* sp. are not listed in the Dictionary of Medicinal Plants (Darwis & Majoindo, 1989) and the Indonesia Medicinal Plant Index (Anonymous, 1995); they are, therefore, new records of medicinal plant species to be added. Nevertheless, research on the potential utilization of these plant species as medicine has been carried out (Akubugwo et al., 2008; Hasan et al., 2016; Sihotang, 2011; Grosvenor et al., 1995; Roosita et al., 2008); Siddhuraju & Becker, 2007). None of the plants are listed in the IUCN Red List.

Table 1. Plants utilization for postpartum care and in a newborn in Cimande, Bogor, West Java

No.	Scientific Name	Family	Local Name	Parts used	Benefit
1.	*Allium cepa*	Amaryllidaceae	Bawang beureum	Tuber	“galogor” potion seasoning
2.	*Allium sativum*	Amaryllidaceae	Bawang bodas	Tuber	“galogor” potion seasoning
3.	*Amaranthus hybridus*	Amaranthaceae	Bayam	Leaves	Breast-milk stimulant
4.	*Arachis hypogaea*	Leguminosae	Suuk	Seed	“galogor” potion
5.	*Areca catechu*	Arecaceae	Jambe	Fruit	Birth control
6.	*Artocarpus heterophyllus*	Moraceae	Nangka	Leaves	To prevent swollen breasts
7.	*Begonia* spp.	Begoniaceae	Calincing	Leaves	Anti flatulence & febrifuge for infants
8.	*Blumea balsamifera*	Compositae	Sembung	Leaves	“godogan 8-15 hari” potion
9.	*Boesenbergia rotunda*	Zingiberaceae	Temu kunci	Rhizome	“godogan 8-15 hari” potion
10.	*Caesalpinia sappan*	Leguminosae	Secang	Flower	To cleanse infant’s eyes
11.	*Capsicum annuum*	Solanaceae	Cabe beureum	Fruit	To restore uterus
12.	*Carica papaya*	Caricaceae	Gedang	Leaves, young fruit	Breast-milk stimulant
13.	*Centella asiatica*	Apiaceae	Antanan ageung	All parts	decoction until day 40
14.	*Citrus aurantiifolia*	Rutaceae	Jeruk nipis	Fruit	To shrink the stomach
15.	*Clitoria ternatea*	Leguminosae	Teleng	Flowers	To cleanse infant’s eyes
16.	*Curcuma aeruginosa*	Zingiberaceae	Koneng hideung	Rhizome	Anti hemorraging agent
17.	*Curcuma longa*	Zingiberaceae	Koneng	Rhizome	Labor stimulant, “godogan 8-15 hari” potion, baby care
18.	*Curcuma* sp.	Zingiberaceae	Koneng tinggang	Rhizome	“pakinum atah” potion
19.	*Curcuma* sp.	Zingiberaceae	Koneng bodas	Rhizome	Potion for anti hemorraging
20.	*Curcuma zanthorrhiza*	Zingiberaceae	Koneng ageung	Rhizome	Labor stimulant
21.	*Cymbopogon nardus*	Poaceae	Sereh	Leaves	“godogan 8-15 hari”” potion
22.	*Elephantopus scaber*	Compositae	Tapak liman	All parts	“godogan s.d. 40 hari” potion
23.	*Etlingera elatior*	Zingiberaceae	Honje	Rhizome	Anti flatulence & febrifuge for infants
24.	*Ficus sagittata*	Moraceae	Ki rapat	Leaves	“godogan s.d. 40 hari” potion
25.	*Gnetum gnemon*	Gnetaceae	Tangkil	Leaves	To prevent swollen breasts
26.	*Hibiscus rosa-sinensis*	Malvaceae	Wera	Leaves	Labor stimulant
27.	*Kaempferia galanga*	Zingiberaceae	Cikur	Rhizome	“godogan 8-15 hari” potion, breast-milk stimulant

(Continue on next page)

Table 1. (Continued)

