journal of

Vol. 15/2018

TROPICAL BIOLOGY & CONSERVATION

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:
Pleurotus djamor var. *roseus* (Photo credit: Foo She Fui)

Date of issue: 2018-10-15

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Journal of Tropical Biology and Conservation 15: 1–14, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

PLB Regeneration of *Paphiopedilum rothschildianum* using Callus and Liquid Culture System

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Abstract

This research was conducted to rapidly propagate *Paphiopedilum rothschildianum* using semi-solid and liquid culture systems. Calli were induced from seed, leaf segments (LS), seed-derived protocorms (SDP) and secondary protocorms (SP) cultured on half-strength semi-solid MS media supplemented with 0-22.6 µM 2,4-dichlorophenoacetic acid (2,4-D) and 4.54 µM 1-phenyl-3-(1,2,3-thiadiazol-5-yl)-urea (TDZ). Regeneration of PLB from callus was optimized on semisolid medium and were evaluated in various concentrations and types of PGRs. PLB regeneration was further optimized using callus originating from a different induction medium, and cultured on different concentrations (0, 15 and 58 mM) of sucrose. For PLB regeneration using liquid culture system, 0.5 g callus were inoculated in a temporary immersion bioreactor system (RITA®) containing 150 ml liquid medium with immersion time of 5 minutes in every 125 minutes. SE, SDP and SP explants produced calli as early as 30 days with the percentages of explant forming callus at 77.0% ± 4.5, 94.4% ± 11.0, and 66.7% ± 14.4 respectively after 90 days of culture. The calli regenerated on medium supplimented with 2.27 µM TDZ and 12.0 µM BAP, but with very low percentage (15.0% ± 13.7 callus produced the average of 3 PLBs). PLB regeneration capacity increased to 37.5% ± 13.7 with the average of 5.9 PLBs for callus originating from an induction medium containing 4.54 µM TDZ, when a lower sucrose concentration (15 mM) was used in the regeneration medium. Callus proliferation using RITA® system showed an almost 2-fold increased in fresh weight and 168 PLBs per gram calli were regenerated. In contrast to semi-solid culture, the regeneration capacity in liquid culture system increased to 190 PLBs per gram calli when sucrose concentration in the medium was elevated from 15 mM to 58 mM.

Keywords: Seed-derived protocorms, secondary protocorms, temporary immersion system, Temporary Immersion System (TIS), RITA® bioreactor system.

Received 07 June 2016
Reviewed 08 November 2017
Accepted 21 December 2017
Published 15 October 2018

Introduction

Paphiopedilum rothschildianum is a rare slipper orchid species which is endemic to the area around Mount Kinabalu, Sabah, Malaysia (Cribb, 1998). Being rare, this species has been classified as endangered and is listed in *Appendix I* of the Convention on International Trade in Endangered Species (CITES; CITES Appendix 2008). According to Arditti & Ernst (1993), the propagation of this orchid species in the natural way takes several years due to its slow growth rate. Hence, multiplying the orchid through tissue culture techniques is seen as the best alternative. Micropropagation has been applied to propagate many orchid species to produced high mass of plants in a considerably short time and with a lower cost (Zeng et al., 2016). Micropropagation through callus culture is now widely used in the mass propagation of orchids, including *Cymbidium ensifolium var. misericors* (Chang & Chang, 1998); *Phaelonopsis* (Ishii et al., 1998; Chen et al., 2004; Tokuhara & Mii, 2001); *Drynaria Quercifolia* (Hegde et al., 2006); *Pleione formosana Hayata* (Lu, 2004); and *Dendrobium fimbriatum* (Roy & Banarjee, 2003). Only a few researches on callus cultures of slipper orchids have been reported. Callus of *Paphiopedilum hybrids* was induced from seed-derived protocorm (Lin et al.,2000) and seed (Hong et al.,2008) as explants. Another slipper orchid, *Cypripedium formosanum* callus culture was also established using seed-derived protocorm (Lee & Lee, 2003). The propagation of *P. rothschildianum* through *in vitro* formation of secondary protocorm-like bodies (PLBs) from primary PLB developed from stem-derived callus was also recently conducted by Ng & Salleh (2011). Liquid culture system is another alternative means to propagate orchids, it provides higher growth rate of explant and less maintenance as compared to semi-solid culture (Mehrotra et al., 2007). Liquid culture system has been widely used for orchid propagation, this includes *Phalaenopsis* (Park et al., 1996); *Cymbidium* (Da Silva et al., 2006); *Epidendrum radicans* (Chen et al., 2002); *Satyrium nepalense* (Mahendran & Narmatha Bai, 2008); *Oncidium* (Kalimuthu et al., 2007); and *Aerides crispum* (Sheelavanthmath et al., 2005). To date, no report on liquid culture for *P. Rothschildianum* has been documented. The culture system has potential for large-scale propagation of *P. rothschildianum*. Therefore, the objective of this work was to develop protocols for propagation of *P. rothschildianum* using callus on semi-solid and liquid culture systems.

Methodology

Plant materials

Paphiopedilum rothschildianum capsules 6-month post pollination were collected from Poring and Kinabalu National Park orchid nurseries. The capsules were surface sterilised with 10% (v/v) chlorox solution containing 1 drop of tween 20 followed by rinsing with sterile distilled water three times. The sterilised capsules were then disected longitudinally and some of the seeds were used as explants for callus induction and designated as Seed Explant (SE). To obtain protocorms, a portion of the seeds were germinated on a half strength MS medium (Murashige & Skooge, 1962) and the resulting protocorms were used as explants designated as Seed-derived Protocorms (SDP). Subsequently, some of the protocorms were cultured on a multiplication medium and secondary protocorms formed on the protocorm explants were designated as Secondary Protocorms (SP). Leaves from *in vitro* plantlets of *P. rothschildianum* were cut into pieces of 1 cm × 1 cm and used as explants designated as Leaf Segments (LS).

Callus induction

To induce callus, the medium based on Hong et al. (2008), was used. The medium was comprised of half strength MS basal medium supplemented with full strength MS Vitamin, 2g/l peptone, 170 mg/l NaH_2PO_4, 58 mM sucrose, various concentration of 2,4-D (0-22.6 µM) with a constant concentration of TDZ (4.54 µM) and 2.2 g/l Gelrite. The pH of medium was adjusted to 5.2 and autoclaved at 121ºC and 15 psi for 20 minutes. The medium was dispensed in 9 cm diameter Petri dishes and four explants of SE, SDP, SP and LS were cultured on each Petri dish. Each treatment was replicated five times and all cultures were maintained at 25 ± 2°C in darkness. Observations were made every 10 days for 90 days and explants were subcultured onto fresh media at four week intervals.

PLB Regeneration

PLB regeneration from callus was first optimized on semi-solid media, and the best medium was subsequently applied on RITA®. To induce PLB, semi-solid media based on Hong et al. (2008) were supplemented with NAA (0-26.85 µM), 2,4-D (0-9.04 µM), TDZ (0-9.08 µM) and BAP (0-22.20 µM) at various concentrations and combinations. To evaluate the effect of origin of callus (induction medium) on PLB regeneration, callus induced from seed explant (SE) cultured on semi-solid half strength MS media containing 4.54 µM TDZ alone or a in combination with 13.6 µM 2,4-D were used. To evaluate the effect of carbon source, medium with sucrose concentration of 0, 15 and 58 mM were

tested. To evaluate PLB regeneration in liquid culture system, 0.5 g callus were inoculated in 1 L RITA® system vessel containing 150 ml liquid medium. The immersion time was set to 5 minutes in every 125 minutes. All cultures were maintained at 25 ± 2°C in a 16 hour photo period. Observations were made every 10 days for 150 days and explants were sub-cultured onto a fresh medium at four week intervals.

Data Collection and Statistical analysis

All data were analyzed using SPSS (Statistical Package for Social Science) version 17.0 and subjected to analysis of variance (ANOVA). Duncan's multiple range tests were conducted for mean comparisons of data collected using $P < 0.05$.

Results and Discussion

Callus Induction

Callus was induced on medium containing a combination of 22.6 µM 2,4-D and 4.54 µM TDZ for SE, SP, and SDP explants. Figure 1 (Plate 1A-H) showed that the callus became visible after 30 days of culture and slowly increased in mass through the 150 days period. The morphology of the callus formed on average was friable and creamy coloured. The auxin to cykonin ratio was considered standard for callus induction experiments for slipper orchids as reported by Lin et al. (2000); Hong et al. (2008); and Lee & Lee (2003) where 22.6 µM 2,4-D and 4.54 µM TDZ gave the highest percentages of explants forming callus. The results indicate that the combination of auxin and cytokinin in the culture medium play important roles in dedifferentiation of plant cell (Pierik, 1997). LS failed to produce callus and died after 40 days of culture (Plate 1I-J). Lin et al. (2000) had reported the failure of stems, root tips and leaves of *Paphiopedilum* hybrids to produce callus when cultured on media containing TDZ, 2,4-D, PBOA, BA, and 2ip. Different types of explants has been used for *in vitro* clonal propagation of *Paphiopedilum* (Chugh et al., 2009). The success rate of the culture depends on the source, types, maturity and treatment of the explants (Trigano & Gray, 1999).

Figure 1. (Plate A-J): Callus induction of *Paphiopedilum rothschildianum* on ½ MS (Murashige & Skoog, 1962) medium in darkness, 25 ± 2ºC. **A)** SDP after 30 days of culture (bar = 0.18 cm); **B)** SDP after 60 days of culture (bar = 0.19 cm); **C)** SDP after 90 days of culture (bar = 0.19 cm); **D)** SP after 30 days of culture (bar = 0.19 cm); **E)** SP after 60 days of culture (bar = 0.19 cm); **F)** SP after 90 days of culture (bar = 0.19 cm); **G)** Seed after 30 days of culture (bar = 0.05 cm); **H)** Seeds after 80 days of culture (bar = 0.21 cm); **I)** LS after 10 days of culture (bar = 0.22 cm); **J)** LS after 40 days of culture (bar = 0.21 cm).

Table 1. Formation of callus by different types of *Paphiopedilum rothschildianum* explants after 90 days of culture on ½ MS (Murashige & Skoog, 1962) medium supplemented with 0-22.6 µM 2,4-D and 4.54 µM TDZ in darkness, 25 ± 2°C.

Plant Growth Regulators (µM)		Explant formed callus (mean%±SD)				Size and quality of callus			
TDZ	2,4-D	SE	SDP	SP	LS	SE	SDP	SP	LS
0	0	33.6 ± 7.6^{f}	25.7 ± 9.8^{f}	40.0 ± 13.7^{ef}	0^{g}	(K,P), c+,f+	(K), c++	(K,P), c++	-
4.54	0	77.0 ± 4.5^{abc}	89.3 ± 13.4^{a}	41.7 ± 14.4^{ef}	0^{g}	(P), f+++	(K,P), c++, f+++	(H, K), n+++, c+++	-
4.54	4.52	76.0 ± 6.5^{abc}	82.1 ± 18.9^{a}	66.7 ± 14.4^{bcd}	0^{g}	(K), c++	(P,K), c+, f+++	(H,K), c+++, n+++	-
4.54	9.04	-	87.5 ± 13.7^{a}	58.3 ± 14.4^{cde}	-	-	(K,P), +, f+++	(H,K), c+++, n+++	-
4.54	13.56	61.0 ± 19.8^{cd}	94.4 ± 11.0^{a}	62.5 ± 14.4^{cd}	0^{g}	(K), c++	(K,P),+, f+++	(H,P), n+++, c+++	-
4.54	18.08	-	82.1 ± 18.9^{a}	55.6 ± 9.6^{de}	-	-	(K,P), c+, f+++	(K,P), c+++	-
4.54	22.6	64.0 ± 20.4^{bcd}	89.3 ± 13.4^{a}	56.3 ± 12.5^{de}	0^{g}	(P), c++	(K,P), c+, f+++	(P,H), c+++, n+++	-

Note: Data were taken from 5 replicates with the same letters are not significantly different at $p<0.05$ using Duncan's Multiple Range Test. SD= Standard Deviation. Callus size and quality index; (K): browning, (P): creamy, (H): greenish, c: compact, f: friable, n: nodular, +: small, ++: average, +++: large.

The highest percentages of explants forming callus after 150 days of culture was SDP (94.4 ± 11.0) followed by SE (77.0 ± 4.5) and SP (66.7 ± 14.4) (Table 1). These results are in contrast with that reported by Lin et al. (2000) which showed seed explants formed better callus than SDP. This may due to the fact that SDP was grown on fresh medium as compared to the dormant nature of the seed explants. These findings suggest that the responsiveness of explant was affected by the maturity and responsiveness of the cell and tissue (Murthy & Pyati, 2001). Table 1 also showed that, on average although SDP showed the highest percentage of explant forming callus, it was SP explants that showed higher mass of callus formed on explants. Better formation of callus by SP explants might be due to the carry over effect of PGR as the SP explants originated from a multiplication medium containing TDZ. This is supported by the finding made by Makara et al. (2010) from their study on banana where higher concentration of endogenous PGRs may increase plant growth and proliferation. They found out that the proliferation rates of shoots originating from basal cycle medium with various TDZ concentrations were

significantly higher than those from 22.2 mM BAP, which suggested that TDZ had a high carry over effect enabling the shoots to continue proliferating on the hormone free medium (Makara et al. 2010).

PLB Regeneration

PLB formation from callus proved to be very slow and low in number. Only 15.0% ± 13.7 callus formed PLB (average of 3 PLBs per callus) on the semi-solid medium supplimented with 2.27 µM TDZ and 13.32 µM BAP after 120 days of culture (Table 2, Figure 2). The other PGRs failed to produce PLB. Lin et al. (2000) suggest that the combination of TDZ with auxin is crucial for the regeneration of PLB for *Paphiopedilum*. However, the results were in contrast with the study conducted by Hong et al. (2008) and Ng & Salleh (2011). Hong et al. (2008) were able to regenerate PLB for *Paphiopedilum* Alma Gavaert using 26.85 µM NAA while Ng & Salleh (2011) used 4.0 µM kinetin to regenerate PLB for *P. rothschildianum*.

Table 2. Formation of PLB on half-strength MS (Murashige & Skoog, 1962) with different types and concentration of PGRs as explants after 150 days of culture.

Plant Growth Regulators (µM)				Callus formed PLB (mean% ± SD)	PLB per explant
NAA	2,4-D	BAP	TDZ		
0	0	0	0	0	0
0	0	4.44	2.27	0	0
0	0	13.32	2.27	15.0±13.7	3
0	0	22.20	2.27	0	0
0.27	0	0	2.27	0	0
2.69	0	0	2.27	0	0
5.37	0	0	2.27	0	0
26.85	0	0	2.27	0	0
0	2.26	0	2.27	0	0
0	4.52	0	2.27	0	0
0	6.79	0	2.27	0	0
0	9.08	0	2.27	0	0
0	2.26	0	4.54	0	0
0	4.52	0	4.54	0	0
0	6.79	0	4.54	0	0
0	9.08	0	4.54	0	0

Note: Data were taken from 5 replicates with the same letters are not significantly different at $p<0.05$ using Duncan's Multiple Range Test. SD= Standard Deviation.

This proved that different PGR were required to succesfully regenerate PLB for different species of *Paphiopedilum*. In addition, PLB regeneration was proven to be difficult for *Paphiopedilum* species as demonstrated by Lin et al. (2000) and Hong et al. (2008). They reported that PLB formation on callus was achieved only after 120 days and 150 days of culture respectively.

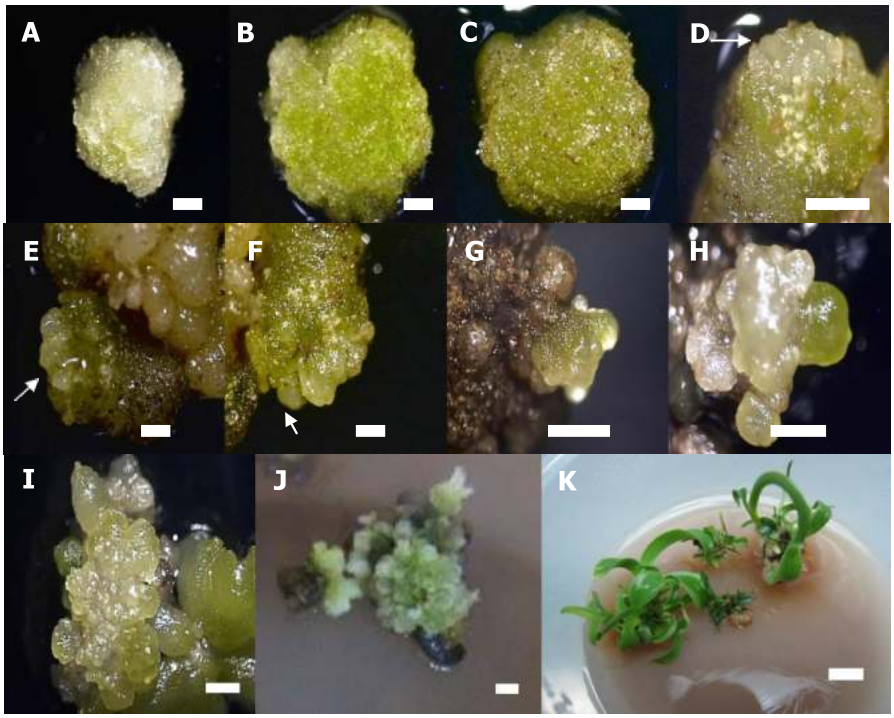

Figure 2. The formation of PLBs from callus cultured on half-strengh MS medium supplemented with 2.27 µM TDZ and 12.0 µM BAP (bar = 1 mm). **(A)** Callus proliferated after 30 days of culture. **(B)** Callus become greenish in colour after 60 days of culture. **(C)** **(C)** Callus become more greenish after 90 days of culture. **(D)** PLB formed on callus after 120 days of culture. **(E)** Formation of PLB become more apparent after 150 days of culture. **(F)** Magnification of the PLB formed. **(G)** Callus turned brown but PLB continue to grow and multiply. **(H)** PLB further multiply after 240 days of culture. **(I)** Multiplied PLBs transferred to development medium. **(J)** PLBs developed into shoot after 14 days of culture on development medium. **(K)** Formation of plantlet after 150 days of culture.

Table 3. Formation of PLB on half-strength MS (Murashige & Skoog, 1962) with different concentration of sucrose using *Paphiopedilum rothschildianum* callus developed from medium supplemented with different PGRs as explants.

Callus source	Sucrose concentration (mM)	Callus regenerate (mean% ± SP)	Average number of PLB	Time taken for PLB formation (Days)
Developed from ½ MS medium supplemented with 4.54 µM TDZ	0	0	-	-
	15	37.5 ± 13.7[a]	5.9	42
	58	33.3 ± 14.4[bc]	2.7	45
Developed from ½ MS medium supplemented with 4.54 µM TDZ and 13.6 µM 2,4-D	0	0	-	-
	15	16.7 ± 14.4[b]	2	66
	58	0	-	-

Note: Data were taken from 5 replicates with the same letters are not significantly different at $p<0.05$ using Duncan's Multiple Range Test. SD= Standard Deviation.

The regeneration capacity increased to 37.5% ± 13.7 callus forming PLB (average of 5.9 PLBs per callus) after only 42 days of culture when lower concentration of sucrose was used in the regeneration medium (Table 3). This showed that lower concentration of sucrose are more suitable for the formation of PLB for *P. rothschildianum* callus. This was supported by Chen & Chang (2002), who reported that higher embryogenic response of *Oncidium* 'Grower Ramsey' leaf explant when they were cultured on the medium containing lower concentration of sucrose. Plant regeneration for cell suspension culture of *Phalaenopsis* species was also more successful using the medium with lower concentration of sucrose (Tokuhara & Mii, 2001). Faria et al. (2004) indicated that high concentration of sucrose resulted in carbohydrate accumulation in the medium and subsequently causing retardation of photosynthesis.

The results in Table 3 also showed that the PGRs used to induce callus affect the callus regeneration capacity. It is observed that lower 2,4-D concentration in the callus induction medium was able to increase the callus regeneration capacity. This was supported by Lu (2004), who indicated that the capability of *Pleione formosana* Hayata callus to regenerate PLB was affected by the concentration of 2,4-D and TDZ in the callus induction medium. Lin et al. (2000) indicated that callus originating from medium supplemented with single treatment of 2,4-D or 2,4-D results in little growth and eventually becomes necrotic. The present study suggests that high concentration of auxin in callus induction medium inhibit PLB regeneration for *P. rothschildianum* callus. This may due to the carry over effect of 2,4-D from induction medium onto the

regeneration medium, similar to the study conducted by Makara et al. (2010). This also proves that high concentration of auxin may inhibit the morphogenesis of plant tissue cultured *in vitro* (Smith, 2013).

For liquid culture experiment, the average PLB formation was higher (4.0 PLBs) on medium with higher sucrose concentration as compared to 3.6 PLBs on medium with lower sucrose concentration (Table 4). The PLBs was then transferred onto a development medium and successfully developed into plantlet after 150 days of culture (Figure 3C-E). The result was different from semi-solid culture, where lower concentration of sucrose was better for PLB formation. This indicates that the optimal sucrose concentration for PLB regeneration of *P. rothschildianum* callus in liquid medium was different compared to a semi-solid medium. This maybe due to efficient distribution and utilisation of nutrients in liquid medium (Sandal et al., 2001). This was supported by Ziv (2005) who stated higher sucrose concentration produced higher biomass of Boston Fern *(Nephrolepis exaltata)*. This finding could also be due to the osmotic pressure in liquid medium not being affected by high concentration of carbon as opposed to semi-solid medium (Pierik, 1997).

Table 4. Formation of PLB from *PaphiopedilPLB um rothschildianum* callus after 30 days of culture in 1 L RITA® system vessel containing 150 ml half-strength MS (Murashige & Skoog, 1962) with different types of carbon source.

Carbon source	Fresh weight increased (gram)	Average of PLB	Total PLB per final weight
58 mM of sucrose	0.30 ± 0.17	4.0	95/0.82 g
15 mM of sucrose	0.06 ± 0.01	3.6	84/0.65 g

Note: Data were taken from 3 replicates. Fresh weight of callus obtained after 30 days of culture. SD= Standard Deviation.

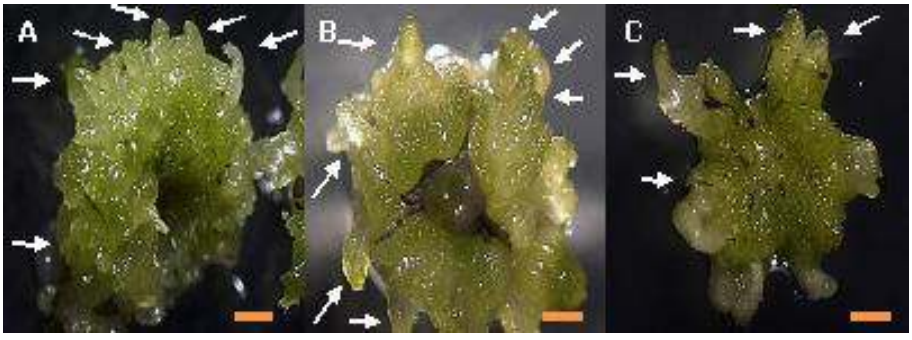

Figure 3. Formation of PLB/shoots on *Paphiopedilum rothschildianum* callus after 30 days of culture in 1 L RITA® system vessel containing 150 ml half-strength MS (Murashige & Skoog, 1962) with different types of carbon source (bar = 1 mm). **A)** PLB/shoots formed in medium with 58 mM sucrose. **B)** PLB/shoots formed in medium with 15 mM sucrose. **C)** PLB/shoots formed in medium with 15 mM glucose.

Table 5. Comparison between regeneration capacity of *Paphiopedilum rothschildianum* callus in liquid culture system and semisolid culture.

Culture system	Carbon source	Regeneration capacity (PLB per gram callus)
Liquid medium in 1 L	15 mM sucrose	168
RITA® vessel	58 mM sucrose	190
Semisolid medium in 9mm	15 mM sucrose	113
Petri plate	58 mM sucrose	45

Note: The calculation of regeneration capacity were based on total number of PLB produced divided by the initial weight of callus used.

For the comparison of the regeneration capacity between liquid culture and semi-solid culture, it showed more than a 2-fold increase in PLB formation on callus cultured in liquid culture as compared to semi-solid culture (Table 5). This is similar to the study conducted by Nayak et al. (2002) on PLB regeneration of *Cymbidium aloifolium* (L.) Sw. and *Dendrobium nobile* Lindl PLB in semi-solid and liquid medium. Their results showed that PLB regeneration of both orchid species was significantly enhanced compared to semi-solid medium. Furthermore, in the liquid culture system, more explants can be cultured in a larger container, thus increasing productivity. This is mainly due to the efficiency of their culturing conditions, media transfer and sterilization procedures. In contrast, the semi-solid culture system is limited by size of the culture container, intensive labour work and poor aseptic condition (Etienne & Berthouly, 2002). The RITA® culture vessel used in the present study was also able to avoid the hyperhydricity problems normally associated with explants in liquid culture, thus producing healthy PLBs (Figure 3A-B). The system used a temporary contact between the explants and liquid medium, which can greatly reduced hyperhyricity (Etienne & Berthouly, 2002). Teherefore, the current work proved that the temporary immersion system provides many advantages that further enhance the PLB regeneration of *P. rothschildianum* callus compared to the conventional system.

Conclusion

Based on the present study, a protocol for propagation of *P. rothschildianum* using callus on semi-solid and liquid culture systems has been established. This work proved that callus can be induced on *P. rothschildianum* explants within 30 days, where SDP explants formed the highest percentage of callus. PLB formation from callus was achieved using 2.27 µM TDZ and 12.0 µM BAP. The regeneration capacity increased by using different concentration of sucrose. Morever, liquid culture system using RITA® further increased the regeneration

capacity; producing up to 190 PLBs per gram calli. Future studies will be directed to further optimise the liquid culture system including volume of media, inoculum density and immersion time.

Acknowledgements

The authors wish to thank Sabah Park Orchid Nursery for supplying *P. rothschildianum* seeds and Universiti Malaysia Sabah for funding the research under the Fundamental Research Grant Scheme (GPS0009-SG-1/2009).

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Journal of Tropical Biology and Conservation 15:15–27, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

The Ethnobotanical Survey of Clove, Pepper, and Nutmeg and Their Utilization by Chinese and Indonesian People

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Abstract

An ethnobotanical survey was conducted in the spices market of two provinces of China. This study aimed to describe the survey of spices sold and the availability of clove, nutmeg, and pepper in China and Indonesia markets. The study documented the knowledge of the utilization of pepper, clove, and nutmeg by Chinese and Indonesian people. Different communities based on their perceptions and experience use the same spice plants for many purposes. Unstructured interviews and literature study were also conducted to complement the data. Cloves, nutmeg, and pepper are the three kinds of main spices for the Chinese people. Indonesian people use nutmeg and clove for one particular type of cuisine, otherwise pepper is used in a variety of dishes, to provide a spicy flavour. Due to the medicinal properties of clove and nutmeg, Indonesian and Chinese people use clove not only as a spice but also for medicine. Apart from using it as a spice and medicine, Indonesian people also use nutmeg for snacks.

Keywords: spices, market, flavouring, medicine

Introduction

People use plants for social, cultural and economic needs. Plants are used for purposes of food, medicine, fuel, industry, ornament, ritual, firewood, construction material, and also as spices. There are many forms of spices such as fresh, dried, or frozen; whole, ground, crushed, pureed, as pastes, and extracts (Raghavan, 2007). There are many other uses of spices, to preserve meats or fish, to eliminate disagreeable odours and to disguise tainted foods. Many spices have medicinal properties such as antioxidants, digestive stimulant action, anti-inflammatory, and antimicrobial (Shan et al., 2005). Indonesia also has great biodiversity and diversity of ethnic groups each with their own social life and different cultures. More than 6,000 species of flowering plants, whether wild or cultivated are recognized and utilized for the purposes of

Received 22 February 2017
Reviewed 21 June 2017
Accepted 01 November 2017
Published 15 October 2018

food, clothing, medicine, protection, and also spices (Walujo, 2011). With the diversity of the tribes that inhabit Indonesia, there are various systems of knowledge on nature and the environment. This knowledge varies from one ethnic group to the other ethnics and depends on where they live, the climate, customs, etiquette, behaviour, and also the pattern of life groups (Walujo et al., 1991). For example, Sumatra people like the food to be spicy, but Javanese people prefer sweet flavours in their food.

China consists of 56 ethnic groups, and each group has traditional knowledge of using plants (Wujisguleng et al., 2012). China is a country with a great diversity of plants, climate and geography. The northern people of China use spices more often compared to southern people. They need spices to warm their bodies. The climate of southern China is warm and humid and rainfall is abundant (Wright, 2011). Northern China is usually relatively dry with less abundant rainfall. The landscape is mostly dry and brown. Dry crops of barley, millet, and wheat grow best in northern China (Wright, 2011). Northern people like a more spicy taste in their food.

Historians have noted that Indonesia is the focus of attention of Chinese, Indian, Arab, and European traders since 300 BC or possibly earlier. They tried to obtain major Indonesian spices of the time, such as cloves (*Syzygium aromaticum* (L). Merr. & L.M. Perry) and nutmeg (*Myristica fragrans* Houtt.) (TREDA, 2012). By the fifth century, there was also a high demand from China for cloves, nutmeg and mace (all from Mollucas), pepper (*Piper nigrum* L.), (from Sumatra and West Java), as well as rhinoceros horn (from Java and Sumatra) and tortoiseshell (from Bali and elsewhere). (Drakeley, 2005). Clove, pepper, and nutmeg are spices with many uses. These are used for traditional medicine, food, ritual materials, to preserve materials, etc. Indonesia is still exporting clove, pepper and nutmeg to China. Indonesia is a producer of cloves and nutmeg, while pepper is also grown in West Java and Sumatra. White pepper, black pepper, and the nutmeg oil were between 2009 and 2014 major export commodities from Indonesia (Statistic Indonesia, 2015). Most spice products were supplied from East Java which reached 49.1 thousand tonnes (32.17 percent), Lampung which reached 34.7 thousand tonnes (22.74 percent), DKI Jakarta which reached 28.1 thousand tonnes (18.41 percent), and North Sumatera which reached 19.6 thousand tonnes (12.83 percent) (Indonesian Foreign Statistic, Volume I, 2015). Until now, clove, nutmeg and pepper are the common spices used by Chinese and Indonesian people. The ethnobotanical study of clove, nutmeg and pepper by Indonesian and Chinese is conducted to document the knowledge of using pepper, clove, and nutmeg

by Chinese and Indonesian people. The survey of spices was conducted in spice markets.

Methods and Material

Study area

A market survey was conducted in two Chinese provinces that have big spice markets. The first survey of a spice market was carried out in Hebei Province, and the second survey was conducted in Guangxi Zhuang Autonomous Region. The first survey of the spice market was conducted in four small spice shops and one big market that has medicinal material and spices in Anguo City, Hebei Province. Anguo is located at the south of Baoding in Hebei province, North China. The Anguo herbal market is opens from 08.00 to 11.00. There are two floors in this market, the first floor mainly sells spices and on the second floor, medicinal material are sold (Figure 1). The medicinal material is not only from plants but also from animals. The second floor sells spices and medicinal material at higher prices (Table 1).

Table 1. Brief sketch of the spice market in Anguo City

First floor	Spices
Second floor	• Medicinal material from plants and animals (higher prices) • Spices (higher prices)

Figure 1. The first floor of the spices market in Anguo City.

The second survey of the spice market was conducted in Yulin City of Guangxi Zhuang Autonomous Region (Figure 2). In Yulin city, we conducted a survey in four big markets, of which two markets solely sell spices. These markets also distribute spices to other provinces in China and also other countries.

Figure 2. The distributor of spices in Yulin City

The third market is the market that usually sells spices packaging. The market is opens between 7:30 and 20:00. In this market, we also found sellers of vegetables, fruits, dried fishes, daily food, tea, and also local food products. The vegetable sellers also sell spices in the fresh form such as ginger, onion, and garlic obtained from Yulin city. The spices sold in Yulin city occur due to several factors, the first one is its location. Yulin is located closer to Southeast Asian countries and people from different provinces and countries visit the city. The second factor is Yulin is traversed by traders or entrepreneurs who come to trade – buying spices before reselling. Usually, spice sellers in this city are those who came from other provinces, such as Guang Dong Province. Local people in Yulin city usually sell the food in some small shops and also sell jeans. Spice selling is done on a larger scale since most people see that selling spices has given them more benefit.

Data Collection

For our data collection, we did surveys and interviews. We also did a literature study on uses of clove, pepper, and nutmeg by Indonesian and Chinese people. The survey was conducted to document spices sold in the market and also the availability of pepper, clove, and nutmeg. The methodological approach was semi-structured interviews with spice sellers. In Anguo City, we interviewed 4 spice sellers at random. In Yulin City, we also did random interviews. In the first spice market, we interviewed 5 spice sellers (2 women and 3 men). In that market, some sellers sell their products directly from home so they do not have to come to the market. In the second market, we interviewed 19 spice sellers by random. Interviews and discussions were undertaken based on questions prepared in English and translated into Chinese. We also collected data about spices in Indonesian markets from literature and did surveys in traditional markets.

Data Analysis

Based on interviews, we produced graphics on the types of spice sellers and their knowledge about the spices. We describe the types of spices sold in the market, how the sellers get the spices, and the use of clove, pepper, and nutmeg by Chinese and Indonesian people. We also analyzed data based on ethnobotany views.

Results

From the interview, we know that some spice sellers know a lot about spices but do not use spices while some know much about spices and use them in their daily lives (Figure 3). Spice sellers received knowledge from their parents, friends, and also from their experience. There are several types of the spice sellers in the market that we visited. The first one is sellers who sell only one kind of spice or the main spices. This is based on the high sales value on those spices as people often use these spices. In other words, there is possibility of the prices of these spices continuing to increase. The second type of the sellers are spice sellers who sell spices produced by their hometown, making it easier to get the spices. They usually sell pepper from Hainan Province. The third type are spice sellers who sell not only the spices but also the medicinal materials (Figure 4).

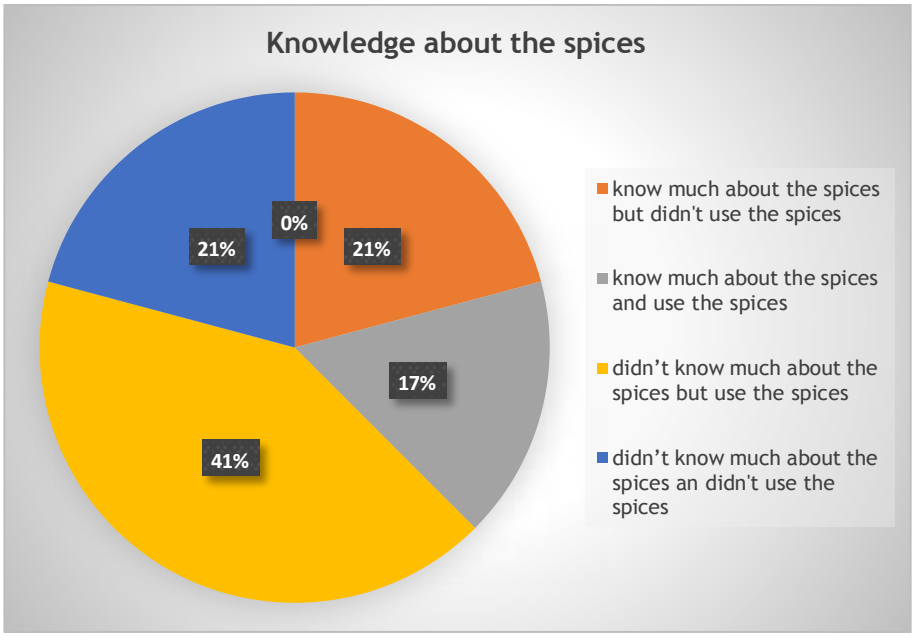

Figure 3. The graphic of knowledge about the spices

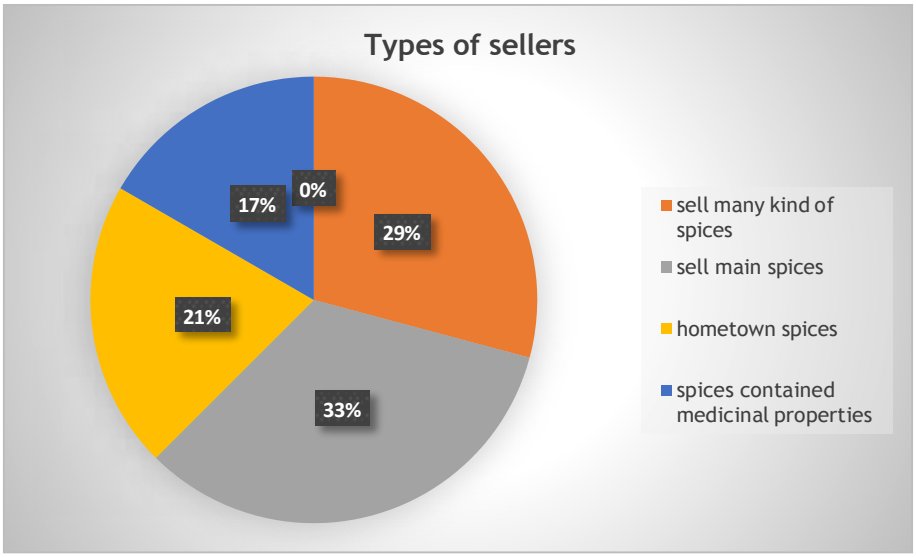

Figure 4. Graphic of types of spice sellers

In China, most spice markets sell spices in the dried form (Figure 5). These dried forms are most frequently used as processed products. The spice sellers also cooperate with medicinal material companies, because these spices can be used as medicine. The spices purchase will be crowded before the Spring Festival. People and restaurants will come to prepare food for the Spring Festival. In Indonesia, spices are sold in the traditional market. The market sells other household needs such as vegetables, meat, fruits, etc.

Figure. 5 Spices sold in Chinese spice markets

The spices sold in Indonesia market are mostly in fresh form. There are also some spice sellers who sell in the dried form. Most spice sellers also sell milled fresh and dried spices. One combination of spices is used specifically for one dish. In Indonesia, purchase of spices will spike before the Idul Fitri and Christmas celebrations. Customers are diverse, ranging from housewives to those who do catering and at food stores to restaurants. There are several methods of how spices are sold in markets. Most farmers will sell spices to a merchant who collects these. When merchants start running out of stock or when prices start to increase, they will directly come to the farmers. The price is determined by the merchant.

In the Chinese spice market, cloves, pepper, and nutmeg are some of the main spices. The other spices that can be found are star anise (*Illicium verum* Hook.f.), ginger (*Zingiber officinale* Roscoe), cinnamon (*Cinnamomum cassia* (L.) J. Presl), cumin (*Cuminum cyminum* L.), citrus skin (*Citrus* sp.), Szechuan pepper (*Zanthoxylum* sp.), long pepper (*Piper longum* L.), cardamom (*Amomum* sp.), aromatic ginger (*Kaempferia galangal* L.), and turmeric (*Curcuma longa* L.). These spices are mostly sold in spice and herbal markets. Based on survey results, nutmeg is imported from Indonesia; clove imported from Indonesia and Africa, while pepper is imported from Indonesia, India, Malaysia, Vietnam, and also brought from Hainan Province, China. The shade of cloves from Indonesia is darker than those from Africa. The stems of cloves are also imported from Indonesia. The reason for importing spices directly from Indonesia is due to the need for Indonesian spices. To import spices from Indonesia, there are people who will connect them with the spice distributors in Indonesia. Some spice sellers also come directly to Indonesia to find spice distributors.

Chili (*Capsicum annum* L.), pepper (*Piper nigrum* L.), nutmeg (*Myristica fragrans* Houtt.), shallot (*Allium cepa* L.), garlic (*Allium sativum* L.), ginger (*Zingiber officinale* Roscoe), turmeric (*Curcuma longa* L.), coriander (*Coriandrum sativum* L.), aromatic ginger (*Kaempferia galanga* L.), clove, candle nut (*Aleurites moluccanus*) are the spices mostly sold in the Indonesian market. The spices are mostly used in daily cooking. There are more variants of spices in the big traditional market, for example in Pasar Induk, Kramat Jati, East Jakarta or in Pasar Senen, Central Jakarta. Spices include cardamom, caraway (*Carum carvi* L.), cinnamon, nutmeg, mace and clove. The spice sellers also import cardamom from India and cinnamon from China (KOMPAS, 2014). In small traditional markets, clove and nutmeg are usually sold in small quantities. Many kinds of spices are sold such as ginger, nutmeg,

white pepper, onion, garlic, chili, turmeric, cumin (*Cuminum cyminum* L.), aromatic ginger, etc. They sell not only spices but also other household needs, such as vegetables and foodstuff.

In general, there are middlemen or stores that collect spices from farmers and sell these in markets. The most expensive spice in the Chinese spice market is pepper and the most expensive spice in the Indonesian market is clove. In the Chinese and Indonesian markets, cloves, pepper and nutmeg are some of the main spices with high demand. The availability of spices sold in the market is influenced mainly by the needs of the community and because of good sales of these spices. Sale is related to profit and if certain spices have stable prices, then these spices will be sold.

Discussion

Cloves, nutmeg, and pepper are the three kinds of main spices for the Chinese people. Indonesian people use nutmeg and clove for one particular type of cuisine, otherwise pepper is used in a variety of dishes, to provide a spicy flavour. Chinese people use cloves, star anise, and citrus skin to eliminate fishy smell in meat dishes. Clove oil can also be used as a natural preservative, it is associated with the usage of spices in the past by the Europeans during winter. Traditionally, the use of spices as a natural preservative is by immersing or smearing it on meat or processed meat. Utilization is usually in the form of a combination of several kinds of spices. Cloves are generally picked by hand, using a ladder or pole of bamboo. It takes four years for clove to mature for harvesting from the time it is planted. Harvesting is done by picking the flower stalk, then putting these in a basket. The age of flower should be old enough and yet in bloom.

Clove is the most expensive spice sold in Indonesia since it is used by many companies as an ingredient of 'Kretek' cigarettes. Kretek are cigarettes with combination of tobacco, cloves and other flavours. Unlike the use of cloves by Chinese society, Indonesian people use cloves for one particular type of cuisine, especially in meat dishes such as 'rendang' and stews. 'Rendang' can last a long time, this is related to the use of cloves as a natural preservative.

As a medicinal material, clove has been used mainly for dental health. This is in line with the days of the Han Dynasty, where the Chinese people were instructed to chew on cloves. Tolaki and Toraja people in Southeast Sulawesi, Indonesia use clove to heal toothache. It is dried and affixed directly on the

sore tooth (Sihotang et al., 2011). Communities in Trunyan village, Bali, use clove and its leaves to treat shortness of breath, fever and tingling. The ethnic of Sunda in Bodogol area, West Java also use clove as medicine for toothache (Sihotang, 2011). Clove is one of the spices with the highest antioxidant capacities. It has potential to be exploited as a natural antioxidant for commercial purposes. Clove contains a high level of phenolic (Shan et al., 2005). Phenolic compounds in these plant materials are closely associated with their antioxidant activity (Shan et al., 2005). Clove oil can also be used as raw material for the manufacture of balsam. Balsam cloves can relieve pain, especially rheumatic pain. In addition, clove also relieves indigestion and vomiting. It is also used in medicinal preparations for asthma, arthritis and sprains

The ground nutmeg which is directly from nutmeg seeds is used for flavouring food. To retain its flavour, nutmeg is usually added in the end of cooking. Some people prefer mace in their cuisine. By using mace, cuisine could be cleaner. In contrast with nutmeg, the food will seem a bit murky. In drying nutmeg, farmers still use a traditional method through which they dry it in the sun for 4-5 days, depending on the level of dryness or moisture, on plastic bags, jute sacks, tarps or cloth material. The use of sack or cloth is to keep the temperature normal. Mace is dried in the sun for a few hours then aerated. It is done repeatedly until the mace is completely dry.

Chinese people use nutmeg in hotpots which is part of Chinese culinary culture. To improve taste and smell of hotpots, spices are always added (Wu, et al., 2012). The Chinese people also consider it to be an aphrodisiac. In Indonesia, nutmeg seed is used for stews, sauces and curries. Nutmeg is used to add a spicy flavour in food, and is able to help warm the body. Nutmeg has religious significance for Maluku people. The nutmeg seed is placed around the neck of a person who is seriously ill and God is then asked to heal the person and decide the person's fate (van Gills, 1994).