No.	Scientific Name	Family	Local Name	Parts used	Benefit
28.	*Lasianthus inodorus*	Rubiaceae	Kahitutan	Leaves	“pakinum atah” potion
29.	*Litsea robusta*	Lauraceae	Tangkalak	Leaves	Breast-milk stimulant
30.	*Macaranga* cf. *triloba*	Euphorbiaceae	Ki saat	Leaves	“godogan s.d. 40 hari” potion
31.	*Morinda citrifolia*	Rubiaceae	Cangkudu	Leaves	Potion “pakinum atah”, to prevent headache
32.	*Moringa oleifera*	Moringaceae	Kelor	Leaves	Breast-milk stimulant
33.	*Oryza sativa*	Poaceae	Ketan hideung	Grain	Potion “galogor”
34.	*Rotheca serrata*	Lamiaceae	Singugu	Leaves	“pakinum atah” potion
35.	*Physalis minima*	Solanaceae	Cecenet	Young leaves	“godogan s.d. 40 hari” potion
36.	*Piper betle*	Piperaceae	Seureuh	Leaves	“godogan 8-15 hari” potion, antiseptic for vagina, potion for anti hemorraging
37.	*Plectranthus scutellarioides*	Lamiaceae	Jawer kotok	Leaves	“pakinum atah” potion , “godogan 8-15 hari” potion, vagina antiseptic, birth control, potion for anti hemorraging
38.	*Pluchea indica*	Compositae	Beluntas	Leaves	“pakinum atah” potion, “godogan 8-15 hari”
39.	*Psophocarpus tetragonolobus*	Leguminosae	Jaat	Seed	Potion “galogor”
40.	*Ricinus communis*	Euphorbiaceae	Jarak beureum	Seed	Birth control
41.	*Rubus rosifolius*	Rosaceae	Hareus	Leaves	“pakinum atah” potion, “godogan 8-15 hari” potion
42.	*Sauropus androgynus*	Phyllanthaceae	Katuk	Leaves	Breast-milk stimulant
43.	*Selaginella plana*	Selaginellaceae	Rane	Leaves	“pakinum atah” potion
44.	*Sesbania grandiflora*	Leguminosae	Gala-gala	Leaves	“godogan s.d. 40 hari” potion
45.	*Vigna radiata*	Leguminosae	Toge	Bean sprout Seed	To boost women’s fertility, Potion “galogor”
46.	*Vigna unguiculata*	Leguminosae	Kacang panjang	Young fruit	To boost men’s fertility
47.	*Vitis* sp.	Vitaceae	Bengbereuman	Leaves	“pakinum atah” potion
48.	*Zea mays*	Poaceae	Jagung	Seed	“galogor” potion
49.	*Zingiber montanum*	Zingiberaceae	Lempuyang	Rhizome	“godogan s.d. 40 hari” potion
50.	*Zingiber officinale*	Zingiberaceae	Jahe	Rhizome	“godogan 8-15 hari” potion, to tighten the vagina

Discussion

"Dukun beranak" or "paraji" is basically a midwife, aged over 40. They acquire their skills by observing, learning, assisting and practicing from their grandmothers who help the labour process. Initially, they used traditional tools during labour, for example, they used a bamboo slat to cut the umbilical cord. Recently the Ministry of Health trained these midwives to use more hygienic tools such as scissors, knife and stainless steel containers. It is expected with these tools, mother and baby will have a safer delivery process. In the research area, women start to consult the paraji in the eighth month of pregnancy.

According to the Paraji, the tradition of banning banana and papaya consumption is not only applied to pregnant women, but also to girls in the Sundanese tribe. Bananas and papaya for unmarried girls or women are believed to cause wet vaginal conditions. Whereas papaya contains enzyme papain, its derivatives the chymopapain enzymes has the ability to soften meat (Khare, 2007; Dewick, 2009; Lim, 2013) and could be harmful for the development of the fetus.

Abortifacient content (substances that cause abortion/miscarriage) in papaya is already recorded (Duke & Bogenschutz-Godwin, 2009). Abortifacient content in bananas has not been reported. The abstinence of eating banana heart during pregnancy prior to 9 months of pregnancy has been reported in the Kerinci community in Jambi (Handayani, 2010), but studies on banana's phytochemicals and biosciences in relation to pregnancy have never been conducted. In this regard, the practice is considered a myth. Kluwih fruit, jackfruit, durian (Khare, 2007), and goat's meat are known for their effect on blood circulation, thus could cause premature labour; in addition, consuming sweet potato tubers could cause flatulence. Further scientific studies about the above traditional restrictions need to be done, to clarify whether these are myths or not.

Research on the utilization of plants for postpartum care by the Sundanese ethnic group in some areas in West Java was conducted. In Pasir Eurih Village, Bogor 43 species were recorded (Setyowati-Indarto & Siagian, 1992); Sukaresmi Village, Bogor 37 species were recorded (Sunarti & Rahayu, 1997); Tapos Village, Bogor 15 species were recorded (Damayanti et al., 2002); in three villages around Gunung Halimun National Park 15 species were recorded (Rahayu & Harada, 2004); Tanjung Lame and Legon Pakis Village, Ujung Kulon-Banten 48 species were recorded (Rahayu et al., 2011). One of the most common plant species used for postpartum care is "katuk" (*Sauropus androgynus)* for increasing breast milk production during lactation. However, the use of plants for newborn care has never been done.

The use of jackfruit leaves (*A. heterophyllus)*, "bengbereuman" leaves (*Vitis* sp.), "wera" leaves (*Hibiscus rosa-sinensis*), "tangkalak" leaves (*Litsea robusta*), "jarak beurum" seeds (*Ricinus communis*), "tangkil" leaves (*Gnetum gnemon*), and "hareus" (*Rubus rosiifolius*) in postpartum care has not been previously reported.

The use of medicinal herbs for postpartum care is divided into several stages depending on the time/day after delivery: Jamu of "pakinum atah" is consumed after 1-7 days of delivery. “Pakinum atah” is a drink made from fine pieces of several medicinal plants brewed with hot water then filtered. Jamu "godogan" potion is taken in the period of 8-15 days. Jamu "godogan" is taken from days 16 to 40. “Godogan” is a drink derived from several species of plants that are cooked in water until it boils and eventually only half of the water remains. The potion of "galogor" is eaten as a light meal while breastfeeding. “Galogor” is a snack made of several kinds of crushed grain fried without oil and given spices. Some plant species can be used alone/single, for example “kelor” leaves (*Moringa oleifera*), “katuk” (*Sauropus androgynus*), jackfruit, "cangkudu" (*Morinda citrifolia*), "tangkalak", jambe (*Areca catechu*), and “jarak beureum” (*Ricinus communis)* (Table 1). The utilization of herbs in postpartum care aims to soothe pain during vaginal bleeding and discharge and eliminate odour, to close the wound and restore or shrink the condition of the uterus and vagina, to prevent headaches, to improve blood circulation and to speed up body fitness.

Figure 3. "Katuk" (*Sauropus androgynus*).

Plants consumed as dishes such as daun kelor and katuk and "galogor" are used to boost breast-milk. Fine crushed of leaves of "tangkalak" (*Litsea robusta*), "tangkil" (*Gnetum gnemon*), or jackfruit (*A. heterophyllus*) are pasted and placed to the breast during the non-breastfeeding period to prevent swelling.

Stewed water, dried fine crushed or sliced jambe nut (*Area cathechu*), and dried "jarak beureum" (*Ricinus communis*) consumption is intended as birth control. The application of "jambe nut" or "pinang" as a natural anti-pregnancy agent has long been practiced in India (Shrestha et al., 2010; Bala et al., 2014). Traditionally in Indonesia, the use of pinang as birth control drug has long been practiced by the Sasak ethnic group in Lombok, Nusa Tenggara Barat (Rahayu et al., 2002). Pinang in Indonesia is found across the country, from Aceh to Papua (Dransfied & Backer, 2006a, 2006b; Dransfields, et al., 2006) and used as a complementary ingredient in the tradition of "makan sirih" or "betel chewing" commonly found in the Asia Pacific region (Anonymous, 2012), it is a well preserved Austronesia tradition until now.

Each 100 gram of jambe contains 21-30 grams of water, 5 to 8 grams of protein, 35 to 40 grams of carbohydrates, 5-10 grams of fat, 11 to 15 grams of fiber and 11 - 18 grams of polyphenols. Alkaloid content is low but significant; one of the eight alkaloid compounds that play an important role is *arecolin*. It is also reported that these compounds contribute in raising blood pressure, liver function, increasing the use of glucose in the brain, and improving the function of consciousness in people with Alzeimer's disease (Brotonegoro et al., 2000; Anonymous, 2012).

A mixture of finely grinded "wera" leaves (*Hibiscus rosa-sinensis*), the "Koneng-ageng" rhizome (*Curcuma zanthorriza*) and chicken eggs are used as a labour stimulant. Flowers of *Hibiscus rosa-sinensis* are widely used in the treatment of diabetes (lowering blood sugar levels), anti-diarrhea, and reducing excessive vaginal fluids and urine; the benzene extracted from the flower is known to cause miscarriage (Khare, 2007). The leaves are also known to have similar property (Dasuki, 2001).