Nutmeg has 6.5% to 16% essential oil (Asgarparnah & Kazemivash, 2012). Mace has 7 to 14% essential oil and about 30% fixed oil (Raghavan, 2007). Because of that, Maluku people rub the oil all over their bodies to keep themselves warm. Nutmeg oil is also rubbed on the abdomen to relieve stomach aches and on the forehead to alleviate headaches (van Gills, 1994). The Iboih people in Aceh use nutmeg as a medicinal plant for treating headaches (Susiarti, 2006). Since ancient times, nutmeg and its oil were used in Chinese and Indian traditional medicine for illnesses related to the nervous and digestive systems. In China,

nutmeg seeds are crushed into a powder and used as a remedy for dysentery in both children and the aged (Mitra et al., 2007). The nutmeg candied fruit also popular as a snack. It can be made into wet candied nutmeg or dried candied nutmeg. In Maluku it is called 'pala gula' (literally, sugar nutmeg), the result of the drying process. In Java it is called 'pala manis' (literally, sweet nutmeg) a sweet candy made from the nutmeg rind.

The bite and pungency in black and white peppers is primarily due to the nonvolatile alkaloids, piperine and chavicin. Piperine contributes to the hotness of pepper (Raghavan, 2007). The pepper-producing areas in Indonesia are Lampung, Bangka Belitung, East Kalimantan and West Kalimantan. Pepper from Lampung province is known as Lampung black pepper and pepper from Bangka - Belitung is known as Muntok white pepper. In the tropical agricultural regions of China, black pepper is one of the important cash crops. Hainan province, a major producer and exporter of black pepper in China, produces 36,000 mg of pepper berry annually and has 22,000 ha in cultivation (C. Zu et al., 2014). It was introduced into Hainan in 1947 from Indonesia (Jiang & Liu, 2011).

Pepper is the most expensive spice in the Chinese market as it is widely used in cooking and also used by fast food restaurants. In addition, pepper is purchased by companies that produce seasoning and food packaging. Chinese people use pepper in cooking beef and dog for flavouring the taste and eliminating the fishy smell. Pepper is one of the instant seasonings for cooking dogs and ducks in Chinese five-spice blend, which consists of *Aidia cochinchinensis* Lour., cumin, star anise, citrus skin, and pepper. It is also included in the instant seasoning (five-spice blend) for cooking fish consisting of chili, white pepper, star anise, cumin, and aromatic ginger. Pepper is also included in the ten-spice blend consisting of star anise, cinammon, Szezhuan pepper, citrus skin, clove, white pepper, lesser galangan (*Alpinia officinarum* Hance), fennel (*Foeniculum vulgar*e Mill.), and shallot (spring onion). This combination is used for vegetables, meat, dumplings, sausages, soup, and pickles. Additionally, pepper is also used in Chinese hotpot (Wu et al., 2012).

Pepper is a spice that is mostly used in Indonesian cuisine and gives a little taste of hot or spicy in dishes. White pepper is very commonly used as a mixture for cuisine. Generally, pepper is used at every soup meal. The combination of pepper, ginger, and cinammon is used for flavouring the taste of fish, vegetables and meat. The combination of cinnamon, nutmeg, cloves, and pepper is used as a seasoning for stews. The Dayak Iban tribe in West

Kalimantan use the roots and seeds from pepper to treat back pain and to recover after childbirth (Meliki et al., 2013). The Seram people also use and drink the mix of pepper fruit, brown sugar, and egg yolks for three days to recover after childbirth (Susiarti, 2015). In Southeast Sulawesi, the Maronene tribe of Rau-Rau village use pepper to treat the disease of vomiting blood (Salibu & Ompo, 2014). The Malay ethnic people of Serambai village in Sanggau, West Kalimantan Indonesia use pepper fruit to cure toothache (Sari et al., 2014).

Conclusion

Spices for sale are related with demand of these spices. The availability of spices in the market is affected by the demand of the spices, their stable prices and also the continuous availability of spices. People have developed knowledge and methods to utilize spice plants. The utilization of spices is part of ethnobotanical information. Based on perceptions and experiences, the different communities in countries across the world have their own knowledge in spice plants to be used with different food. Cloves, nutmeg and pepper are the three kinds of main spices for the Chinese people. Indonesian people use nutmeg and clove for one particular type of cuisine. Because of the medicinal properties of clove and nutmeg, Indonesian and Chinese people use clove not only for spices but also for medicine. Indonesian people also use nutmeg for a snack called ‘pala manis’. Both Indonesian and Chinese people use pepper in a variety of dishes, to provide a spicy flavour. Pepper is also included in Chinese spices blend specifically used for meat and fish dishes.

Acknowledgement

We would like to thank Talented Young Scientist Program supported by Ministry of Science and Technology, P.R China. Without financial support from this program, this study could not be done. Sincere thanks to the National Resource Center for Chinese Materia Medica-China Academy of Medical Sciences which supported the field trip and thanks go to local people who helped us find and share information.

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Journal of Tropical Biology and Conservation 15: 29–41, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Short Communication

The Role of wildlife-viewing activity at Tabin Wildlife Reserve

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Abstract

The Tabin Wildlife Reserve is the only gazetted wildlife reserve in Sabah. For it to remain sustainable, tourism is strategically taken as a socio-economy instrument with wildlife-viewing currently being the most important tourism activity. But tourism could also be used as a conservation instrument, and a scientific research was carried out to determine the role of wildlife-viewing in conservation at the Reserve. This research comprises a social media content analysis, on-site observations and interviews with the Reserve's tourists, and findings show that the Reserve has the basic facilities for tourists to view wildlife. Besides this, the findings also show that the Reserve's tourists were satisfied with their wildlife-viewing experiences. However, the findings also reveal that the tourists' viewing experiences and satisfactions did not influence their understanding of conservation. Instead, the experiences and satisfactions prompted them to revisit and promote the destination to other people. These findings suggest the wildlife-viewing as an activity enforces conservation interest rather than increases conservation interest, which brings the understanding that tourism is a selective conservation instrument.

Keywords: Conservation, Environmental Awareness, Tourism

Introduction

On the eastern part of Sabah where large scale alienation of land for agricultural purposes has taken place, the Tabin Wildlife Reserve was established under the Sabah Forestry Enactment 1968. Much like an island, it is surrounded by alienated land. The Reserve was established to manage the human-wildlife relationship of the area; and under the gaze of conservation, tourism was used from 1999 onwards as a management strategy.

Received 13 March 2017
Reviewed 23 May 2018
Accepted 01 August 2018
Published 15 October 2018

Wildlife tourism is a tourism niche that focuses on travels relating to the viewing or searching of wild animals. It is about tourists wanting to interact with the animals (Peter, 2011), and it has been extremely popular among Europeans as safari tours in African nations since the early 19th century. This tourism niche has expanded greatly over the past decades generally in Malaysia, and particularly in Tabin Wildlife Reserve. An increased understanding about the relationship between tourists and wildlife would contribute to the conservation of wildlife as well as the sustainability of tourism (Rodger & Moore, 2004). Among the two matters that need better understanding include the influences to tourists' behaviour during visits (Orams & Hill, 1998) and tourists' motivations to contribute monetarily and non-monetarily towards the environment (Powell & Ham, 2008). With regards to tourism at Tabin Wildlife Reserve and the involvement of many stakeholders, and the fact that there are only a handful of tourism related studies to support nature related tourism expansion (Peters, 2000), the two matters have already become a complex social phenomenon to manage. Thus, understanding about the tourists-wildlife relationship is crucial to a place like Tabin Wildlife Reserve.

Literature about wildlife tourism and the relationship between tourists and wildlife are available. Many of these are tourism market reports that outline market factors (Fredline & Faulkner, 2001). Interestingly, central to the literature is the ability and reliability of sighting wildlife. The lack of viewing opportunities would give a negative impact on tourists' experiences and thus affect their overall decision to return to a particular destination or tell someone about the destination (Kuhar et al., 2010). As noted in the literature, anything can affect that sighting opportunity. The structure of a forest could affect the reliability of sighting wildlife. Height and width of tropical rainforest trees would have an impact on one's ability to see certain animals (Marshall, Lovett, & White, 2008). Tourists face a certain amount of risk when wanting to see a wild animal up close. For example, in Tabin's two decade tourist-wildlife relationship history, a tourist was killed in 2011 when the victim viewed a lone male bull Borneo pygmy elephant up close ("Fatal elephant attack the first in Sabah Resort," 2011). This incident raised the question about wildlife visibility and proximity as an essentiality of wildlife tourism; which is baseless under certain strict conditions. If an animal is endangered, not being able to see it during a tour does nothing for the development of wildlife tourism (Saikim, 2008). Being able to see an animal is not everything.

Tourists' satisfaction is about the feelings and attitudes of tourists after having experienced a particular tourism product. The question about what satisfies tourists has been asked repeatedly. This is an important question because under wildlife tourism the concept of satisfaction is linked to the tourists' affinity towards a certain environment (Tonge & Moore, 2007). Information about tourists' satisfaction is important to tourism services providers. Such information could affect the attractiveness of a particular tourism destination, which in turn increases the possibility of repeat visits and bring about the sustainability of the place as a destination (Spenceley & Snyman, 2017; Tonge & Moore, 2007). The act of revisiting or promoting could indicate a person's attitude towards the conservation of that attraction (Karppinen, 2005; Reynolds & Braithwaite, 2001; Tisdell & Wilson, 2004). And yet, in a Kenyan case study, tourists' arrivals at certain destinations were in decline although the tourists were very satisfied (Akama & Kieti, 2003). Because of this limitation and earlier understanding about the essentiality of wildlife visibility (Akama & Kieti, 2003), it is unclear if viewing satisfactions would promote better conservation understanding particularly in Sabah's Tabin Wildlife Reserve.

Tourism service providers need to know about tourists' wildlife-viewing satisfaction so that they can provide services in a safe manner while fulfilling conservation roles. What are the factors that make wildlife tourism so satisfying? Does a person become more aware about conservation after having the opportunity to see a particular animal? Or, would the person only become aware on conservation when he or she is satisfied? For a place like Tabin Wildlife Reserve to take tourism as a conservation tool, these are relevant questions. To answer these questions, this study explored tourists' animal-viewing expectations, satisfaction level and its role in conservation at Tabin Wildlife Reserve.

Methods and Materials

As introduced, Tabin Wildlife Reserve was established in 1968 as a reserve for large mammals. Three largest and endangered species of North Borneo i.e. Borneo Pygmy Elephant *(*Latin: *Elephas maximus)*, Sumatran Rhinoceros *(*Latin: *Dicerorhinus sumatrensis)* and Tembadau (Latin: *Bos javanicus*) are found here. It is situated at Sabah's Dent Peninsular and the location is illustrated in the following figure.

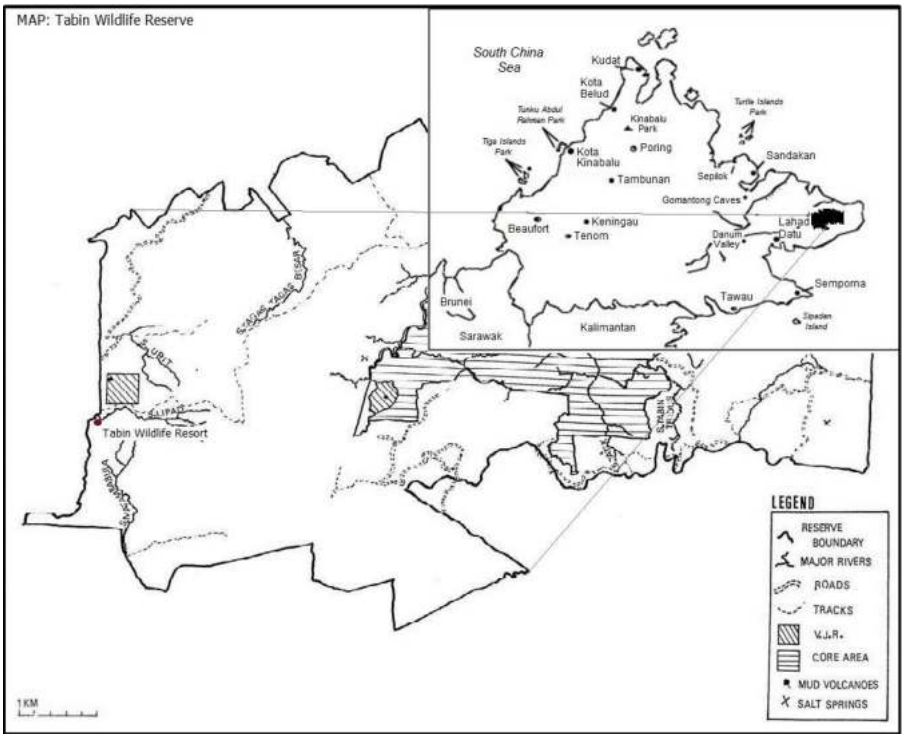

Figure 1. Illustrative map of the research location

Tourists' satisfaction at the research location is the result of many factors. Apart from factors such as the quality of services, feelings and attitudes of tourists; wildlife viewing is also a factor. These factors and thus the satisfaction of tourists can change over a period of time. This is the nature of a tourism destination; it evolves (Butler, 1980). Because of this, a case study is needed.

As a method, a case study allows an investigation to retain the holistic and meaningful charateristics of real-life events. It arises out of the desire to understand complex social phenomena (Yin, 2009). Wildlife tourism is a complex social phenomenon. In this case study, data collection was carried out through three different methods namely a) the documentation method, b) direct observation method and c) the interview method. The documentation method was carried out on reports in the print and electronic media about Tabin Wildlife Reserve until December 2016. Direct observation method was carried out during routine tourists' engagements e.g. guest registration and walks in the first half of 2015. The interview method was carried out on

tourists using a category and 5-point Likert Scale structured questionnaire at a tourism facility within the research location in the first half of 2015. The questionnaire contained questions about the tourists' demographic background, tourists' satisfaction concerning the visibility of wildlife, tourists' travel intentions and perceptions, and their awareness on the conservation of wildlife. The collected data from the interview was analysed statistically to determine tourists' wildlife related expectations, experiences, satisfaction, and motivation to support conservation. These analyses were then used in an explanation-building approach to determine the social phenomenon that exist in the reseach location (Yin, 2009). The results are provided in the following section.

Results and Discussion

Tabin Wildlife Reserve was gazetted by the Sabah Government in 1968. The main tourism attractions of the Reserve to date are its wildlife, mud volcano and a waterfall adjacent to the mud volcano. In 1999, about two (2) decades later, the only tourism facility known as the Tabin Wildlife Resort (TWResort) was established within the Reserve; and it is managed by Tabin Wildlife Holiday Pte Ltd. This facility was given the privilege of exclusivity on the understanding that market competition could be detrimental to conservation efforts, an understanding that has already been documented elsewhere (Spenceley & Snyman, 2017). Through direct observation, the Resort comprises of a main structure that houses a restaurant, a souvenir shop and a reservation section. Apart from the main structure, there are a number of cabins connected to the main structure by a network of elevated boardwalks. There is a 13KM trail walk system and an observation tower belonging to the Sabah State Wildlife Department i.e. Reserve Manager. The tourism environment of Tabin is as follows:

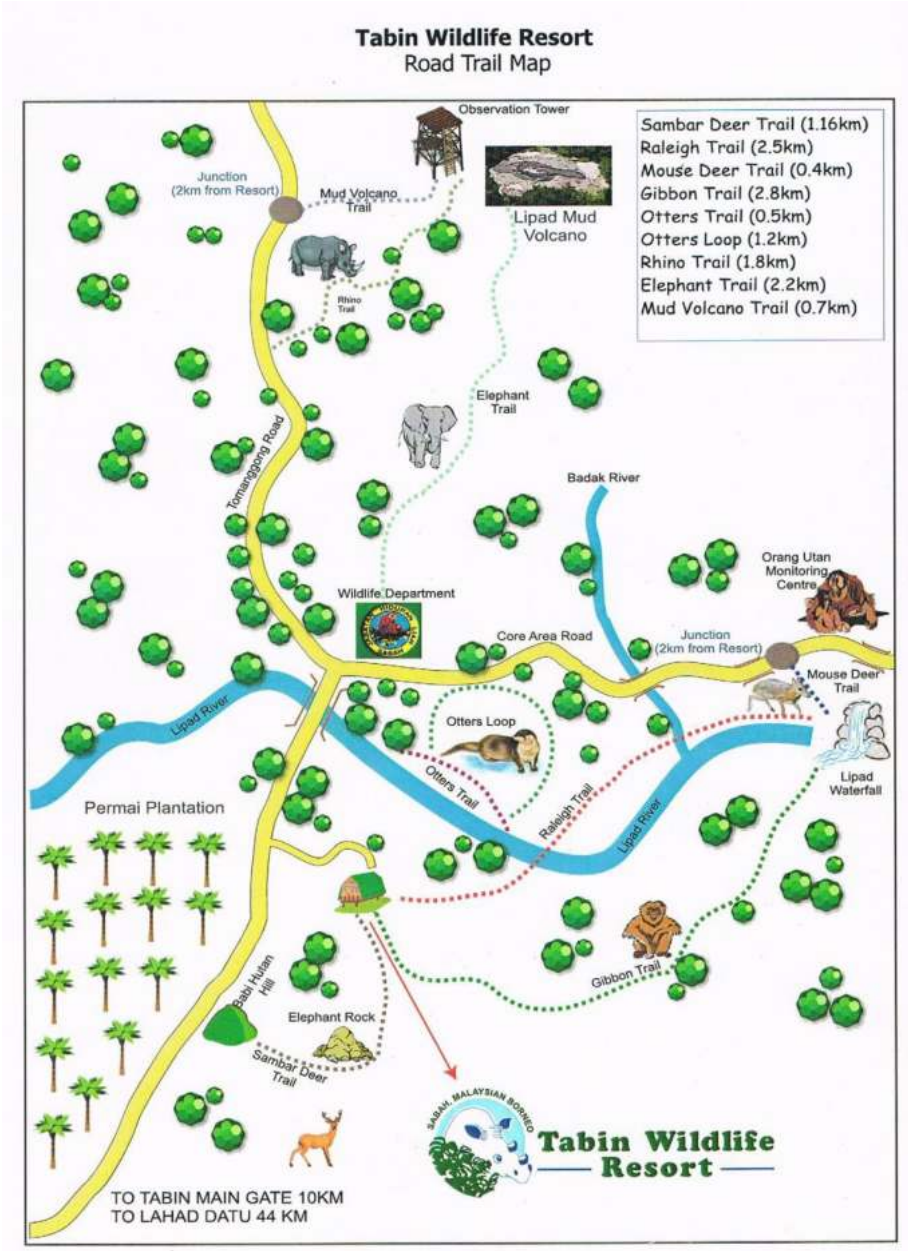

Figure 2. Tabin tourist map Illustration retrieved on 07/03/2017 from http://www.abctours.com.my/uploads//Tabin%20Wildlife/Tabin%20Trail%20map%20large.jpg

Through the documentation method of print media, among activities that are carried within the Reserve include jungle trekking, trail walks, night drive/safari, night walks, swimming, picnic, wildlife sensoring, birdwatching and environmental education. Tourists at Tabin Wildlife Reserve would take part in seemingly standardised three-day-two-nights (3D/2N) tour package. The package at Tabin is designed to give tourists a variety of experiences. This package includes trail walks, mud volcanos and night drives on the first day. On the second day, the package focuses on visiting the viewing platform, waterfalls, and night walks; while on the last it the package is based on the either leisure or water activities. Overall, the activities allow tourists to have a chance to view both diurnal and nocturnal animals.

Through documentation method of electronic media using the popular electronic media tripadvisor® Malaysia, a total of 131 reviews about TWResort up to 2016 were noted. The reviews started coming in since late 2008. At least 65% of those reviews ranged from average to excellent. The positive reviews were in relation to the authenticity of the forest experience and wildlife-viewing experiences and guiding services to promote that authenticity. Negative reviews were mainly about the tourism facility.

On the ground, a total of 77 tourists were interviewed. The respondents' size was not large, and this was because tourists stated that they had to address logistic and financial challenges before visiting it. This finding is similar to other wildlife tourism related researches (Lindsey, Alexander, Mills, Romanach, & Woodroffe, 2007). Foreigners constituted 97.4% of the respondents whereby 77.9% were of the female gender. Some 58.4% of the 77 respondents were above 51 years of age, while 1.3% of the respondents were below 20 years of age. In relation to their education level, 47.3% of the 77 respondents possess tertiary education with the rest having secondary education. In terms of the respondents' demographic characteristic, the findings of this study are similar to findings from other wildlife tourism related studies conducted elsewhere (Kirchberg, 1996; Lemelin & Smale, 2006). In terms of the respondants' answers to questions prepared using the 5-point Likert Scale, Spearman Correlation Analysis was used to analyse the compiled data. From the collected data and analysis, foreigners toured TWResort because they wanted to see wildlife animals, and what drove them to support wildlife conservation was their viewing satisfaction. This study found that the tourists had satisfactory viewing experiences and this is largely due to viewing conditions and the help from local guides. While that satisfaction may motivate tourists to revisit or promote the Reserve, it does not make the

tourists loyalists or essentially strong supporters of conservation. The viewing statisfaction does not change the tourists' understanding about conservation of wildlife. Nevertheless, tourists are willing to pay for conservation.

Good environmental conditions and guiding assistance ensured tourists' satisfaction of their viewing experiences.

People visit a wildlife tourism destination for many reasons. In this study, 68.6% of the respondents visited Tabin Wildlife Reserve to see animals. Twelve per cent of the respondents visited the Reserve because they were curious and wanted to get involved with conservation work, while 18.60% respondents travelled for a holiday. From those who visited TWR, 96.1% of them expected to see wildlife, while 3.9% of the respondents did not expect anything. Of those interviewed, 84.4% of the respondents expressed their satisfication, 11.7% responded indifferently, while 1.3% was not happy with their wildlife-viewing experiences and 3.9% of the respodents were dissatisfied of their wildlife viewing experiences. This finding is similar to an Australian wildlife-viewing satisfaction study that was conducted in 2001. In that study, 81.4% of respondents were satisfied with their experience and they were satisfied because they had good wildlife-sighting experiences (Fredline & Faulkner, 2001). While there are many factors observed in this study, the most dominating factor was the weather (27.3%); followed by the quality of tourist guides (24.7%) due to the belief that the ability of tourists guides in wildlife-spotting assists in increasing tourist' wildlife-viewing experience. Not only do those guides provide some sort of safety and security services, the guides also increased the reliability of the sighting. This finding supports the understanding that the professionalism of tourist guides are crucial in wildlife-based tourism (J. A. Bennett, Jooste, & Strydom, 2005). The next dominating factor is vegetation foliage (19.5%), while other contributing factors included the planned activities, distance from wildlife, wildlife behaviour, and tourist group size. This suggests that Tabin Wildlife Reserve is a tourism destination with certain world class charateristics; it has good conditions for viewing wildlife and it is operatored by capable people i.e. local guides.

The relationship between wildlife-viewing satisfaction and the motivation to promote or revisit a destination.

A tourist's affinity towards a particular natural attraction is associated with many factors. A tourist's viewing satisfaction is notioned to affect the tourist's affinity towards a particular place (Tonge & Moore, 2007). Using Spearman Correlation Analysis, the correlation efficient i.e. relationship between tourists' wildlife-viewing satisfaction and the motivation to revisit and

promote Tabin Wildlife Reserve were valued at 0.400 and 0.396 respectively. While these average values show that viewing satisfaction does motivate tourists to revisit and promote TWR, it shows that satisfaction does not overly motivate tourists to revisit or promote the destination. The finding is consistent with an understanding that satisfaction does not necessarily equate with loyalty (R. Bennett & Rundle-Thiele, 2004). But more importantly, as noted in literature about tourists' act to revisit or promote a destination as an indicator of the tourists' attitude towards the conservation (Karppinen, 2005; Reynolds & Braithwaite, 2001; Tisdell & Wilson, 2004), the finding suggests that tourists may not want to support conservation by revisiting the destination.

Satisfaction rather than awareness affects tourists' willingness to support wildlife conservation.

It is largely accepted that there is a direct relationship between the economy and conservation of wildlife; a person with a high awareness level might provide that economic support when he or she is satisfied (Trauger et al., 2003; Žabkar, Brenčič, & Dmitrović, 2010). In this study, the tourists were asked if they would like to conserve wildlife and if they are aware about the threates that endangered wildlife faced. Using Spearman Correlation Analysis, the correlation efficient i.e. relationship between tourists' viewing satisfaction and the interest in conservation is valued at 0.529 whereas the relationship value between tourists' viewing satisfaction and the awareness about the threat to wildlife is 0.173. These findings show tourists may be aware of the threats that wildlife face but that awareness does not affect the tourists' wildlife viewing satistfaction in any way. In this case study, 66.7% respondents claimed that their wildlife conservation awareness would not be affected by the ability or inability of viewing a particular wildlife. Thus, similar to the findings of Saikim and Prideaux (2014), this study confirms that wildlife-viewing satisfaction does not influence the understanding of wildlife conservation.

In relation with tourists' willingness to participate or pay for conservation at Tabin Wildlife Reserve, the respondents were asked if they would voluntarily carry out wildlife conservation related activities and if they would contribute financially to support the conservation in Tabin Wildlife Reserve. Using the Spearman Correlation Analysis, the correlation efficient of tourists' willingness-to-participate and tourists' willingness-to-pay were determined at 0.399 and 0.853 respectively. Since 81% of the respondents were found to be satisfied with their viewing experience, these findings show that satisfied

tourists, though unwilling to participate in a conservation activity, they were willing to pay for so that other people could do conservation work. Based on these findings, which support Žabkar et al. (2010) findings, a key factor of conservation contribution comes of tourists' satisfaction rather then from their awareness about the threats to wildlife.

Conclusion

Tabin Wildlife Reserve was designated by the Sabah Government in 1968 to preserve a population of Sabah's wildlife from the threat of extinction resulting from deforestation. As tourists' demand to experience Sabah's rich biological diversity steadily rose in the early 1980s, a certain part of the Reserve was developed as a wildlife tourism destination with tourism funding management of the Reserve.

Tourists take part in wildlife tourism to see wild animals. In context of wildlife conservation, it is generally understood that tourists' expectations and satisfactions could act as a platform to increase the tourists' wildlife conservation awareness. It is often stated that tourists could be educated so that their environmental awareness increases and they exhibit positive behaviour towards wild animals and their habitat (Duffus & Dearden, 1992). Wildlife-related education tours can cause a behavioural change and an increase in knowledge, which subsequently promote responsible actions towards wild animals and the natural surroundings, and encourage conservation research and contribution. To address the needs of tourists, two strategies were taken, namely the increment of the reliability of sighting and the engagement of quality tourist guides.

From this investigation, Tabin's tourists were satisfied with their visit. They were satisfied because they received good services and were able to see wild animals. Also, from this investigation, the tourists' satisfaction motivated them to share their experiences with their friends and to revisit the Reserve. Future visitors' arrival at the Tabin Wildlife Reserve is anticipated to increase. Nevertheless, this study revealed that Tabin's tourists' wildlife-viewing satisfaction did not influence the tourists' awareness level or their interest to do conservation. Wildlife-viewing at Tabin Wildlife Reserve has an impact only on financing the conservation of wildlife. While wildlife-viewing is good for tourism, it may not be necessary to the conservation awareness of a particular wildlife. This was because the tourists were already aware about wildlife conservation before visiting the Reserve. Instead, wildlife-viewing affected the

tourists' relationship with locals. The effects were positive, and it was because there is a sharing of information between tourists and locals. Wildlife-viewing satisfaction could improve hospitality between hosts and guests; and for this, further investigation is needed.

Acknowledgement

This study was made possible through the support of both the management of Tabin Wildlife Resort and the Sabah State Wildlife Department, and the researchers acknowledge this.

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Journal of Tropical Biology and Conservation 15: 43–60, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Invasive Apple Snails in Wetlands of Selangor, Malaysia: Species, Distribution, and Ecological Associations

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Abstract

Apple snails in the genus *Pomacea* are among the worst invasive species in Southeast Asia. Our objectives were to survey a selection of different wetlands in Selangor for *Pomacea,* verify which species of *Pomacea* occurred in that location, and assess basic environmental parameters associated with their presence and relative abundance. Aquatic parameters including pH and concentrations of selected electrolytes were measured at 25 wetland sites distributed among eight localities in Selangor. DNA from snails collected at each locality was extracted and the mitochondrial cytochrome c oxidase subunit I (COI) was sequenced. We detected two of the most successful invaders of this genus: *P. canaliculata* was found in five localities and *P. maculata* in two. Both pH and calcium ion concentrations were negatively associated with *Pomacea* presence. *Pomacea* were absent in brackish wetlands with high pH and calcium concentrations reflecting possible physiological intolerance or that dispersal into these habitats has yet to occur. *P. maculata* is reported to tolerate pH as low as 4.5-6; thus most freshwater wetlands in Selangor and most of Malaysia can potentially be invaded. *Pomacea canaliculata* and *P. maculata* have demonstrated remarkable capacity for depleting aquatic macrophytes and may cause rapid changes in aquatic plant communities with potential impacts to wetland state and function. Public awareness and environmentally safe recommendations to mitigate the reproduction and spread of this invasive snail is needed for protecting the biodiversity and health of natural wetlands.

Keywords: *Pomacea canaliculata*; *Pomacea maculata*; environmental parameters; *cox*1 gene; invasive species; wetlands; Selangor

Introduction

Some of the most aggressive invaders in freshwater systems are ampullariids that are indigenous to humid tropical regions of South and Central America (Qiu & Kwong, 2009). The most species-rich genus in the Ampullariidae is

Received 22 March 2017
Reviewed 04 October 2017
Accepted 16 November 2017
Published 15 October 2018

Pomacea with 96 nominal species, although the actual number of species is estimated at 50 (Hayes et al., 2015). Certain species of *Pomacea* are notorious for their voracious appetite for macrophytes, causing significant damage to rice fields and other wetland agriculture (Horgan et al., 2014). *Pomacea canaliculata* is listed among the world's 100 worst invasive species (Lowe et al., 2000), but reports of this species have been frequently confounded with its morphologically similar congener, *P. maculata* (Rawlings et al., 2007; Matsukara et al., 2008; Hayes et al., 2012). Thus, reports of damage by "golden apple snails", which were previously thought to be one species (Hayes et al., 2008; Hayes et al., 2012) may have alluded to either *P. canaliculata* or *P. maculata*. Unless specified, use of the name *Pomacea* in the remainder of this manuscript will refer to these two invasive species.

Pomacea was introduced to Malaysia in the late 1980's (Cowie, 2002), and was later identified as *P. canaliculata* using molecular diagnosis (Hayes et al., 2008). The snails may have been introduced to Southeast Asia with the purpose of becoming a local food and export item. Eventually escaping into agricultural wetlands, the snails quickly spread through the Asian rice irrigation system into natural wetlands, and thrived in these new habitats (Naylor, 1996). Agricultural impacts by invasive species of *Pomacea* have received far more attention to date than its spread and impacts on wetland ecosystems. However, a few studies indicate dramatic losses to wetland macrophytes (Carlsson et al., 2004), shifts in the state and function of natural wetlands (Horgan et al., 2014), and possible cascading effects including the decline and extirpation of native ampullariids (Cowie, 2000; Horgan et al., 2014). In Malaysia, two species of *Pomacea* have been reported: *P. canaliculata* (Yahaya et al., 2006; Salleh et al., 2012) and *P. maculata* (Arfan et al., 2014), but to our knowledge, there was no molecular confirmation of the species.

Wetlands provide ecosystem services, such as water purification, nutrient cycling, and flood control, of tremendous ecological and economic significance (Sather & Smith, 1984). Costanza et al. (1997) estimated the per hectare economic value of wetlands to be twice that of lakes and rivers, four times that of coastal ecosystems, and seven times that of tropical forests. Wetland macrophytes play an important role in water purification through erosion control and pollutant retention. A diversity of flora and fauna rely on wetland habitats for survival. Because apple snails feed predominantly on fresh macrophytes, have high growth rates, large body masses and high reproductive output, they can cause rapid changes to the macrophyte community structure,

including increased water turbidity and shifts in wetland ecosystem function (Sheldon et al., 2003; Horgan et al., 2014). In Southeast Asia, wetlands invaded by *P. canaliculata* shifted from macrophyte dominance to phytoplankton-dominant, which resulted in higher levels of aquatic nitrogen and phosphorus (Carlsson et al., 2004).

Knowledge of the distribution and species of *Pomacea*, including associated environmental factors is needed to predict the types of habitats in Peninsular Malaysia that are vulnerable to invasion, and for developing effective management strategies (Rawlings et al., 2007; Hayes et al., 2012; Horgan et al., 2014). Morphologically, some species of *Pomacea* demonstrate high intraspecific variability and interspecific overlap, making identification to species difficult (Hayes et al., 2012). Using a single-locus genetic approach to confirm species identity, we provide the first information on the species of *Pomacea*, their distribution, and associated environmental parameters in natural and agricultural wetlands in Selangor, Malaysia. Specifically we asked the following questions: 1) Which species of *Pomacea* occur in Selangor; and 2) Which aquatic parameters are associated with the presence and abundance of *Pomacea spp.* in Selangor wetlands.

Materials and Methods

Study Area

Selangor is the most populous state in Malaysia with approximately 800,000 ha of land and 5.8 million people (Department of Statistics Malaysia, 2008-2015). Monthly mean temperature and rainfall ranges from 23 °C to 33 °C and 90 mm to 300 mm. Northeast and eastern Selangor is covered with undulating hills, whereas the central and coastal regions are relatively flat (Abdullah & Nakagoshi, 2008). These relatively flat regions support abundant wetland habitats and abandoned tin mine lakes, including the northern coastal region in Sekinchan, which supports intensive rice agriculture. Based on shell morphology, Arfan et al. (2014) reported the presence of *P. canaliculata* and *P. maculata* in rice fields in Peninsular Malaysia (Arfan et al., 2014); thus these, including other species of *Pomacea*, could potentially occur in other types of wetland habitats.

Fieldwork

Twenty-five sampling sites distributed among eight localities (Figure 1) were surveyed for *Pomacea* presence/absence and measured for a set of aquatic

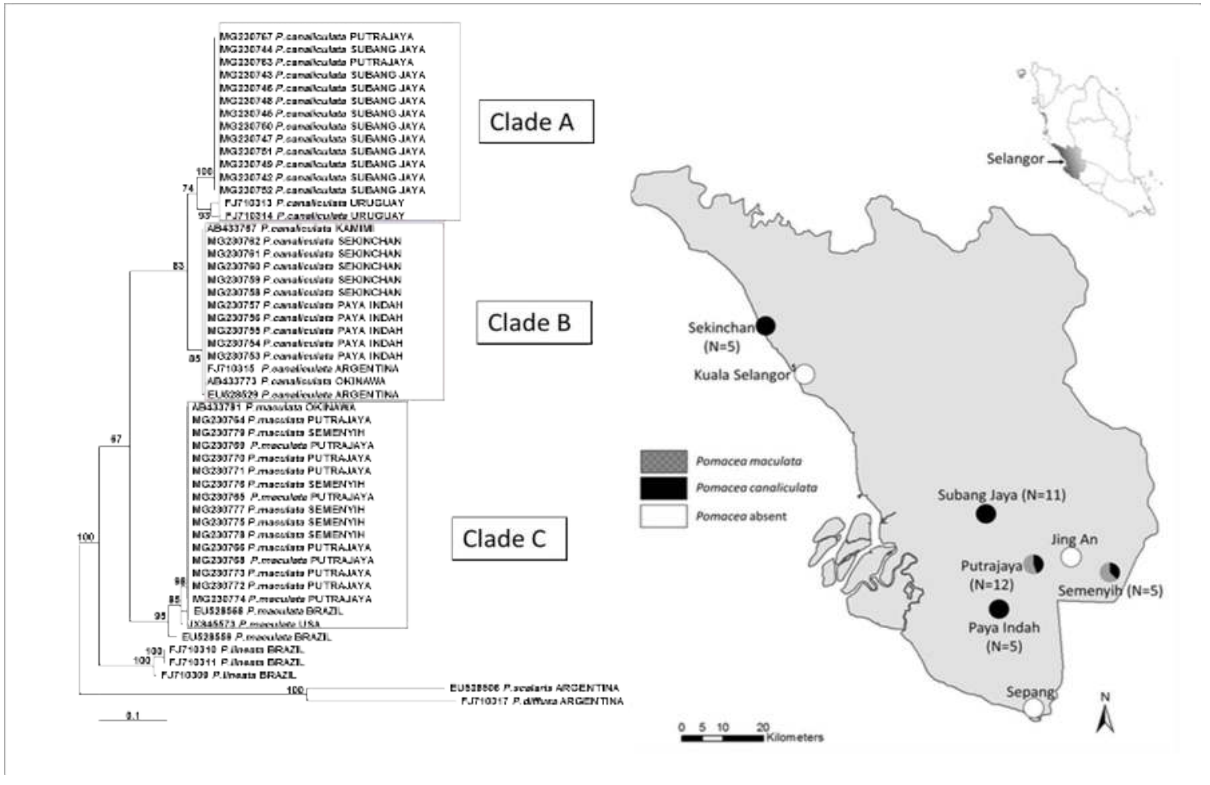

Figure 1. Sampling localities for *P. canaliculata* and *P. maculata* in Selangor, Peninsular Malaysia, September – November 2016. Phylogenetic tree was based on the mitochondrial *cytochrome c* oxidase subunit I (COI) gene. Node values represent 1000 bootstrap replicates (> 50%) under maximum likelihood. *Pomacea scalaris* and *P. diffusa* were selected as outgroup taxa. GenBank accession numbers for all individuals sequenced in this study are included.

variables, namely pH, salinity, conductivity, nitrate, calcium and potassium, that we considered important for aquatic snails. Population density of aquatic organisms tends to decrease as pH becomes more acidic, suggesting low pH levels affect biodiversity and productivity (Bemvenuti et al., 2003). Aquatic pH is one of the principle variables affecting distribution and survivorship of *Pomacea* (Ito 2002, 2003; Byers et al., 2013; Pierre, 2015). *Pomacea* distribution and physiological functions are limited by high salinity (Costil et al., 2001). Conductivity provides an insight to the total amount of ions in water that allows electrical flow (Horiba Scientific, 2016). High nitrate, phosphate and potassium levels indicate potential eutrophication that leads to algal proliferation and eventually low oxygen levels in water bodies, which may not be suitable environments for oxygen-dependent organisms, and could possibly limit survival (Smith et al., 1999). Calcium is important as it plays a role in maintaining shell thickness of *Pomacea* and preventing shell erosion (Glass & Darby 2009; Kwong et al., 2008).

We surveyed a map of Selangor to identify eight locations in different regions of the state that had wetlands or rice fields. Sampling was conducted from Sep 15th to Nov 7th 2016 from 9am - 3pm. Sample sites at these locations consisted of ponds, lakes, rice fields, or roadside ditches. At each locality, we surveyed one to six sites that were spatially separated by at least 30 m (i.e., different ponds/lakes/irrigation systems) so that a variety of mesohabitats were sampled. Three water samples were collected at each site, concentrations of selected electrolytes were measured using the LAQUAtwin water probes (HORIBA Instruments Inc, U.S.A), and the mean value was used for subsequent analyses. We collected 5–10 adult *Pomacea* snails at different sites for subsequent species identification using genetic analysis.

We conducted counts of *Pomacea* snails (>2 cm shell height) and egg masses at selected locations to obtain an index of relative abundance among sites. *Pomacea maculata* and *P. canaliculata* exhibit high interspecific similarity and intraspecific variability in shell and external morphology (Hayes et al., 2012); thus, we did not attempt to distinguish between species of *Pomacea* during counts. Counts were conducted along four 10 × 5 m transects selected at random points along the shoreline at each sampling site. Two individuals performed independent counts of abundance and the average of those counts were reported. Shells of collected specimens were cleaned, photographed and deposited in a reference collection at Sunway University.

Data Analysis

(i) Statistical Analysis

We explored associations between individual aquatic variables and snail counts (and egg mass counts) using Spearman rank correlation tests. We used binary logistic regression (Hosmer & Lemeshow, 1989) to assess the relationship between aquatic parameters and *Pomacea* presence/absence at 25 sample sites. We developed a set of eight *a priori* models that included one or more aquatic variables that we considered important predictors of *Pomacea* presence or absence and used Akaike's Information Criterion corrected for small sample sizes (AIC_c) to select the models best supported by the data (Burnham & Anderson, 2002). Because sample sizes were small, we limited the number of predictor variables to <2 in any single model and did not include interaction terms. Analyses were performed in Program R (v.3.3.1; R Development Core Team 2007).

(ii) Genomic Analysis

Genomic DNA was extracted from approximately 1 to 5 mg of foot tissue of individual snails using the NucleoSpin® Tissue kit (Macherey-Nagel, Germany). A portion of the mitochondrial cytochrome *c* oxidase subunit I (COI) was amplified and sequenced using the primers and thermocycle protocol published by Cooke et al. (2012). The PCR products were visualized in a 1% agarose gel before sequencing by MyTACG Biosciences Enterprise. Samples were sequenced using the BigDye® Terminator v1.1, v3.0 and v3.1 Sequencing Kit and analysed with Applied Biosystems 3730xl DNA Analyser. Sequencing data were analysed and edited using ChromasPro version 1.42 (2003-2008 Technelysium Pty Ltd) and BioEdit Sequence Alignment Editor Version 7.0.9.0 (Hall, 1999) software. Edited sequences were aligned using CLUSTAL X alignment (Thompson et al., 1997) and visually checked before conducting phylogenetic analyses.

Sequences for the COI gene of *Pomacea* were downloaded from the GenBank sequence database provided by the National Center for Biotechnology Information (NCBI). The generated partial COI mt DNA gene of *Pomacea* from selected localities together with the COI sequences downloaded from GenBank were used for the phylogenetic analysis. Maximum likelihood analysis was performed using Treefinder version October 2008 (Jobb et al., 2004) to construct phylogenetic trees (Swofford, 2002). Kakusan version 3 (Tanabe, 2007) was used to find the models with the best fit. We used Treefinder to build a phylogram of maximum likelihood using 1000 bootstrap replicates.

Results

Pomacea occurred in 5 of the 8 localities sampled and at 15 of 25 surveyed sites (Figure 1). Genomic analyses of 38 snails revealed two species of *Pomacea*: *P. canaliculata* and *P. maculata*. *Pomacea canaliculata* occurred in 5 of the 8 localities sampled (Figure 1) and co-occurred with *P. maculata* at two localities (Semenyih and Putrajaya). Fifteen sequences of *P. maculata (*synonymous with *P. insularum)*, *P. lineata*, and *P. canaliculata* were obtained from GenBank (Table 1). *Pomacea scalaris* and *P. diffusa* were used as outgroups in this study (Figure 1). *Pomacea canaliculata* was the more abundant and widespread of the two species, whereas *P. maculata* was documented only at Putrajaya and Semenyih, sympatric with *P. canaliculata.* The maximum likelihood tree produced 3 clades (A, B and C). *Pomacea canaliculata* from Putrajaya and Subang Jaya grouped with *P. canaliculata* sequences from Uruguay (Figure 1, Clade A) with 74% bootstrap support. *Pomacea canaliculata* from Sekinchan and Paya Indah clustered with *P. canaliculata* sequences from Argentina and Okinawa, Japan (Figure 1, Clade B) with 85% bootstrap support. *Pomacea maculata* sequences from Semenyih and Putrajaya clustered with *P. insularum* sequences from Okinawa, Japan; USA and Brazil (Figure 1, Clade C) with 98% bootstrap support. Sequences for the specimens used in this study were uploaded to GenBank.

Variation in depth, turbidity and vegetation at sample sites sometimes affected visibility and reliable snail counts. Counts of snails and egg masses ranged from 2–27 snails (averaged for two observers) and 5–50 egg masses, but

Table 1. GenBank Accession numbers for *Pomacea* species from different geographic locations.

Species	Location	GenBank Accession Number
P. maculata	Mato Grosso do Sul, Brazil	EU528559
P. maculata	Mato Grosso, Brazil	EU528568
P. maculata	Okinawa, Japan	AB433781
P. maculata	USA	JX845573
P. lineata	Algoas, Brazil	FJ710309
P. lineata	Rio de Janeiro, Brazil	FJ710310
P. lineata	Rio de Janeiro, Brazil	FJ710311
P. canaliculata	Maldonado, Uruguay	FJ710313
P. canaliculata	Buenos Aires, Argentina	EU528529
P. canaliculata	La Leonesa, Argentina	FJ710314
P. canaliculata	Buenos Aires, Argentina	FJ710315
P. canaliculata	Okinaw, Japan	AB433773
P. canaliculata	Kamimi, Japan	AB433767
P. scalaris	Buenos Aires, Argentina	EU528506
P. diffusa	Amazonas, Brazil	FJ710317

egg mass counts were only slightly correlated with snail counts (Figure 2; N = 15, Spearman's rho = 0.50, p = 0.08). Except for Semenyih, all sites with *Pomacea* had greater mean counts of egg masses than mean counts of snails (Figure 2). Variation was high among counts taken along different transects at the same wetland site, ranging from 8 to 30 snails and 5–53 egg masses. Consequently, snail counts and egg mass counts were not correlated with any aquatic parameter. Thus, we used presence/absence of *Pomacea* to investigate further relationships. The highest values for all measured parameters were from Sepang and Kuala Selangor (Figure 3). *Pomacea* were absent at these two sites, including Jing An, where water parameters were not substantially different from other sample sites. Marked differences in aquatic parameters existed between sites with and without *Pomacea*, sites without *Pomacea* had higher pH and electrolyte concentrations (Figure 4).