The importance of the genus *Curcuma*, including *C. zanthorriza*, in traditional medication is well documented (Duke, 2003; Ramadevi et al., 2007; Skornickova et al., 2007). Its role especially during childbirth, postpartum care and blood circulation is associated with the enzyme content of curcumin and its derivatives. Local name of “ki rapat” in Indonesia generally refers to *Parameria laevigata* (Kamiya et al., 2001); however the Sundanese in Cimande use the same name for *Ficus sagittata*. These two plants which came from two different genera are used for the same purpose, that is to tighten the vagina and uterus after birth. Research on the phytochemical content of *P. laevigata* has been conducted by Kamiya et al. (2001) and van Valkenburg (2001). Similar research by Rojo et al. (1999) showed narcotic compound in *F. sagittata* leaves.

The traditional use of “antanan ageung” leaves (*Centella asiatica*) by the Sundanese in Cimande as a “blood purifier” in post partum care is not unusual (Priyadi, et al., 2010), as the plant is widely used by communities across the Pacific side of Austronesia (Wiart, 2006). It is known to contain the enzyme *asiaticosides,* which acts as a blood purifier (Khare, 2007; Soerahso et al., 1992). In addition, it also contains anti bacterial agent of *citroneal*, *linalool*, *neral*, *mentol*, *linaol acetat*, and *sitronelil acetal* (Sait et al., 1992; Khare, 2007).

Data analysis showes that the rhizome of “koneng” (*Curcuma longa*), leaves of “seureuh” (*Piper betle*) and leaves of “jawer kotok” (*Plectranthus scutellarioides*) have more than two categories of use. Among the four postpartum medicinal herbs, jamu godogan used for days 8-15 is made up of the most variety of plants (11 species), followed by “pakinum atah” herb and the jamu godogan for days 16-40 (9 species), and “galogor” herb (8 species). The importance of *C. longa* and *P. betle* in traditional medicine as well as pharmaceutical industry is widely known (Dyer & Palmer, 2004; Seidemann, 2005; Ramadevi et al., 2007; Skornickova et al., 2007). *P. scutellarioides* was reported to contain mild hallucinogens (Schultes, 1976), but its medicinal potential is not yet known, unlike its more famous relatives *P. Amboinicus* (Seidemann, 2005).

Unlike researches on the benefit of medicinal plants for postpartum care, research on utilization of medicinal plants in newborns is not much known. The data analysis shows two plants species are used as fever-lowering agents and two species are used to cleanse baby's eyes. To reduce fever, leaves of the plant are manually crushed or grinded , mixed with coconut oil and applied on to the infant's fontanelle and applied to the body with a gentle massage.

To cleanse the infant's eyes, flowers of "teleng" (*Clitoria ternatea*) or tree bark of "secang" (*Caesalpinia sappan*) are soaked in water, the bluish water (from teleng) is then dripped into the infant's eyes. This seems to be a typical common traditional practice found only in Indonesia (Sastroamidjojo, 1968; Mardisiswojo & Radjakmangunsudarsom 1968; Heyne, 1987), it is not known in India (Khare, 2007) or even in China. It is suggested that saponin and poliphenol contribute to the cleansing property (Hutapea, 1993).

Figure 4. "Teleng" (*Clitoria ternatea*)

The active compounds that might have medicinal properties in the species of *Hibiscus rosa-sinensis*, *Litsea robusta*, *Rubus rosifolius* and *Vitis* sp. need further studies in order to increase and strengthen their potential value as medicinal plants.

Conclusion

The role of dukun beranak or paraji in some villages in Caringin Subdistrict, Bogor Regency in assisting during childbirth is still indispensable, although these villages have access to health facilities. Local wisdom and knowledge of the Cimande community in postpartum care is demonstrated by the diverse information on the benefits and use of each drug-beneficial species. Fifty plant species were used in postpartum care in Cimande. There are four species that are used for the care of the newborns; two species are commonly used; and seven species have never been previously reported.

The potential of these medicinal plants in postpartum care need further studies to improve/strengthen their benefits. Moreover, full government support is important for the recognition of "Indonesian Traditional/Natural Medicine" that uses herbal ingredients.

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Journal of Tropical Biology and Conservation

Vol. 16 https://jurcon.ums.edu.my/ojums/index.php/jtbc 2019

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E-ISSN 2550 - 1909

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ISSN 1823 - 3902

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