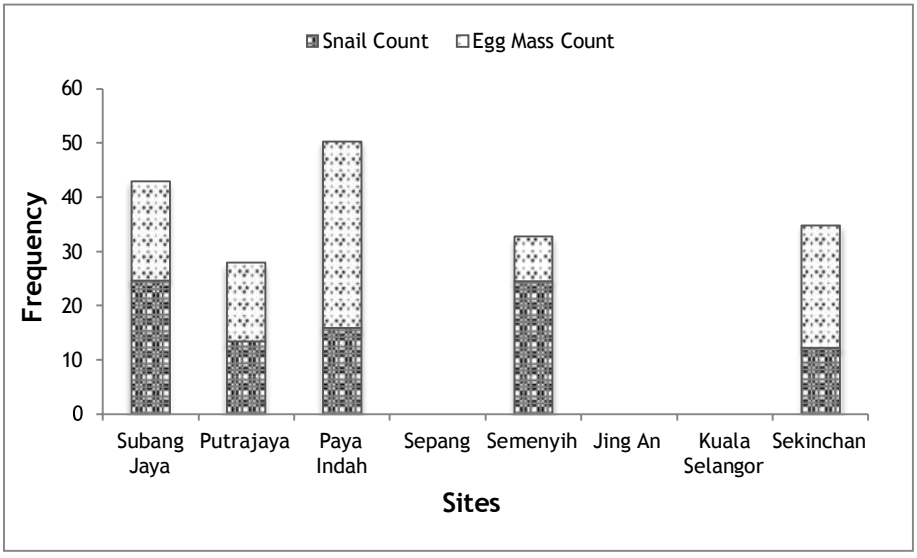

Figure 2. Mean counts of *Pomacea* snails and egg masses observed at eight different sites in Selangor, Peninsular Malaysia from September to November 2016.

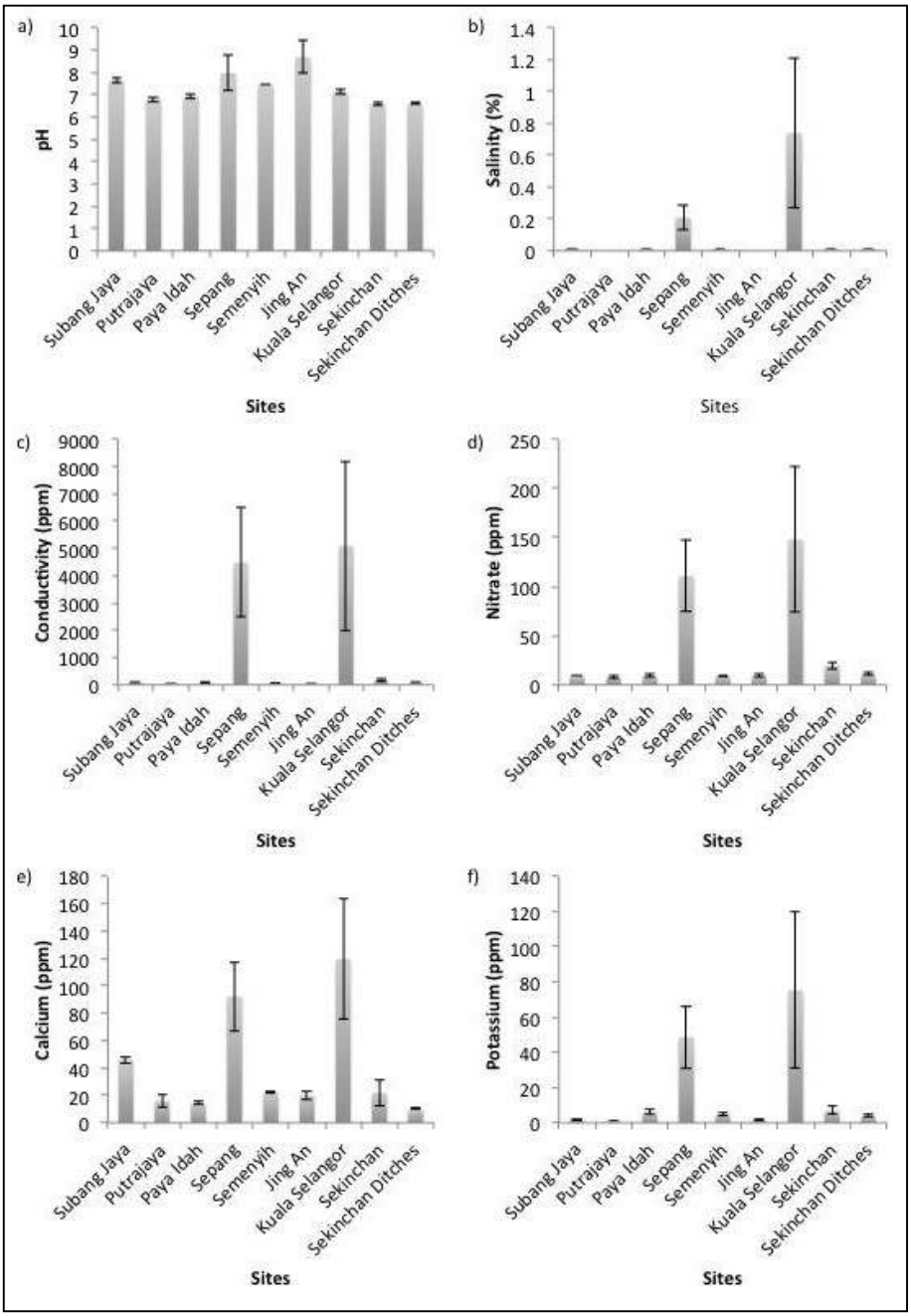

Figure 3. Comparison of means of different aquatic parameters among sampling localities in Selangor. Mean values for roadside ditches at Sekinchan are reported separately from those collected from rice fields. Error bars represent standard errors of the mean.

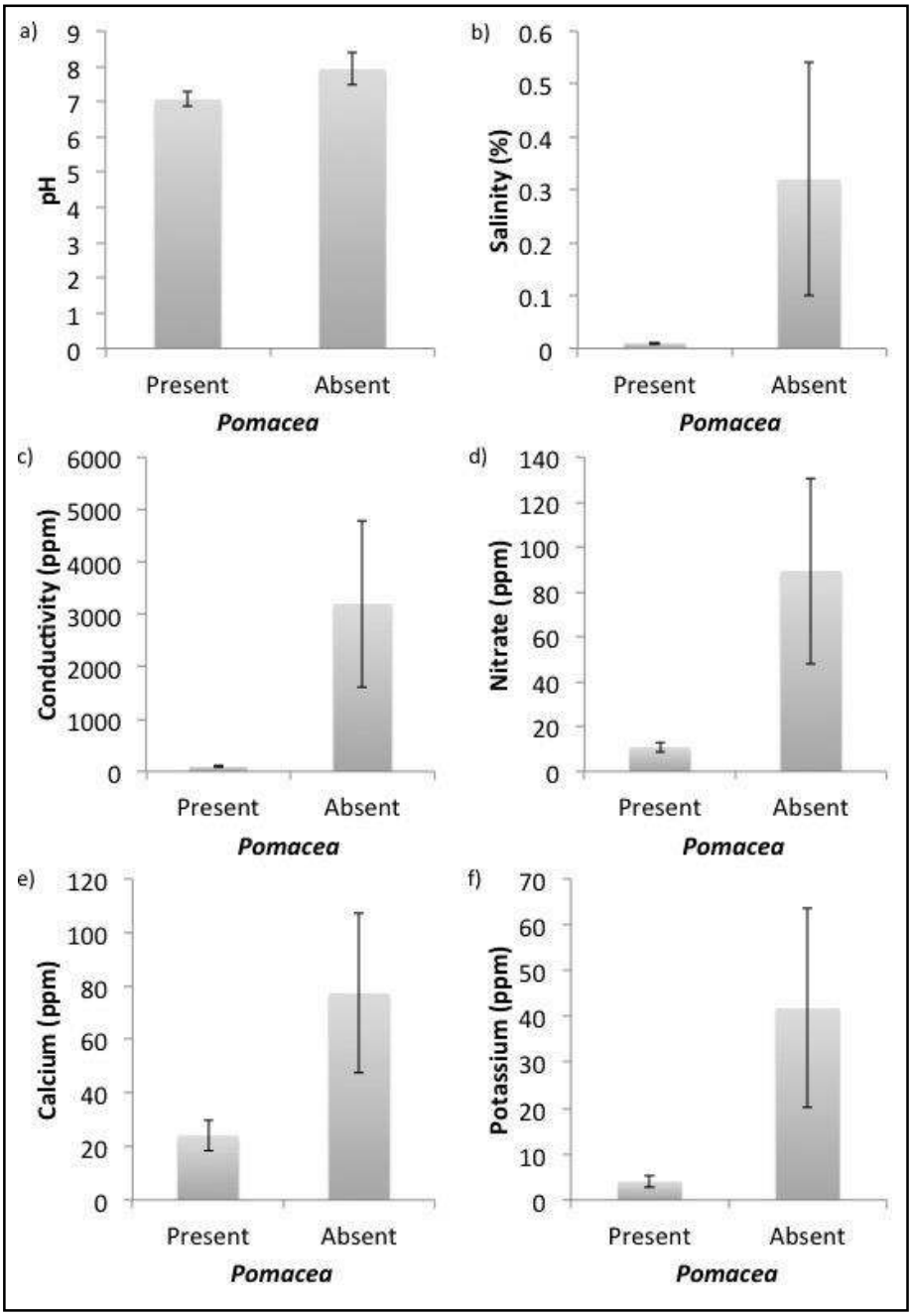

Figure 4. Relationship between the overall means of different aquatic parameters across all localities and presence/absence of *Pomacea*. Error bars represent standard errors of the mean.

We developed 8 logistic regression models representing the association between one or a combination of two aquatic parameters and presence or absence of *Pomacea* (Table 2). The most supported model was the combined model of pH and calcium concentration (lowest AIC_C value and a ΔAIC_C >2 compared to the next ranked model; Table 2). The parameter estimates for pH and calcium were negative, although not significant for calcium (Table 3). Data indicated that *Pomacea* occurred at sites with lower pH (6.5 – 7.5) and lower calcium ion concentrations (16-46 ppm). Sites with high pH (>8) and calcium concentrations (> 90 ppm) were associated with *Pomacea* absence. *Pomacea* were absent at sites with the highest levels of all aquatic variables measured, although models with those variables had poor fit, possibly because the distribution of those variables were distinctly skewed (Figure 3). Calcium concentrations were strongly correlated with salinity (Spearman's rho = 0.73, p = 0.0001) and conductivity (Spearman's rho = 0.76, p = 0.0001); thus overall aquatic ion concentrations were high where calcium and salinity levels were high.

Table 2. AIC-based logistic regression model selection for the presence/absence of *Pomacea* evaluated against six different aquatic parameters. Akaike Information Criterion corrected for small sample size (AIC_c), AIC_c differences (ΔAIC_c), Akaike weights (w_i), and number of estimable parameters (K).

Model	AIC_c	ΔAIC_c	w_i	K
1. pH + calcium	24.420	0.000	0.510	3
2. Conductivity	26.841	2.421	0.152	2
3. Nitrate	27.721	3.301	0.098	2
4. Salinity	28.069	3.649	0.082	2
5. Potassium	28.327	3.907	0.072	2
6. pH	29.339	4.919	0.044	2
7. Potassium + nitrate	30.285	5.865	0.027	3
8. Calcium	31.433	7.013	0.015	2

Table 3. Coefficients of top ranking logistic regression model for parameters predicting *Pomacea* presence/absence. Calcium concentration and pH were negatively associated with snail presence.

	Estimate	Standard error	z value	Probability
Intercept	17.097	7.207	2.372	0.018
pH	-2.063	0.958	-2.152	0.031
Calcium	-0.037	0.019	-1.917	0.055

Discussion

Phylogenetic analysis confirmed the presence of the two most invasive species in the genus *Pomacea* in wetlands of Selangor. *Pomacea canaliculata* occurred in approximately five localities and *P. maculata* in two (Putrajaya and Semenyih) where it co-occurred with *P. canaliculata*. Genetically, the two species separated clearly with no shared haplotypes. *Pomacea canaliculata* occurred at 15 of the 25 sample sites and *P. maculata* at just four. Based on this limited survey, *P. canaliculata* seems the more widespread of the two species in Selangor.

The strongest predictors of *Pomacea* presence/absence were pH and calcium ion concentration where the probability of *Pomacea* presence was low when pH and calcium concentrations were high. All aquatic parameters were noticeably higher at sites in Sepang and Kuala Selangor where *Pomacea* was absent, suggesting that *Pomacea* may be physiologically limited by the total amount of dissolved salts in the water, which causes osmotic stress (Costil et al., 2001; Ramakrishnan, 2007). Under laboratory conditions, *P. bridgesi* and *P. maculata* tolerate salinities between 0 – 6.8% quite well (Ramakrishnan, 2007; Jordan & Deaton, 1999). In Hong Kong, freshwater locations inhabited by *P. canaliculata* had high alkalinity, high levels of phosphate and high salinity (Kwong et al., 2008; Chaichana & Sumpan, 2015). Furthermore, Rossi (2012) commented that salinity appears to not limit *Pomacea*'s establishment in an area. In this study, the highest salinities recorded at sites in Sepang and Kuala Selangor did not exceed 1.64%. Sites at Sepang were isolated from tidal influence, but sites at Kuala Selangor may experience spikes in salinity that *Pomacea* cannot tolerate. The absence of *P. canaliculata* and *P. maculata* in Sepang and Kuala Selangor may also mean that introduction to these sites or dispersal via canals and drainages has not yet occurred.

Both high and low aquatic pH influences aquatic biodiversity and productivity (Bemvenuti et al., 2003). *Pomacea* may be exceptional in its ability to tolerate aquatic pH as low as 4.5 in lab settings (Ramakrishnan, 2007), but whether survival and reproduction occurs over the long term under these conditions, is unknown. In temperate regions like Japan, with marked seasonal fluctuations in temperature, low water velocity, high dissolved oxygen and low pH (6.29-6.63) is associated with greater over-winter survivorship of *P. canaliculata* (Ito, 2002, 2003). Most sites surveyed in this study had pH levels ranging from 6.5–7. The possibility that low pH habitats such as peat wetlands may be vulnerable to invasion should be considered, although Byers et al. (2013) reported absence of *P. maculata* in southeastern U.S. waters with pH <5.5 and Pierre

(2015) reported decreasing survivorship of translocated *P. maculata* at aquatic pH <6. Generally, low pH affects the ability of gastropods to deposit calcium in their shells, and calcium plays a crucial role in maintaining shell thickness in gastropods and preventing shell erosion (Glass & Darby, 2009). The lowest calcium levels were recorded at Paya Indah and Putrajaya wetlands where *Pomacea* populations were abundant, but where pH levels were between 6.5-7.0. Lab experiments and a greater coverage of these parameters in Malaysian wetlands may help to elucidate the relationship between pH, calcium, and *Pomacea* presence/absence. This is the first report of aquatic parameters associated with the presence/absence of *P. canaliculata* and *P. maculata* in Malaysian wetlands. Similar kinds of information from different parts of its introduced range will help establish its full physiological range of tolerance and invasive potential.

Obtaining crude relative abundance estimates of *Pomacea* populations using egg mass counts needs to be further explored. Our data suggested that snail counts and egg mass counts were weakly correlated. *Pomacea*'s conspicuously pink coloured egg masses are deposited above the waterline on the stalks of emergent aquatic plants or inanimate structures, making accurate counts possible. Snail counts, on the other hand, may be hampered by turbid water conditions or cryptic shell coloration and pattern (Burks et al., 2010). We observed that snails were often hidden among aquatic plants, and were well camouflaged. Surveying for the presence of egg masses is an efficient and practical way to ascertain presence of the snail, but egg mass counts were only weakly correlated with snail counts. Seasonal patterns of egg deposition must be investigated against snail counts to use egg mass counts as an index of abundance.

The success of *Pomacea* as an invader owes largely to its ecological competence and high adaptability to a range of aquatic habitats and environmental conditions, including seasonally dry lands (Chaichana & Sumpan, 2015; Hayes et al., 2015; Glasheen et al., 2017). *Pomacea maculata* and *P. canaliculata* may be quite opportunistic, with a highly variable diet ranging from macrophytes to animal carcasses (Carlsson & Brönmark, 2006). Both *P. maculata* and *P. canaliculata* possess remarkable reproductive capacity and introduced populations expand rapidly at the expense of native species. In Southeast Asia, *Pomacea canaliculata* reproduces three times faster under the warm climate as compared to its native cold and seasonal South American habitat (Carlsson & Lacoursiere, 2005). Snails in the genus *Pomacea* may be capable of surviving harsh environments including pollution, low oxygen levels,

or lack of water, because ampullariids are equipped with lungs and gills to accommodate aerial and aquatic breathing (Baloch et al., 2012; Hayes et al., 2015). Both *P. canaliculata* and *P. maculata* possess a remarkably flexible operculum that acts as a trapdoor to tightly seal the snail from the exterior, thus protecting it from desiccation for months at a time (Kwong et al., 2009). In combination, these characteristics have contributed to the spread and destructiveness of these two species on a global scale.

Conclusion

Two of the most invasive species in the genus *Pomacea* occur in Selangor. We did not include peat swamp habitats in this study and preliminary surveys have not detected *Pomacea* in the Northern Selangor peat swamp, which is upstream and adjacent to Sekinchan where *Pomacea* occurs. *Pomacea*'s reported tolerance for low pH suggests that peat swamp habitats may be vulnerable to invasion and should be monitored. Osmotic stress may limit *Pomacea*'s ability to invade brackish environments such as alkaline lakes and mangrove habitats. *Pomacea canaliculata* and *P. maculata* are capable of dispersing large distances, drifting along currents in natural and artificial drainages, and *Pomacea* eggs and hatchlings may be transported from one region to another via aquatic plants used for landscaping and aquaria (Pierre, 2015; Ng et al., 2017). It is important that public awareness via educational brochures, digital media and magazine/journal articles is increased so that people have information to 1) identify invasive apple snails, 2) recognize activities that can inadvertently contribute to the spread of invasive snails, and 3) use environmentally safe ways to control their spread and reproduction.

Acknowledgements

We thank the Department of Biological Sciences, Sunway University, for providing us with the facilities and equipment to carry out our research. We thank Thor Seng Liew for advice on the systematic work, and the Malaysian Nature Society and PERHILITAN for providing access to wetlands under their purview.

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Journal of Tropical Biology and Conservation 15: 61–80, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Jackfruit (*Artocarpus heterophyllus*) and Breadfruit (*A. altilis*): Phytochemistry, Pharmacology, Commercial Uses and Perspectives for Human Nourishment

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Abstract

The *Artocarpus* J. R. & G. Forster genus is comprised of about 50 species. *Artocarpus* is derived from the Greek word *artos*, meaning bread while *karpos* means fruit. There are two species that are widely distributed in tropical regions, *Artocarpus heterophyllus* Lam., known as jackfruit, and *Artocarpus altilis* (Parkinson) Fosberg, known as breadfruit, both in the Moraceae or mulberry family. Both of these *Artocarpus* species have medicinal properties and biological activities that are derived from almost every part of the tree, fruit, seed, wood, bark, leaves and sap. This review examines the limited work that has been conducted on the biology and biotechnology of these two *Artocarpus* species with the hope that this knowledge may spur further basic and applied research.

Keywords: fruit, medicine, Moraceae, secondary metabolites, tropical tree

Introduction

The genus *Artocarpus* (Moraceae), which contains food-producing plants that are spread throughout tropical and subtropical regions of the world, consists of about 50 species (Motley, 2014), but The Plant List (2018) lists 193 accepted names for *Artocarpus*, although many of them are synonymous and unresolved species. The word *Artocarpus* is a compilation of two Greek words, *artos*, which means bread, and *karpos*, which means fruit (Jones et al., 2011).

Received 30 May 2017
Reviewed 04 October 2017
Accepted 13 October 2017
Published 15 October 2018

The species epithet of jackfruit, *heterophyllus*, is a compilation of two Greek words, *hetero*, meaning different, and *phyllus*, which means leaf (Gupta, 2011). This implies existing variation in the shape and size of the leaves. Jackfruit, which typically grows in the form of a tree, provides edible fruit and medically potential secondary metabolites, is a source of timber, and has been cultivated throughout China, Sri Lanka, India and Southeast Asia, but is also found in Africa, the Caribbean islands, Brazil, Suriname and tropical parts of Australia (Thaman & Ali, 1993). Jackfruit is known as *nangka* in Indonesia and has various ethnobotanical properties that derive from its ripe fruit which serve as ingredients for local sweets such as *kolak* and *dodol* in Java, young fruit is consumed as a vegetable, and its leaves are used as cattle feed (Lim, 2012). Ash of leaves can be used to treat wounds and serve as medication to treat ulcers (Gogte, 2000). Jackfruit timber is a good wood for furniture, construction material, and musical instruments since it resists bacterial, fungal and termite attacks (Orwa et al., 2009).

The methanolic extract of stem, root bark and heartwood, leaves, fruit, and seed have multiple antibacterial compounds (Khan et al., 2003). One of those compounds, artocarpin, is used as an antitermite agent (Shibutani et al., 2006). The basal part of the fruit, which is fleshy, fibrous and rich in sugar, provides a good natural source of carbohydrates and minerals such as calcium, iron, magnesium, carboxylic acids, and vitamins A, C and E, primarily ascorbic acid and thiamine (Rahman et al., 1999). Mature seed are edible when dried or after cooking by boiling and roasting. Fresh mature seed contain 25 IU/100 g of vitamin A, 4.3-6.6 g/100 g of protein, 23-25 g/100 g of calcium, 80-126 mg/100 g of phosphorus, and 10-17 mg/100 g of ascorbic acid (Acedo, 1992). In fresh (raw) fruit, there are 23.25 g/100 g of carbohydrate, 24 mg/100 g of calcium, 0.23 mg/100 g of iron, 29 mg/100 g of magnesium, 110 IU/100 g of vitamin A, 13.7 mg/100 g of vitamin C (ascorbic acid), and 0.34 mg/100 g of vitamin E (α-tocopherol) (USDA, 2016). The latex which also has anti-syphilitic and vermifuge properties, contains 71.8% resin, 63.3% of which are yellow fluavilles and 8.5% white albanes that are useful for varnishes (Rao et al., 2014). A study conducted in New Delhi and Kerala, India by Suba Rao (1983) showed that jackfruit is symbiotically asociated with *Azotobacter* and *Beijerinckia*, 35 and 4 $\times 10^4$/g soil, respectively at pH 6.8-7.5, and 14 and 18 $\times 10^4$/g soil, respectively at pH 3.5-5.5. According to Prakash et al. (2009), a hot water extract of jackfruit leaves when consumed orally by humans at 20 g/kg of the patient's weight, improves glucose tolerance for mature-onset diabetic patients, while the crude methanolic extract of jackfruit parts (stem, root heartwood, bark, leaves, fruits and seeds) and their subsequent partitioning with petrol,

dichloromethane, ethyl acetate and butanol gave fractions that exhibited broad spectrum antibacterial activity, the most active fraction being the butanolic extract of fruits and root bark. An extract from jackfruit shoots also revealed nematicidal activity against *Rotylenchulus reniformis*, *Tylenchorynchus brassicae*, *Tylenchus filiformis* and *Meloidogyne incognita* (Sharma & Trivedi, 1995 cit. Prakash et al., 2009).

The species epithet of *A. altilis*, the word *altilis* itself is a Greek word that means fat, refers to the fruit shape (Small, 2011). Breadfruit, also a source of food, was first cultivated in the Western Pacific about 3,000 years ago and is native to the eastern part of Indonesia, New Guinea, Malaysia and the Philippines (Orwa et al., 2009). The migration of Polynesians to South and South America, Africa (Senegal, Ghana, and Liberia), India, Maldives and Sri Lanka contributed to the distribution of breadfruit (Deivanai & Bhore, 2010). The breadfruit tree is often employed in a mixed cropping system with yams, banana, black pepper and coffee, although details of these cropping systems are lacking (Ragone, 1997). The fruit of ripe breadfruit can be eaten fresh or cooked by steaming, roasting and frying (Ragone, 1997). Leaves and the non-edible part of fruit can be used as cattle feed while tree bark can also serve as feed for horses (Morton, 1987). In Samoa and several Pacific Islands, bark is used to cure headaches, in Java and Malaya the toasted flower is used to treat toothache, while in the Bahamas, leaves of *A. altilis* are used to relieve headaches (Kuete et al., 2011). In Indonesia, the methanolic or dichloromethane extracts of leaves have medicinal properties and are used to cure liver cirrhosis, hypertension and diabetes (Kasahara & Hemmi, 1988; Arung et al., 2009). Similar to jackfruit, breadfruit trunk wood is good for construction and furniture, and its sap can be used to trap birds and houseflies or to treat human skin and fungal diseases (Ragone, 1997).

Jackfruit and breadfruit are tropical fruits with potential beneficial uses as food, timber and ethnomedicines, but this requires scientific testing. This paper, in a bid to expand research of these trees, and expand their sustainable use and production through biotechnological interventions, highlights their basic biology such as morphology, medicinal properties and propagation (both in classical and biotechnological approaches). In this paper, we highlight research that has been conducted on two species, *A. heterophyllus* Lam. (syn: *A. integrifolia* Linn.) or jackfruit, and *A. altilis* (Parkinson) Fosberg (syn: *A. communis* J.R. Forst & G. Forst; *A. incisus* (Thunb.) L.f.), or breadfruit.

Morphology

Jackfruit is an evergreen tree 8-25 m in height and with a trunk diameter of 30-80 cm that can live up to 100 years. Young trees grow with a conical or pyramidal canopy shape that turns into a dome-shaped canopy as the plant grows older. Canopy diameter which can reach 10 m, is close to the ground and provides dense shade (Elevitch & Manner, 2006). Wood of jackfruit is categorized as medium hardwood with a specific gravity of 0.6-0.7 (Orwa et al., 2009). When the tree ages, wood turns from yellow to red or brown. Breadfruit is also an evergreen tree 15-20 m in height and with a 1-2 m diameter trunk whose bark is smooth, thick and light-grey while wood is golden although, after exposure to air, it darkens (Ragone, 1997).

Jackfruit inflorescences sprout from a short, thick stalk and emerge from the lateral side of the main stem and thick branches (Backer & Bakhuizen, 1965). The male inflorescence forms in the axil of the apical branch with a cylindrical to conic-ellipsoid shape 2-7 cm in diameter and a 1-5 cm long peduncle with a tubular calyx that has a two-lobed apex 1-1.5 mm in diameter, pubescent texture, straight filament and ellipsoid anther while the female inflorescence has a globose fleshy rachis with a tubular calyx, lobed apex and a one-celled ovary (Zhou & Gabriel, 2006). Some parts of the male inflorescence are sterile. As in jackfruit, the breadfruit inflorescence emerges from the apical trunk (Figure 1).

Figure 1. Jackfruit young fruit (left) and mature fruit (right). White scale bar = 10 cm. Unpublished figure.

The breadfruit inflorescence has a cylindric-clavate shaped flower with a 3-6 cm long peduncle and globose or ellipsoid inflorescence shape with a diameter up to 20-30 cm. It has a tubular calyx that is pubescent, has two lobes on its apical surface and has a lanceolate-shaped lobe while the anthers are elliptic. Female breadfruit flowers have a tubular calyx, an ovoid ovary with a long style and two branches on the apex. Each flower consists of a reduced tubular perianth that covers a single stamen with a two-lobed anther on a thick filament (Sharma, 1962).

Both jackfruit and breadfruit exude a sticky white latex from the injured parts of the plant (Rahman & Khanom, 2013), and forms part of the plants' defense against herbivory (Agrawal & Konno, 2009). The phyllotaxis (i.e., leaf arrangement) of jackfruit and breadfruit is distichous or spiral with simple, leathery leaf blades with a full margin and plants are monoecious (i.e., male and female flowers on the same tree) with inflorescences growing from the main branch or trunk (cauliflory) for jackfruit but sprouting from the apex of the main branch, also where new leaves emerge, and arising from simple, pseudomonomerous ovaries as in other Moraceae species (Singh, 2016). Both jackfruit and breadfruit form a single leaf blade that is lobed, but mature jackfruit leaves become entire and lose their lobes, hence the species epithet, *heterophyllus*. The leaves of jackfruit and breadfruit have stipulate leaf types, with an ovate form for jackfruit and a lanceolate to broadly lanceolate form for breadfruit. Jackfruit leaves are spirally arranged with an elliptic to obovate leaf blade, leathery, leaf margins are lobed in seedlings but entire in mature trees, with pale green on the lower leaf surfaces displaying scattered globose to ellipsoid resin cells while the axial surface is dark green, smooth and glossy (Zhou & Gilbert, 2003) with a cuneate, subdecurrent base, firmly coriaceous, leaf size is 10-20 × 5-10 cm (l × b), the stipule is 1.5-5 cm, and the petiole is 2-4 cm long (Backer & Bakhuizen, 1965). Breadfruit leaves are also spirally arranged, elliptic in shape with a broadly cuneate or obtuse base, up to 3-7 lobed along each margin, lobes are oblong, long-acuminate – acute, the stipule is 16-20 cm long, the petiole is 2-4 cm ling, and leaves are 30-100 cm × 25-65 cm (Backer & Bakhuizen, 1965).

Jackfruit and breadfruit have a compound fruit or syncarp that is classified as a compound false fruit or pseudofruit that forms from the enlargement of the stigma, and the inflorescence is composed of 1,500-2,000 flowers attached to the fruit's axis (Jarret, 1976). The fruit of jackfruit can weigh 4.5-30 Kg and can reach 30-40 cm in length, with an oblong-cylindrical shape and dark green coloration when young that turns greenish-yellow or brownish when mature.

The fruit grows and matures on the trunk for 90-180 days (Elevitch & Manner, 2006). Some jackfruit achenes contain multiple fruits, each with a bulk composed of seed and with a waxy and soft texture, golden-yellow with a sweet and aromatic aril (Orwa et al., 2009). The fruit of breadfruit is formed from the fused flower perianth, except for the base (Reeve, 1974), young fruit is light-green but turns yellowish-green when mature, and as the fruit develops, perianths fuse, becoming the fleshy edible portion of the fruit (Ragone, 1977). When sliced, breadfruit has a white flesh composed of dense perianths (Figure 2).

Figure 2. Breadfruit: whole (left) and sliced (right). Scale bar = 5 cm. Unpublished figure.

Jackfruit seed are semi-round, light brown to brown, 2-3 cm in length and 1-1.5 cm in diameter, wrapped in a whitish seed coat/testa, and a yellow aril (Figure 3). The seed is recalcitrant and can be stored for up to a month in humid conditions (Elevitch & Manner, 2006). Adelina et al. (2014) air-dried seeds for 0 h (control) to 5 h (treatments separated by 1 h) at 28°C and 70% humidity, noticing that water content was reduced from 75.03% to 22.95%, seed respiration rate declined from 7.189 mg CO_2/kg h to 5.32 mg CO_2/kg h, and seed viability dropped after 14 days of germination from 97.33% to 24.67%. The seed of breadfruit is brown, round or obovoid in shape with a thin wall 1-2 cm thick with reduced or no endosperm, hence its recalcitrance to storage or desiccation (Ragone, 1997). Some modern bread breadfruit cultivars are seedless (Devanai & Bhore, 2010). The male inflorescence of seedless cultivars produces less viable pollen than fertile, less-seeded cultivars and only few flowers in the male inflorescence produce and release pollen (Devanai & Bhore, 2010). In seedless breadfruit cultivars, nectar is only produced in male flowers but not in female flowers (Ragone, 1997). In general, the loss of

fertility in breadfruit is caused by triploidy (*2n* = *3x* = ~84) or by sterile diploids (*2n* = *2x* = 56) that result from hybridization (Ragone, 2001).

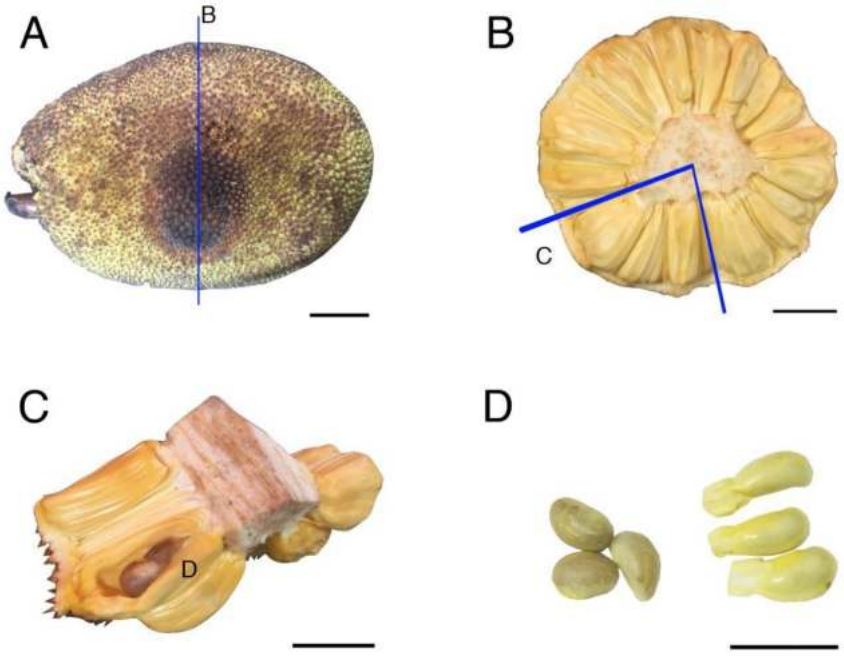

Figure 3. Mature fruit of jackfruit **(A)**, sliced **(B)**, part of the fruit with arils and the seed covered with testa **(C)**, and jackfruit seeds with testa (left) and still wrapped with aril (right). Blue lines indicate the direction of cuts. Scale bar = 5 cm. Unpublished figure.

Medicinal properties

Artocarpus produces various secondary metabolites and biologically active compounds, particularly phenolic compounds such as flavonoids (Table 1), stilbenoids, and arylbenzofurans (Hakim et al., 2006), extracted from leaves, the stem, fruit and bark, which have ethnomedicinal uses and antibacterial (Khan et al., 2003), antiviral (Likhitwitayawuid et al., 2005; 2006), antifungal towards Herpes Simplex Virus (HSV) and Human Immunodeficiency Virus (HIV) (Jayasinghe et al., 2004; Trindade et al., 2006), antiplatelet (inhibitory of thromboxane formation) (Weng et al., 2006), antiarthritic (Ngoc et al., 2005), tryrosinase inhibitory (Arung et al., 2006; Likhitwitayawuid & Sritularak, 2001) and cytotoxicity properties (Hakim et al., 2006) (reviewed in greater detail by

Table 1. Typical flavonoids, modified flavonoids, and flavonoid-derived xanthones found in *Artocarpus* (Hakim et al., 2006)

Compound class	Typical group found
Flavonoids	Chalcone
	Flavanone
	Flavone
	Flavan-3-ol
	3-Prenylflavone
Modified flavonoids	Oxipinoflavone
	Pyranoflavone
	Dihydrobenzoxanthone
	Furanodihydrobenzoxanthone
	Pyranodihydrobenzoxanthone
Flavonoid-derived xanthones	Quinonoxanthone
	Cyclopentenoxanthone
	Xanthonolide
	Dihydroxanthone
	Cyclopentenochromone

Jagtap & Bapat, 2010). Jacalin, which is a tetrameric two-chain lectin extracted from *A. heterophyllus*, has strong mitogenic activity against human $CD4^+$ T lymphocytes, serving as an immunobiological diagnosis agent for HIV-1 patients (Kabir, 1998).

Jackfruit contains various components used for medical benefits. Some flavonoids (Table 2) are used as antinflammatory agents (Wei et al., 2005). Fang et al. (2008) extracted three phenolic compounds from the ethyl acetate fraction of jackfruit fruit: artocarpesin (5,7,2',4'-tetrahydroxy-6-ß-methylbut-3-enyl flavone), norartocarpetin (5,7,2'4'-tetrahydroxyflavone), and oxyresveratrol (*trans*-2,4,3',5'tetrahydroxystilbene). All three compounds showed a potent anti-inflammatory property after inhibiting lipopolysaccharide-activated RAW 264.7 murine macrophage cells. Other

Table 2. Flavonoids with anti-inflammatory properties (Wei et al., 2005)

Flavonoid Compounds
Cycloartomunin
Cyclomorusin
Dihydrocycloartomunin
Dihydroisocycloartomunin
Cudraflavone A
Cyclocommunin
Artomunoxanthone
Cycloheterohyllin
Artonin A and B
Artocarpanone A
Heteroflavone A, B, and C

compounds, cycloheterophyllin and artonins A and B, showed antioxidant properties as they inhibited iron-induced lipid peroxidation after exposure to oxygen radicals in more than 60% of a rat brain homogenate after the addition of 1 µM of each of the three compounds and in more than 80% when 3 µM was used (Ko et al., 1998). A chitin-binding lectin, jackin, which was purified from a saline crude extract of jackfruit seed, displayed anti-fungal properties, inhibiting the growth of *Fusarium moniliforme* and *Aspergillus niger* cultures (2.25 mg/ml, but no effect for *A. niger* at 4.5 mg/ml) and induced hemagglutination against human and rabbit erythrocytes (with at least 0.15 mg/ml) (Trindade et al., 2006). Jacalin, a 65 kDA two-chain lectin, has potential as an immunomodulatory agent, having shown mitogenicity against human $CD4^+$ T lymphocytes when added at 100 µg/ml (Blasco et al., 1995). The addition of 10, 20, 30, and 40 µg/ml of jackfruit lectin displayed *in vitro* inhibitory activity against herpes simplex virus type HSV-2, varicellazoster virus (VSZ), and cytomegalovirus (CMV) via a cytopathic effect, and inhibited HIV-1 infection *in vitro* by preventing the binding of the virus to host cells (Wetprasit et al., 2000; Swami et al., 2012).

The methanolic and ethyl acetate extracts from breadfruit fruit contain steroids, phenolics and flavonoids that can inhibit the growth of human pathogenic bacteria like *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans* and *Pseudomonas aeruginosa* by establishing a defense mechanism (Pradhan et al., 2013). During a test on mice, the methanolic extract of breadfruit fruit and leaves (500 µg/ml each) was used to treat inflammation by lowering the intensity of leukocyte infiltration by preventing skin tumor growth and angiogenesis induced by carcinogenic chemicals 30 minutes after treatment (Lin et al., 2014). Fruitackin, a lectin isolated from the saline crude extract of breadfruit seed, induced hemagglutination against human and rabbit erythrocytes when added at 0.15 mg/ml and exhibits antifungal activity against *Fusarium moniliforme* and *Aspergilus niger* at the same concentration as used for jackin (2.25 mg/ml, but no effect on *A. niger* at 4.5 mg/ml) (Trindade et al., 2006).

Propagation (classical and biotechnological)

Conventional vegetative propagation using cuttings, grafting and rootstocks have unsuccessfully been used to propagate *A. heterophyllus* and *A. altilis*, thus seed serve as an effective choice to propagate *A. heterophyllus* (Roy et al., 1993). *In vitro* culture is an effective solution to cultivate and mass-produce both species. Roy et al. (1993) first washed adventitious shoot buds in 100 ml of 0.7% polyvinylpyrrolidone (PVP) with 2% sucrose, shook them at 100

rpm for 3 minutes then washed buds with tap water to remove PVP. Buds were disinfected in 0.2% $HgCl_2$ for 5 minutes then rinsed with sterile double-distilled water (SDW) for 3 minutes and this procedure was repeated 3-5 times. Buds cultured on Difco bacto-agar-solidified Murashige & Skoog (1962) (MS) basal medium supplemented with 8.88 µM 6-benzyladenine (BA) and 2.68 µM α-naphthaleneacetic acid (NAA) induced 10 shoots/explant after the 7th subculture. Shoots were elongated on MS medium with 4.44 µM BA, 0.54 µM NAA and 10% (v/v) coconut milk. Shoots were rooted *in vitro* on half-strength MS medium with 5.37 µM NAA and 4.92 µM indole-3-butyric acid (IBA), 80% of shoots being able to root. Plantlets were transplanted into earthen pots containing sterile sand, soil and humus (1:2:1, v/v/v), and 75% survived after 30 days.

Amin & Jaiswal (1993) used 10-20 days' old terminal buds from an *A. heterophyllus* trunk from a 30-50 year-old tree grown from seeds. Stems were washed in running tap water, treated with 1% (v/v) Cevalon® (an antiseptic and detergent), disinfected in 0.1% $HgCl_2$ for 5 minutes, then rinsed with SDW 4-5 times. Explants (5-10 mm denuded buds) were prepared by removing the outer cover of green stipules and excising inner buds encased by creamy-white stipules before implanting them vertically on growth medium, and placing cultures at 26±1°C, a 16-h photoperiod (50-70 µmol m^{-2} s^{-1}), and subculturing them every 4-5 weeks. MS basal medium with four concentrations (4.5, 9.0, 18.0, and 36.0 µM) of BA and kinetin (Kin) and a combination of BA and Kin (4.5 µM each) were used to induce shoots while MS with two concentrations of BA (4.5 µM and 9.0 µM) and BA with Kin (4.5 µM each) were used to multiply shoots. Roots were successfully induced from shoots with four combinations (0.5, 5.0, 10.0, and 25.0 µM) each of NAA and IBA, or two combinations (5.0 + 5.0 and 10.0 + 10.0 µM of NAA and IBA). The highest percentage of bud break resulted from 9.0 µM BA (82 ± 6%) while BA + Kin (4.5 µM each) resulted in 90 ± 7%. The highest number of shoots/explants formed with 4.5 µM BA (3.5±0.6), or 38±1.1 for BA + Kin (4.5 µM each). Under *ex vitro* conditions, the survival percentage of regenerated plantlets was 50%.

A. altilis can be propagated vegetatively *in vivo* and *in vitro*. *In vivo* vegetative propagation can be achieved by cuttings and air layering of branches by removing the ring bark, covering the wound with peat moss and then encapsulating in plastic to induce rooting before being cut and placed on soil (Deivanai & Bhore, 2010), although details about how long it takes to achieve each step was not explained. *In vitro* propagation of *A. altilis* can be achieved using shoot tips (Rouse-Miller & Duncan, 2000; Murch et al., 2008).

Rouse-Miller & Duncan (2000) collected shoot tips from a 6-7 year-old tree during the dry season (December to April in Trinidad-Tobago). Explants with one or two expanded leaves and 3-6 cm of associated stem were collected and placed in water (period of time not specified). Expanded leaves and bracts surrounding the shoot tip were removed and shoots were rinsed in tap water before cleansing in 70% ethanol for 1 minute. Shoots were reduced to 1 cm, dipped in 70% ethanol for 30 seconds, 10% household bleach (5.25% available chlorine) for 10 minutes and rinsed three times in sterile distilled water. The Rouse-Miller & Duncan (2000) study used Margara (1978) nutrients (Table 3).
For shoot induction, N5K and N15K macronutrients (Margara, 1978), MS micronutrients and vitamins with 3% sucrose, 0.8% agar and 4.4 µM BA were necessary. Shoot proliferation required Margara (1978) $N30NH_4$ macronutrients, MS micronutrients, vitamins, 3% sucrose and 2.2 µM zeatin. Rooting required $N30NH_4$ macronutrients, vitamins, 2% sucrose, with 0.5, 1.0, 1.5, 2.0, and 2.5 µM IBA. However, IBA alone could not induce roots, and 60% of shoots formed roots in auxin-free medium ($N3ONH_4$ in Table 3; Margara, 1978). Murch et al. (2008) used MS or B_5 (Gamborg et al., 1968) media with 2.5 g/L gelrite and 3% sucrose, 2 µM BA and 3 µM Kin to induce shoots in *A. altilis* within one week and 1 µM IAA to induce roots.

Table 3. Margara (1978) nutrient lists according to Karla da Silva (2010).

Medium	Macronutrients (mg/L)							
	KNO_3	$NaNO_3$	NH_4NO_3	$Ca(NO_3)_2 \cdot 4H_2O$	$CaCl_2 \cdot 2H_2O$	$MgSO_4 \cdot 7H_2O$	KCl	KH_2PO_4
N5Ca			80	354	292	246	149	136
N30Ca	808		480	1180		246	74.5	136
N30K	1313		480	590		246	74.5	136
N15K*	606		240	354		246	149	136
N15Ca	101		240	944		246	149	136
N45K	1818	85	720	944		246	372.5	136
N5K*	75.8		80	265.5		246	372.5	136
$N3ONH_4$*	606		800	472		246	372.5	136
edium	Micronutrients (µg/L)							
	$MnCl_2$	$ZnSO_4 \cdot H_2O$	H_3BO_3	KI	$CuSO_4 \cdot 5H_2O$	$NaMoO_4 \cdot H_2O$	$FeSO_4 \cdot 7H_2O$	NaEDTA$\cdot 2H_2O$
All	157	500	500	10	100	59	35000	30000

* only the macronutrients were used in the Rouse-Miller and Duncan (2000) study

Molecular advances and future perspectives

Molecular studies of both jackfruit and breadfruit offer promising prospects for exploiting biotechnology- and industry-derived benefits. Breadfruit molecular genetics has been studied more than in jackfruit. Studies on the genetic identification and profiling of breadfruit used microsatellite or short sequence repeats, identifying around 65 loci for nuclear genomic DNA (Witherup et al., 2013; De Bellis et al., 2016) or 15 loci for chloroplast genomic DNA (Elliot et al., 2015). Multi-access identification key software to identify breadfruit cultivars has been developed from a prototype version on a Lucid 3.3 platform based on quantitative and qualitative traits (Jones et al., 2013). Amplified fragment length polymorphism (AFLP) has been used to identify and track the origin of breadfruit cultivars as linked to the routes of human migration in Oceania (Zerega et al., 2004), or to assess genetic diversity (Shyamalamma et al., 2008). Random amplified polymorphic DNA (RAPD) was also used to assess genetic diversity (Prasad et al., 2014) and fruit cracking in jackfruit (Singh et al., 2011). Chloroplast and nuclear DNA were used to assess the phylogeny of 60 Moraceae taxa, including the *Artocarpus* genus (Zerega et al., 2010). Gibberellin 20-oxidase genes isolated from breadfruit allowed for the detection of sequence variants, their role in stem elongation after cuttings were treated with paclobutrazol (a GA inhibitor), and their regulation of abiotic stress, namely salinity and drought (Zhou & Underhill, 2015, 2016). Future research needs to identify breadfruit and jackfruit genetic diversity more precisely while studies on molecular genetics related to metabolic biosynthetic pathways, for example the elucidation of genes coding for artocarpatin synthesis, would allow for applications in the pharmaceutical industry.

Jackfruit and breadfruit are still known locally and may be good sources of nutrients ranging from carbohydrates to secondary metabolites. These fruits could be useful germplasm in future plant breeding projects for improving fruit, such as fortifying stress tolerance. Roy et al. (1993) bred flood-resistance jackfruit plants *in vitro* as a way to solve the problem of annual flooding in Bangladesh. A breeding programme conducted in South Florida aimed to improve jackfruit aroma, edible percentage, flesh firmness, colour and flavour (Campbell et al., 2004). A red-fleshed variant of jackfruit exists in India (International Tropical Fruits Network, 2011). These colour variants can be used to attract more consumers and thus achieve the maximum benefits of jackfruit, thus breeding for more colourful fruit flesh could be important. For the nutraceutical and pharmaceutical industries, future jackfruit breeding for higher content of specific metabolites can be achieved in a similar way as "Gama Melon Parfum," a melon cultivar that was developed in Indonesia to

obtain higher yield of sesquiterpenes aimed for perfume production (Maryanto et al., 2014). Breadfruit colouration is mostly only white, but it has some shape variants ranging from oval to long fruits (McCormack, 2007). As breadfruit appears to have potential as a better source of starch used in drug tablets than cornstarch (Adebayo et al., 2006), a breeding programme to produce a higher yield of starch in breadfruit could be a good prospect. Similar prospects for jackfruit could also be applied to breadfruit in future by creating colour variants for increased appeal or to improve metabolite content for the food, pharmaceutical and nutraceutical industries. As one example, breadfruit flour was found to be a good substitute for wheat flour when used as a composite breadfruit-wheat flour mix for donuts, with a larger ratio of breadfruit flour resulting in lighter donuts, apparently as a result if its lower gluten content, although panelists preferred the color, aroma, taste, and texture of donuts with more wheat flour in the dough (Oke et al., 2018).

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Journal of Tropical Biology and Conservation 15: 81–95, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Mosquito Diversity between Logged and Unlogged Forest Areas in Kalabakan Forest Reserve, Sabah

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Abstracts

Mosquitoes were sampled in an undisturbed area within the trail in the forest near the Maliau Basin Conservation Area (MBCA) and in a disturbed area within the Logged Forest Experimental (LFE) area near the SAFE Project camp site. A total of 48 days of sampling was done in both areas in a bi-monthly sampling starting June 2016 to April 2017. The aims of this study were to investigate the species diversity and peak biting hours of mosquitoes in both sites. A total of 807 individuals from 17 species were caught using manual collection method of Human Landing Catch (HLC). 15 species were collected in MBCA while only 9 species were collected in LFE. Based on Generalized Linear Mixed Model (GLMM), there was a significant difference of mosquito abundance between LFE and MBCA ($p<0.05$) and mosquito day biting time ($p<0.05$). Also, based on Independent T-test analysis, there was a significance difference in terms of mosquito diversity level and abundance ($p<0.05$). In this study, LFE had higher mosquito abundance with a total of 563 mosquito individuals caught compared to MBCA with 244 mosquito individuals. In both areas, more species were recorded during day time samplings than night time samplings. *Anopheles balabacensis*, *Aedes albopictus*, *Heizmannia scintillans* and *Culex vishnui* were among the predominant species collected in LFE while in MBCA species collected were *Heizmannia scintillans*, *Anopheles umbrosus*, *Aedes albopictus* and *Armigeres jugraensis*. LFE had peak biting hours around 2:00 p.m., 5:00 p.m., 7.00 p.m. and 9:00 p.m. while for MBCA, the peak biting hours were between 2:00 - 3:00 p.m. and 6.00 p.m.

Keywords: mosquito, Maliau Basin, diversity, logged and unlogged forest, HLC

Introduction

Mosquitoes are classified under family Culicidae in order Diptera. They are mostly found in moderate climate and tropic regions. There are currently about 42 genera and around 3,500 mosquito species that can be found in the world (Service, 2008; Rueda 2008; Harbach & Besansky, 2014).

Received 22 June 2017
Reviewed 26 October 2017
Accepted 16 March 2018
Published 15 October 2018

Even though there about 3,500 named mosquito species, only a number of this very diverse family are considered medically important and bring nuisance to humans (Fang, 2010). In Malaysia particularly, there are about four medically important mosquito genera which are *Aedes*, *Anopheles*, *Culex* and *Mansonia* (Rahman et al., 1997). Mosquitoes also serve important functions in numerous ecosystems they live in (Fang, 2010). However, mosquito importance is mainly due to its medical reasons towards human health as it has capability in becoming a vector for several dangerous pathogens to humans (Foottit & Adler, 2009). The diseases that are commonly transmitted by these small vectors are malaria, dengue fever, yellow fever, chikungunya, filariasis (Service, 2008) and recently Zika virus (Nhan & Musso, 2015).

The environment plays a major role in affecting mosquito diversity and abundance. Mosquito communities may change across a landscape including when there are changes in habitat which can affect species relative abundance and the invasion of new species (Thongsripong et al., 2013). A changing environment possesses negative impact to human health especially through the transmission of disease especially by a vector mosquito (Vanwambeke et al., 2007).

This study was conducted to determine and compare adult mosquito species diversity and biting activity between disturbed areas within the Logged Forest Experimental (LFE) area near the SAFE Project camp site and undisturbed primary forest in Maliau Basin Conservation Area (MBCA). The objectives of this study were; (a) to investigate the mosquito species that are present in disturbed and undisturbed site; (b) to compare the diversity and abundance in disturbed and undisturbed sites; and (c) to determine the peak biting time of mosquito between disturbed and undisturbed sites. Although previously several mosquito studies had been conducted in the SAFE Project site (Brant et al. 2011; Brant et al., 2016), this study is hopefully able to complement existing data as well as provide new information on mosquito diversity at Kalabakan Forest reserve area.

Materials and Methods

This study was carried out in the ‘Stability of Altered Forest Ecosystems' (SAFE) Project area which includes the Kalabakan Forest Reserve at Benta Wawasan and Maliau Basin Conservation Area (Figure 1). SAFE Project is considered as one of the largest ecological study sites in the world. The SAFE Project has been recognized as a Class II Forest Reserve except for the Virgin Jungle Reserve (VJR)

which is Class IV Forest Reserve while Maliau Basin Conservation Area is a Class I Forest Reserve (Chung et al., 2010). Maliau Basin Conservation Area is fully protected and has a very broad primary forest area which has never been logged (Hardwick et al., 2015). Samplings were divided into two types of areas; disturbed and undisturbed. The disturbed area was within the Logged Forest Experimental (LFE) area near the SAFE Project camp site while the undisturbed area was within the trail in the forest near the Maliau Basin Conservation Area (MBCA).

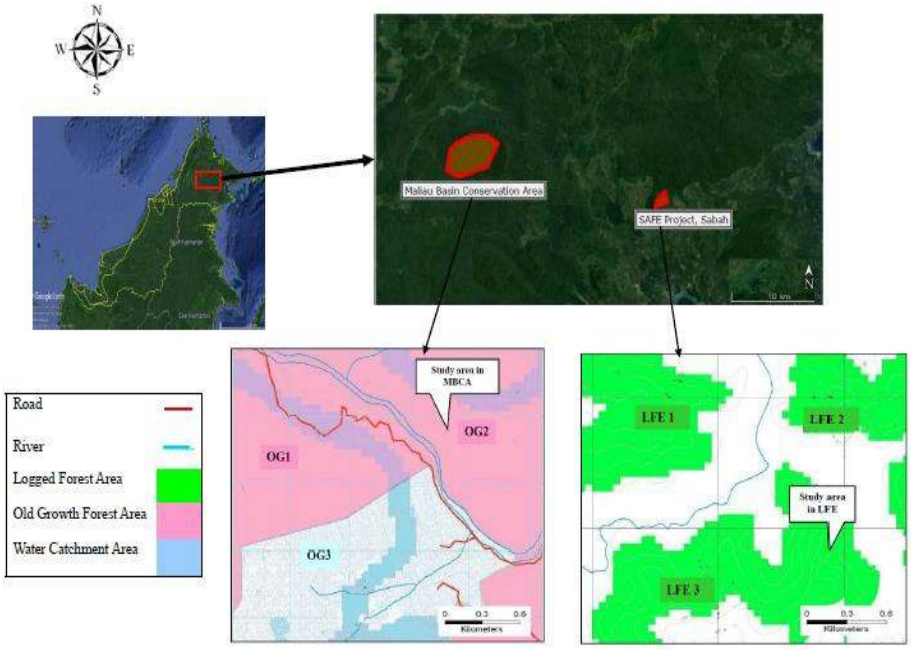

Figure 1 : Location of the study area
(Source: i) Google Earth ii) http://www.safeproject.net/concept/maps/)

Adult mosquitoes were collected using human landing catches (HLC). Anti-malaria pills were taken one week before the sampling started. In this method, legs and hands of the collector were exposed in order to attract the mosquitoes. Mosquitoes that landed on or bit the exposed skin were caught using a vial or Eppendorf tubes. The samplings were divided into two different time frames; day-time and night-time samplings. Each time frame was conducted for two days. Altogether, a total of 48 days of sampling efforts was

made in both areas through bi-monthly sampling starting in June 2016 until April 2017. The day-time sampling was conducted from 6 a.m. until 6 p.m. while the night-time sampling was carried out from 6 p.m. until 12 a.m. The purpose of a 12-hours day time mosquito collection in this study was to determine the peak biting time of mosquitoes since different mosquitoes have different peak biting periods (Varnado et al., 2012). Due to logistics and safety issues, night-time sampling usually ended at 12 a.m. Collected mosquitoes were killed using ethyl acetate solution. Mounted specimens were dried for three days in a drying oven. Later, all of the specimens were stored in the specimen box. All mosquitoes were identified using a compound microscope and were identified to genus and up to species level using the Southeast Asia identification key by Ratanarathikul et al., (2005a,b; 2006a,b), Stojanovich & Scott (1966) and Reid (1968). Mosquitoes that could not be identified to species level were written as "sp" after their genus name and if the unidentified species belong to the same genus, a number was written at the end of the 'sp' to represent the species name.

In this study, Generalized Linear Mixed Models were used to detect mosquito abundance differences using sites, sampling time frame (day-time and night-time), month of sampling, average temperature and average humidity of each month in each site as factors. Shannon-Weiner diversity index value was also used to get the diversity value of mosquitoes caught in each site. Then, independent T-test using Statistical Package for Social Science (SPSS) Version 20 was used to compare the mosquito diversity and abundance between the disturbed and undisturbed areas. For peak biting hour record, the numbers of mosquitoes caught were recorded according to the hour of the collection in order to sort and determine the peak biting hour.

Results

A total of 807 mosquito individuals representing 7 genera and up to 17 species were collected using the HCL technique. Figure 2 shows the species of mosquitoes collected. In LFE, the most collected species were *Anopheles balabacensis*, *Aedes albopictus*, *Heizmannia scintillans* and *Culex vishnui* while in MBCA the species were *Heizmannia scintillans*, *Anopheles umbrosus* and *Armigeres jugraensis*. *Aedes albopictus*, *Anopheles balabacensis*, *Heizmannia scintillans* were among the species found in both areas. Fifteen species were recorded in the undisturbed area compared to 9 species in the disturbed area (LFE). Table 1 shows the list of mosquito species caught during day and night sampling in the two study sites.

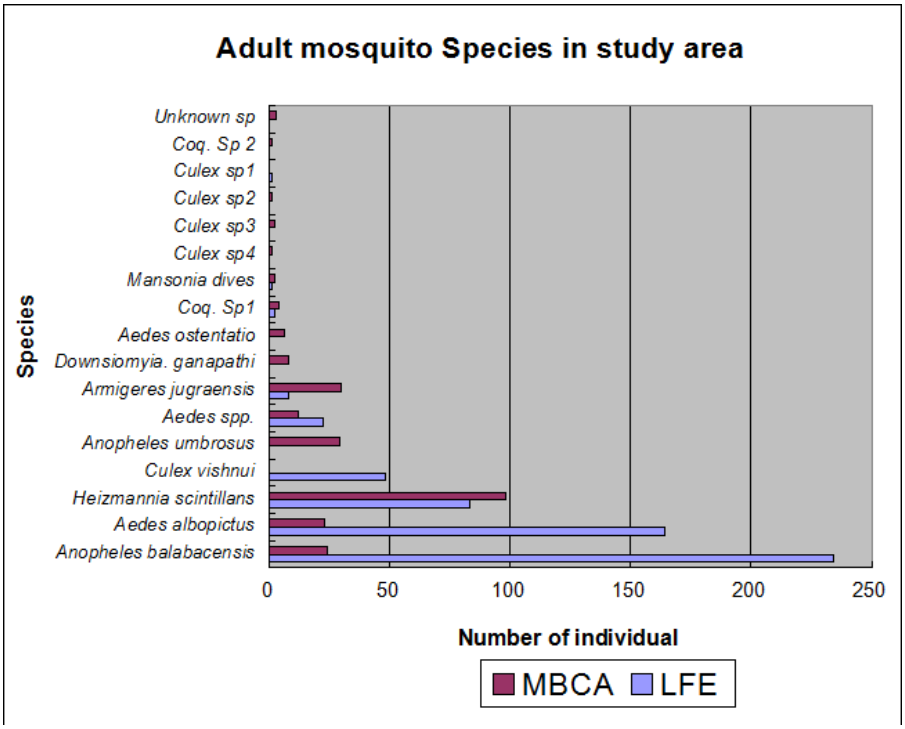

Figure 2: Mosquito species in study sites

Table 1. List of mosquito species caught according to day or night sampling in LFE and MBCA

LFE (Logged Forest)		MBCA (Primary Forest)	
Day	**Night**	**Day**	**Night**
Aedes albopictus	*Culex vishnui*	*Anopheles umbrosus*	*Aedes albopictus*
Aedes spp.	*Aedes albopictus*	*Armigeres jugraensis*	*Anopheles balabacensis*
Coquillettidia sp 1	*Anopheles balabacensis*	*Aedes ostentatio*	*Anopheles umbrosus*
Culex sp1		*Downsiomyia ganapathi*	*Coquillettidia sp2*
Armigeres jugraensis		*Heizmannia scintillans*	*Mansonia dives*
Heizmannia scintillans		*Aedes albopictus*	*Unknown sp.*
Mansonia dives		*Aedes spp*	
		Coquillettidia sp1	
		Culex sp2	
		Culex sp3	
		Culex sp4	

Table 2. Effect of Parameter on Mosquito Abundance using Generalized Linear Mixed Model

Model Term	Coefficient	Std. error	t	Sig.	95% Confidence Interval	
					Lower	Upper
Intercept	427.142	316.448	1.350	.199	-251.570	1,105.855
Site=1	26.620	8.821	3.018	.009*	7.702	45.539
Site=2	0[a]					
Month=1	33.770	16.081	2.100	.054	-0.719	68.260
Month=2	10.744	18.591	0.578	.572	-29.129	50.618
Month=3	3.625	15.176	0.239	.815	-28.924	36.174
Month=4	-1.466	14.857	-0.099	.923	-33.331	30.398
Month=5	-10.178	17.975	-0.566	.580	-48.732	28.375
Month=6	0[a]					
Time=1	39.578	18.259	2.168	.048*	0.416	78.741
Time=2	0[a]					

Probability distribution:Normal, Link Function: Identity
a Coefficient is set to zero because it is redundant
*Significant value <0.05

Mosquito abundance was significantly associated with site factor in this study between LFE and MBCA (GLMM, Site=1, $p<0.05$) (Table 2). Mosquito abundance was much higher in LFE (disturbed site) rather than in MBCA (undisturbed area). Also, day biting activity collection was significantly different than night biting collection (GLMM,Time=1, $p<0.05$). From the biting activity graph (Figure 4 & Figure 5) we can see that the mosquito biting activity was significantly active during the day time compared to night time.

Based on Independent T-test analysis, there was a significant difference between mosquito diversity value in disturbed area and undisturbed area ($t(10)= -2.88$ $p=0.017$, $d=0.19$, 95% [-0.96, -0.12]. The mean for disturbed area ($M=1.07$ $SD=0.34$) was significantly different compared to the undisturbed area ($M=1.61$ $SD=0.32$). These results show that mosquito diversity was much higher in the undisturbed area than in the disturbed area. Using the same analysis, mosquito abundance was also significantly different in disturbed and undisturbed areas ($t(22)=2.8$ $p=0.01$, $d=8.8$, 95% [6.43, 43.07]. The mean for disturbed area ($M=45.75$ $SD=26.22$) was significantly different to the undisturbed area ($M=21.0$ $SD=15.79$). These results show that mosquito abundance was much higher in the disturbed area than in the undisturbed area.

Figure 3 shows the graph for bi-monthly mosquito collection data. Based on Figure 3, the highest collection was recorded in June 2016 while the lowest collection made was recorded in August 2016 where the total collection was only 80 individuals.

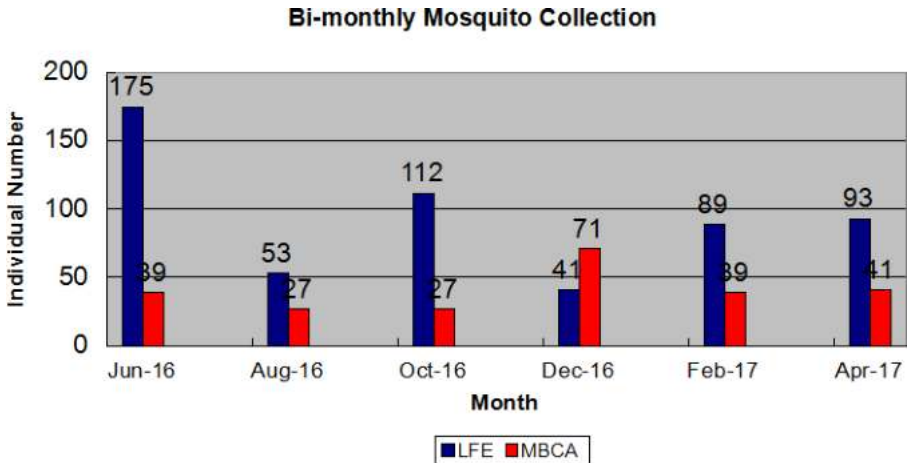

Figure 3. Mosquito Bi-monthly Collection

Table 3. Mean temperature and relative humidity for day and night sampling in study site.

Site	Mean temperature (°C)		Mean relative humidity (%)	
	Day	Night	Day	Night
LFE	26.9	23.5	84.4	96.6
MBCA	25.9	24.2	88.9	97.1

Table 3 shows the total mean temperature and relative humidity for day-time and night-time sampling using one-way ANOVA analysis. LFE had slightly higher temperature and low humidity for day-time and night-time sampling compared to MBCA. However, the temperature in LFE area at night during this study was lower than in the MBCA area, which may have been caused by canopy gap presence in the logged forest area.

For peak biting hour during the sampling periods, LFE had peak biting hours around 2:00 p.m., 5:00 p.m., 7.00 p.m. and 9:00 p.m. while for MBCA, the peak biting hours were between 2:00 - 3:00 p.m. and 6.00 p.m. (Figure 4 & Figure 5).

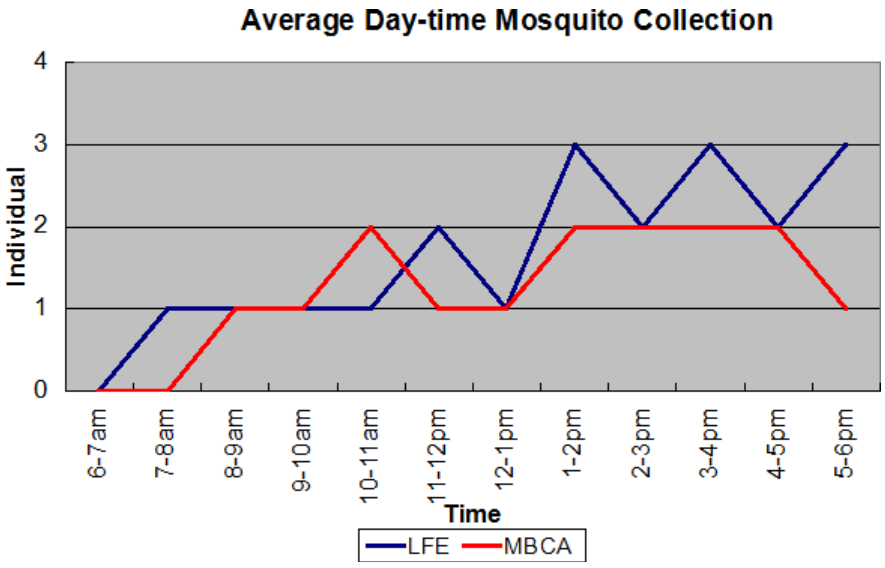

Figure 4. Mosquito biting graph during day sampling

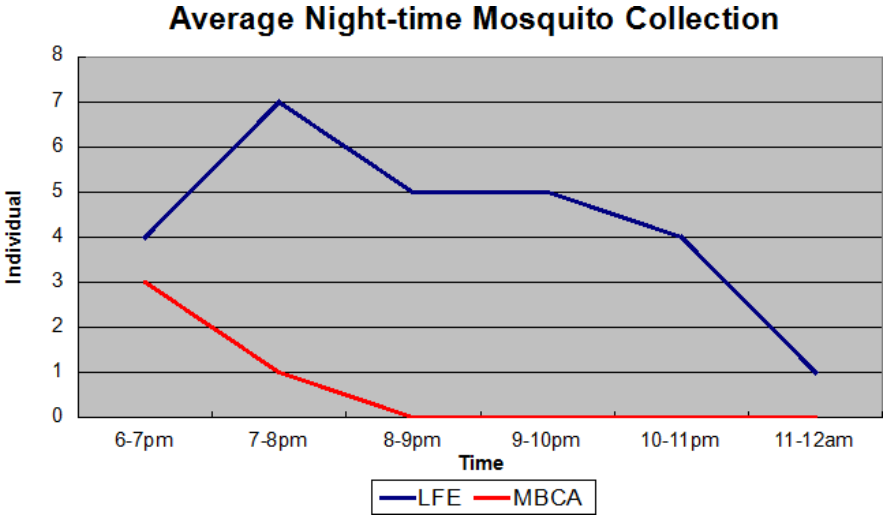

Figure 5. Mosquito biting graph during night sampling

Discussion

Habitat disturbance such as forest modification inevitably caused ecological disturbance in the forest ecosystem and has become a great threat to forest biodiversity (Fitzherbert et al., 2008). Changing environments pose negative impact on human health especially through the transmission of disease by a vector-borne mosquito (Vanwambeke et al, 2007). Mosquito communities may change across landscapes including changes in habitat which can affect species relative abundance and the invasion of new species (Thongsripong et al., 2013). In this study, mosquitoes were much more diverse in the undisturbed area (MBCA) than in the disturbed area (LFE). The possible reasons are undisturbed forests have lots of suitable natural habitat for the mosquitoes to breed and feed on hosts. Also, the old growth forest site in MBCA was cooler compared to the logged forest area (Brant et al., 2011). This was probably due to canopy height differences between LFE and MBCA where in MBCA it consists of lots of higher primary forest trees than in LFE. Canopy height can be the contributing factor that influences forest microclimate (Hardwick, et al., 2015). Previous studies by Chen et al. (1993) and Williams-Linera et al. (1998) show that the air temperature within a forest canopy has a higher relative humidity compared to air in the open area. The open canopy area usually has higher air temperature due to low sunlight cover (Fayle et al., 2010).

MBCA is a protected area and still has lots of primary forest areas which have never been logged before (Hardwick et al., 2015). The difference in terms of number of mosquito species present are related to the disturbance level in an area. The higher the intensity of disturbance, the lower the diversity of organisms in an area as only certain species are able to tolerate the surrounding environment (Haddad et al., 2008; Moretti & Legg, 2009). Loss of natural habitat in a disturbed area due to high intensity of disturbance can lead to loss of certain species and food resources (McCabe & Gotelli, 2000). According to Schowalter (2011), individuals or species that are not tolerant to certain changes in the habitat environment will face a decrease in number and then undergo extinction. Instead, tolerant species will gain benefits in terms of reducing the number of predators.

In this study, the number of mosquito species was lower in the disturbed forest area compared to the undisturbed area. Habitat in the disturbed area usually has been damaged or destroyed which causes the area to be unsuitable for species survival. Human activities such as deforestation and development have changed habitats which result in declining mosquito species numbers in the disturbed area at the SAFE Project. Diversity level in a certain area will increase

if the disturbance occurs in low spatial scale while diversity level will decrease if the disturbance occurs in high spatial scale (Dumbrell et al., 2008). In this study, mosquito abundance was higher in logged over forests compared to primary forests. The results seem to complement the previous study by Brant et al. (2016) which found the abundance of landing mosquitoes were higher in a logged forest compared to primary forest area. However, in terms of the number of possible vector mosquito presence, both LFE and MBCA had almost the same vector species. *Aedes albopictus*, *Anopheles balabacensis*, and *Culex vishnui* can be found in LFE while for MBCA, only *Culex vishnui* was not present. Both *Aedes albopictus* and *Anopheles balabacensis* were present in both study sites. *Aedes albopictus* population seems to spread very widely due to its ability to live in all ecotypes in towns, villages, forest fringes and coastal areas. *Ae. albopictus* is also known as dengue vector in Malaysia (Rohani et al., 2008). *Anopheles balabacensis* which was the most common and the most predominant *Anopheles* species found has been incriminated as a malaria vector in Sabah (Reid, 1968; Wong et al., 2015).

The biting activity of mosquito was studied as it provides to our understanding of the biting cycles of some mosquito species, nuisance level determination and possible disease transmission detection (Rohani et al., 2013). In this study, we can see two different biting time behaviour between day-time and night-time samplings. For day-time sampling usually dominated by *Aedes* and *Heizmannia* mosquito groups showed their biting activity was around 2:00 p.m and near dusk which was around 5:00 p.m. This result was almost similar to a previous study where *Aedes* mosquito usually peaks at dusk 5:00-6:00 p.m. (Rogozi et al., 2012; Sahani et al., 2012). For night-time samplings, the biting activity peaked at 7:00-8:00 p.m and 9.00-10:00 p.m. when *Anopheles* mosquitoes were active. Only few *Aedes* mosquito were collected during night-time sampling. Similar to the previous study by Brant et al. (2016), *Anopheles balabacensis* started biting as early as 6:00-7:00 p.m. Since *Anopheles balabacensis* live within forested area of Sabah and readily bite human and monkey hosts, it is no wonder that it is considered as one of the dangerous simian malaria vectors (Vythillingam, 2010). Biting time of mosquitoes in this study showed decreasing biting activity as the night progressed. This situation was almost similar to the study by Chen et al. (2014) and Mahanta et al. (1999) that showed a reduction in mosquito activities towards midnight.

For day sampling in LFE, the peak biting hours were at 2:00 p.m. and 5:00 p.m. and usually were made up of mosquito species from *Heizmannia scintillans* and *Aedes albopictus*. However, *Aedes* mosquito tends to be more active at dusk

than other mosquito species caught in this study. A study by Marques & Gomes (1997) reported that the biting activity of *Aedes albopictus* usually peaked at 6:00-7:00 a.m., 1:00-2:00 p.m. and highest activity during 4:00-5:00 p.m.. For the night sampling in LFE, biting activity was highest at 7:00 p.m. and 9:00 p.m., dominated by *Anopheles balabacensis* and *Culex vishnui*. A study by Wong et al. (2015), showed that the biting activity of *Anopheles balabacensis* can be as early as 6:00 p.m. up to 8:00 p.m. On the other hand, the day sampling in MBCA had a peak biting hour at 2:00-3:00 p.m where mosquito species like *Heizmannia scintillans* and *Armigeres jugraensis* were caught during this hour. However, for the night sampling in MBCA, the biting activity only peaked at 6:00 p.m. and the species collected during the hour were *Anopheles umbrosus*, *Anopheles balabacensis* and a few other species from genus *Aedes* and *Mansonia*. The reasons for different peak biting hours between the two sites were probably due to different environment conditions that the mosquito live in and type of species present in the areas. Based on the mean temperature and humidity data, the environment in MBCA was cooler compared to LFE. In terms of abundance of mosquito, LFE has higher abundance of mosquito and vector species from Anopheles, Aedes and Culex groups compared to MBCA which lead to the biting activity in LFE to be more active than in MBCA.

Changes in land use such as deforestation and other development activities have a direct impact on mosquito abundance, species biodiversity, biting behaviour and vector competency (Rohani et al., 2016). In addition, the effects of land modification also cause changes in temperature and moisture which in turn result in increasing vector population and transmission rates (Geist, 2006). MBCA in this study was a good example of the effect of forest modification towards mosquito biodiversity and biting behaviour. Also, the transition of forest land from its previous primary forest state can result in environmental stress due to microclimatic changes (Edwards et al., 2013). For example, a study by Kweka et al., (2016) showed that deforestation affects microclimate conditions and mosquito survivorship where an increase in malaria vector reproductive rate was associated with an increase in temperature. The influence of landscape change on microclimate condition of an area can be the key to determining the effect on diversity, abundance and survivorship of the mosquitoes (Patz & Olson, 2006). In a nutshell, changes in mosquito diversity can affect the risk of infectious diseases in a system by disrupting their normal host and pathogen relationships. By understanding the vector community that lives in an area that has undergone anthropogenic changes, a basis could be formed for understanding the emergence and persistence of mosquito-borne diseases (Thongsripong et al., 2013).

Conclusion

There are differences in diversity of mosquitoes between disturbed and undisturbed areas. Undisturbed area possesses a higher number of mosquito species than disturbed areas. Based on this study, we can see that *Anopheles balabacensis* was the most predominant species for night-time catch and *Aedes albopictus* was the most predominant species for day-time catch. Overall, a good understanding on the ecology and behaviour of these mosquitoes would give us an advantage in managing and controlling the vectors.

Acknowledgement

This project was supported by UMS-Great Research Grant GUG0007-ST-M-1/2016. We are very grateful to SAFE Project and Maliau Basin Conservation Area for giving their permission and for extending their cooperation.

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Journal of Tropical Biology and Conservation 15: 97–100, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Short Communication

Codonoboea kjellbergii (Gesneriaceae) in Buru Island, Maluku: A New Genus Record for the Island

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Abstract

Codonoboea kjellbergii (B.L.Burtt) Karton., recently discovered in Buru Island, Maluku, is a new genus record for the island. The species was observed from only a single location. It grows on rocky, more or less nutrient poor soil. A description, figure and illustration are provided.

Keywords: *Codonoboea kjellbergii*, Gesneriaceae, new record, Maluku, Buru Island

Introduction

Codonoboea Ridl. is a genus distributed from Southern Thailand and throughout Malaysia with about 120 species named (Middleton et al., 2013). Its centre of distribution is Peninsular Malaysia in West Malaysia (Kartonagoro & Potter 2014).

Codonoboea kjellbergii (B.L.Burtt) Karton. is distributed in Sulawesi, Maluku (Ambon & Seram) and New Guinea (Kartonagoro, 2012; Kartonagoro & Potter, 2014). It was originally described as *Henckelia kjellbergii* B.L. Burtt based on a specimen from southeast Sulawesi (Burtt, 1998). On a recent visit in 2014 to Buru Island, Maluku, this species was discovered and is the first record of this species and also the genus in Buru Island, and the third locality within the Maluku Islands.

Material and Methods

The plant was collected in May 2014 from the road to Lake Rana, Fena Leisela District, Buru Regency, Maluku Province (Figure 1). It was preserved as a dried herbarium specimen and deposited in the Bogoriense Herbarium (BO).

Received 21 October 2017
Reviewed 24 August 2018
Accepted 12 September 2018
Published 15 October 2018

Figure 1. Locality of *Codonoboea kjellbergii* in Buru Island, Maluku (marked by star-shaped polygon).

Identification is based on Burtt (1998), Kartonagoro (2012) and Kartonagoro & Potter (2014).

Results and Discussion

Codonoboea Ridl., Fl. Malay Penins. 2: 533 (1923); Middleton, Weber, Yao, Sontag & Moller, Edinburgh J. Bot. 70, 3: 387 (2013). – Type: *Codonoboea leucocodon* (Ridl.) Ridl.

Perennial herbs, caulescent or rosette forming, sometimes creeping, somewhat woody. Leaves opposite or alternate, or are crowded at the top, petioled or sessile, blades lanceolate, margin dentate to entire; petiole channeled. Inflorescence axillary, one- to few-flowered, pedunculate, flowers 5-merous. Calyx deeply 5-lobed, reaching the base. Corolla tubular, campanulate, lobes short, 2-lipped, lower lip 3-lobed, longer, upper lip 2-lobed, shorter than lower lip. Stamens 2. Nectary a flat-topped or lobed ring, sometimes not a complete ring or lacking. Style with stigma peltate or clavate. Capsule slender, cylindric. A genus of about 120 species, from Southern Thailand and throughout Malaysia (Middleton et al., 2013).

Codonoboea kjellbergii (B.L.Burtt) Karton., Edinburgh J. Bot. 69, 2: 360. 2012; Kartonagoro & Potter, Reinwardtia 14, 1: 4. 2014; Middleton, Weber, Yao, Sontag & Muller, Edinburgh J. Bot. 70, 3: 399. 2013. – Basionym: *Henckelia kjellbergii* B.L.Burtt, Beitr. Biol. Pflanzen 70: 378. 1998. – Type: Indonesia, Celebes (=Sulawesi), Boeloe Watoewila, 1500 m, 24 iii 1929, *Kjellberg* 1092 (holo S n.v.; iso BO n.v.).

Herb to 20 cm tall. Stem unbranched, 2.5–2.75 mm diameter at the base, densely pilose, less dense in very old parts. Leaves spirally arranged, distinctly spaced, 7–16 mm apart, blades elliptic-oblong, slightly obovate, 7.5–11.25 × 2.8–3.45 cm, base cuneate, margin finely serrate, apex acute or acuminate, both sides pilose on lower side chiefly on veins, mixed with shorter hairs, petiole short, pilose, to ca. 2.5 mm long. Peduncle axillary, pilose; flowers solitary or usually paired, one well-developed ca. 5.25 cm long accompanied by a shorter one, a pair of bracteoles inserted at ca. 3/4 of its length, 1.8–2.5 mm long, pilose as the peduncle. Calyx 5-partite, lobes ca. 2 mm long, acute, hairy. Corolla tubular, total length ca. 23 mm, thinly pubescent, glandular hairs also present, tube white, inside around and at the mouth with two yellow bands, limbs rounded with apex rounded, light purple, darker at the margin, upper 2 lobes partially connate. Fertile stamens 2, arising from the basal part of corolla, filaments ca. 7 mm long, glabrous, anthers 2 mm at most. Nectary short cylindric, ca. 0.6 mm long, not lobed. Ovary and style hairy, stigma ca. 1.4 mm wide. Fruit ca. 5.6 cm long, persistent and sparsely pubescent (figure 2).

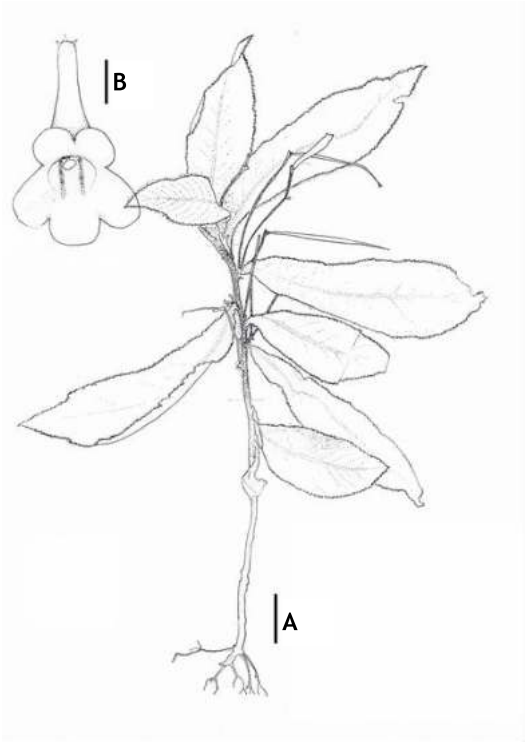

Figure 2. *Codonoboea kjellbergii*. **A)** habit; **B)** flower. Scale bar: A= 2 cm, B= 5 mm. (All from *Taofik Hidayat 37* (BO). Prepared by W.A. Mustaqim).

Specimen examined: INDONESIA. Maluku, Buru Island, near road from Wamlana to Lake Rana, 950 m (S 3 8' 53.8" E 126 35' 2.8") *Taofik Hidayat* 37 (BO!).
Distribution: Sulawesi, Maluku (Ambon, Buru, Seram) and New Guinea.
Habitat and ecology: Found once in sandstone-derived soils, at about 950 m elevation. The soil was probably nutrient-poor as indicated by the presence of *Nepenthes maxima* in the surrounding area.
Notes: The description above is based on a single plant preserved as a dried herbarium specimen. Kartonagoro & Potter (2012) noted that this species is easily recognized from its flower that has a white tube and purple lobes, its oblong pubescent leaves and long narrow capsule. These characters are ease to recognize even in the field. The discovery of this species on Buru Island fills a gap in its geographical distribution, which was previously thought to be disjunct.

Acknowledgements

Thanks are due to Major Agus Sutomo, chief commander of Ekspedisi NKRI Koridor Maluku & Maluku Utara 2014, who supported the plant exploration to remote areas of Buru Island, Maluku. Also to Capt. Dedy Dwi Cahyadi, chief commander of subkorwil-03/Namlea, who provided facilities and gave invaluable help during the field excursion. Also to Sergeant Taofik Hidayat for the material used in this study, La Herman Buton, Ayu P.N. Muda, Khoirunnisa M. Fatwa for the help in the field. The author is also indebted to Abdulrokhman Kartonagoro (BO), who provided literature used in this study and Inggit P. Astuti (Bogor Botanical Garden) for providing help in many ways.

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Journal of Tropical Biology and Conservation 15:101–120, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Genetic Variability and Relationship of Banana Cultivars (*Musa* L.) From East Java, Indonesia based on the Internal Transcribed Spacer Region nrDNA Sequences

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Abstract

A genetic variability and relationship study subjected to 41 banana cultivars from East Java based on nrDNA sequences of the ITS region was conducted. It would be useful to consider the genomic identification, genetic conservation strategy, and for further banana improvement. ITSL and ITS4 primers were used to amplify the ITS region. Results show that the ITS region DNA sequences length of 41 banana cultivars examined varied from 631 bp to 651 bp. It showed high variability with conservation level G+C content of 62.79%. Total aligned and selected ITS region DNA sequences was 656 bp comprising 346 positions (52.74%) as conserved region, 223 positions (33.99%) as variable sites (polymorphic) and 87 positions (13.26%) alignment gaps. About 143 positions (64.13%) of the variable positions were potentially parsimony informative and 80 positions (35.87%) were singleton variables. The singletone variation sequences spesific to certain banana cultivars may be proposed as identification barcodes. The haplotype diversity was very rich (Hd=1.00), resulted 41 haplotypes with none of haplogroup. Haplotype distribution map revealed the lineage pattern of banana cultivars from East Java. They were presummably derived from common ancestors and the same population in East Java mainland which then experienced an evolution process, dispersed by human migration both in and out, and became isolated to the islands. Genetic relationship reconstruction using NJ algorithm resulted in a tree and classification better than MP algorithm. It was clustered according to its geomic group, into 3 main clades *i.e.* AA/AAA, AAB and ABB. The ITS region nrDNA sequences was proven powerful in classifying until cultivar level of bananas. All 41 banana cultivars examined are recommended for genetics conservation.

Keywords: banana cultivar, East Java, genetic diversity, Internal Transcribed Spacer, molecular marker

Received 17 July 2017
Reviewed 09 April 2018
Accepted 18 July 2018
Published 15 October 2018

Introduction

The diversity of bananas (*Musa*ceae) in Indonesia is very high, being part of the primary origin and diversity centre for both wild seeded species and edible cultivated varieties (Nasution & Yamada, 2001). Wild seeded bananas are not economically utilized much but are linked to their role as genetic resources to improve banana quality in the future (Nasution, 1991). Meanwhile, edible banana is a popular fruit plant worldwide; and it has importance in food security (Hapsari, 2011a), socio-economic and cultural values particularly in rural communities and in developing countries (Megia, 2005; Suhartanto et al., 2009; Hapsari et al., 2017). Edible banana cultivars contain high nutrient values (high carbohydrates, total sugar, vitamin C and potassium; moderate protein and low fat); it is a recommended food for people of all ages (Hapsari & Lestari, 2016). There are approximately at least 325 cultivars recognized in Southeast Asia (Valmayor et al, 2002), of which about 200 cultivars are available in Indonesia (Nasution & Yamada, 2001); and not less than 90 cultivars reported in East Java (Hapsari et al., 2015a; Hapsari et al., 2017). All those local banana cultivars are valuable resources with their own potential characteristics which are necessary to be conserved for further use.

Banana cultivars were putatively derived from natural hybridization between wild diploid *Musa* species. *Musa acuminata* (A genome, x=11) and *Musa balbisiana* (B genome, x=11) are believed to be the ancestors of most banana cultivars (Simmonds & Shepherd, 1955); also *Musa schizocarpa* (S genome, x=11) and *Musa textilis* (T genome, x=11) in a few of them (Simmonds, 1959; Singh et al., 2001). Elucidating the phylogenetic and domestication of bananas is important to provide valuable information for further banana improvement. Studies on phylogeny and domestication of bananas revealed that current banana cultivars are the result of evolution through hybridization, mutation, domestication and adaptation that occurred over thousands of years; the processess are very complex involving multiple stages and separated by time and places. It led to great phenotype and genotype variation amongst cultivars in the region *e.g.* AA, AAA, AAB, ABB, BB, AT, AS, *etc.* (Carreel et al., 2002; De Langhe et al., 2009; Hřibová et al., 2011).

Morphological characteristics have long and often been used in many diversity studies of various organisms for classification, identification, description, characterization and to reconstruct relationships. In addition to morphological characters, various molecular techniques based on PCR by utilizing the deoxyribo nucleic acid (DNA) data has also been conducted to confirm and support the morphology results (De Jesus et al., 2013; Zulfahmi, 2013).

Molecular marker represents the presence of the nucleotide sequences which encodes a trait or specific characteristic. It provides information about the existence of conserved sequences in the genome which can be used in evolutionary biology to study how different organisms are related and how they evolved. To date, optimization and assortment of molecular markers has been developed to visualize the DNA polymorphism amongst species until sub species level (Chase et al., 1993; Hidayat & Pancoro, 2008; Ubaidillah & Sutrisno, 2009).

Source of DNA can be obtained from nuclear, chloroplasts, and mitochondrial genomes. Nuclear genome that is often used for genetic analysis is ribosomal DNA (rDNA). Ribosomal DNA is the coding region of the genome RNA component of the ribosome. It has three types of units including 18S (Small Sub-Unit), 5.8S and 26S (Large Sub Unit) that is encoded by a single transcription and separated into units by an internal transcribed spacer (ITS) (Baldwin et al., 1995; Vanderpoorten et al., 2006). The ITS region as a non-coding region has mild functional problems therefore its evolution occured in a more neutral and natural manner (Álvarez & Wendel, 2003). ITS region is easy to be amplified using universal primers, has high sensitivity due to its small size (300-800 bp) and high copy number in the genome (100-200 copies) (Baldwin et al.,1995). It has proven to be a useful source of characters for genetic variability and phylogenetic studies in many angiosperm families (Baldwin et al.,1995; Álvarez & Wendel, 2003); including Musaceae (De Jesus et al., 2013; Nwakanma et al., 2003; Irishs et al., 2009; Li et al, 2010; Liu et al, 2010; Ravishankar, et al., 2011; Hřibová et al., 2011; Ekasari et al., 2012; Jingyi et al., 2013; Sulistyaningsih et al., 2014)

The present study aims to analyze and evaluate the genetic variability and relationship amongst banana cultivars from East Java based on sequences of ITS region nrDNA which contains entire ITS region (ITS1 and ITS2) including the intervening 5.8S sub-unit (ITS1-5.8S-ITS2). The natural landscape of East Java Province is interesting, it is fragmented into several islands, including the mainland and some small/very small islands. Better knowledge and understanding of genetic variability and relationship of bananas from East Java will be useful to consider its genetic conservation strategy and for further banana cultivar improvement. Genetic variability is the basic material for adaptation, flexibility and responsiveness of evolution in facing various pressures including environmental stresses due to climate change, new pests or diseases, pollution, habitat destructions and fragmentation, and/or other ever-changing environmental conditions (Sumarno & Zuraida, 2008).

Nowadays, proper documentation of genetic variability of biological resources through bio-informatic tools are important in supporting both *in-situ* and *ex-situ* conservation. Storage of genetic information through a centralized GenBank DNA database will allow biodiversity data to be preserved and provide intellectual property protection and establish commercial benefits to owners of biological resources. This study also aimed to fill in the gaps of bio-informatics data of local banana cultivars from East Java, Indonesia.

Material and Methods

Plant materials

A total of 41 banana cultivars originating from East Java were sampled from the living collections at Purwodadi Botanic Garden – Indonesian Institute of Sciences, Pasuruan, East Java. The locality of banana cultivars from areas covering 15 regencies in East Java Province (Figure 1). It represented four genomic groups *i.e.* AA, AAA, AAB and ABB (Table 1). The leaves sample was selected from one individual per cultivar.

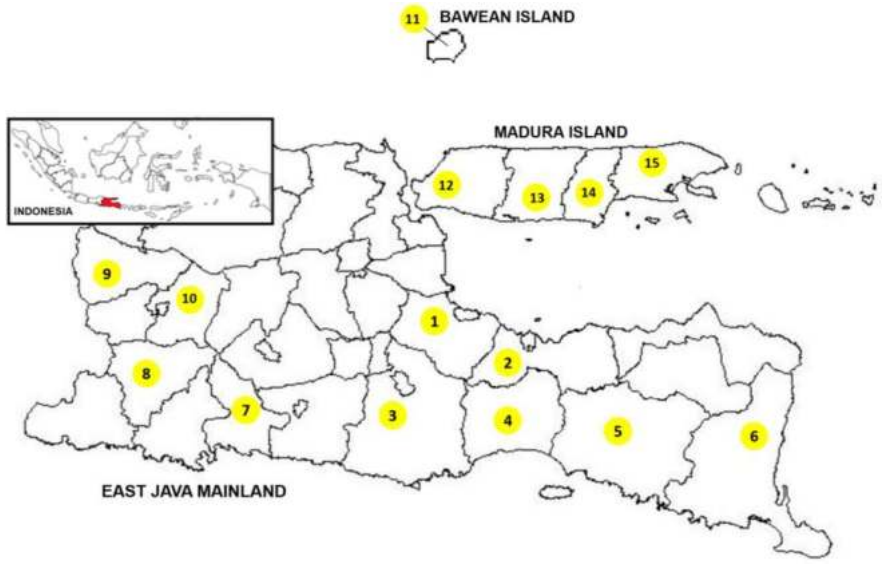

Figure 1. Map of banana cultivars locality from East Java Province: **1.** Pasuruan, **2.** Probolinggo, **3.** Malang, **4.** Lumajang, **5.** Jember, **6.** Banyuwangi, **7.** Tulungagung, **8.** Ponorogo, **9.** Ngawi, **10.** Madiun, **11.** Sangkapura (Bawean Island), **12.** Bangkalan (Madura Island), **13.** Sampang (Madura Island), **14.** Pamekasan (Madura Island), **15.** Sumenep (Madura Island).

Table 1. List of 41 banana cultivars from East Java examined

No.	Banana cultivars	Genomic group	Locality	Locality code
1	Sri	ABB	Pasuruan	PAS
2	Kates	ABB	Tutur, Pasuruan	PAS
3	Raja Prentel	ABB	Nongkojajar, Pasuruan	PAS
4	Sobo Londo	ABB	Purwodadi, Pasuruan	PAS
5	Sobo Awu	ABB	Purwodadi, Pasuruan	PAS
6	Raja Wesi	ABB	Ngawi	NGWI
7	Ebung	ABB	Siman, Ponorogo	PNRG
8	Gajih Bali	ABB	Kedungjajang, Jember	JBR
9	Susu Gabug	ABB	Sangkapura, Bawean Island, Gresik	BWN
10	Kusta Putih	ABB	Batuputih, Sumenep, Madura Island	SMNP
11	Sabeh Biru	ABB	Camplong, Sampang, Madura Island	SMPNG
12	Belindang	AAB	Batuputih, Sumenep, Madura Island	SMNP
13	Ongkap	AAB	Karang Penang, Sampang, Madura Island	SMPNG
14	Dokare	AA	Purwosari, Pasuruan	PAS
15	Jambe	AA	Tulungagung	TLGAG
16	Grito	AAA	Krucil, Probolinggo	PROB
17	Lilin	AA	Kabat, Banyuwangi	BWI
18	Satroli	AA	Krucil, Probolinggo	PROB
19	Raja Sri	AA	Glagah, Banyuwangi	BWI
20	Mas	AA	Kalibaru, Banyuwangi	BWI
21	Mas Kripik	AA	Senduro, Lumajang	LMJG
22	Gading	AA	Tragah, Bangkalan, Madura Island	BGKLN
23	Mas	AA	Galis, Bangkalan, Madura Island	BGKLN
24	Masang	AA	Tragah, Bangkalan, Madura Island	BGKLN
25	Madu	AA	Batuputih, Sumenep, Madura Island	SMNP
26	Pakak Merah	AA	Omben, Sampang, Madura Island	SMPNG
27	Kayu	AAA	Nongkojajar, Pasuruan	PAS
28	Santen	AAA	Nongkojajar, Pasuruan	PAS
29	Nangka	AAA	Purwodadi, Pasuruan	PAS
30	Agung	AAA	Krucil, Probolinggo	PROB
31	Kongkong	AAA	Lawang, Malang	MLG
32	Morosebo	AAA	Sarangan, Madiun	MDUN
33	Kidang	AAA	Kalisat, Jember	JBR
34	Embug	AAA	Ledokombo, Jember	JBR
35	Celakat	AAA	Glagah, Banyuwangi	BWI
36	Lumut	AAA	Batuputih, Sumenep, Madura Island	SMNP
37	Jabol	AAA	Lenteng, Sumenep, Madura Island	SMNP
38	Rosok	AAA	Arosbaya, Bangkalan, Madura Island	BGKLN
39	Nangkah	AAA	Waru, Pamekasan, Madura Island	PMKSN
40	Elang	AAA	Pegantenan, Pamekasan, Madura Island	PMKSN
41	Pakak Santen	AAA	Kadur, Pamekasan, Madura Island	PMKSN

Genomic group reference: Hapsari et al., 2015

Molecular analysis

Total genomic DNAs were extracted from fresh cigar leaf tissues using Promega Wizard® Genomic DNA Purification Kit (Madison, Wisconsin, USA) following the manufacturer's protocols. Amplification of the ITS region (ITS1 + 5.8S + ITS2) was accomplished using primer pairs of ITSL (5'-TCG TAA CAA GGT TTC CGT AGG TG-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al., 1990; Hsiao et al., 1994; Nwakanma et al., 2003). PCR reactions were conducted in a 30 µL volume contains of 15 µL of DreamTaq Green PCR Master Mix (2x) from Thermo Scientific, California, USA (Taq DNA polymerase, 2x DreamTaq Green buffer, 0.4 mM each of dNTPs and 4 mM $MgCl_2$), 3 µl of 5 pmol each of forward and reverse primers and 3 µl of nuclease-free water. PCR thermal cycling program used for ITS amplification consists of initial denaturation temperature at 95 °C for 3 minutes; followed by 25 cycles of denaturation for 30 seconds at 95 °C, annealing for 30 seconds at 53 ºC, and extension for 30 seconds at 72 ºC. Final extension carried out for 7 minutes at 72 ºC. Amplified products were then purified and sequenced at 1st BASE Laboratories Sdn Bhd, Malaysia using ABI PRISM 3730xl Genetic Analyzer developed by Applied Biosystems, USA.

Data analysis

ITS region DNA sequences data were evaluated using ABI sequences Scanner v.10. Multiple sequences alignments were performed using ClustalW program followed by visual adjustment (MEGA5.03 software). We used unrooted genetic relationship to reconstruct both the network and trees. Unrooted trees (no outgroup) are useful (and informative) to establish the conservancy and the variability among a set of sequences, also to draw a network of relationships among taxonomical units, in accordance with the purpose of this study. In addition, it is also not necessary to root a haplotype network by an outgroup. Outgroups provide the connection of the ingroup populations (species, genera, *etc.*) to a broader phylogeny. However, the relationships among the haplotype network will not change (Olalde et al., 2002). Genetic variability including nucleotide mutations were analyzed with DnaSP ver. 5.10.01. Median Joining analysis was employed using Haplotype Network 4.6.1.2. to reconstruct haplotype distribution map. Genetic relationship reconstructions were performed using MEGA5.03 based on evolution model of Kimura 2 parameter (K2P) using Neighbor Joining (NJ) and Maximum Parsimony (MP) algorithms with 1000 bootsrap replications; a pairwise distance analysis was also performed to generate genetic distances (Tamura et al., 2011; Seltman et al., 2003). Bootsrap support was categorized as strong (>85%), moderate (70-85%), weak (50-69%), and poor (<50%) (Kress et al., 2002).

Results and Discussion

ITS region amplifications & DNA sequences

Amplification of the ITS region using ITSL and ITS4 primers were successfully carried out on the 41 banana cultivars examined. Visualization on 1.5% agarose gel electrophoresis was shown by the presence of a specific DNA band in the sample lane at the length of 600 bp to 700 bp (Figure 2). DNA sequences length of ITS region in Angiosperms varied between 400 bp to 800 bp (Baldwin et al., 1995). The amplicon size of banana cultivars were similar to monocot plants such as rice, sorghum and wheat *i.e.* 591 bp, 588 bp and 603 bp respectively (Nwakanma et al., 2003; Hsiao et al., 1994).

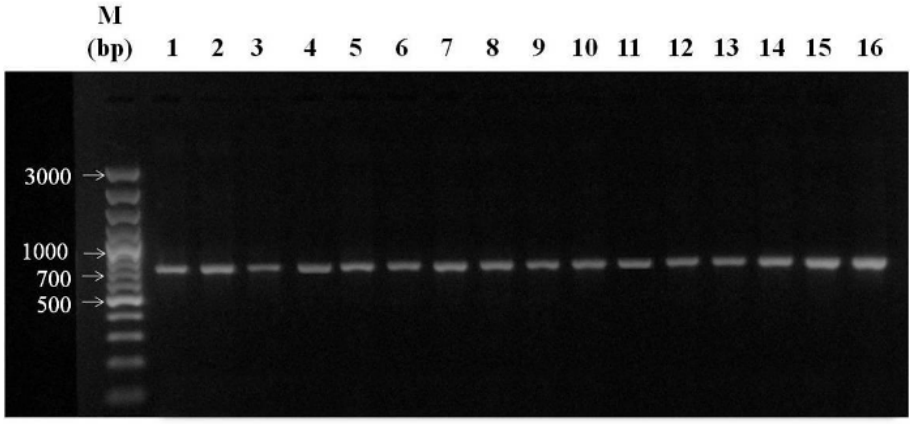

Figure 2. Electrophoregram amplicons of ITS region of some banana cultivars examined on agarose gel 1,5 %. M= marker. Lane 1= Susu Gabug, 2=Madu, 3=Pakak Santen, 4=Kusta Putih, 5=Belindang, 6=Lumut, 7=Jabol, 8=Nangkah, 9=Elang, 10=Sabeh Biru, 11=Pakak Merah, 12=Ongkap, 13=Rosok, 14=Masang, 15=Gading, 16=Mas.

Sequencing on ITS region amplicons of 41 banana cultivars from this study produced DNA sequences with sizes of 631 bp to 651 bp (Table 2). The ITS sequence size of banana cultivars was in accordance with the ITS sequence size of wild bananas which ranged 599 bp to 697 bp (Sulistyaningsih et al., 2014). Based on Basic Local Alignment Search Tool (BLAST) on NCBI, all data DNA sequences of 41 banana cultivars were homologues with ITS region in Musaceae family (similarity ≥92%). It was not found with contaminant of endophytic fungi. All 41 banana cultivars ITS sequences were subjected to genetic variability analysis and relationship reconstruction.

Table 2. Nucleotide composition of 41 banana cultivars from East Java

No.	Banana cultivars	Total sequences	Base (%)			
			T(U)	C	A	G
1	Sri (ABB) PAS	650	17,4	29,2	20,2	33,2
2	Kates (ABB) PAS	645	17,5	30,1	20,0	32,4
3	Raja Prentel (ABB) PAS	650	17,2	29,2	19,5	34,0
4	Sobo Londo (ABB) PAS	643	17,4	29,4	19,0	34,2
5	Sobo Awu (ABB) PAS	648	17,4	29,5	19,9	33,2
6	Raja Wesi (ABB) NGWI	650	18,5	29,4	20,2	32,0
7	Ebung (ABB) PNRG	649	18,0	30,7	21,0	30,4
8	Gajih Bali (ABB) JBR	650	17,8	29,2	20,5	32,5
9	Susu Gabug (ABB) BWN	648	17,1	29,0	19,8	34,1
10	Kusta Putih (ABB) SMNP	648	17,1	29,2	19,3	34,4
11	Sabeh Biru (ABB) SMPNG	648	17,1	29,3	19,3	34,3
12	Belindang (AAB) SMNP	651	16,9	29,3	20,4	33,3
13	Ongkap (AAB) SMPNG	650	16,8	29,2	20,3	33,7
14	Dokare (AA) PAS	649	17,4	29,0	20,3	33,3
15	Jambe (AA) TLGAG	640	17,2	29,7	20,6	32,5
16	Grito (AAA) PROB	650	15,7	30,0	20,9	33,4
17	Lilin (AA) BWI	650	17,2	28,6	19,5	34,6
18	Satroli (AA) PROB	645	18,3	30,1	18,4	33,2
19	Raja Sri (AA) BWI	649	18,0	29,0	19,6	33,4
20	Mas (AA) BWI	649	17,1	29,6	19,4	33,9
21	Mas Kripik (AA) LMJG	648	17,6	29,8	19,1	33,5
22	Gading (AA) BGKLN	650	17,8	28,5	20,2	33,5
23	Mas (AA) BGKLN	650	17,1	29,4	19,7	33,8
24	Masang (AA) BGKLN	631	17,1	29,3	19,2	34,4
25	Madu (AA) SMNP	647	17,0	29,5	19,9	33,5
26	Pakak Merah (AA) SMPNG	643	17,4	28,1	20,5	33,9
27	Kayu (AAA) PAS	646	16,4	30,5	20,9	32,2
28	Santen (AAA) PAS	647	16,8	29,2	21,6	32,3
29	Nangka (AAA) PAS	647	18,1	29,1	21,2	31,7
30	Agung (AAA) PROB	645	17,7	30,5	19,4	32,4
31	Kongkong (AAA) MLG	648	14,8	29,8	20,4	35,0
32	Morosebo (AAA) MDUN	643	15,6	29,7	20,4	34,4
33	Kidang (AAA) JBR	651	17,4	29,8	20,0	32,9
34	Embug (AAA) JBR	640	16,6	29,8	19,2	34,4
35	Celakat (AAA) BWI	651	16,6	30,1	20,3	33,0
36	Lumut (AAA) SMNP	646	18,1	29,4	22,9	29,6
37	Jabol (AAA) SMNP	649	16,8	31,1	20,2	31,9
38	Rosok (AAA) BGKLAN	630	15,7	29,4	20,6	34,3
39	Nangkah (AAA) PMSKN	640	16,4	29,1	20,3	34,2
40	Elang (AAA) PMSKN	643	16,3	30,3	19,1	34,2
41	Pakak Santen (AAA) PMKSN	648	16,5	29,8	21,0	32,7
Average		646,5	17,1	29,5	20,1	33,3

Notes: G = Guanin, A= Adenin, C= Cytosin, T= Thymin, U= Uracil

Genetic variability

The total aligned and selected ITS region DNA sequences length of 41 banana cultivars was 656 bp positions. Of those, 346 positions (52.74%) were identified as conserved region (invariable/monomorphic), 223 positions (33.99%) were potential variable sites (polymorphic) and 87 positions (13.26%) were alignment gaps or missing data. About 143 positions (64.13%) of the variable positions were potentially parsimony informative and 80 positions (35.87%) were singleton variables. The ITS region DNA sequences of 41 banana cultivars showed high variability with neutral conservation level so that become valuable characters to reconstruct genetic relationships.

The nucleotide composition of ITS region in 41 banana cultivars were high in G+C bases content with an average of 62.62% in *Musa* ABB group, 62.80% in *Musa* AAB group, 62.89% in *Musa* AA group and 62.85% in *Musa* AAA group. At average, G+C bases content of 41 banana cultivars was 62.79% (Table 2). ITS region as non-coding region or intron was known to have high G+C content because it was associated with their relative functions in transcription and translation. DNA sequences with higher G+C content are hotspots of mutation, C base is often methylated and occured errors during multiplication. DNA methylation is an addition reaction of a methyl group at the 5' end of C base covalently. Methylation was induced substitution mutation in the form of transition *i.e.* C base to T/U (Ubaidillah & Sutrisno, 2009; Nusifera, 2007). Methylation events and/or the addition of other alkyls in the DNA are the major causes of mutations in many organisms.

DNA sequences with singletone variable positions is DNA sequence variation where point mutation occured only in one operating taxonomic unit (OTU) (Hidayat & Pancoro, 2008). Mutation events analysis in singleton variable positions revealed that variation of ITS sequences amongst banana cultivars in this study were mostly contributed by point mutations in form of substitution (Table 3). About 73 positions of the singleton variables comprised of two variants point mutations and in 7 positions there were three variants point mutations. The subtitution events were 51 sites in form of transversion *i.e.* the substitution of a (two rings) purine for a (one ring) pyrimidine or vice versa; and 49 sites in form of transition *i.e.* a point mutation that changes a purine nucleotide to another purine (A ↔ G) or a pyrimidine nucleotide to another pyrimidine (C ↔ T). About 8 mutation events from C to T occured pressumable due to methylation (Table 3). It was reported that only minor proportion of indels affected to the variation of ITS sequences in Angiosperms (Baldwin et al., 1995).

Table 3. Singletone variations of ITS region DNA sequences of 26 banana cultivars

No.	Banana cultivars	Base position	Base mutation	Total
1	Lumut (AAA) SMNP	92, 101, 104, 108, 113, 116, 136, 168, 178,218, 133, 316, 418, 329	C → T, G → A, G → A, G → A, G → A, G → A, C → T, G → A, G → A, G → C, G → T, G → T, A → G	14
2	Elang (AAA) PMSKN	358, 530, 593, 537, 638, 543, 563, 545, 565, 462, 474, 556, 596	C → G, C → A, C → A, C → T, C → T, G → A, G → A, A → C, A → C, T → G, G → C, G → C, A → G	13
3	Nangka (AAA) PAS	243, 244, 341, 429, 245, 377, 269, 281, 362	G → T, G → C, G → C, G → C, C → T, C → T, C → A, C → G	9
4	Santen (AAA) PAS	536, 574, 564, 573, 598, 593, 596, 638	G → T, G → T, G → A, C → G, C → G, C → A, A → G, C → T	8
5	Kates (ABB) PAS	1, 6, 7, 264, 311, 530	T → C, G → A, A → G, C → A, A → C, C → T	6
6	Masang (AA) BGKLN	471, 630, 590, 624, 634, 636	A → G, A → G, C → T, C → T, T → A, T → G	6
7	Ebung (ABB) PNRG	108, 243, 110, 170, 270	G → C, G → C, G → A, G → A, G → A	5
8	Satroli (AA) PROB	218, 304, 410, 642, 647	G → T, G → C, G → C, T → C, A → G	5
9	Pakak Merah (AA) SMPNG	31, 32, 223, 379	G → T, A → G, C → T, C → T	4
10	Gajih Bali (ABB) JBR	130, 151, 157, 265	G → C, C → A, C → A, G → A	4
11	Raja Wesi (ABB) NGWI	200, 309, 464	C → T, C → A, G → C	3
12	Rosok (AAA) BGKLAN	481, 516, 572	C → G, T → C, T → C	3
13	Kongkong (AAA) MLG	499, 640, 642	A → G, T → A, T → A	3
14	Susu Gabug (ABB) BWN	14, 63	C → T, T → A	2
15	Morosebo (AAA) MDUN	82, 341	T → G, G → A	2
16	Jabol (AAA) SMNP	205, 234	G → C, G → C	2
17	Agung (AAA) PROB	482, 635	G → T, A → T	2
18	Nangkah (AAA) PMSKN	537, 620	C → G, C → T	2
19	Kidang (AAA) JBR	266	T → A	1
20	Embug (AAA) JBR	288	T → G	1
21	Lilin (AA) BWI	353	A → G	1
22	Raja Sri (AA) BWI	383	C → T	1
23	Mas (AA) BWI	569	A → C	1
24	Kayu (AAA) PAS	460	G → T	1
25	Dokare (AA) PAS	465	C → G	1
26	Jambe (AA) TLGAG	555	G → A	1

Notes: G = Guanin, A= Adenin, C= Cytosin, T= Thymin

Singletone variations were found in 26 out of 41 banana cultivars ITS sequences. Those singletone variation sequences are presented in Table 3. It may be proposed as identification barcodes for each particular banana cultivar. Pisang Lumut is a cultivar with the highest singletone variation events (14 events) followed by Pisang Elang (13 events). Interestingly, they both originate from Madura Island but in different regions, *i.e.* Sumenep and Pamekasan respectively. In addition, Pisang Nangka (Pasuruan), Pisang Santen (Pasuruan), Pisang Kates (Pasuruan) and Pisang Masang (Bangkalan, Madura) also have high singletone variations with mutation events of 9, 8, 6, and 6 respectively (Table 3).

Mutations are essential to evolution. Mutations can be caused by high-energy sources such as radiation, chemicals, high pressures of environment, or appear spontaneously during DNA replication, *etc*. Beneficial mutations allow an organism to reproduce more effectively and adapt well to a changing environment (Carlin, 2011). Banana cultivar is asexually reproductive by corms, therefore it can maintain the mutated genetic constituent. However, domestication, human selection and migration also add evolution aspects in banana cultivars. Therefore, It is presumed that banana cultivars with high singletone variables found in this study may be carrying adaptive alleles to their specific habitats, and resulted variation. Particularly in Madura Island, which has a dry ecotype in tropical weather, limy land, low rainfall, and low soil productivity (Rochana, 2012).

Haplotype diversity and distribution

Haplotype is a set of specific DNA sequences in the cluster associated with a particular gene on a chromosome that is most likely inherited together or comes from a the common ancestor (Seltman et al., 2003). Haplotype network represent evolutionary relationships (genealogies) to intraspesific level (Mardulyn, 2012). Haplotype (gene) diversity based on ITS region DNA sequences of 41 banana cultivars from East Java was very high (Hd = 1.00) with variance 0,00003 and standard deviation 0,005. It resulted in 41 haplotypes with none of the haplogroup (Figure 3). This study revealed the lineage pattern of banana cultivars from East Java Province including East Java mainland, Bawean Island and Madura Island; it was inter-connected and clustered according to its genomic group into 3 clusters.

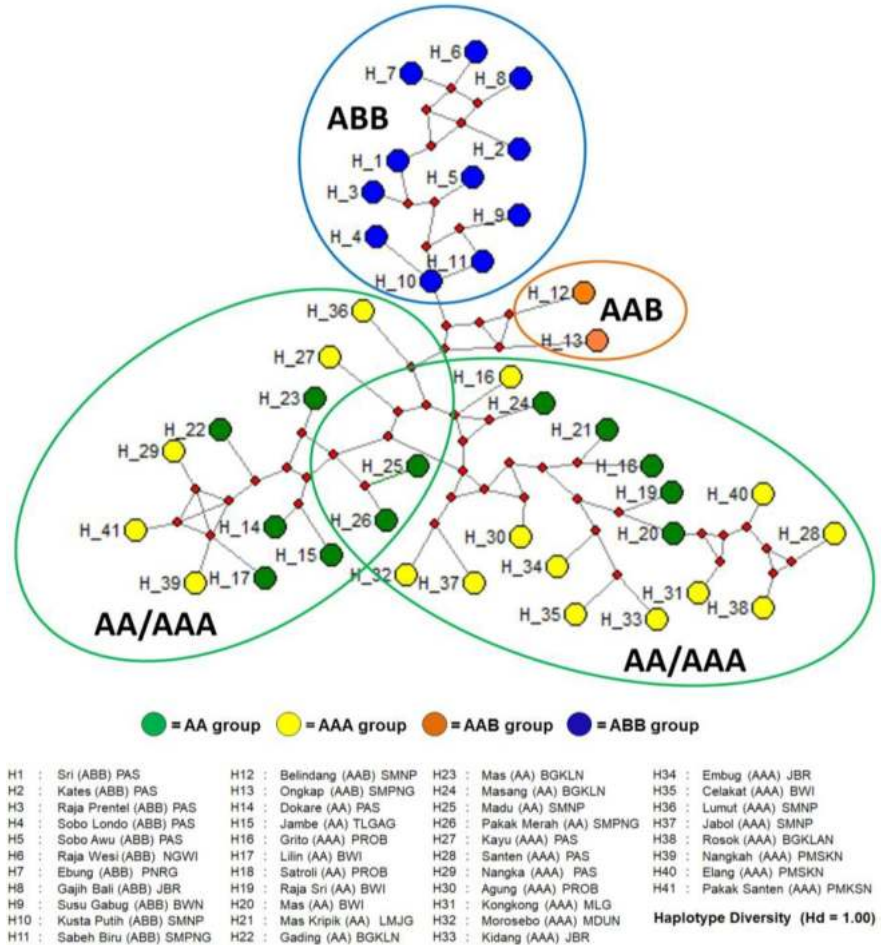

Figure 3. Haplotype distribution map of 41 banana cultivars from East Java based on ITS region DNA sequences.

Geologically, East Java mainland also the islands of Madura and Bawean (the study areas) are included as Great Sunda Island plate/ Sundaland (Verbeek & Fennema, 1896; Van Bemellen, 1949; Usman, 2012). Madura Island is a continuation of the Solo limestone mountains which is now separated by Madura Strait. Whereas Bawean Island is formed from the remains of an old volcano located near its centre which is now separated by Java Island (Verbeek & Fennema, 1896; Rochana 2012; Figure 1). Island biogeography is the study of the factors affecting species diversity of natural communities. According to the island biogeography theory by MacArthur & Wilson (1967), the

species diversity on the island was determined by the island area, also equilibrium numbers between the average rate of local extinction with the rate of migration to the islands as well as the island's isolation level. Since then, all 41 banana cultivars examined were presummably derived from common ancestors and same population in East Java mainland which then experienced evolution process, dispersed by human migration both in and out, and got isolated to the islands consequently resulting in rich haplotype diversity (Figure 3).

The haplotype distribution map showed that banana cultivars of AA were nested together with AAA group. Furthermore, the network of banana cultivars of AA and AAA groups were directly connected with banana cultivars of AAB group, and AAB group was directly connected with banana cultivars of ABB group. Hence, the AAB group bananas served as intermediate bananas which connect both B genome bananas and A genome bananas. These findings support previous studies by De Langhe et al. (2009) that during evolution, banana cultivars may first exist from clone selection of wild cultivated population and then in regions where the diffusion of plants occurred (exchange or via human migration) hybridization between wild cultivated populations and partly fertile clones from different origins led to the generation of more sterile AA diploids, and the more vigorous and nearly sterile triploids. The *Musa* AA group (edible diploid) emerged first, followed by *Musa* AAA group, *Musa* AAB group and later on *Musa* ABB group. Selection among those new diploid and triploid populations produced new cultivars. Those new cultivars can undergo somatic mutation, thus leading to new derived cultivars.

According to the haplotype network map, there are two main sub-groups of *Musa* AA group *i.e*. Sub-group 1 consist of Pisang Lilin, Gading, Dokare, Jambe, Mas Bangkalan, Madu and Pakak Merah; and Sub-group 2 consists of Pisang Mas Kripik, Raja Sri, Satroli, Mas Banyuwangi, Grito and Masang (Figure 3). Pisang Masang appeared as a derived cultivar of Pisang Mas. In addition, Pisang Mas from Bangkalan (Madura Island) was separated into a different sub-group with Pisang Mas from Banyuwangi (East Java Mainland); it is indicated that they both are genetically different due to variation although they have same name.

Musa AAA group consists of two main sub-groups *i.e*. Sub-group 1 comprises of Pisang Nangka, Nangkah, Kayu, Lumut, Morosebo and Jabol; and Sub-group 2 comprises of Pisang Celakat, Kidang, Embug, Agung, Elang, Rosok, Santen and Kongkong (Figure 3). Pisang Nangka from Pasuruan were placed in the same

sub-group and are directly connected with Pisang Nangkah from Pamekasan, Madura Island; it is indicated that they both have the same lineage, are separated by distance, but experienced less of variation. Meanwhile, Musa AAB group number was very limited to only 2 cultivars *i.e*. Pisang Belindang and Ongkap, both originating from Madura Island (Figure 3). Based on previous studies it was known that many of bananas morphologically identified as AAB, once confirmed molecularly were identified as AAA (Hapsari et al. 2015b). In addition, all banana cultivars of ABB were consistantly clustered in one sub-group, in which Pisang Kusta Putih appeared become the basic cultivar of banana ABB group studied because all of other ABB bananas were connected to it (Figure 3).

Genetic relationship

ITS region is bi-parental inherited and is more informative in revealing the evolutionary history of organisms that naturally involves crossing both inter and intra species, also occurrence of polyploidy and hybrid speciation events like in Musaceae family (Alvarez & Wendel, 2003). This study has proven that ITS region nrDNA sequences were powerful to classify to the cultivars level of bananas.

NJ algorithm analyze the data by converting the DNA sequences into distance matrix based on differences in pairs between sequences while MP algorithm using sequences character directly in which total number of character-state changes is to be preferred (Ubaidillah & Sutrisno, 2009; Tamura et al., 2011; Hall, 2008). Based on the trees resulting from this study, both algorithms can be used to explain the relationship of banana cultivars from East Java. They both resulted in trees which were separated and clustered according to genomic group. However, comparatively NJ algorithm constructed a genetic relationship tree that was better than MP algorithm in classifying and clustering of bananas from East Java (Figure 4).

Genetic relationship analysis of banana cultivars from East Java based on ITS sequences using NJ algorithm resulted in a tree which separated into 3 main clades in accordance to its genomic group classification. The first clade consists of cultivars of ABB group, and it became the outgroup and supported by strong bootstrap. The second clade consists of 2 cultivars of AAB group supported by moderate bootsrap. Third group consists of cultivars of AA and AAA groups, and supported by low bootstrap (Figure 4A). The relationship tree using MP algorithm was separated only into 2 main clades, in which the AAB group is nested in the second clade together with AA and AAA groups. The

separation of the second clade (AA, AAA and AAB groups) is supported by strong bootsrap but low bootrap support in its sub-clades separation (Figure 4B).

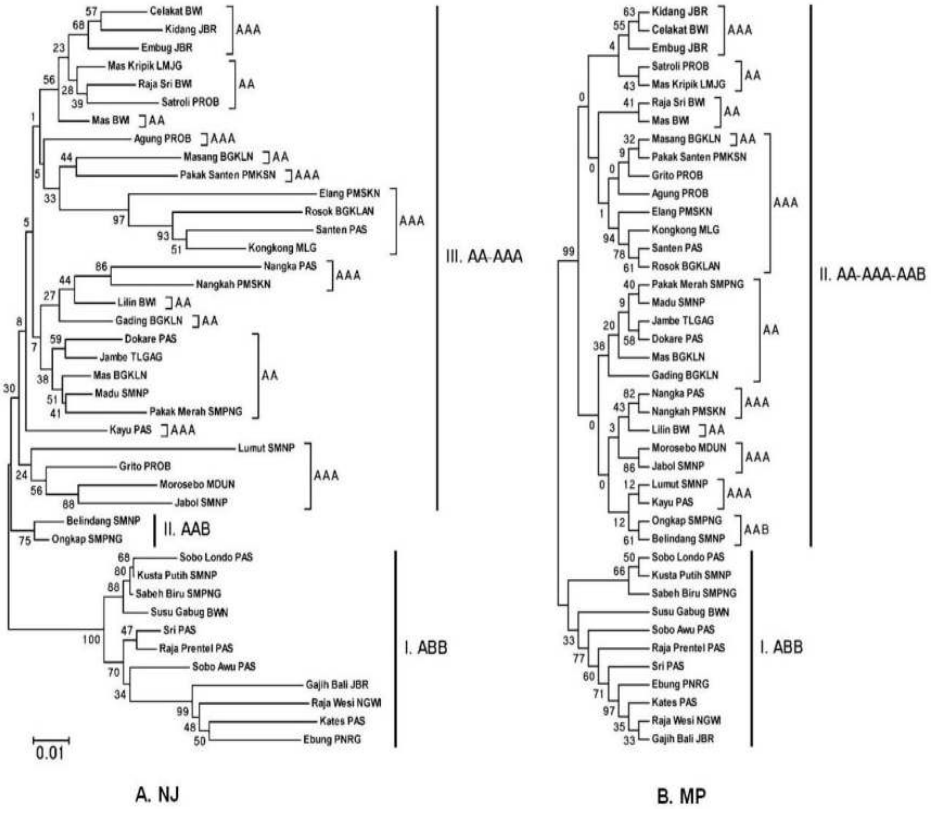

Figure 4. Relationship trees of 41 banana cultivars from East Java based on ITS region nrDNA sequences: **A)** Neighbor Joining (NJ) and **B)** Maximum Parsimony (MP)

The ABB clade was separated into 2 sub-clades according to its genetic similarity (Figure 4). Genetic similarity amongst members of Musa ABB group were 90.65% to 99.82%, in which Pisang Sabeh Biru x Kusta Putih was the closest pair while Pisang Gajih Putih x Sobo Londo was the the farthest pair. Banana cultivars with A genome were not clearly separated, *Musa* AA group

was nested together with *Musa* AAA group. However, their relationship tree had tended to separated into two main sub-groups similar to haplotype's grouping (Figure 4). Genetic similarity of AA/AAA group were 88.20% to 97.50%, in which Pisang Embug (AAA) and Mas Kripik (AA) was the closest pair whereas Pisang Santen (AAA) and Pakak Merah (AA) was the farthest pair. Pisang Belindang and Ongkap as AAB clade was related closely to AA/AAA clade with genetic similarity of 88.63% to 97.31% compared to the ABB group with genetic similarity of 89.23% to 95.28%.

The assessment of genetic variation and distance provides important information for conservation on a genetic basis. In terms of conservation genetics, it is focused mainly on the protection and maintanance of genetic variation for further evolvability (Woodruf, 2001). Banana cultivars with high genetic variability and far genetic distance or low similarity are prioritized for conservation. Referring to the genetic similarity value of Pisang Santen (AAA) x Pisang Kates (ABB), these were the farthest pair with genetic similarity 81.51% followed by Pisang Masang (AA) x Pisang Kates (ABB) with genetic similarity of 86.52%. Therefore, those banana cultivars were prioritized for conservation both *in-situ*/*on-farm* and *ex-situ*. In addition, if conservation resources are limited, any banana cultivars which are very closely related (high similarity) should be chosen with one of them as representative. Pisang Sabeh Biru (ABB) x Kusta Putih (ABB) is the closest pair with genetic similarity 99.82%, and are both from Madura Island. However, since they both had different haplotype, it is necessary for these to be conserved.

Conclusions

This study has proven that ITS region nrDNA sequences was useful as a source of characters for genetic variability and phylogenetic studies of bananas. It was powerful to classify until the cultivar level of bananas. Genetic variability of 41 banana cultivars from East Java based on ITS region DNA sequences were very rich. Haplotype analysis resulted in 41 haplotypes with none of haplogroup. Haplotype distribution and genetic relationship analyses revealed the lineage pattern of banana cultivars from East Java. It was connected and clustered according to its genomic group. They were presummably derived from the common ancestors and same population in East Java mainland which then experienced evolution process, dispersed by human migration both in and out, and got isolated to the islands. Genetic relationship analyses resulted in trees which were also clustered according to their geomic group. However, NJ

algorithm method constructed a relationship tree and classification better than MP algorithm in classifying and clustering of bananas from East Java.

ITS sequences data of 41 banana cultivars from East Java from this study were deposited at the National Center for Biotechnology Information (NCBI) with GenBank accessions numbers KT696446 to KT696490. It will allow the genetic diversity of local bananas data to remain preserved and also to provide intellectual property protection. This study recommended that all of the 41 banana cultivars from East Java are necessary for genetics conservation. Conservation efforts both *in-situ* and *ex-situ* to all local *Musa* germplasms are needed to maintain the genetic resources for further banana improvement.

Acknowledgements

This research was funded by the Indonesia Endowment Fund for Education, Ministry of Finance, Republic of Indonesia with Graduate Research Fellowship grant number PRJ-541/LPDP/2013. The authors also greatly acknowledge Purwodadi Botanic Garden, Indonesian Institute of Sciences for providing the plant materials studied and Plant Physiology Laboratory of Biology Department, University of Brawijaya for the molecular and genetic facilities. Sincere thanks to Didik Wahyudi, M.Si. for all technical help and valuable discussions during the study.

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Journal of Tropical Biology and Conservation 15:121–137, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Assemblage Structure of Palaeotropical Frugivorous Bats at Mineral Licks Sites in Deramakot and Tangkulap Forest Reserve, Sabah

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Abstract

Few studies have been done on natural mineral licks visitation on bat fauna, particularly in Borneo and Southeast Asia in general. Little is known about the assemblage of bats using mineral licks and this study was done to determine assemblage structure of Palaeotropical bats at six established mineral licks in Deramakot and Tangkulap Forest Reserve, Sabah. The main findings of the present study revealed that Palaeotropical frugivorous bats were using mineral licks, observed through their behaviour of drinking from mineral licks, supported by their high species occurrences at mineral licks and higher concentration of water insoluble soil tracer elements, Al and Si detected in their faeces in comparison with non-visitor bats. The five species of bats *Macroglos susminimus* (n=3), *Balionycteris maculata* (n=2), *Cynopterus brachyotis* (n=1), *Megaerops ecaudatus* (n=2) and *Penthetor lucasii n=(1*) were observed drinking from mineral licks. Four species of frugivorous bats (*M. minimus, B. maculata, C. brachyotis* and *P. lucasii*) frequently occurred at all six sites at mineral licks. In addition, there were higher enrichment Al and Si in *M. minimus* faeces (n=5) in comparison with non-visitor bats suggesting that frugivorous bats got those elements from ingestion of mineral lick muddy water.

Keywords: Palaeotropical frugivorous bats; mineral licks; Deramakot and Tangkulap Forest Reserve; Sabah

Introduction

Mineral licks are distinct elements in the natural landscape which are present in both temperate and tropical ecosystems (Link et al., 2011; Molina et al., 2013), arctic ecosystem (Ramachandran 1995; Calef & Grant, 1975) and in montane ecosystem (Ramachandran, 1995). Mineral licks are considered keystone resources and act as limiting resources in a particular habitat for

Received 10 January 2018
Reviewed 21 February 2018
Accepted 09 April 2018
Published 15 October 2018

many wildlife species (Montenegro, 2004). Thus, they are ecologically important for various wildlife (Molina et al., 2013; Rea et al., 2004; Panichev et al., 2002). Generally, mineral licks are mineral-rich places that are long lasting and seasonally stable where animals frequently and actively visit to consume earthly minerals (Hon & Shibata, 2013; Ping et al., 2011; Link et al., 2011; Bravo et al., 2010b).

Animals do lick from clay-enriched muddy spring water or eat mineral-rich soils in order to obtain minerals such as sodium, calcium, potassium, magnesium and clay minerals (Brightsmith et al., 2008; Burger & Gochfeld, 2003; Klaus & Schmid, 1998). The most common reason for this behaviour is as strategy for mineral nutrient supplementation. Studies state that soils enriched with minerals are important for physiological processes of the body, such as pregnancy and lactation (Voigt et al., 2007). Other than that, mineral lick soil or water provide essential elements that aid in detoxification of noxious or unpalatable compounds present in the diet through absorbtion of dietary toxins and plant metabolites, aid in the digestive tract such as alleviate gastrointestinal upsets like diarrhoea, means of dealing with excess acidity in the digestive tract, and ease the digestion process of animals (Slamova et al., 2011).

Other than physiological benefits, mineral licks have conservation implications (Rea et al., 2004) since licks may affect the distribution (Panichev et al., 2002), density (Molina et al., 2003; Ping et al., 2011) and temporal structure of animal populations (Panichev et al., 2002; Rea et al., 2004; Ghanem, 2012). Furthermore, mineral licks are reported to provide a social role in inducing visitations of animals to mineral licks including a variety of terrestrial vertebrates (mammals, birds, reptiles) and also invertebrates (Blake et al., 2010; Morales, 2009; Voigt et al., 2008; Wilson, 2003).

In Neotropical regions, Neotropical frugivorous bats were reported to frequently use mineral licks (Ghanem, 2012; Bravo et al., 2012; Bravo et al., 2010a; Bravo et al., 2010b; Bravo et al., 2008; Voigt et al., 2008). Insectivorous bats were not reported to use mineral licks (Gnahem, 2013; Voigt et al., 2008). However, such information is scarce in this region. Since animal response toward licks vary seasonally and geographically (Rice et al., 2010), studies on visitation of mineral licks by bats across regions are essential to further understand its utilization.

There are a multitude of knowledge gaps in mineral lick utilization and significance, particularly in Southeast Asian bats. The presence, visitation and usage of mineral licks for bats remain unclear in Southeast Asia, particularly in Borneo. Soil of Bornean tropical rain forests tend to be nutrient-poor (Matsubayashi et al., 2007a; Klaus et al., 1998). Thus, plants that grow on such soils do not contain as much minerals such as sodium. Therefore, the mammals of tropical rain forests, especially herbivores and frugivores utilise mineral licks for mineral supplement. Matsubayashi et al. (2007b) and (2011) also state the importance of mineral licks for reproductive support of mammals. Studies on mineral licks and mammals were done in Deramakot Forest Reserve but bats were excluded (Ishige et al., 2017; Matsubayashi et al., 2011; 2007a; 2007b).

Palaeotropical bat assemblage at mineral licks in Deramakot and Tangkulap Forest Reserve, Sabah was documented to evince the utilisation of mineral licks by bats, and to identify the bat community that visit these mineral licks. The results from this study are reported herein. In this study, frugivorous bats refer to herbivorous bats that have fruit and nectar diets.

Methodology

Nine sampling sites were identified: six representing mineral lick sites, two representing control sites and one site in the forest. The mineral lick sites were located in Deramakot Forest Reserve (five sites) and Tangkulap Forest Reserve (one site). Mineral licks in the respective sites were labelled as ML1 (5°27'N, 117°12'E), ML2 (5°19'N, 117°28'E), ML3 (5°22'N, 117°29'E), ML4 (5°20'N, 117°30'E), ML5 and ML6 (5°29'N, 117°34'E) (Figure 1). The control wallow site, CW1, was located approximately 800m from ML2 while CW2 was located approximately 1km away from ML5 and ML6. Both sites were surrounded by thick shrubs and yam plants with stagnant water appearing on surface.

Bats were sampled using two methods: mist net and 4-bank harp trap. At each sampling site, 2 mist-nets and 1 harp trap were established covering the pools and following the paths of bats around the sites. The mist net used in this study was the Khon Kean Fishing Net mist net, Twine number 2, measuring 2.5 x 9 x 4 with three shelves, and the mesh size was 2.5 mm. The mist nets were tied with adjustable poles for support. Meanwhile, the harp trap used consisted of four-bank, with monofilament-fishing lines (0.22 mm, 10 lb) strung vertically and spaced 2-3 cm apart. Mist nets were used to catch

Megachiroptera bats while the harp trap targeted Microchiroptera bats. Captured bats were placed inside individual bags, sexed, measured and weighed. Bat identification was done following Struebig and Sujarno (2006), Yasuma et al., (2005a), Yasuma et al., (2005b) and Payne and Francis (2007). Bats were marked on the right wing using a 3mm biopsy punch, which allow recaptures to be recognized. No sample tissues were taken during this process. Bats were released at the point of capture within a 12 hour period. For faeces collection, bats caught during the sampling session in the mineral lick and forest sites were kept inside the cloth bags for 1-3 hours to collect their faeces, one bag per individual. The faeces were collected using forceps, and each individual faeces was placed in a labelled eppendorf tube 1.5ml with 70% alcohol until further analysis.

Methods that were used to determine the captured bats using the mineral licks were behaviour observations, species occurrences and insoluble soil tracer test in bats faeces. For behaviour observation, behaviours of bats at mineral licks were recorded using the *ad. libitum* sampling method. This method aimed to determine the behaviour of bats while using the mineral licks. Any behaviour performed by bats at mineral licks was individually recorded together with time (Altman, 1974). One hour was spent per night for observations in each mineral lick site. Another observation was made after bats had been processed and released at the mineral lick.

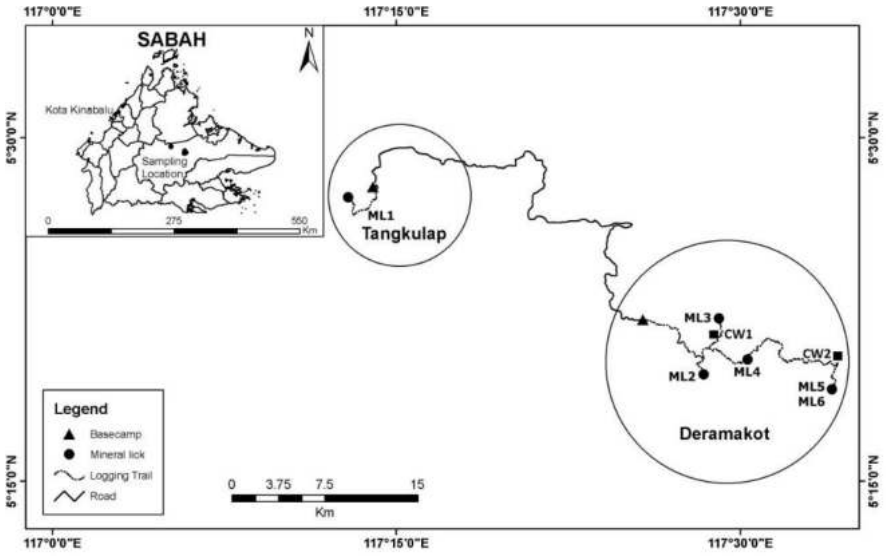

Figure 1. Location of mineral lick sampling sites in Sabah, Borneo, Malaysia.
*Mineral lick sites: ML1, ML2, ML3, ML4, ML5, and ML6; Wallow sites: CW1 and CW2.

All bats caught at mineral licks may not use mineral licks as few bats were seen drinking from mineral licks. Through this, bats that randomly flew around mineral licks and mineral licks users were distinguished. The first method was through species occurrences. Control sites were determined as a part of field sampling design in order to make a comparison between bats occurrences at mineral and non-mineral licks. In this study, wallows were chosen as the controls as their attributes are similar to wet licks. However, muddy depression of wallows created by ungulates is not made specifically for earth consumption. Common bats at mineral licks were expected to be caught more at mineral licks (as they frequently occurred mineral licks) and scored higher species occurrences similiarity in comparison with bats caught at wallow sites. This was adapted from Bravo et al. (2010) and Bravo et al. (2008) where bat-capture frequency was higher at mineral licks compared to non-mineral lick sites, hence indicating that they are mineral lick users.

The second method was through faecal analysis of bats caught at mineral licks and forest control sites. In Neotropics, insectivorous bats were not reported to use mineral licks (Gnahem, 2013; Gnahem et al., 2013; Bravo et al., 2008). Faeces of insectivorous bats caught at mineral licks and forest site were used as the control. Faecal analysis was done by using insoluble soil tracer elements, aluminium, Al (Gnahem, 2013) and silica, Si (Panichev et al., 2002) to detect soil consumption by bats. Al and Si are elements that are commonly used to determine the consumption of soil in humans because these elements are not metabolized or are poorly absorbed in the gut (Abraham, 2013; Darvis & Mirick, 2006). Bats which use mineral licks were expected to ingest soils, and contain higher concentration of insoluble soil tracer elements in their faeces (Gnahem, 2013). In a study conducted by Gnahem (2013a), faecal analysis was used to measure concentrations of insoluble soil tracer in bats. In this study, faeces samples 0.07g were used, labelled and underwent a series of acid digestion and heat using hydrogen peroxide (analytical grade), nitric acid (70%), hydroflouric acid (40%), and perchloric acid (analytical grade). Next, the solutions were filtered using 0.45µm (JET BIOFIL) and then further analysed in inductively coupled plasma-optical emission spectrometer (ICP-OES).

Data analysis

The correlation between mineral lick sites and species occurrences for both frugivorous bats and insectivorous bats were determined using the non-parametric test, Spearman's Rank Order Correlation, which was generated using SPSS v.21 (Gnahem, 2013; Bravo et al., 2008). The pattern of species similiarities among all six mineral lick sites were determined by using Bray-

curtis similiarity index generated using estimateS (Gnahem, 2013; Bravo et al., 2008). The capture rate index of bats was calculated after the following equation, adapted from Gnahem (2013a) and Bravo et al. (2008) to examine the overall occurrences of bats at mineral lick and wallow sites.

$$\text{Capture rate} = \frac{\text{Bats}}{\text{total number of traps}} \Big/ \text{total number of sampling nights}$$

Results and Discussion

The overall sampling effort recorded was 42 trapping nights, pooled across all sampling sites. Total bats caught was 94 bats (91 individuals at mineral lick sites and 3 individuals at wallow sites). No recaptured individuals were recorded during the sampling sessions.

Behaviour Observation

A total of nine individuals from five species of frugivorous bats were observed flying close to the ground and licking mineral lick pools. These were *Macroglossus minimus, Balionycteris maculata, Cynopterus brachyotis, Penthetor lucasii* and *Megaerops ecaudatus*. Observation was conducted in the morning upon the release of the captured bats, where they then descended to drink from the licks. Their behaviour is presented in figure 2 and figure 3. The first type of behaviour observed was when the bats were flying close to the surface of the mineral licks descending and ascending to drink from the licks (Figure 2). The other behaviour observed was that the bats were flying very low, close enough to the water pool and perched on any structure in order to drink from the mineral licks (Figure 3).

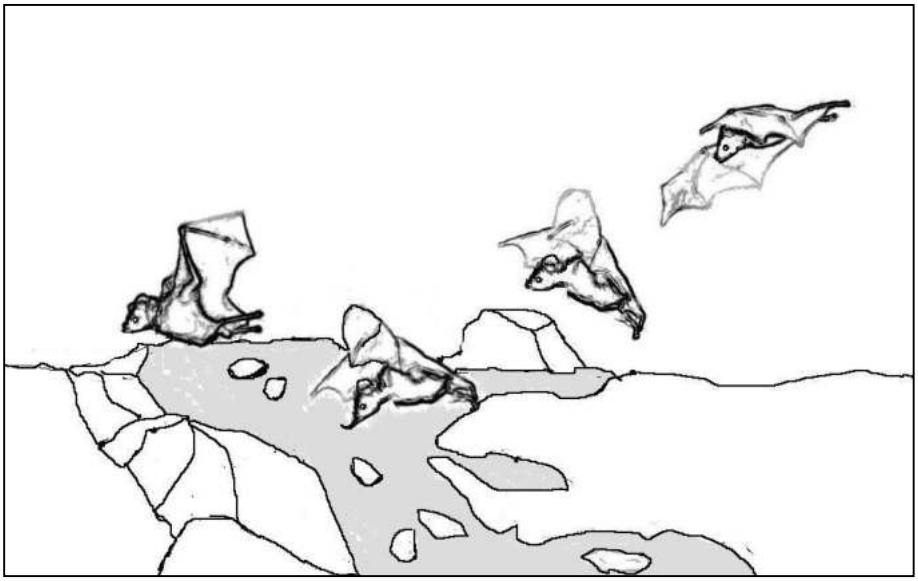

Figure 2. Behaviours of bats' drinking from mineral lick puddles: drink on wing.

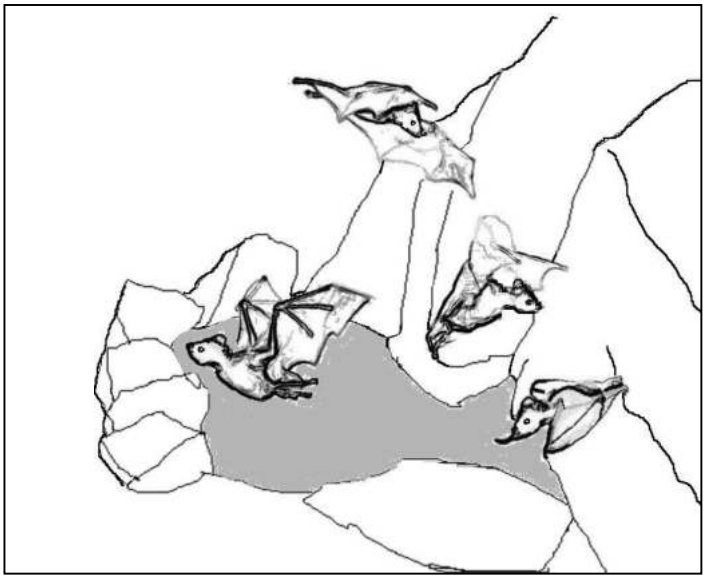

Figure 3. Behaviours of bats' drinking from mineral lick puddles: cling and drink.

Table 2. Summary of the observed behaviour of bats at mineral licks in Deramakot and Tangkulap Forest Reserve

Species of Bats	Estimated Recorded time (s) of indv.	n of indv. Observed ($\Sigma n = 9$)	Mean of Estimated Time (s)±SD	Location of Observation	Behaviour Observed
Macroglossus minimus	20,21,22	3	21.67±5.77	ML5, ML6	Cling & drink
Balionycteris maculata	18,22	2	13.33±11.7	ML5	Cling & drink
Cynopterus brachyotis	18	1	6.00±10.39	ML4	Drink on wing
Penthetor lucasii	25, 23	2	16..00±13.89	ML5, ML6	Cling & drink
Megaeropse caudatus	16	1	5.33±9.24	ML3	Drink on wing

The foraging spaces for the five species of frugivorous bats were known at the upper and the middle storey (Yasuma et al., 2005a) where most fruits are more abundant in the canopy. This may explain why pteropodid bats are more readily captured in the higher forest strata than in the understorey level (Tan et al. 1998). This is also suggested by studies done in Neotropics, where mineral licks may attract species of bats that normally fly high in the forest by drawing them down and getting captured at ground levels (Bravo et al., 2008; Emmons et al., 2006). This may support the observations where bats that fly close to mineral licks intentionally visit licks for resources.

Direct observations of their behaviour was possible during the day where observations were made after releasing these bats and they flew back to drink from the mineral licks. The released methods depend on the condition of bats especially in the morning session. It was either by hanging them on the nearby tree (for the weak, vulnerable bats) or gently releasing and letting them fly away (active bats). Based on the observation of nine frugivorous bats, they flew back to drink from mineral licks after they were released.

The time duration that each bat spent utilising the licks was less than 20 seconds per individual. This starts from the time the bats approach, drink and leave the lick. Table 2 summarises the observed behaviour of bats at mineral licks.

This drinking behaviour ending in a short time was potentially due to their anti-predator strategy at mineral licks. In addition, their short drinking time at mineral licks was due to the fact that they only needed 1ml to 2ml of mineral licks water for daily consumption as suggested to be sufficient for bats by Ghanem et al. (2013). In other words, they do not need to take too much time drinking from mineral licks as they only need small amount of mineral lick water for their consumption.

Species Occurrences

A total of 91 bats were caught at mineral licks comprising of 14 species. There were 81 individuals of frugivorous bats comprising five species and 10 individuals of insectivorous bats that comprised of nine species. Bat occurrences at mineral licks were dominated by frugivorous bats (86.81%) where each frugivorous bat species can be found in at least two mineral licks.

Captured bats ranged from 1 to 7 bats per night for all sites. Sites ML6 and ML3 recorded the highest capture index score while sites ML1 and ML2 scored the lowest capture index (Table 3). There was no group of bats seen congregating to drink at mineral lick sites as noted in previous studies (Gnahem, 2013; Gnahem et al., 2013). The visitations of bats observed in this study were individual-based visitations and in small groups (<three individuals) based on the low capture index at all mineral lick sites.

Table 3. Capture rate index of bats caught in mineral licks and control sites

Site	n bats	n of traps per night	Sampling nights	Capture Rate bats/traps/night
ML1	7	3	6	2.33
ML2	6	3	6	1.99
ML3	25	3	6	7
ML4	14	3	6	4.66
ML5	16	3	4	5.67
ML6	23	3	4	7.34
CW1	1	3	6	0.33
CW2	2	3	4	0.66

This study revealed that Palaeotropical frugivorous bats in Deramakot Forest and Tangkulap Forest Reserve had different response properties toward utilisation of mineral licks. From this study, bat activities at licks were in lower intensity, less than a hundred individuals from several species. Bats were caught less than seven bats/trap/night (capture rate) and they were not observed congregating at mineral licks. In contrast with the Neotropical region, there were hundreds of individuals from several species of frugivorous

bats that were reported to visit mineral licks in Peruvian and Ecuadorian Amazon, indicating higher activity of bats at mineral licks (Bravo et. al., 2008). In a study conducted by Bravo et al. (2008), bats were caught slightly more than ten bats/net/hours.

There was a modest, positive correlation between mineral lick sites and species occurrences, rho=0. 447, n=81, $p<0.005$. There was no bat caught at wallow sites. This indicated that most of the frugivorous bats caught at those lick sites were commonly found at mineral licks as they utilised mineral licks, and their capture was not by chance. Species *Macroglossus minimus* occurred at all sites while *Megaerops ecaudatus* were only found at ML4 and ML6. Species *Balionycteris maculata* (ML1, ML3 and ML5) and *Penthetor lucasii* (ML4, ML5, ML6) can be found at three sites each, while *Cynopterus brachyotis* can be found at two sites (ML1 and ML3). *Macroglossus minimus* occurred at all sites indicating this species is a frequent mineral lick visitor and its presence at mineral licks was not by chance.

In this study, insectivorous bat occurrences at mineral licks sites could be a random event. There was no correlation between mineral lick sites and species abundance of insectivorous bats (rho=-0.09, n=10, $p>0.005$). Among the nine species of insectivorous bats caught at mineral licks, none of them were recorded more than once at mineral lick sites. They were also not seen drinking from mineral licks and occurred in low occurrences in both mineral licks and wallow sites. All nine species of insectivorous bats identified in this study are species that commonly fly at the under storey level (Yasuma et al., 2005a; 2005b). This makes it easy for them to hit those traps while flying around study sites as the mineral licks and wallow sites were within their foraging range.

The similarity index (Table 4) showed 33% to 67% of bat species similarity occurred at most mineral lick sites. This is due to the fact that many frugivorous bats caught at most mineral lick sites were from the same species and this increases the percentage of species similarity among all mineral lick sites. The wallow CW1 and CW2 showed a similar pattern of species occurrence. Both sites had low species similarities in comparison to all of the mineral lick sites (<19%). This same pattern was also reported in Bravo et. al., (2008) where there were low species similarities between mineral lick and non-mineral lick forest sites.

Table 4. Bray-Curtis Similarity Index among six mineral licks and two wallow sites

Sites	ML1	ML2	ML3	ML4	ML5	ML6	CW1	CW2
ML1								
ML2	0.31							
ML3	0.38	0.39						
ML4	0.20	0.63	0.42					
ML5	0.25	0.52	0.52	0.67				
ML6	**0.13**	0.41	0.33	0.61	0.60			
CW1	0.25	**0**	**0**	**0**	**0**	**0**		
CW2	0.25	**0**	**0**	**0**	**0**	**0**	1.00	

*Notice in bold indicates low similarities (<0.2) between licks and wallows

Insolube Soil Tracer Test

In all, there were 12 faecal samples of bats collected for this laboratory test analysis. The number of insectivorous bats caught at mineral licks was limited, as one species had at the most, two individuals at mineral lick sites. Faeces of *R. borneensis* (n=2) were used for faecal analysis since the other species occurred only once at each mineral lick site (Table 6). Faecal samples of frugivorous bats *M. minimus* (n=5) were from the same species from the same site. Similarly, faecal samples for insectivorous bats, *Hipposideros cervinus* were collected at forest control (n=5). The concentration of Al and Si were higher in the faeces for frugivorous bats (bold in Table 5) compared to faeces of insectivorous bats caught at mineral lick and forest control sites.

The higher concentrations of Al and Si in their faeces suggest that frugivorous bats consume soil while they are utilising water puddles at mineral licks. Those elements were not part of their diet and thus excreted through their faeces. This makes their faeces enriched with those elements. Al and Si are elements present in high concentrations in soils but poorly absorbed through the gastrointestinal tract, thus should not be part of bat diet (Gnahem, 2013; Gnahem et al., 2013; Abraham, 2012). Meanwhile, samples of faeces from insectivorous bats, *R. borneensis* and *H. cervinus* were not enriched with Al and Si. Both species are cave dwelling bats (Yasuma et al., 2015a) and the smaller amount of concentration of insoluble soil tracer in their faeces may come from their behaviour of drinking water from small limestone caves.

Table 5. Concentration of element Al and Si in faeces of frugivorous bats and insectivorous bats caught at mineral licks and forest control

	Element concentration (Mean±SD)			
	Mineral Licks		Forest Control	
Bats (No. Of samples)	Al (ppm)	Si (ppm)	Al (ppm)	Si (ppm)
Frugivorous Bats				
Macroglossus minimus (5)	**206.05±57.50**	**156.47±25.26**	-	-
Insectivorous bats				
Rhinolophus borneensis (2)	0.27±0.21	13.71±1.81		
Hipposideros cervinus (5)			0.10±0.08	11.73±0.56

*Notice in bold, higher concentration of elements, Al and Si in faeces of *Macroglossus minimus*.

Assemblage Structure

Species composition of bats caught at licks were dominantly frugivorous bats (86.81%). The most common species (>5% relative abundance) caught at all mineral licks in this study were *Macroglossus minimus* (58.24%), *Balionycteris maculata* (12.09%), *Cynopterus brachyotis* (9.89%), and *Penthetor lucasii* (6.59%). The frugivorous bats consisted of subfamily Cynopterinae (*Balionycteris maculata*, *Cynopterus brachyotis*, *Megaerops ecaudatus* and *Penthetor lucasii*) and subfamily Macroglossinae (*Macroglossus minimus*). In this study, the assemblage of species from subfamily Macroglossinae, *Macroglossus minimus*, was well presented at mineral licks. Meanwhile the number of insectivores bats caught at mineral licks were small at <2.2% per site, represented by three families namely, Vespertilionidae Hipposideridae and Rhinolophidae.

Table 6. Species composition and relative abundance of bats caught at mineral licks

Families	Common name	Sp. Name	Captured percentages (%)	Site
Pteropodidae	Long-tongue nectar bat	*Macroglossus minimus*	58.24	ML1, ML2, ML3, ML4, ML5, ML6
	Spotted wing fruit bat	*Balionycteris maculata*	12.09	ML1, ML3,ML5
	Short-nosed fruit bat	*Cynopterus brachyotis*	9.89	ML1,ML3
	Dusky fruit bat	*Penthetor lucasii*	6.59	ML4,ML5, ML6
	Tailless fruit bat	*Megaerops ecaudatus*	2.2	ML4, ML6
Vespertilionidae	Clear-winged woolly bat	*Kerivoula pellucida*	1.1	ML1
	Lesser woolly bat	*Kerivoula minuta*	1.1	ML4
	Lesser tube-nosed bat	*Murina suilla*	1.1	ML3
Hipposideridae	Dayak roundleaf bat	*Hipposideros dyacorum*	1.1	ML5
	Ridley's Roundleaf bat	*Hipposideros ridleyi*	2.2	ML5
Rhinolophidae	Acuminate horseshoe bat	*Rhinolophus acuminatus*	1.1	ML2
	Borneon horseshoe bat	*Rhinolophus borneensis*	2.2	ML3
	Creagh's horseshoe bat	*Rhinolophus creaghi*	1.1	ML6
	Lesser wolly horseshoe bat	*Rhinolophus seduluus*	1.1	ML4

Total individual of bats = 91 (Fruit bats = 81; Insectivorous bats = 10);
Total species =14 (Fruit bats = 5; Insectivorous bats = 9)

In Southeast Asia, Paleotropical bat assemblages are dominated by members of families Rhinolophidae, Hipposideridae and Vespertilionidae (Struebig et al. 2008). Although their assemblages are bigger than family Pteropodidae, they make up only a small percentage of bats caught in study sites especially mineral licks. In this study, there was lack of evidence to prove that bats from insectivorous families utilise mineral licks. Meanwhile frugivorous bats dominated the bat assemblages in mineral licks as they utilise mineral licks. This same pattern was also documented in studies on bats and mineral licks conducted in the Neotropics region (Ghanem, 2013a; Gnahem et al., 2013; Bravo et al., 2012; Bravo et al., 2010a; Bravo et al., 2010b; Bravo et al., 2008; Voigt et al., 2008; Voigt et al., 2007).

The underlying causes of frugivorous bats dominating bat assemblages in mineral licks still remain unclear (Gnahem, 2013). Nonetheless, there are hypotheses that have been proposed to explain their motives in consuming muddy water from licks (Gnahem, 2013; Bravo et al., 2010). Their motives are not limited to one particular hypothesis as many hypotheses can be used to explain their geophagous behaviour due to multifunction benefits of using mineral licks (Brightsmith et al., 2008). Lick water may provide minerals (Brightsmith et al., 2008), antidiarrhoeal components (Slamova et al., 2011), or clay for binding potential dietary toxins (Gilardi et al., 1999). Further studies are needed to test these hypotheses covering Paleotropical bats.

In all, this study gives a general tentative on structure of bats caught at mineral licks and can be used as a baseline for other studies. For instance, a study of bats visiting pattern to licks throughout the year. Pattern of bats visitation may relate to the reproduction season of bats, fruiting/flowering season, and climatic season such as the wet and dry seasons. More studies involving the visitation of bats across time related to the reproduction season of bat species, fruiting/flowering, wet and dry seasons in Borneo are important to reveal the underlying reasons and understanding the seasonal visiting pattern of bats visiting mineral licks in Borneo.

Conclusion

Five species of Old World frugivorous bats (*Macroglossus minimus*, *Balionycteris maculata*, *Cynopterus brachyotis*, *Megaerops ecaudatus* and *Penthetor lucasii*) made up the assemblages of bats visiting mineral licks in DFD. Species compositions of bats caught at licks were dominanted by frugivorous bats (86.81%), and the common visitors are *M. minimus, B.*

maculata, and *C. brachyotis*. Old World frugivorous bats were confirmed as visiting mineral licks in the Deramakot and Tangkulap Forest Reserve as these five species of frugivorous bats were observed drinking from mineral licks (*M. susminimus*, n=3; *B. maculata* n=2, *C. brachyotis,* n=1, *Megaerops ecaudatus*, n=2; *Penthetor lucasii,* n=1). These observations were supported with the species occurrences data across all mineral lick sites where four species of frugivorous bats (*M. minimus, B. maculata*, *C. brachyotis* and *P. lucasii*) were commonly found and frequently occurred at all six sites at mineral licks. Meanwhile, for species *M. ecaudatus,* this species was observed drinking at mineral licks. Frugivorous bats ingested soil from mineral licks. The concentrations of insoluble soil tracer elements, Al and Si, in frugivorous bat species (represented by species *M. minimus*) were higher compared to the concentrations of those elements in the faeces of insectivorous bats caught at mineral lick and forest sites. Indeed, Al and Si elements excreted from their faeces were not from their fruit diet, but were highly found in the soil.

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Journal of Tropical Biology and Conservation 15: 139–154, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Selectively Logging Old Growth Rain Forest Twice Changes Canopy Ant Species Composition, While Conversion to Oil Palm Changes Composition and Reduces Species Richness and Diversity

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Abstract

Tropical forests around the world, and particularly in Southeast Asia, are being affected by anthropogenic habitat conversion and degradation. Ants, an ecologically important group in the rainforest canopy, have previously been demonstrated to be robust to a single round of selective logging, but are strongly affected by conversion to oil palm. However, the impacts of multiple rounds of selective logging on canopy ants remain unexplored. We studied the ant assemblages across a habitat gradient comprising old growth forest, twice-logged forest and oil palm plantation in Sabah, Malaysian Borneo. Canopy ants were collected using insecticide fogging across 36 sampling sites. Old growth forest and twice-logged forest had similar species richness and Shannon species diversity. These two forest habitats were significantly higher in species richness and Shannon diversity than oil palm plantation. Abundance of canopy ants was similar across all three habitats. There was a significant difference in species composition between all pairs of habitats. Leaf litter depth on the ground was positively related to ant species richness, while canopy cover was positively related to ant abundance. Hence, multiple rounds of logging cause shifts in ant species composition, while forest conversion to oil palm additionally causes reductions in ant diversity. This is of concern, since forests in Sabah and elsewhere are becoming increasingly degraded. Our results indicate that both old growth and twice-logged rain forests can be useful for conservation of canopy ants.

Keywords: Formicidae, SAFE Project, land-use change, canopy ants

Received 04 January 2018
Reviewed 02 March 2018
Accepted 25 May 2018
Published 15 October 2018

Introduction

A major threat to rainforest biodiversity is logging and forest conversion to agriculture (Gibson et al., 2011). Southeast Asia is a global biodiversity hotspot, and is also under threat from habitat change, with a reduction in forest cover of 12% during the decade of 2000-2010 (Stibig et al., 2014). Malaysia in particular has seen reductions of 23% in the period between 2011-2016 (www.globalforestwatch.org), due to expansion of agricultural and agro forests. Malaysia is the second highest producer of palm oil in the world, after Indonesia, and increases in area planted with oil palm often results in loss of forested habitats (Koh & Wilcove, 2008). As tropical forests are increasingly vulnerable to such conversion, it is important to study the effects of these land use changes.

Rain forest canopies are highly diverse habitats, yet are relatively poorly known (Nakamura et al., 2017). The direct impact of logging of tropical forest is the alteration of canopy layers and the loss of the closed canopy (Whitmore, 1998). Specifically, this involves reduction in canopy height, canopy surface area, and crown sizes for individual trees (Okuda et al., 2003). Despite these changes, logged forests still support reasonable numbers of species of a range of groups from primary forest (Edwards et al., 2010). However, it is unlikely that all species could be conserved using this approach (Gibson et al., 2011). Oil palm primarily has a less complex physical structure than natural forest, with many fewer plant species, a hotter and drier microclimate, and experiences other direct human impacts, such as application of pesticides (Foster et al., 2011). Hence it supports substantially lower biodiversity than forested habitats (even those that have been heavily logged) for many groups (Fitzherbert et al., 2008; Foster et al., 2011).

Ants are dominant insects in tropical lowland forest (Turner & Foster, 2009). Approximately 50% of all ant species are at least partially reliant on the canopy in the tropics, making them ideal insects to study effects of changing forest landscapes in relation to arthropod assemblages (Floren, Wetzel & Staab, 2014). Compared to leaf litter on the ground, as a three-dimensional arboreal space the canopy supports a different composition of ant species (Hashimoto et al., 2006), and the species present are usually highly adapted to arboreal life with specialization of diet and other niches. Ants are also a useful focal group (Philpott et al., 2010) due to their high total biomass (Folgarait, 1998). They may influence the whole ecosystem as they play various important roles in terms of ecosystem services, as decomposers (McGlynn & Poirson, 2012), biological control agents (Hölldobler & Wilson, 1990; Navarrete et al., 2013), seed dispersers, mutualists (Hashimoto et al., 1999), soil engineers, scavengers or

predators, and drivers of nutrient re-distribution (Fayle et al., 2011; Folgarait 1998; Philpott et al., 2010).

In Malaysian Borneo, the impacts of logging and conversion to oil palm vary widely both between taxonomic groups (Foster et al., 2011) and as a result of differences in logging intensity (Edwards et al., 2014). Although oil palm clearly supports fewer canopy ant species and different communities than primary forest (Fayle et al., 2010), the situation regarding logged forest is less clear. Previous work has shown in a forest that was selectively logged only once in Danum Valley, canopy ant species richness and abundance did not differ from the nearby primary forest (Widodo et al., 2004). Furthermore, although species diversity metrics were higher in primary forest than in one once-logged forest area, they did not differ between primary forest and a second area of once-logged forest. Elsewhere, in Papua New Guinea, primary forest supports a higher species richness of canopy ants than secondary forests that have regrown following complete clearance (Klimes et al., 2012). Hence, the importance of degraded forest as a habitat for ant species depends greatly on the particular management that the forest has undergone. In Sabah, the majority of logged forests have now been logged more than once, and so the results of Widodo et al. (2004) are not representative of the value of logged forest in the area more broadly. Furthermore, work on leaf litter ants has shown that twice-logged forest supports fewer species than primary forest at small scales, but that the total number of species remains the same (Woodcock et al., 2011). Here we look at species richness, diversity, abundance and species composition of canopy ants using fogging in a primary forest, a twice-logged forest and an oil palm plantation in Sabah, Malaysia. We also record environmental variables to see how they affect the ant assemblages.

Materials and Methods

Study site

Sampling took place in lowland tropical rain forest and oil palm plantation in Sabah, Malaysia, located in north-east Borneo. Average annual temperature in the area (recorded in nearby primary forest in Danum Valley) is 26.7°C, while average annual rainfall is 2669 mm (Walsh & Newbery, 1999). Sampling was conducted from January 28, 2015 until November 6, 2015. Three survey habitats were selected: old growth forest in Maliau Basin Conservation Area, twice-logged forest and oil palm plantation. In each habitat, 12 survey sites were

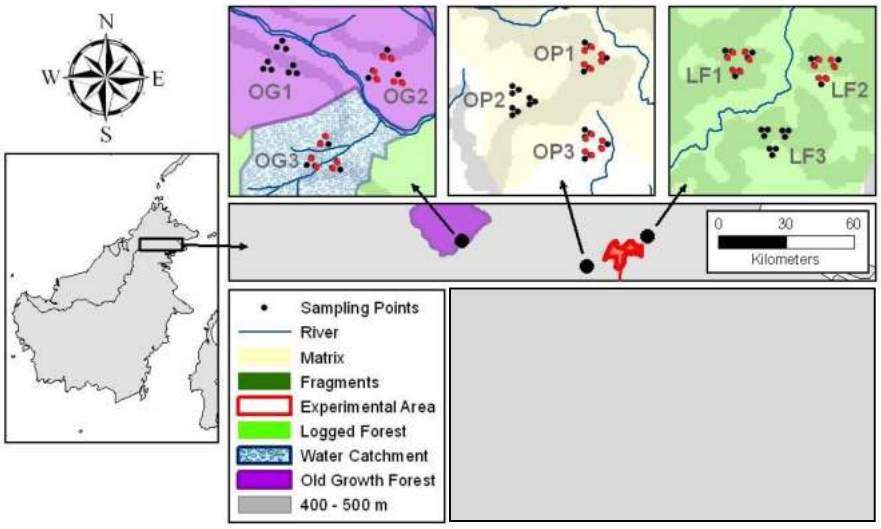

Figure S1. Map of sampling points in Malaysian Borneo (denoted in red). Adapted from the SAFE project website (www.safeproject.net). Note that the SAFE experimental area was not sampled, and so is not presented in this map.

established as part of the Stability of Altered Forest Ecosystems (SAFE) Project (Figure S1) and were used with collections being conducted at "second order" (referring to the fractal sampling design) SAFE survey sites (Ewers et al., 2011). Old growth forest (4º 41'-4º65'N 116º4'-117 º4'E) comprised six sites that have never been logged, and six sites that experienced very low intensity logging in the 1970s and 1990s around the field centre area for construction purposes. However, these low intensity logged sites retained a structure similar to that of pristine primary forest (Ewers et al., 2011). Twice-logged forest was situated to the north of the experimental area of SAFE Project. It is a continuous forest but has been selectively logged twice, once during the 1970s and again from the late 1990s to 2000s (Hardwick et al., 2015). Oil palm plantation (4º33'E, 117º28'24.41"E) was a monoculture of *Elaeis guineensis* with sites planted in either 2000 or 2006 (Ewers et al., 2011). Although the sampling sites for different habitat types were not spatially interspersed (Figure S1), they were representative in terms of these kinds of habitats in Sabah.

Sampling methods

Fogging was used to sample the canopy ants with synthetic pyrethrum insecticide (active compound: alphacypermethrin with synergist 2.27%) diluted in diesel by a ratio of 15:1 (Yusah et al., 2012). Four circular 1 m^2 area collecting

trays were laid out and suspended using ropes tied to trees or oil palm trunks at each site (a total of 144 m^2 of sampled canopy area across 36 samplings sites). In plantations, the only large trees at the sites were oil palm. Trays were placed as close as possible to the pre-designated sampling point, regardless of the presence of vegetation. Fogging was carried out around 06:00 am to avoid strong lateral drift of insecticide fog. Days with wind or rain were avoided. The fogging machine was run for four minutes and then the site was left for two hours for the insecticide to act before collection of ants, which were brushed into pots of 70% ethanol suspended from apertures at the centre of the trays. Ants were identified to genus (Fayle et al., 2011), sorted to morphospecies, and then species names assigned where possible. Voucher specimens were deposited at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. We excluded reproductive individuals, major workers and callow ants from counts to avoid misidentification and subsequent inflation of species counts. In each habitat, four environmental variables were recorded: leaf litter depth, canopy cover, dead wood coverage and climber coverage. These variables are potentially important for ant communities (Wilkie, Mertl & Traniello, 2010). Climbers are important for providing a three-dimensional structure in the forest canopy, allowing spatial partitioning of canopy ant communities (Tanaka, Yamane & Itioka, 2010) and also providing connectivity between different canopy strata (Powell et al., 2011). The average leaf litter depth was obtained from three readings within a 1 m^2 quadrat. The canopy cover, dead wood and climber coverage measures were based on the standard scale used for SAFE project vegetation plots, ranging from very poor or few (one) to very good or abundant (five): 1 = up to 20% canopy cover, absent dead wood or lianas; 2 = up to 40% canopy cover, one or a few occurrences of dead wood and lianas; 3 = up to 60% of canopy cover, moderately abundant dead wood or lianas; 4 = up to 80% of canopy cover, abundant dead wood or lianas; 5 = full coverage of canopy, very abundant dead wood or lianas. These variables were estimated within a 20 m by 20 m area, centred on the sampling point, with one estimate made per variable per sampling point.

Statistical analyses

A series of linear models were run to test for differences in species diversity (Shannon diversity index), species richness and ant abundance along the habitat gradient using the *lm* function in the R 3.3.0. Data from the four fogging trays were pooled prior to analysis. We used a $\log_{10}(x)$ data transformation for abundance to normalize the data. To determine the relationship between the environmental variables and the species diversity we ran models twice: once with only the habitat as a predictor, and a second time with all environmental

variables as predictors as well. Full models were included first, and then simplified by stepwise removal of predictors (using the *drop1* function in R 3.3.0), such that AIC was minimised. Post-hoc tests for differences between habitats were conducted where a significant effect of habitat was found using the linear models, using Tukey honest significant differences (*TukeyHSD* function in R 3.3.0). A non-metric multidimensional scaling (NMDS) ordination was used to illustrate differences in species composition between habitat types, with pairwise ANOSIM tests being conducted to test statistically for differences between all habitat pairs (*metaMDS* and *anosim* functions run on presence-absence data in the R vegan package). The analyses for species composition were conducted on ant presence/absence data.

Results

We sampled a total of 9,002 individual ants, 199 morphospecies and 44 genera from all habitats across the 36 sampling sites. The total number of subfamilies, genera and morphospecies recorded was highest in the old growth forest, followed by twice-logged forest and then oil palm plantation (Table 1). Across all the habitats combined, *Polyrhachis* was the most species-rich genus (33 species) followed by *Camponotus* and *Crematogaster,* both with 23 species each. Myrmicinae was the most diverse subfamily representing 42.4% of the total species sampled. Mean species richness in old growth forest and twice-logged forest was significantly higher than in oil palm plantation (Linear model: $F=10.29$, $df=2,33$, $p<0.001$; Tukey HSD: old growth-oil palm, $p<0.001$; twice logged-oil palm, $p=0.037$) but not different between old growth forest and twice-logged forest (Tukey HSD, old growth-twice logged, $p=0.144$). The mean species richness in old growth forest was five times higher than in oil palm plantation (Figure 1a).

Table 1. Composition of canopy ants in old growth forest, twice-logged forest and oil palm plantation.

Subfamily	Old growth		Twice-logged forest		Oil palm	
	Genera	Species	Genera	Species	Genera	Species
Formicinae	10	54	10	41	7	13
Myrmicinae	19	61	13	32	8	11
Dolichoderinae	3	16	3	8	2	2
Ponerinae	8	8	1	2	0	0
Pseudomyrmecinae	1	3	1	4	0	0
Ectatomminae	1	1	1	1	0	0
Proceratiinae	1	1	0	0	0	0
TOTAL	**43**	**144**	**29**	**88**	**17**	**26**

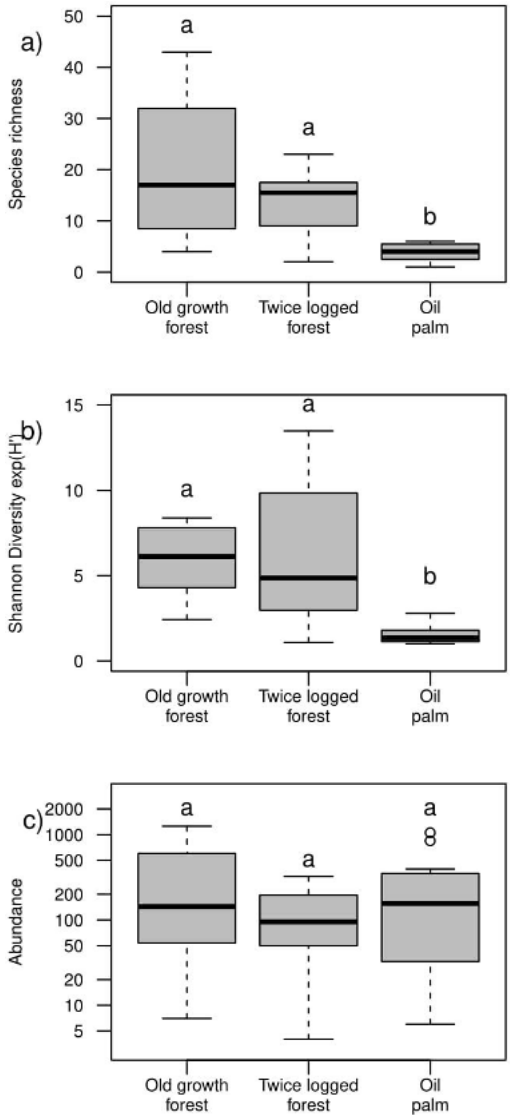

Figure 1. a) Species richness, **b)** Shannon diversity and **c)** abundance (note logarithmic scale on y-axis) per sampling point in old growth forest, twice-logged forest and oil palm plantation. Different letters indicate statistically significant differences between habitats when only habitat type is included in the linear model. Boxplots show medians (thick horizontal line), interquartile ranges (grey boxes), and full ranges (whiskers). Outliers more than 1.5 times the interquartile range from the 25th or 75th percentiles denoted as unfilled circular points.

Similarly, old growth forest and twice-logged forest had a significantly higher Shannon species diversity than oil palm plantation but there was no difference between old growth forest and twice-logged forest (Linear model, $F=10.6$, $df=2,33$, $p<0.001$; Tukey HSD: old growth-twice-logged, $p=0.776$; old growth-oil palm, $p<0.001$; twice-logged-oil palm, $p<0.001$). Species diversity was highest in old growth forest followed by twice-logged forest, with oil palm plantation having lowest diversity. There was no significant difference in mean ant abundance between the three habitats (Linear model, $F=1.70$, $df=2,33$, $p=0.198$).

When environmental variables were also included in starting models, for species richness, leaf litter depth was the only variable remaining in the final model and was positively related to richness (linear model: $t=5.71$, $p<0.001$, Figure 2a). Habitat was not included in this final model, and leaf litter depth was a significant predictor of species richness even when habitat and leaf litter were included in the same model. For species diversity, no environmental variables were present in the final model, with habitat being the only predictor remaining (see model in paragraph above). For ant abundance, the final model included both habitat and canopy cover (linear models: habitat: old growth-twice logged $t=-1.15$, $p=0.259$, old growth-oil palm $t=1.07$, $p=0.293$, twice logged-oil palm $t=2.23$, $p=0.032$, canopy cover: $t=2.09$, $p=0.053$, Figure 2b).

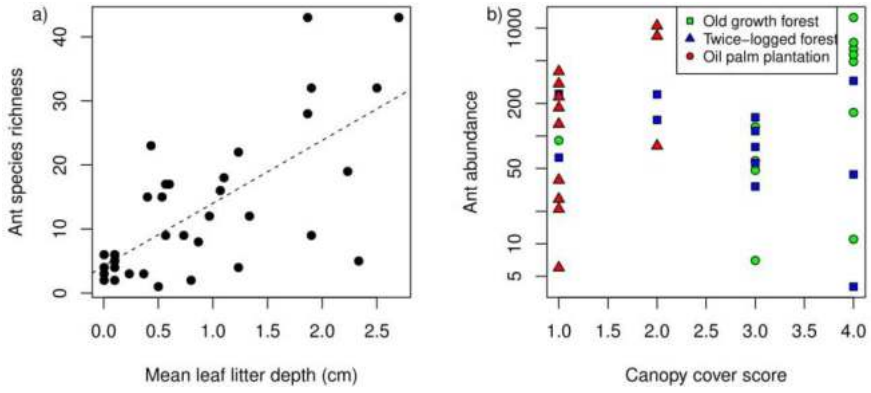

Figure 2. Statistically significant relationships between environmental variables and aspects of canopy ant community structure. **a)** Relationship between leaf litter depth and ant species richness. **b)** Relationship between canopy cover/habitat and ant abundance.

Canopy cover had a positive relationship with ant abundance, and twice-logged forest had lower ant abundance than oil palm plantation, when also accounting for differences in canopy cover. All of the environmental variables varied between the habitats (Figure 3).

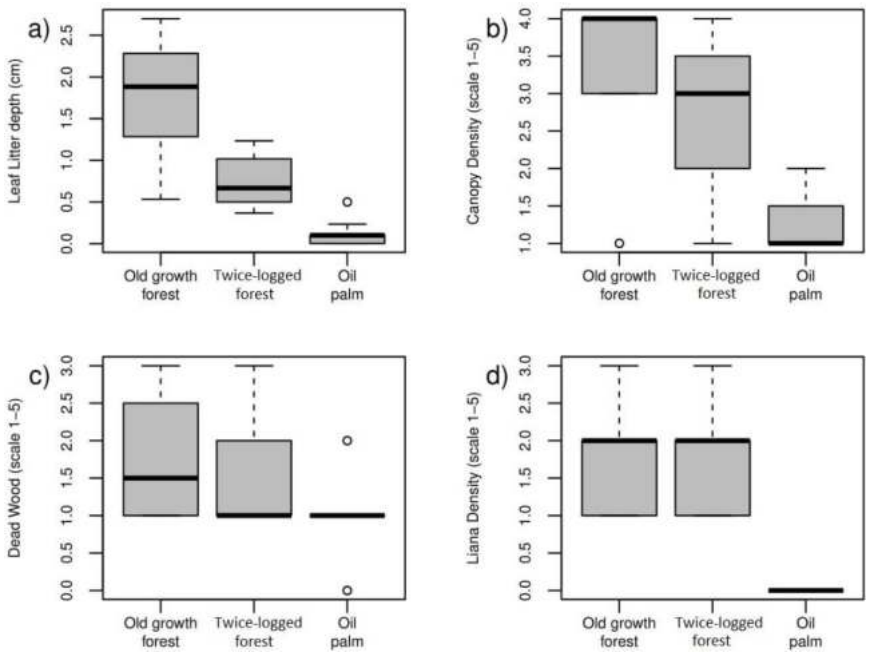

Figure 3. Boxplots for all environmental variables measured across the three different habitats. **a)** Leaf litter depth. **b)** Canopy density. **c)** Dead wood. **d)** Liana density.

From 199 morphospecies sampled, 94, 39 and 12 species were found only in old growth forest, twice-logged forest and oil palm plantation respectively. Only seven species were present across all habitats, while 38 species appeared in both old growth and twice-logged forest. There were significant differences in species composition between all habitats (ANOSIM: old growth -twice logged, R=0.270, p=0.001; old growth -oil palm, R=0.531, p=0.001; twice-logged -oil palm, R=0.549, p=0.001; Figure 4).

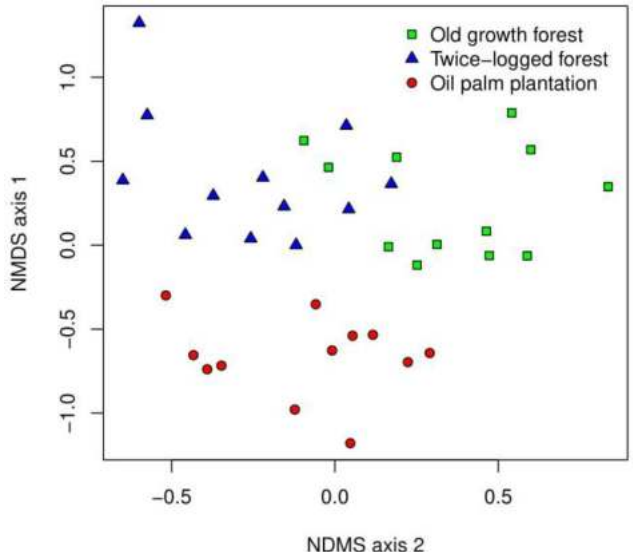

Figure 4. NMDS ordination plot showing the species composition according to habitat type (stress = 0.145) There were significant differences in species composition between all three habitat types (see text for details).

Discussion

Old growth forest and twice-logged forest did not differ in terms of mean species richness and Shannon diversity of ants. This broadly follows findings from other biogeographic regions (e.g. Schulz & Wagner, 2002), and from other microhabitats in Sabah (Woodcock et al., 2011). The results suggest that at the scale of a single sampling site, the two different forest types provide similar amounts of habitat for ants, and can hence support similar numbers at these smaller scales. Depending on the age of forest regeneration, a logged forest may provide intermediate conditions for nesting and niches for ants (Luke et al., 2014). The continuous (unfragmented) nature of old growth and twice-logged forest in the present study sites allow ants to move at a larger scale without restriction. Types of trees may also affect the distribution of canopy ants (Foster et al., 2011; Schulz & Wagner, 2002) especially trees with extra-floral nectaries available (Blüthgen, Verhaagh & Goitía, 2000). Hence, it might be expected that tree community changes due to logging would impact ant communities. However, ant-plant interactions take place on myrmecophytes such as *Macaranga* sp. that have higher abundance in secondary forest than in old growth forest (Tanaka et al., 2008), which may help to offset any effects of vegetation simplification due to logging. The presence of epiphytes such as ferns on trees provide an additional niche for canopy arthropods, including ants, in the tropics

(Fayle et al., 2009; Turner & Foster, 2009). However, the majority of ant-plant interactions are not particularly specific at the level of entire forests (Klimes et al., 2012), and hence the impacts of tree community composition on ant species persistence are likely to be complex. Furthermore, old growth forests are mostly occupied by large trees and thus have a non-uniform canopy layer. Although the larger trees have a different composition of ant species, at least within old growth forest in the region (Yusah & Foster, 2016), impacts of tree size on local ant species richness can be moderated if there are high levels of connectivity via lianas (Adams et al., 2016).

Despite the robustness of the rainforest to two rounds of logging in terms of number of canopy ant species per sampling site, the total number of species collected was greater in the primary forest. The greater number of species at larger scales in old growth forest indicate some kind of habitat heterogeneity at these larger scales that is not present in twice-logged forest. This hypothesis is supported by the fact that twice-logged forest also differed from old growth forest in terms of ant species composition. This change in composition may have been driven by adaptation of dominant genera such as *Camponotus*, *Crematogaster* and *Polyrhachis* to particular types of nesting sites (Floren, Wetzel & Staab, 2014). Our results are important because they demonstrate that two rounds of selective logging can affect canopy ant species composition, whereas previous work on logged areas that had undergone one round of logging did not show major effects (Widodo et al., 2004). This is of concern because the majority of degraded forests in Sabah have been logged multiple times, and also because tropical forests more widely are becoming more degraded. Further research could study an even wider range of different degrees of logging intensity, over greater spatial ranges (to overcome any biases in our data introduced from lack of interspersion of sampling sites in different habitats), and with a more comprehensive range of canopy environmental measurements.

In contrast, in oil palm plantation, there were reductions in number of species, and changes in species composition relative to both old growth and twice-logged forest. This agrees with previous work comparing canopy ants from old growth forest with those in oil palm plantation (Fayle et al., 2010). In our study, most ant species in oil palm plantation could not be found in old growth forest or twice-logged forest and vice versa. This suggests that the ability to live under environmental conditions differs between ant species. In particular, the harsh microclimate in oil palm plantation (Turner & Foster, 2009) in combination with the low diversity of trees is likely to negatively affect ant communities. We also found a high abundance of non-native species in oil palm plantation, such as

Anoplolepis gracilipes, as has been previously noted by Brühl and Eltz (2010). Another species with high abundance in oil palm plantation recorded in this study was *Oecophylla smaragdina*, which is associated with human disturbance. Although fogging is efficient in sampling canopy arthropods, this method does not necessarily exclude all ground nesting but canopy foraging ant species (Weiser et al., 2010). However, this study focused on ants that use canopy in general (rather than only those that nest there), and hence we can still conclude that losing the canopy affects ant composition. The differences between forested habitats and oil palm plantation indicate that conversion of any kind of forest, even that which has undergone multiple rounds of logging, is likely to have negative impacts on ant biodiversity.

In terms of directly measured environmental variables, both ground leaf litter and canopy density affected ant communities. Depth of ground leaf litter was positively related to site ant species richness, both when accounting for overall differences in richness between habitats, and when analysed by itself. There are two possible explanations for this. First, that ground conditions are important because there is some movement of ants from ground to the canopy and vice versa. For example in Bornean forests, some ant species nest at ground level and forage into the canopy (e.g. *Dinomyrmex gigas*, the giant forest ant (Pfeiffer & Linsenmair, 2000)). Second, that the depth of leaf litter is an indicator of some aspect of canopy complexity that we did not measure. Ant abundance was positively related to canopy density, and once canopy density was accounted for, there were significantly lower ant abundances in twice-logged forest than in oil palm plantation. The overall relationship with canopy density suggests that, generally, more dense canopies provide a greater amount of habitat for canopy ants. However, because this effect is observed for overall ant abundance and not site species richness, this indicates that the average colony size is larger in less degraded habitats. The difference between twice-logged forest and oil palm plantation indicates that for a given amount of canopy cover, there are more ants in twice-logged forest. This may relate either to the change in microclimate, or simply to the fact that for any given canopy density in oil palm, the vast majority of that canopy will comprise only one plant species.

In summary, multiple rounds of logging changes canopy ant species composition, and conversion to oil palm causes further shifts in ant composition accompanied by reduction in species richness and species evenness, and increases in the occurrence of non-native ant species. These impacts on canopy ants may shift the ecological functioning of the whole canopy ecosystem. Our results suggest that twice-logged forest is likely to be of use for conserving canopy ant

biodiversity, but that some species are likely to require maintenance of areas of unlogged forest.

Acknowledgements

We are thankful for SAFE project team, especially the research assistants who helped us a lot: Mainus, Denny, Mike, Zinin, Ling and James. We are also grateful to Maliau Basin Conservation Area staff for allowing us to stay and do our fieldwork at the centre. Amelia Joyce Philip was funded by a Sabah State Scholarship. This manuscript was greatly improved by the comments of two anonymous reviewers and Martin Pfeiffer, to whom we are grateful. Tom Maurice Fayle was supported by a Czech Science Foundation Centrum of Excellence Grant (14-36098G). Kalsum M. Yusah was supported by Malaysian Ministry of Higher Education (FRG-0373-STWN-1/2014). We are also grateful to the South East Asian Rainforest Research Partnership, in particular Datuk Dr Glen Reynolds, and the Stability of Altered Forest Ecosystems project run by Prof Rob Ewers for their support.

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Journal of Tropical Biology and Conservation 15: 155–162, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

Notes on Congregating Fireflies (Coleoptera, Lampyridae) of Binsulok River, Sabah

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Abstract

A brief survey on congregating fireflies of Binsulok River was conducted on September 9 and 16, 2017. Altogether nine sampling stations were selected among the display trees of *Rhizophora apiculata*. Water quality parameters were also recorded close to each sampling station. This is the first record where all five *Pteroptyx* species recorded in Sabah were found in a single area on one species of display tree. *P. bearni* was the predominant species sampled with 33 male individuals, followed by *P. tener* (5 males), *P. valida* (5 males), *P. gelasina* (2 males) and *P. malaccae* (1 male). In terms of water quality, only water pH (which was mostly acidic, mean pH 4.51 ± 0.03), and low dissolved oxygen (D.O., mean 3.36 ± 0.64 mg/L), can be considered as not suitable for aquatic life, which could contribute to the decreasing population of fireflies, as larvae of fireflies feed on river snails. An aerial survey of the area by a drone showed that there was some encroachment and land use change from its original mangrove forest. However, these results could not be quantifiable but this survey suggested that the land usage could contribute to the decline in firefly population.

Keywords: fireflies, *Pteroptyx*, mangrove forest, conservation

Received 05 February 2018
Reviewed 16 May 2018
Accepted 17 May 2018
Published 15 October 2018

Introduction

Fireflies are beetles (Order Coleoptera) under the family Lampyridae. Beetles in this family have the ability to emit light from luminous organs located at the tip of their abdomen. Fireflies use their flashing signals to attract the opposite sex of the same species (Ohba & Sim, 1994; Ohba, 1999). Their ability to produce rhythmic synchronous flashing light in large population densities has made them an attraction (Buck, 1988). The loss of their natural habitat, the mangrove forests, has caused their extinction in several places, making them a subject for serious studies (Ballantyne et al., 2011). The firefly that has the potential as an ecotourism product is the one from the genus *Pteroptyx* (Mahadimenakbar et al., 2009). There are currently five *Pteroptyx* species, namely *P. bearni*, *P. tener*, *P. malaccae*, *P gelasina*, and *P. valida*, that can be found in Sabah (Mahadimenakbar & Fiffy, 2016). *P. similis* Ballantyne, which was thought to be endemic to Sabah (Ballantyne, 2001) is now synonymised with *P. bearni* (Ballantyne & Lambkin, 2013).

Each species has a unique flashing frequency and their courtship involves an exchange of flashing signals at dusk or once it is dark. In general, they can be divided into three different groups, which are: 1) the congregating synchronous flashing type; 2) the congregating non-synchronous flashing type; and 3) the solitary fireflies. The congregating firefly is commonly found in the mangrove regions of Southeast Asia (Hogarth, 1999). In the Oriental region, the congregation of these magnificent insects can only be found principally at mangrove trees along brackish rivers (Buck & Buck, 1968). All synchronous displays occur in trees or shrubs along tidal rivers in mangrove-nypa swamps (Ballantyne & McLean, 1970).

Malaysia is blessed with an abundance of these congregating species in most of the mangrove inter-tidal rivers where the numbers depend wholly on the health of the riparian forest and the water quality. In order to ensure the fireflies can survive in their natural habitats, it is crucial to conserve the habitats that the insects reside in (Foo & Mahadimenakbar, 2015). Assessments of the area and baseline scientific studies are needed to determine the population status of the fireflies for conservation planning and development (Foo & Mahadimenakbar, 2017).

Methodology

Binsulok River is located in Klias Peninsula. It is a potentially a good destination for nature tourism and environmental education (Mohamed et. al.,

2000). An initial population survey was conducted on 9 September 2017, followed by sampling on 16 September 2017 in Binsulok River. Prior to the sampling, aerial photos were taken from a drone at selected points of the surveyed area to study potential threats to the population of fireflies. In the evening, fireflies were surveyed from the boat berth (N 05°31'27.0" E 115°43'03.2'') where tourists start their journey on the river cruising up to the end point (N 05°31'50.3" E 115°42'05.0'').

The surveyed area was divided into three sections and three display trees (sampling stations) with the highest congregation of fireflies were sampled at each section. Fireflies were sampled by using an aerial net for approximately two minutes. Specimens collected from each display tree were placed in separate plastic bags (Foo & Mahadimenakbar, 2016).

The plastic bags were later brought to the lab and kept in a freezer overnight to kill the specimens. Specimens were later transferred to vials containing ethanol solution 75%. Specimens were then identified based on reference collections in Universiti Malaysia Sabah. In addition, there were six aquatic (pH, water temperature, dissolved oxygen (DO), conductivity, salinity and total dissolved solid (TDS)) and four terrestrial parameters (wind speed, relative humidity (RH), ambient temperature and light intensity (LI) from 3 sections (1) Boat berth – Starting point (N 05°31'27.0" E 115°43'03.2''), (2) Midpoint (N 05°31'34.9" E 115°42'34.2'') and (3) Endpoint (N 05°31'50.3" E 115°42'05.0'') were recorded with three replicates (A-I) for each section. Aquatic variables were recorded using Eutech Instruments PCD650 Multiparameter Meter while other terrestrial variables were recorded using the Kestrel 5500 Portable Weather Meter.

Results and Discussion

Species Diversity

In terms of firefly species, all five *Pteroptyx* fireflies that are recorded in Sabah were found in the area and these are *P. bearni*, *P. tener*, *P. malaccae*, *P gelasina*, and *P. valida*. This was the first record in Sabah where all five *Pteroptyx* species were recorded in the same area (Table 1). Binsulok can be considered a good place for congregating fireflies as in this short study, (one sampling occasion on nine display trees) can generate all five *Pteroptyx* species found in Sabah. The display trees were all from the same species, *Rhizophora apiculata* (Nilus et al., 2010).

Table 1. The number of samples collected on each display tree

Station		*P. bearni*	*P. tener*	*P. valida*	*P. gelasina*	*P. malaccae*
	A	5	0	3	0	0
1	B	5	0	0	0	0
	C	2	0	0	1	0
	D	3	0	0	1	0
2	E	2	0	2	0	0
	F	0	1	0	0	1
	G	3	1	0	0	0
3	H	10	0	0	0	0
	I	3	3	0	0	0
Total		**33**	**5**	**5**	**2**	**1**

This result suggested that Binsulok River has the highest number of species in one area in Sabah compared to other firefly sites reported earlier such as Klias, Paitan, Sepilok, Tuaran, Beaufort, and Pulau Sakar (Chey, 2004; Chey, 2006; Chey, 2008; Chey, 2009; Chey, 2010; Chey, 2011).

Throughout Malaysia, there are only seven species of *Pteroptyx* recorded, and in Sabah, only five have been confirmed. All five *Pteroptyx* species in Sabah can be found in Binsulok River. This result also suggests that Binsulok River has the richest species of congregating fireflies in Sabah, hence the need to conserve the area.

Land Use

The drone survey showed that there is a small patch of oil palm, a poultry farm, a few small watermelon farms and disused village garden patches. The drone survey also revealed that there is significant construction of roadwork in the pristine area of the mangrove across the Binsulok Nature Resort. These results could not be quantified but this survey suggests that these land use changes could contribute to a decline in the population of fireflies. However, from the researchers' point of view, this only has a small effect, as the visibility of fireflies sighted during research was still high compared to other fireflies research sites in Sabah.

Aquatic and Terrestrial Parameters

The survey for aquatic and terrestrial parameters suggest that most organisms living in estuaries prefer a pH of between 6.5 and 8.5. If the pH drops below 5.0 or goes above 9.0, many marine organisms will have trouble surviving (Robertson-Bryan, 2004). In Binsulok, the water pH recorded from all stations

were below 5.0. Water temperature recorded was between 28.2-28.4°C and this is considered normal in tropical areas (Table 2).

Table 2. The results of the aquatic and terrestrial parameters. Standard error of the means were calculated.

Station		Parameters									
		pH	Water Temp (°C)	DO (mg/L)	EC (µS/cm)	Salinity (mg/L)	TDS (mg/L)	Wind Speed (m/s)	RH (%)	Ambient Temp (°C)	LI (lux)
1	A	4.77	28.3	4.14	92.76	130	59.22	0.0	82.9	28.3	0.0
	B	4.54	28.2	4.82	92.54	150	60.15	0.0	82.0	28.2	0.0
	C	4.53	28.3	7.75	91.49	180	58.55	0.0	82.9	28.3	0.0
2	D	4.48	28.4	2.29	104.6	170	66.94	0.0	85.8	27.9	0.0
	E	4.47	28.4	1.44	115.1	110	73.66	0.0	85.8	27.8	0.0
	F	4.47	28.4	2.14	109.4	170	70.01	0.0	85.8	27.9	0.0
3	G	4.46	28.4	2.69	116.4	140	74.49	0.0	88.7	27.3	0.0
	H	4.46	28.4	2.69	118.5	130	75.84	0.0	87.2	27.6	0.0
	I	4.45	28.4	2.31	115.8	150	74.11	0.6	86.7	27.7	0.0
Mean ± SE		**4.51 ± 0.03**	**28.36 ± 0.02**	**3.36 ± 0.64**	**106.29 ± 3.77**	**147.78 ± 7.60**	**68.11 ± 2.37**	**0.07 ± 0.07**	**85.3 1 ± 0.75**	**27.89 ± 0.11**	**0.00**

Data on dissolved oxygen DO (mg/L) showed that dissolved oxygen in Sungai Binsulok was quite low. Most stations gave readings lower than 5 mg/L. Only station 1 C gave a reading above 5 mg/L. This indicated that Binsulok River has low dissolved oxygen which is crucial for aquatic organisms to survive. Dissolved oxygen above 5 mg/l is needed for most marine plants and animals to survive as they need plenty of oxygen to breath. When the dissolved oxygen is low, below 3 mg/l, the water is called hypoxic. If all the dissolved oxygen is used up and is below 0.5 mg/l, the water is called anoxic. Under hypoxic conditions, many marine plants and animals may not survive. No marine plants and animals that require oxygen can survive in anoxic conditions. However, further water quality analysis has to be done in order to ensure the status of water quality of the river as the data obtained from this study was too minimal to determine the status.

Electric Conductivity (µS/cm) readings showed Binsulok River is not very salty since readings from all stations were between 118.5 (max) – 91.49 (min) µS/cm. The more ions that are present, the higher the conductivity of water.

Most fresh drinking water will have less than 100 µS/cm conductivity. Very brackish water could be around 27,000 µS/cm. Seawater has conductivity of around 54,000 µS/cm.

The average ocean salinity is 35ppt (35,000 mg/L) and the average river water salinity is 0.5ppt (500 mg/L) or less. Due to the fact that water in estuaries is a mix of fresh and sea water, the salinity in most estuaries is less than in the open sea. The salinity of Binsulok River was between 110 to 180 mg/L. Total dissolved solids (TDS) is defined as all inorganic and organic substances contained in water that can pass through a 2 micron filter. TDS is anything—other than the pure water—in water that cannot be seen. This could include any salt, metal or mineral, and the lower the TDS level is, the purer the water. The range of TDS of Sungai Binsulok was between 58.88 - 75.84 mg/L and this level is considered excellent.

All other terrestrial parameters (wind speed, relative humidity, air temperature and light intensity) did not show any peculiar patterns. Light intensity did not show readings because measurements were made at night, indicating that there was no light pollution at the display trees. Only two tests out of 10 came back with negative results. The low pH (acidic) and the low dissolved oxygen (hypoxic) of the river water could contribute to a decrease in the population of fireflies. Since the eight other tests have shown positive results, this means the river is still healthy hence the high visibility of firefly sightings.

Conclusions

Binsulok River has the highest species richness of congregating fireflies compared to other studied areas in Sabah. All five species are available and were recorded in a short study period. From the water quality study, two variables, pH and dissolved oxygen (DO) showed a range of values that are of concern as these variables are important for the survival of many species. Photos taken from the drone showed that there were some anthropological disturbances of the natural habitat. This, if not controlled, could give a considerable impact to the firefly population as fireflies are very dependent on their natural habitat for survival since they need swampy areas as breeding grounds, good and healthy mangrove trees as their display trees and a high abundance of snails as source of food for the firefly larvae.

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Journal of Tropical Biology and Conservation 15: 163–171, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Short Communication

A Checklist of Bats at Ulu Senagang, Keningau, Sabah

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Abstract

A bat survey was conducted at Crocker Range Substation Ulu Senagang, Keningau, Sabah, from February 19 to 23, 2018. Harp trap and mist nets were used to capture bats. The four trapping nights resulted in the capture of five species of bats belonging to three families namely, Pteropodidae, Emballonunidae and Vespertilionidae. Three species were captured from the old logged-over forest, whereas two species were captured in the modified landscape. Generally, the recorded bat species are classified as Least Concern by IUCN.

Keywords: Bats, diversity, landscape, logged-over forest, modified

Introduction

Borneo is the third largest island in the world and is recognized as one of the world's mega-biodiversity regions. One of the significant factions of Borneo's biodiversity is the mammalian fauna with about 288 extant species of terrestrial mammals (Payne et al., 1985). Among the most interesting group of mammals in Borneo are bats, which represent at least 31.9% of the terrestrial mammals of Borneo (Payne et al., 1985), although still deemed as underrepresented (Khan et al., 2008). Among the type of bats that can be found in Borneo are the Old-World fruit bats and insectivorous bats. They are a significant portion in the ecosystem contributing significantly to local forest ecology as pollinators, seed dispersers and regulator of insect populations (Kunz et al., 2011). Furthermore, the distinctive adaptations and specialization make them as eminent models for ecophysiology, ecomorphology, trophic interactions, biogeography, emerging diseases, and conservation studies (Kunz & Fenton, 2005). However, most abundant animals in the world are threatened by habitat loss, which significantly contributes to the declining of bat populations locally, regionally and globally (Kunz et al., 2011).

Received 25 May 2018
Reviewed 19 July 2018
Accepted 14 August 2018
Published 15 October 2018

Numerous surveys have revealed interesting information on the diversity of bats in Borneo (Suyanto & Struebig, 2007; Khan et al., 2008; Struebig et al., 2010; Struebig et al., 2012), although there are many more areas that are yet to be explored. One such place, for example, is Ulu Senagang, Keningau. To date, there is no published information on bats from this area, instigating this survey. The main aim of this study is to produce a taxonomic checklist of bats in Ulu Senagang, Keningau, as a baseline information on bats in the area. The results from the survey are reported herein.

Materials and Methods

Study Area

A bat survey was conducted in Ulu Senagang Substation, Keningau, which is situated at N 5º 21' 2", E 116º 1' 41" (Figure 1). The sampling site is close to the boundaries of Tenom and Keningau districts, and separated by the Mosolog River. Interestingly, this area is managed through a joint effort between Sabah Parks and the local communities of Ulu Senagang, identified as the Community Use Zone (CUZ). The study area is mainly covered by old logged-over forest and a mixed landscape of secondary forest and mixed-crop plantation, which include

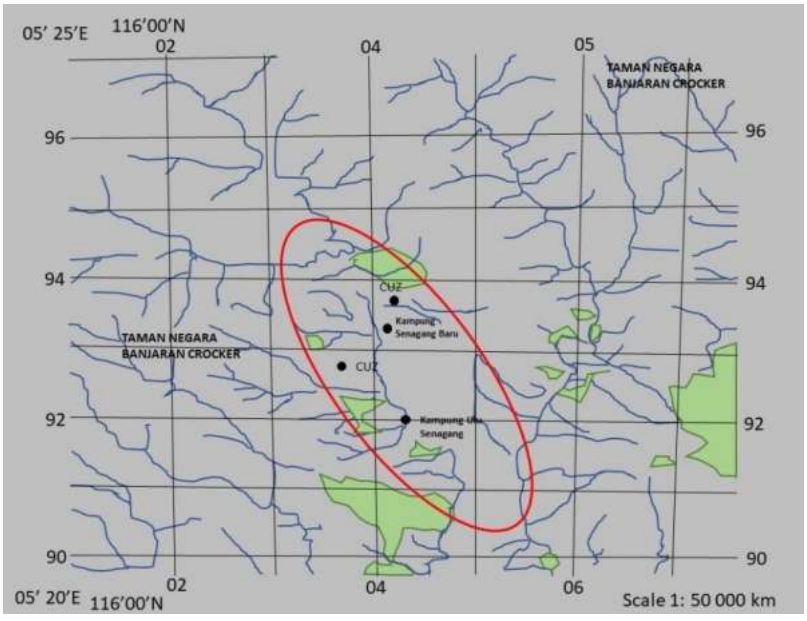

Figure 1. Map of the study area at Crocker Range Substation Ulu Senagang, Keningau.

rambutan (*Nephelium* spp.), mango (*Mangifera* sp.), durian (*Durio* spp.), tarap (*Artocarpus* sp.), cocoa (*Theobroma cocoa*) and mangosteen (*Garcinia mangostana*). The mixed landscape area is referred to as modified landscape in the current study.

Trapping methods and Sample Processing

Four nights of bat trapping was done using mist nets and harp traps that were set at distinctive landscapes, which are the old logged-over forest and a modified landscape (Table 1). The trap and nets were set up at potential bat flyways in the forest under storey and across small streams. The nets were opened from 1800 hours until 2100 hours and checked every 15 minutes to avoid casualties and over-stressing the captured bats. Meanwhile, harp traps were checked for every 1-2 hours until 2100 hours and left overnight and rechecked again at 0600 hours the following day.

All captured bats were identified to species level based on Payne et al. (1985) and Philips and Philips (2016). Information such as forearm length (mm), weight (g), sex and reproductive status were recorded (Brunet-Rossinni & Wilkinson, 2009). There were four categories of reproductive status assigned for female bats including nulliparous, pregnant, lactating and post-lactating. Measurements were taken using Vernier caliper and the bats were weighed using a Pesola spring balance. The total length of the cartilaginous region (total epiphyseal gap) between the bony diaphysis was used to estimate the age of bats (Brunet-Rossinni & Wilkinson, 2009).

Table 1. Coordinates of the respective locations where trapping were conducted.

	Trapping Site	Coordinate		Trapping Site	Coordinate
Old Logged-over Forest	A	5° 21' 58" N 116° 1' 34" E	Modified Landscape	G	5° 20' 37'' N 116° 1' 59'' E
	B	5° 21' 56" N 116° 1' 32" E		H	5° 20' 31'' N 116° 1' 50'' E
	C	5° 21' 56" N 116° 1' 34" E		I	5° 20' 33'' N 116° 1' 53'' E
	D	5° 21' 54" N 116° 1' 32" E		J	5° 20' 30'' N 116° 1' 49'' E
	E	5° 21' 54" N 116° 1' 32" E		K	5° 20' 27'' N 116° 1' 59'' E
	F	5° 21' 51" N 116° 1' 32" E		G	5° 20' 37'' N 116° 1' 59'' E

Results

A total of 11 individuals of bats representing five species were recorded from the four nights of sampling. The five species of bats represented three families namely, Pteropodidae (two genera, three species), Emballonuridae

(one genus, one species) and Vespertilionidae (one genus, one species) (Table 2; Figure 2).

Table 2. Taxonomic checklist of bats at Substation Ulu Senagang, Crocker Range Park Keningau, Sabah

Family	Species	Common Name	Relative Abundance (%)	Conservation Status	
				WCE 1997	IUCN Red List
Pteropodidae	*Cynopterus* cf. *minutus*	Forest Short-nosed fruit bat	18.2	NL	LC
	Cynopterus cf. *brachyotis*	Sunda Short-nosed fruit bat	18.2	NL	LC
	Balionycteris maculata	Spotted-winged fruit bat	27.3	NL	LC
Emballonuridae	*Saccolaimus saccalaimus*	Pouched Tomb bat	27.3	NL	LC
Vespertilionidae	*Glischropus tylopus*	Thick-thumbed Pipistrelle	9.1	NL	LC
Number of Families			3		
Number of Species			5		
Number of Individuals			11		

***NL - Not Listed**
****LC - Least Concerned**

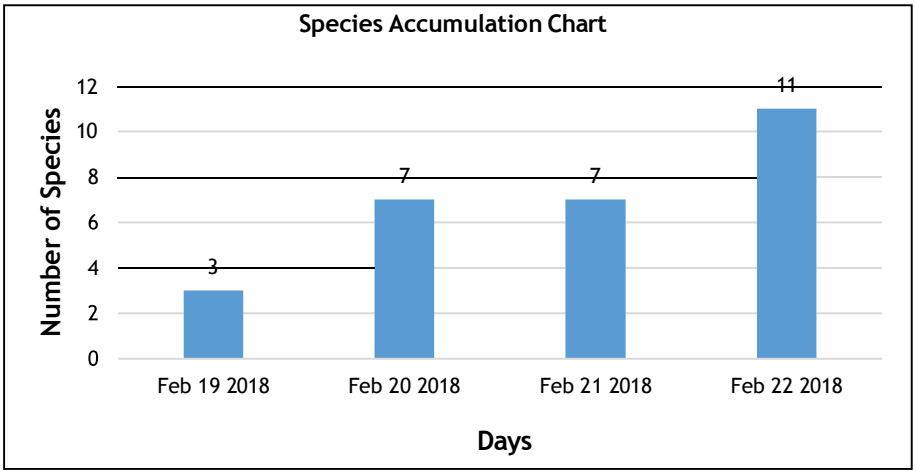

Figure 2. Species Accumulation Chart of Bats in Ulu Senagang, Keningau.

From the survey, *Balionycteris maculata* was the most abundant species, followed by *Cynopterus* cf. *brachyotis*. A colony of 30 – 40 individuals of *Saccolaimus saccolaimus* were observed in one of the buildings at SK Ulu Senagang. However, only three individuals were captured and examined. One species, *Glischropus tylopus* was caught in singleton during the survey.

However, the species accumulation graph generated from the results of the current survey did not reach asymptote (Figure 3).

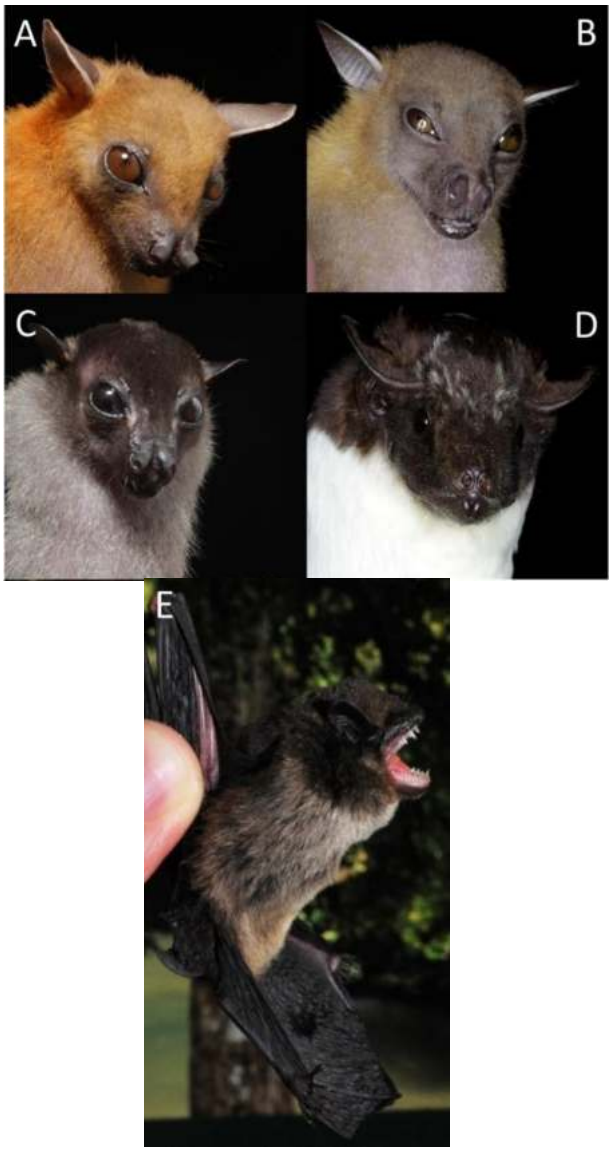

Figure 3. Photo **A)** *Cynopterus minutus*, **B)** *Cynopterus brachyotis*, **C)** *Balionycteris maculata*, **D)** *Saccolaimus saccolaimus*, **E)** *Glischropus tylopus*

Species Account

Family Pteropodidae

Cynopterus* cf. *minutus Miller 1906 (Forest Short-nosed Fruit Bat)

A total of two individuals were captured at the old logged over forest nearest to the substation. *Cynopterus* cf. *minutus* can be distinguished from other members of genus *Cynopterus* using forearm (FA) measurement. Both adult individuals have FA length of less than 60mm (Phillips & Phillips, 2016). This species feed on various fruits, leaves and nectars (Phillips & Phillips, 2016). This species is listed as of Least Concern by IUCN 2018.

Cynopterus* cf. *brachyotis Müller 1838 (Sunda Short-nosed Fruit Bat)

Two adult individuals were captured in the old logged over forest nearest to the substation. Similar their smaller counterpart, *C*. cf. *brachyotis* can be distinguished using their forearm (FA) length. The two captured individuals have a forearm length of more than 60 mm (Phillips & Phillips, 2016). This species is a medium-sized fruit bat with a short muzzle, brown with brighter collar which dark orange in adult males, yellowish in females (Yasuma et al., 2003). Their diet is mainly small fruits, where they were reported to be sucking on the juice and soft pulps (Yasuma et al., 2003). This species is listed as of Least Concern by IUCN.

Balionycteris maculata Thomas 1893 (Spotted-winged Fruit Bat)

A total of three individuals were captured from the old logged over forest nearest to the substation. The captured individuals have FA length between 40-45 mm. This species is easily recognized by the pale spots on their wings and face (Phillips & Phillips, 2016). They forage at all strata of the forest (Phillips & Phillips, 2016). This species is known to occur at a variety of habitat. Roosts include trees, crowns of palms and clumps of epiphytic ferns (Yasuma et al., 2003). *B. maculata* is listed as Least Concern by IUCN.

Family Emballonunidae

Saccolaimus saccalaimus Temminck 1838 (Pouched Tomb Bat)

A total of three individuals were captured from one of the buildings at SK Ulu Senagang. It is among the larger species of the tomb bat in Borneo (Phillips & Phillips, 2016). *S. saccalaimus* can be distinguished with the bare skin on their face, which normally appears black or dark grey. This is one of the distinct species where their upperparts are dark black or brown with white spot markings (Payne et al., 1985; Phillips & Phillips, 2016). Both sexes have a glandular chin-pouch. There were about 30 – 40 individuals of *S. saccolaimus* estimated to be roosting in the building. This species is listed as Least Concern by IUCN.

Family Vespertilionidae

Glischropus tylopus Dobson 1875 (Thick-thumbed Pipistrelle)

This is the only species caught in singleton in the harp trap placed across small streams in the modified landscape of Ulu Senagang. The capture area was covered with patch of secondary forests, with various fruiting trees and plants such as banana, rambutan and cocoa. *Glischropus tylopus* can be distinguished from similar species such as *Hesperoptenus blanfordi* and *Tylonycteris* spp. through pale to pinkish pads on their thumbs and feet (Payne et al., 1985; Phillips & Phillips, 2016). This species is known to occur in lowland dipterocarp forests and they usually roost in dead or damaged bamboo stems, as well as rock crevices or new banana leaves (Yasuma et al., 2003). This insectivorous bat is listed as Least Concern by IUCN.

Discussion

This survey has recorded five species of bats in Ulu Senagang, Keningau. The recorded bat species were listed as Least Concern in the IUCN Red List of Threatened Taxa 2018. However, the number of species recorded in the current study were low. In the current study, mist nets were more successful in catching bats compared to harp traps. There were only two species of insectivorous bats recorded however, only one species was captured using harp traps, which was placed across small streams in the modified landscape. Additionally, three individuals of *S. saccolaimus* were captured using mist nets erected at their roosting area. This however does not question the efficiency of harp traps in catching insectivorous bats over mist nets (Mohd-Azlan et al., 2005). Mist nets can be useful for catching insectivorous bats in certain situations. To improve the capture of insectivorous bats, nets should be checked regularly to ensure bats do not escape. Insectivorous bats are known to be able to escape from mist nets by chewing the nets using their sharp, fine teeth (Abdullah et al., 1997).

The physical presence of human impact on the landscape and its inhabitants in Ulu Senagang were quite apparent. Although there was no clear evidence of hunting in the area due to its Community Use Zone status, landscape modifications for agriculture is prominent and is a major source of income for the locals. This leads to a speculation consistent with Struebig et al. (2009) that habitat loss a major threat to bat populations in Borneo. The Paleotropical bats are very dependent on natural landscapes such as forests as it provides them with resources such as roosting areas and food. In relation to this, the suitability of an area as a roosting site is often judged as a sanctuary for bats as it

safeguards them from abrasive and unpredictable environmental conditions and predators (Kunz, 1982).

Ecosystem services provided by the pteropodids and insectivorous bats are increasingly recognized in Southeast Asia. Various bat researchers have conducted studies at varying degrees to further apprehend the role of bats and the impacts of the provided ecosystem services, both to the local ecosystem as well as the economy (see Bumrungsri et al., 2009; Bumrungsri et al., 2013; Acharya et al., 2015; Aziz et al., 2017). Modification of natural landscape driven by industrialization and urbanization in Southeast Asia will not ameliorate the situation that bats are facing at present. It is anticipated that constant modification of Ulu Senagang's landscape will aggravate the diminishing local bat populations and eventually will have a discernible effect on the surrounding forests as well as the local economy. However, a more comprehensive study is needed to reassert the assumptions derived from the observed intensification of landscape conversion towards the bat community of Ulu Senagang.

Acknowledgements

This study would not have been possible without the financial and logistic support from University College Sabah Foundation. We are thankful to Sabah Parks and the villagers of Ulu Senagang for permitting us to conduct our work at Ulu Senagang and ensuring our needs were met throughout the duration of the survey. We would like to extend our gratitude to various personnel including students and lecturers of FNSS who have provided continuous effort in facilitating the acquirement of data for the current study, of which is significant in enabling the completion of this manuscript.

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Journal of Tropical Biology and Conservation 15: 173–188, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

An Inventory of Flora in Urban Forests of Universiti Malaysia Sabah Campus, Sabah, Malaysia

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Abstract

Species diversity is one of the most important measures for estimating the sustainability of forest communities. This study aims to compare plant diversity between two secondary forest sites namely the UMS Hill and ODEC in Universiti Malaysia Sabah (UMS) and to update the list of flora in UMS forests. A plot of 50 m x 50 m (0.25 ha) was set up at each forest site. Temperature, light intensity and relative humidity were measured in both study plots with HOBO data loggers. A total of 5,301 individuals, 84 species and 48 families were recorded in both plots. The family Zingiberaceae was represented by a single species *Alpinia aquatica* which dominated other families and species by having the highest abundance, contributing to 19.79% of the total density while the family Rubiaceae was the most speciose in both plots. There was no significant difference between plant species diversity in UMS Hill (*H*'= 3.355, Hill's number=29) and ODEC (*H*'= 3.290, Hill's number=27) (t=1.827; p = 0.0677). Species in UMS Hill (*E* = 0.792) was distributed more evenly compared to ODEC (*E* = 0.785). Measured climate parameters have slight variation in both plots which is attributed to microhabitat influence within each study plot. Similar environmental conditions in both study plots contribute to relatively similar plant diversity and composition in the study plots. The study added 26 species as new records to the existing flora checklist thus giving a total of 302 plant species for the UMS forest.

Keywords: Species diversity, biodiversity, secondary forest, tropical, conservation

Introduction

The lowland evergreen tropical rainforest is the most fertile forest, diverse in species and has a large and complex structure (Whitmore, 1991). Undisturbed forest or primary forest is the most biologically diverse type of forest, relatively unaffected by human activities, and still exists in its original condition (Butler, 1994). Meanwhile, secondary forest is a rainforest that has been disturbed in some ways, naturally or unnaturally. Generally, secondary forest is

Received 08 June 2018
Reviewed 18 July 2018
Accepted 14 August 2018
Published 15 October 2018

characterized (depending on its level of degradation) by less developed canopy structure, smaller trees and less diversity (Butler, 1994). Chokkalingam & de Jong (2001) stated that secondary forest relates to successional forests which develop after clearing of the original forest, and secondary succession is complete when they develop again into climax communities or primary forests.

Plant diversity can be affected by many factors, indirectly by elevation as environmental condition changes along elevational gradient (Korner, 2007; Brown, 2001; Malik, 2008), soil fertility (Takyu et al., 2002; Kumar et al., 2010), geological substrate (Aiba et al., 2002), disturbance (Denslow, 1995; Cayuela et al., 2006) and climate (Hidore & Oliver, 1993). Elevation gradients create varied climate, along with resultant soil differentiation and promote the diversification of plant species (Brown, 2001). Changes in climate could be expected to alter the regeneration success, growth and mortality rate of tree species (Malik, 2008). Climate may also affect the distribution of individual species and community (Hidore & Oliver, 1993). Changes in forest structure due to disturbance alter the environment within the forest, subsequently the diversity and composition of the forest also changes. According to Whitmore (1991), the forest can be replaced by a less fertile forest with medium sized trees and fewer species when environmental conditions become deficient.

Plants may be a renewable resource, but plant diversity is not. Anthropogenic activities such as deforestation, logging activities, and slash-and-burn clearing of forests for agriculture and infrastructure development pose a major threat to plant diversity. As a result, primary tropical rainforests are replaced with a secondary forest and open woodland patches or grassland (Whitmore, 1991). The loss of plant species is often accompanied with the loss of insects and animals. With the expansion of secondary forests in place of virgin forests in tropical countries, managing these forests has never been more relevant for the conservation of forest biodiversity. Regenerated forests or secondary forests are valued for their roles as refuge for flora and fauna, thus preventing extinction (Chazdon et al., 2009; Dent & Wright, 2009) and for their contribution to carbon pool recovery (Martin et al., 2013). The Universiti Malaysia Sabah (UMS) Hill and Out-Door Development Center (ODEC) forests are patches of secondary forests which form part of the extensive vegetation cover in UMS campus. For the past 23 years after the establishment of the campus, these patches of forest have been slowly recovering and a few notable species of fauna have been observed in UMS Hill forest. These patches of secondary forests attract animals by providing habitat and food sources.

The study aims to determine the composition and diversity of flora in the secondary forests on UMS Hill and ODEC. The only study on the flora of UMS Hill forest was conducted along the trail leading up to the peak of the hill that resulted in a plant checklist (Sugawara et al., 2009). No flora surveys were ever conducted in other forest areas in UMS campus. The UMS forests are an important source of biodiversity in the urban area and may act as refuge for flora as well as fauna, thus mitigating the effects of species loss such as local extinction. The enhancement of quality of the forests is deemed necessary and thus findings from this study may contribute to effective rehabilitation process of the forests. The presence or absence of dominant and rare species in a forest provide valuable information about the quality of the forest, for instance, dominance of climax tree species suggest that the forest has recovered and reached a stable climax forest community. On the other hand, dominance of secondary forest species implies the forest is still regenerating or recovering from past disturbances. Ecological restoration such as rehabilitation activities can accelerate the recovery process of the forest.

Materials and Methods

Study Site

UMS is located in Kota Kinabalu, Sabah with a total area of 4.04 km^2, facing the South China Sea at Sepanggar Bay. Prior to infrastructure development of the UMS campus in 1995, the landscape of the area was overlaid with a mosaic of human settlements, agricultural land and some vegetation cover. Since the establishment of the campus and operation of the university in its new campus in 2000, the undeveloped part of the land has regenerated resulting in the spread of vegetation cover to bare lands, some areas dominated by stands of *Acacia* species. This area is located northwest of the campus, and is a place covering approximately 1.2 km^2 (120 ha), and which has patches of vegetation (Sugawara et al., 2009). Other small forest patches isolated from the 1.2 km^2 area also exist within the campus. Within the context of our study, only the 1.2 km^2 is referred to as UMS forest. UMS forest is an extensive area with diverse landscape from fragmented secondary forest to open canopy areas which are primarily covered with bushes and grasses. The vegetation is reminiscent of typical lowland forest and mangrove forest in several areas extending seaward. The topography is flat undulating to hilly with steep slopes in some areas. The highest peak is UMS Hill peak at 190 m asl. The UMS forest experiences a typical equatorial climate, with constant temperature and considerable amount of rain and high humidity. The average annual rainfall is 2,700 mm and the average annual temperature is 28 °C.

This survey was carried out in selected forest areas in UMS forest, the first site was the forest in UMS Hill (hereafter called the UMS Hill) (06° 02' 15.59" N; 116° 06' 55.26" E) and the second site was in ODEC (hereafter called the ODEC) (06° 02' 42.0" N; 116° 06' 46.0" E) (Figure 1). UMS Hill site at 114 m asl is dominated by a secondary forest with hills, ridges and secondary ridges shaping its terrain. The ODEC site is located in a flat coastal area with an elevation of 29 m asl. The vegetation of the ODEC site consists of secondary forest and bushes.

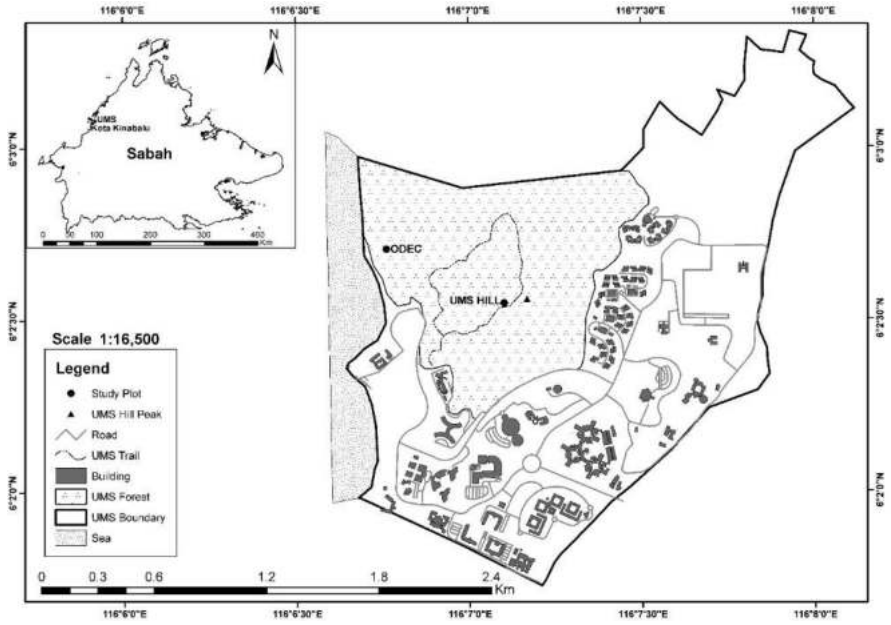

Figure 1. Location of the UMS forest and study plots in UMS campus

Data collection

To determine plant species diversity and composition, a plot of 50 m x 50 m was each set up at UMS Hill and ODEC. The plot was divided into 10 subplots, each measuring 10 m x 25 m. All vascular plants (trees, shrubs, herbs, grasses, ferns and palms) within the subplots were counted, recorded and identified. All plants with a minimum height of 3 m and ≥10 cm DBH were considered as trees. This included treelets with less than 10 cm DBH but reaching the height of 3 m. Plants with hard stem, less than 3 m height and ≤ 10 cm DBH were categorized as shrubs. Herbs included small forest floor plants with tender stem whereas grasses, ferns and palms were characteristically grouped based on their own distinctive features.

Voucher of fertile specimens were made in duplicates and deposited in Borneensis Herbarium (BORH) at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. Fertile specimens included plants bearing either flowers or fruit or both were collected while mature fern with fronds bearing spores were prioritized for collection. Identification of plants is based on the Tree Flora of Sabah and Sarawak Volume 1 – 7 (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) and Buku Panduan Hutan Bukit UMS (Sugawara et al., 2009). Additionally, identification of plants was performed by comparing voucher specimens in the BORH and SAN herbariums (Forest Research Centre, Sandakan).

HOBO Pendant Temperature/Light Data Logger (UA-002-64) and HOBO Pro v2 Logger (U23-002) were used to detect and record the data of temperature and light intensity for five months (November 2011 - March 2012), and relative humidity for four months (November 2011 - February 2012) in the two plots. The devices were set up high above the ground (>1.5 m) and secured with durable string on tree branches or tree trunks. Five data loggers (three U23-002 and two UA-002-64) were set up in each study plot. The data loggers were distributed at an interval of approximately 10 – 20 m apart along a transect line from north to south in the middle of each plot.

Data Analysis

To compare plant species diversity and composition, Shannon-Weiner Diversity Index, Sorensen's Similarity Index and Pielou's Evenness Index were determined for the two plots. Hutcheson's t-test for significance on Shannon-Wiener Indices (Hutcheson, 1970) was performed to test the difference between plant species diversity of the two plots. The Shannon Diversity Index was converted to effective number of species (also referred to as Hill's number) (Jost, 2006) to determine the magnitude of the difference between study sites in relation to plant species diversity.

Shannnon-Wiener Index:

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

$$Pi = \frac{ni}{N}$$

n_i= individual number of i species

N=total number of all individuals

Hutcheson t-test:

$$t = \frac{H_a - H_b}{\sqrt{s^2_{H_a} + s^2_{H_b}}}$$

Ha = Shannon Index for sample a
Hb = Shannon Index for sample b
S = variance

Variance of the Shannon diversity is computed using the formula below:

$$s^2_H = \frac{\sum p.(\ln p)^2 - \left(\sum p.\ln p\right)^2}{N} + \frac{S-1}{2N^2}$$

S = species richness
N = total number of individuals
p = proportion that each species makes towards the total

Sorenson's Similarity Index (Ss):

$$Ss = \frac{2a}{2a + b + c}$$

a= number of species common to both samples
b= number of species in sample 1
c= number of species in sample 2

Pielou's Evenness Index (*E*):

$$E = \frac{H'}{\ln S}$$

H'= the number derived from the Shannon Diversity Index
S = maximum value of *H*'

Results and Discussion

Forest micro-climate

No obvious variation of temperature, light intensity and relative humidity was observed in both study plots (Figure 2). The average monthly temperature from November 2011 to March 2012 in the study plots was similar (25ºC - 27ºC), a typical temperature for equatorial tropical forests (Hess, 2014; Peterson et al., 2015). The temperature was high in November 2011 and decreased in December

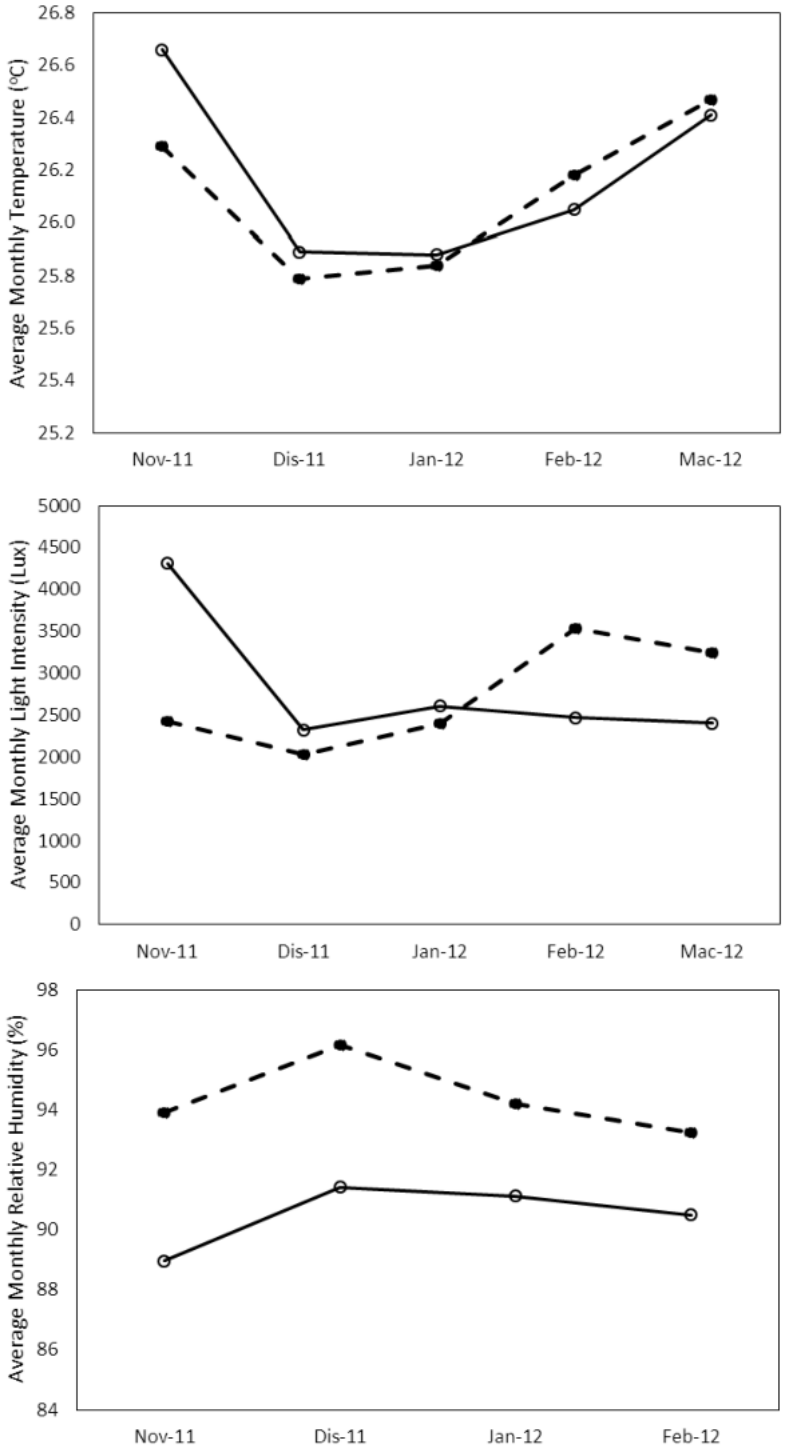

Figure 2. Fluctuation of temperature, light intensity and relative humidity in UMS Hill and ODEC (UMS Hill; ODEC)

2011 and January 2012 with the onset of the rainy season. The temperature started to rise in February and March 2012 because it was the beginning of the dry season. The study plots recorded the lowest light intensity in December 2011. The relative humidity was always interrelated with light intensity. There was a strong negative correlation between relative humidity and light intensity at each study plot (r = -0.943 for UMS Hill and r = -0.801 for ODEC). Both study plots recorded the highest relative humidity in December which coincided with the peak of the rainy season. During this time, the high rainfall coupled with proximity of the plots to the sea created a more humid air environment than usual. The average relative humidity was higher in ODEC as compared to UMS Hill and this was probably due to its location. ODEC was located nearer to the sea and received stronger sea breeze especially during the monsoon season and the air humidity around the coastal region was higher compared to other regions.

Species diversity and composition

A total of 5,301 individuals, 84 species and 48 families were enumerated from the plots. A majority of plant species recorded were classified as trees (including treelets) with 57 species, followed by shrubs with 11 species, herbs with six species, lianas with five species, grasses and ferns each with two species, and palm with one species (Figure 3).

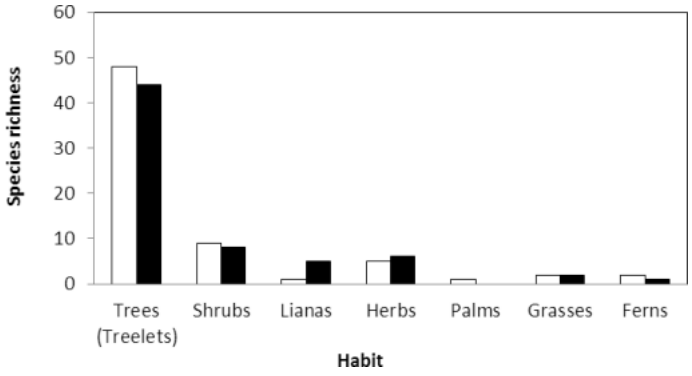

Figure 3. Comparison of plant types between study plots (□ UMS Hill; ■ ODEC)

Zingiberaceae was the most dominant family in term of density (19.79%), followed by Cyperaceae (16.32%), Myrtaceae (6.47%) and Poaceae (5.28%). Myristicaceae, Rhamnaceae (both 0.06%) and Taccaceae (0.04%) were rare. The most abundant species was a member of the Zingiberaceae family, *Alpinia aquatica*, which was the only ginger species recorded. *Alpinia aquatica* can

adapt to a wide range of ecological conditions. An open area can be overgrown by this species in a relatively short period of time (Gobilik & Limbawang, 2010). Other abundant species were *Scleria sumatrensis*, *Carex* sp., *Syzygium leucoxylon, Acroceras* sp. and *Lygodium circinnatum*. In terms of species richness, many plant families enumerated in the study sites were less speciose with less than ten species (Figure 4). A majority of the plant families consisted of only a single species. Shannon-Weiner's diversity index for UMS Hill (H'= 3.355) was not significantly different from ODEC (H'= 3.289), indicating that the secondary forest in UMS Hill was apparently similar to the ODEC forest (t=1.827, *p*=0.0677). The actual numbers of species for UMS Hill and ODEC were 68 and 66 respectively. The effective number of species (Hill's number), which relates to equally abundant species, varied by only two species between the plots (29 species in UMS Hill versus 27 species in ODEC) (Table 1). In addition, Pielou's evenness index was similar for the two plots (*E*=0.795 for UMS Hill versus *E*=0.785 for ODEC). The species rank abundance curve also showed a similar pattern (Figure 5). UMS Hill and ODEC were moderately even in terms of species abundance. Some forest floor species such as *A. aquatica* and *S. sumatrensis* were common species that dominated both plots by having high abundance, whilst *Hypserpa nitida,* a woody climbing vine, was considered rare in UMS Hill plot, the only species that has two individuals. In ODEC plot, *Timonius villamilii*, *Fagraea cuspidata*, *Ficus septica* and *Tacca borneensis* have the lowest abundance with two individuals.

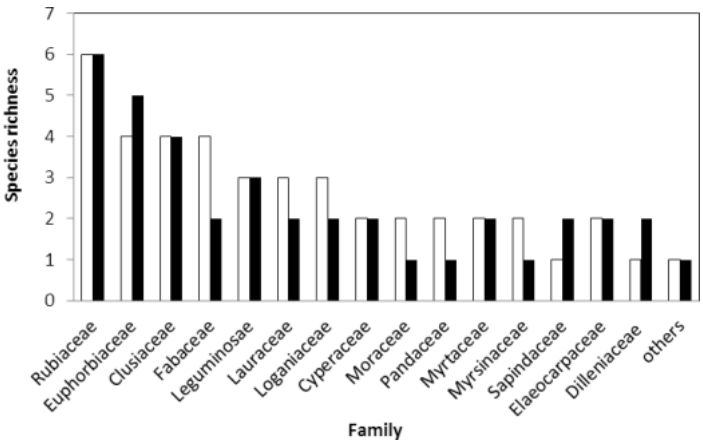

Figure 4. Species richness of enumerated plant family in UMS Hill and ODEC (□ UMS Hill; ■ ODEC)

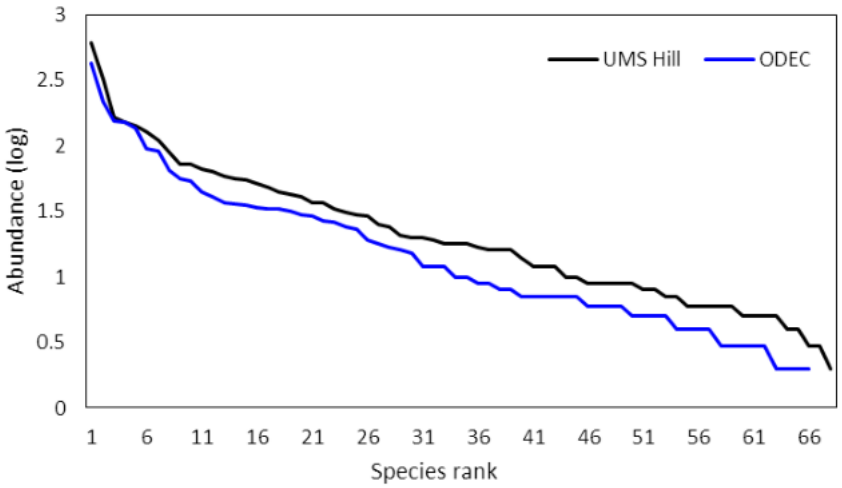

Figure 5. Species rank abundance curve of UMS Hill and ODEC

The calculated Sorenson similarity index showed that 74.5% of the total species recorded were common in both plots. From the total of 84 species identified, 50 species were found in both plots. Forests in both plots have similar environmental conditions such as climate, with little difference in elevation. The other 25.5% of species only occupied either one of the plots. Eighteen species were only observed in UMS Hill whereas 16 species only occurred in ODEC. Nevertheless, these species may be encountered in either plots if the magnitude of sampling effort is increased such as increasing the plot size to capture more plant species. Many of the tree species encountered in both plots are considered as constituents of secondary or disturbed forests. Previous studies have shown that the composition of species in a secondary forest is influenced by many factors, such as previous type of land-use, the degree and different type of forest degradation across the landscapes (Chazdon, 2003) as well as the level of forest succession, i.e. regeneration since disturbed, and previous vegetation (Brearley et al., 2004). Kessler et al. (2005) enumerated trees in 0.02 ha of a five year old secondary forest in Central Sulawesi and found

Table 1. Measures of plant species diversity of forest community in UMS Hill and ODEC

Elements of diversity	UMS Hill	ODEC
Species richness	68	66
Hill's Number	29	27
Number of family	40	44
Total number of individual	3,063	2,238
Shannon Diversity Index, *H*'	3.355	3.290
Pielou's Evenness Index, *E*	0.795	0.785

6 to 17 tree species. In contrast, Brearley et al. (2004) recorded an average of 55 tree species in six plots of 0.25 ha in a 55 year old secondary forest in West Kalimantan. Our study recorded an average of 67 species (66 species in ODEC and 68 in UMS Hill) in two plots of 0.25 ha, of which an average of 46 species were trees. A higher species richness was the result of including all vascular plant types except epiphytes in our survey. In addition, the UMS forest, inclusive of the study plots, has been regenerating and has been re-colonized for the past 23 years since the campus was developed. During that time, remnants of forest patches within the UMS forest may have already existed, and provided a source of plant propagules for regeneration in the open and disturbed areas. Monitoring of the forest is therefore crucial to determine the extent of natural and assisted forest succession. Thus, increasing the number and size of plots is deemed necessary in order to capture the actual species diversity and composition at different successional stages.

Species indicators of forest disturbance such as *Mallotus* and *Macaranga* (Slik et al., 2003) were absent in both plots which was in contrast to findings by Sugawara et al. (2009) that recorded *Mallotus paniculatus* and *Macaranga tanarius* in their survey. Their survey was conducted along forest trails which were in an open area and received abundant sunlight, a condition suitable for the growth of these species. On the other hand, our study plots were established in areas with dense canopy and no forest edges, thus it is likely that *M. paniculatus* and *M. tanarius* have been replaced by other plant species during the forest succession period. In a 55 year old secondary forest in Central Kalimantan, Indonesia, Brearley et al. (2004) recorded higher dominance by the tree species *Pternandra coerulescens* (Melastomaceae), a species regarded as a pre-disturbance remnant. Although not in abundance, the species occurred in both of our study plots suggesting the existing individuals were regenerated species or remnants of prior vegetation. Both plots were dominated by a tree species, *Syzygium leucoxylon* (Myrtaceae), which is a common tree species in coastal forests and along estuaries (Soepadmo et al., 2011), and can also be found in peat swamp forests (Siregar & Sambas, 2000).

Several species recorded in the plots were non-native such as *Hevea brasiliensis*, *Acacia auriculiformis* and *Acacia mangium* which were introduced for agricultural purposes in the past. Land use history such as human settlement, disturbance and cultivation may pose a significant effect on the pattern of plant diversity in the UMS forests. The rubber tree could be the remnant of cultivated crops which existed in the area. *A. auriculiformis* and *A. mangium* appeared abundant in forest patches within the campus area but not in the study plots.

Acacia species comprised only 2% of the total abundance enumerated from the plots. The species are non-native species that have become naturalized through intentional and unintentional introduction. Aguiar et al. (2013) reported that individuals of *Acacia* species growing underneath the forest canopy have greater potential to grow to adult phase compared to individuals growing outside of the canopy cover as the existing native trees can create a conducive micro-environment for *A. mangium*. However, lush ground vegetation overgrown with seedlings and sapling may hinder the invasion of *A. mangium* due to competition for light (Osunkoya et al., 2005). Both plots are dominated by dense undergrowth such as *Alpinia aquatica* and grass species that may out compete seedlings of *A. mangium*, thus individuals of the species were seldom encountered in the study plots.

The differences in plant species composition between the UMS Hill and ODEC plots may be influenced by the microclimatic condition within the forests. The interaction of temperature and light with humidity creates favourable condition for certain types of plants. The air humidity decreases as temperature or light increases in the environment as heat causes evaporation of the air. Forests in both study sites contain higher richness of small trees with less canopy structures. Due to lack of a full canopy, more light reaches the forest floor and support vigorous ground vegetation (Whitmore, 1991). UMS Hill plot has higher light intensity as compared to ODEC plot, this is due to the existence of open canopy and less canopy structures in the forest, allowing more sunlight to reach the forest floor. Higher diversity of plant species and abundance in tropical forests may be attributed to higher light intensity (Subramanyam & Sambamurty, 2006; Cavusoglu & Kabar, 2007). Additionally, the features of landscape in an area may act together with the within-site variations that could affect species occurrence and abundance (Johnson et al., 2000; Moran et al., 2000; Zarin et al., 2005), thus influencing the composition and abundance of species in the plots.

In a previous floristic survey along the trail to UMS Hill peak, a total of 276 plant species were recorded in UMS Hill (Sugawara et al., 2009). The current study has added 26 plant species (Table 2) to the existing list, giving a total checklist of 302 plant species in the UMS forest. A larger area (0.5 ha) was covered in this study, thus increasing the potential of discovering new records of plant species in the area.

Table 2. A list of new records of plant species found in UMS Hill and ODEC

Family	Species
Annonaceae	*Desmos teysmannii* *Polyathia angustissima* *Uvaria curtisii*
Aquifoliaceae	*Ilex cymosa*
Clusiaceae	*Calopyhllum obliquinervium* *Garcinia caudiculata* *Garcinia microphylla* *Garcinia penangiana*
Cyperaceae	*Scleria sumatrensis*
Euphorbiaceae	*Breynia racemosa*
Elaecarpaceae	*Elaeocarpus nitidus*
Fabaceae	*Airyantha borneensis* *Spatholobus gyrocarpus*
Lauraceae	*Litsea cylindocarpa* *Neolitsea cassia*
Menispermaceae	*Hypserpa nitida*
Myrsinaceae	*Ardisia macrocalyx* *Rapanea borneensis*
Oleaceae	*Chionantus pluriflorus*
Pandaceae	*Galearia stenophylla* *Microdesmis casearifolia*
Rubiaceae	*Aidia borneensis* *Metadina trichotoma* *Oxyceros longiflorus*
Rutaceae	*Clausena excavata*
Sapotaceae	*Palaquium gutta*
Total = 15 Families	**Total = 26 Species**

Conclusion

Species diversity is similar for UMS Hill and ODEC plots but species composition and abundance are different between the plots. Similar geographic location, elevation, land use history and climate could be the contributing factors that influence plant diversity in the plots. The present survey contributes a total of 26 new records of plant species to the current checklist by Sugawara et al. (2009), thus giving a total of 302 plant species found in the UMS forest. The new update of plant species checklist and plant specimens collected during the present study can be used as a reference or guideline for a future study in the UMS forest. To know the exact richness and composition of plant species, expansion of plot size and increasing the number of plots is necessary. Understanding the structural and floristic vegetation are important for proper management of forest rehabilitation process in the UMS forest. The UMS forest is still in the regenerating phase and faster recovery can be facilitated through a specific rehabilitation programme. In future, any rehabilitation programme of

UMS forests should consider matching habitat suitability with native species that are of ecological match to the forest type in UMS.

Acknowledgements

The authors would like to acknowledge the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Malaysia and Forest Research Centre, Sandakan, Sabah for the use of their facilities and logistic support. Special thanks to Ms. Arnie Abdul Hamid for her assistance in creating a map of the study area.

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Journal of Tropical Biology and Conservation 15: 189–201, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Seagrass Meadow Impacts on Universiti Malaysia Sabah (UMS) Beach, Kota Kinabalu Sabah (Malaysia)

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Abstract

Seagrass meadow is known for its contribution as a habitat, ability to attenuate the wave's energy and as natural protection for the beach. The coastline of Universiti Malaysia Sabah (UMS) consists of important coral reef and seagrass ecosystems. Seagrass meadow is found at the northern part of the UMS beach at an area known as the Outdoor Development Centre (ODEC) while patchy coral reefs are found at the deeper part of the shoreline. The objectives of this study are to determine the beach profile and sediment characteristics of UMS-ODEC beach during the Southwest and Northeast monsoons and to compare the beach profile at areas where seagrass meadow is present and exposed beach area to the open sea. Beach profiling measurement was done at the three transects placed perpendicular to the UMS-ODEC beach. Transects 1 was with presence of seagrass and transects 2 and 3 were without seagrass. Sediment samples were taken at each transect for sediment characteristics identification. The assessment was done at an interval of every two month starting from May 2016 to March 2017. Beach profile elevation of UMS-ODEC Beach is higher during the Northeast monsoon than during the Southwest monsoon. It was also identified that large accretion happened at transects 1 and 2 between the month of July 2016 and September 2016 and between the month of January 2017 and March 2017. The erosion process happened at all transects between the month of September 2016 and November 2016 during the peak of the Southwest monsoon and continued to erode between the month of November 2016 and January 2017 during the Northeast monsoon.

Received 02 July 2018
Reviewed 07 August 2018
Accepted 13 September 2018
Published 15 October 2018

Sediment characteristics of mean, sorting, skewness at all transects were categorized under fine sand, moderately sorted and coarsely skewed, respectively during the Northeast monsoon and vary sediment characteristics except skewness of at all transects during the southwest monsoon. Based on this one year measurement of beach profile and sediment characteristics, there is no clear impact of the seagrass meadow as a natural coastal protection for the UMS-ODEC beach.

Keywords: seagrass, beach profile, seasonal monsoon, sediment characteristics, Universiti Malaysia Sabah

Introduction

Seagrass beds are known for their ability to attenuate the wave's energy at coastal areas. It contributes to the protection of shorelines from strong waves and current. The extensive root systems and leaves help to control subtidal sediment erosion (Fronseca & Fisher, 1986). Development of coastal protection by incorporating ecology and ecosystem services has gained a strong interest over the last decades (Borsje et al., 2011). There is a need to minimize the impacts of coastal protection structure on ecosystems and also a strong need for an innovative, sustainable and cost-effective way to control subtidal erosion. Based on a nearshore numerical model done, there is a significant reduction of wave energy flux at the shoreline where seagrass is present (Chen et al., 2007). Thus, in certain coastal environments, seagrasses can be established at a much lower cost as a non-structural alternative for shoreline protection compared to man-made structures such as jetties and bulkheads. Seagrass meadow could also bring in added benefits by producing a highly productive biological community.

Universiti Malaysia Sabah (UMS) beach is located in Sepanggar Bay, Kota Kinabalu at the west coast of Sabah, Malaysia (Figure 1). Sepanggar Bay plays an important role for the coastal ecology socio-economic development of the area. The marine ecosystem within the UMS beach is characterised by coral reefs and seagrass meadow. Seagrass species identified are *Enhalus acoroides, Cymodocea rotundata, Halodule uninervis, Halophila ovalis* and *Thalassia hemprichii*. The coverage area is approximately 2,700 m^2 scattered around Tg. Tarak Tarak (Yang, 2017). However, rapid urbanization along the coastal area such as the expansion of UMS and Sepanggar Port contribute to the degradation of marine ecosystems.

Generally, the west coast of Sabah is affected by two seasonal monsoons. The Northeast Monsoon occurs from the months of November to March, while the Southwest monsoon occurs during the months of May to September while the

Inter-monsoons occur successively between the months of April to May and September to October (Suhaila, et al., 2010). Ho, et al. (2013) reported that Kota Kinabalu is not directly affected by winds during the Northeast monsoon due to blockage by the Crocker Range. The seasonal monsoons that occur in this area contribute in a change of the beach profile and variation of sediment characteristics. However, the profile of the beach and sediment characteristics may vary at the seagrass meadows area and the beach directly exposed to an open area of the South China Sea. The objectives of this study are to (i) determine the beach profile and sediment characteristics of the UMS Outdoor Development Centre (ODEC) beach during the Southwest to Northeast monsoons, and to (ii) compare the beach profile at area where seagrass meadow is present and area where the beach is exposed to the open sea.

Materials and Methods

Study area

The UMS shoreline forms a sandy beach that is approximately 1,407 m in length. The beach is divided into two parts; the beach located between the ODEC and Tg Tarak Tarak located in the North; and the beach near the UMS Hatchery building in the South (Figure 1).

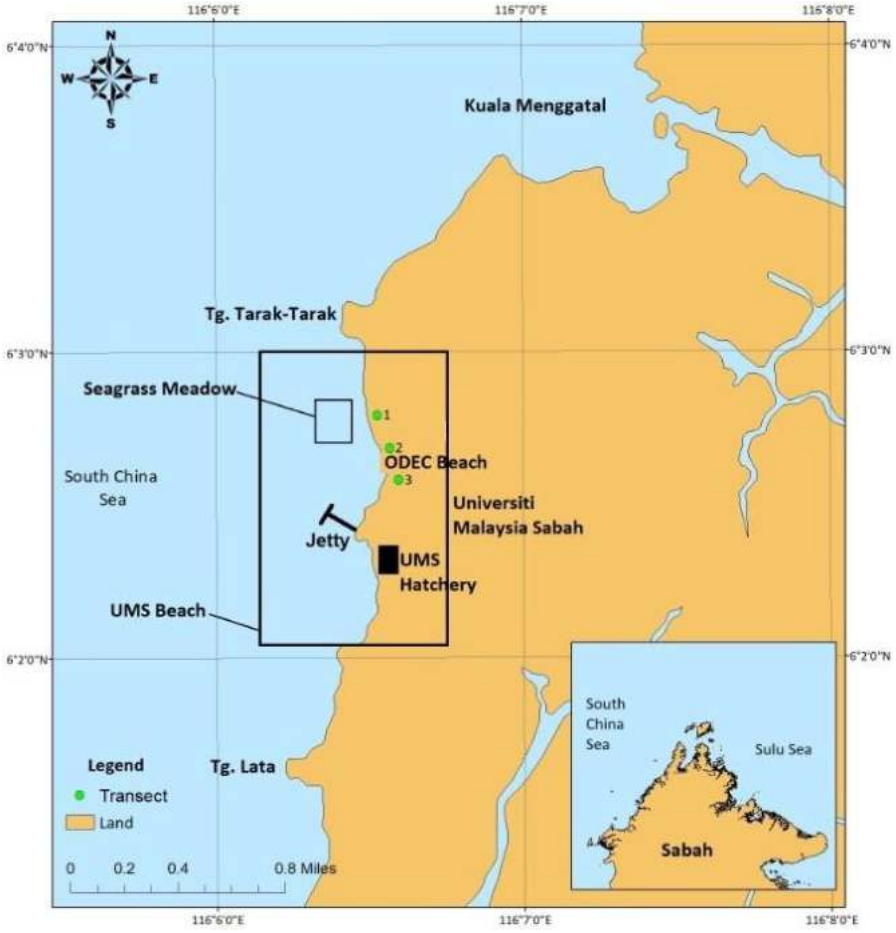

Figure 1. Location of the study area and sampling station.

The seagrass meadow is present only at northern part of ODEC beach and this is an area that accommodates picnics, beach sports and retreat programmes. The main threat to the beach is erosion. Currently, coastal protection such as stone revetments have been built nearby to reduce the impact of beach erosion. Seagrass meadow is located at transect 1 near the Tg. Tarak Tarak (North 6° 2'51.25", East 116° 6'35.98").

Beach Profile Measurements and Sample Collection

Beach profiling measurements are set up perpendicular to the UMS-ODEC beach. The measurements start from the high tide zone to the low tide zone area. Three

replicates of beach profiling were done in each transect (Table 1) to increase accuracy. The locations of each transect replicates were marked and recorded using a Global Positioning System (GPS).

Table 1. Coordinates of beach profile transects at UMS ODEC Beach.

Transect	Replicate	Latitude (N)	Longitude (E)
1	1	006°002'79"	116°006'67"
	2	006°002'83"	116°006'67"
	3	006°002'89"	116°006'66"
2	1	006°002'68"	116°006'69"
	2	006°002'71"	116°006'68"
	3	006°002'74"	116°006'67"
3	1	006°002'58"	116°006'70"
	2	006°002'62"	116°006'70"
	3	006°002'63"	116°006'69"

Permanent markers such as trees or structural landmarks (lamp post) were identified as landmarks for the beach profiling transect. The Automatic level type TOPCON series AT-G6 sighting staff was set up at high tide mark and the 3 m intervals reading of the measuring rod along the transects line were recorded using data sheet. The procedures were repeated for each beach profile transect. Sediment samples for sediment characteristics analysis were also collected at the middle tidal zone and in the lower tidal zone of the beach. The samples were labelled according to the point of each sampling transect and were brought to the Borneo Marine Research Institute laboratory for further analysis.

The beach profile measurement was taken at an interval of every 2 months starting from May 2016 to March 2017. Measurements were done on 19th May 2016, 14th July 2016 and 13th September 2016 to represent the Southwest monsoon and on 19th November 2016, 13th January 2017 and 25th March 2017 for the Northeast monsoon.

Data Analysis

Beach profile measurement was plotted to determine the changes of the beach during the Northeast and Southwest monsoons. A decline of the beach profile based on the previous measurement would indicate that the erosion process is taking place while an increase of the beach profile would indicate the accretion process. The beach's profiling was also compared among three transects to identify the role of seagrass meadow for shoreline protection.

The sediment samples were analysed for mean, sorting, skewness and kurtosis parameters to describe the sediment characteristics using the dry sieving method. All sediment samples were dried in the open air. Dry sediment samples were placed at 850 µm, 500 µm, 355 µm, 250 µm, 125 µm, 106 µm and 63 µm sieve size then sieved and filtered in the automatic shaker for 10 minutes. The sediment samples on each sieve were removed and weighed using the analytical balance. The raw grain-size date was run in the GRADISTAT program version 8.0 software. This software would determine the fraction of sediment from each sample by size category based on the modified Wentworth (1922) size scale. The sediment samples were then characterized in terms of mean size, sorting, skewness and kurtosis following the Folk and Ward (1957) method for the Southeast and Northeast monsoons.

Results

Beach Profile Measurement

The ODEC beach has a higher beach profile level during the Southwest monsoon compared to the Northeast monsoon (Figure 2). A steeper high tide zone followed by flatter mid to low tide zones were identified at transects 1 and 2 (Figure 2a-b) while beach profile at high and mid tide zones of transect 3 was at a higher level compared to the other two transects (Figure 2c). The width of the beach profiles were about 45 m during the Southwest monsoon and decreased to 35 m during the Northeast monsoon.

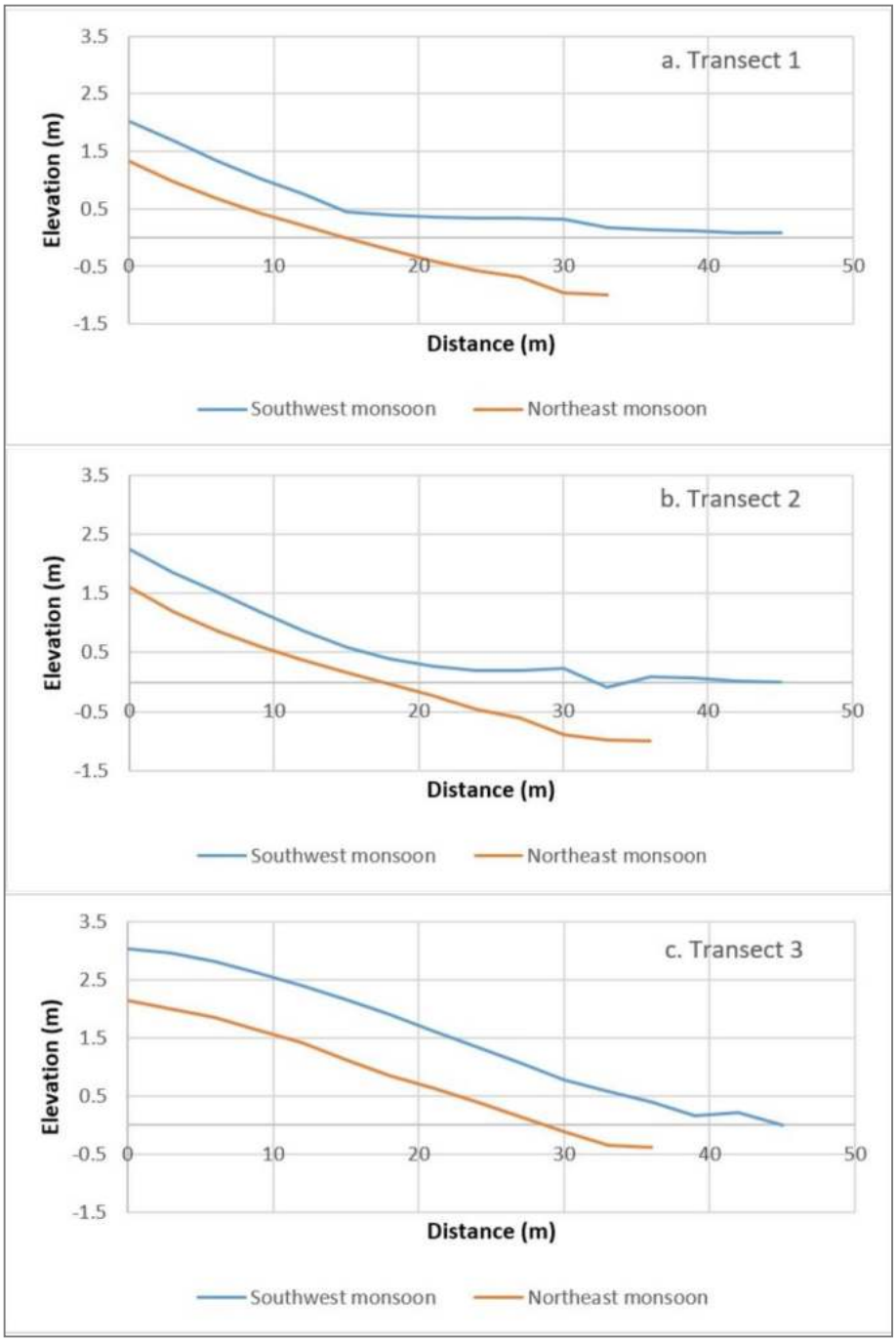

Figure 2. Comparison of beach profile of transect 1(a), transect 2(b) and transect 3(c) during the Northeast and Southwest monsoon.

The comparison of every two months beach profiles measurement are summarized in Table 2. Beach erosion happened between the month of May and July at all transects. There was a small sign of erosion that had occurred at transect 3 (Table 2). However, between the month of July and September, the accretion process took place at all transects with a large amount of sediment accumulated at transects 1 and 2. There was a large amount of sand loss (erosion) at all transects between the month of September and November (peak of Southwest monsoon). Loss of beach sediment continued between the months of November and January at all transects. Accretion took place again between the month of January and March for all transects with a large accumulation of sediment at transects 1 and 2.

Table 2. Summary of beach profile changes between two months of samplings.

Transects	May-July	July-September	September-November	November-January	January-March
1	Erosion (Small)	Accretion	Erosion (Large)	Erosion	Accretion
2	Erosion	Accretion (Large)	Erosion (Large)	Erosion	Accretion (Large)
3	Erosion	Accretion (Large)	Erosion (Large)	Erosion	Accretion (Large)

Sediment Characteristics of UMS-ODEC Beach

The average values of mean size, standard deviation (sorting), skewness and kurtosis of sediments in each transect was tabulated to see the dynamics of the UMS-ODEC beach (Table 3). Mean size of sediments for all transects during the Southwest monsoon is fine sand (FS), while during the Northeast monsoon the mean size of sediments is FS in transect 1 and very fine sand (VFS) in other transects. Sediment during the Southwest monsoon is moderately sorted (MS) in all transects (Table 3). However, during the Northeast monsoon, the sorting of sediment is MS in transect 1 and moderately well sorted (MWS) in transects 2 and 3. The skewness of sediments for both monsoons in all transects is coarsely skewed (CSk). Kurtosis of sediment is leptokurtic (LK) in transects 1 and 3 while in transect 2 is mesokurtic (MK) during the Southwest monsoon. There was LK kurtosis type in transects 1 and 2 while transect 3 had MK during the Northeast monsoon (Table 3).

Table 3. Average values of mean size standard deviation (sorting), skewness and kurtosis of sediments in each transect during the Southwest Northeast monsoons.

Transect	Sediment Character	Southwest Monsoon		Northeast monsoon	
1	Mean	2.84	FS	2.68	FS
	Sorting	0.74	MS	0.87	MS
	Skewness	-0.27	CSk	-0.25	CSk
	Kurtosis	1.35	LK	1.26	LK
2	Mean	2.69	FS	3.28	VFS
	Sorting	0.91	MS	0.61	MWS
	Skewness	-0.27	CSk	-0.10	CSk
	Kurtosis	0.92	MK	1.43	LK
3	Mean	2.75	FS	3.08	VFS
	Sorting	0.71	MS	0.65	MWS
	Skewness	-0.19	CSk	-0.25	CSk
	Kurtosis	1.15	LK	1.07	MK

Notes: FS-fine sand, VFS-very fine sand, MS-moderately sorted, MWS-moderately well sorted, CSk-coarsely skewed, LK-leptokurtic

Discussion

Beach profile comparison

Erosion and deposition of sediment are natural processes at the beach. Beach erosion is the offshore movement of sediment from the upper part (high tide zone) of the beach during storms as waves move sand from the beach and dunes to offshore storm bars (DLWC, 2001). When the calm weather comes back and deposition begins, the sand from the offshore bar moves back onshore to establish the beach. Generally, monthly erosion and deposition process occurs at UMS-ODEC beach mainly due to natural forces such as wave action generated by the seasonal monsoon.

The beach profile elevation of ODEC Beach is higher during the Northeast monsoon compared to the Southwest monsoon (Figure 2). Hoque et al. (2010) reported that the water current during the Northeast monsoon shows less steady current velocity compared to the Southwest monsoon measurement. This indicates that generally stronger and more severe wave conditions are found during the Northeast monsoon. Similar condition applied to UMS-ODEC Beach where higher wave energy reached the beach thus bringing out more sediment to the sea during this season. Yakof (2008) recorded the width of the UMS-ODEC beach can be up to 70 m at transects 1 and 2 and only 25 m at transect 3 during low neap tides.

Transects 1 and 2 have almost the same beach morphology (Figure 2a-b). The beach profile is steeper on the high tide zone and is flattened towards the low tide zone. These two transects might be naturally protected by the nearby Tg. Tarak Tarak and islands (Gaya and Sepanggar islands). Higher usage of the beach also affects the beach's geomorphology. The erosion process of this beach may not only be caused by the natural phenomenon but also by sea traffic at Kota Kinabalu coastal waters that produce wake waves at surrounding area (Jalihah & Nor-Hafizah, 2016; Bilkovic, et al. 2017). Transect 3 located in front of the UMS ODEC is exposed to the open sea and shows steeper and higher elevation compared to the other two transects (Figure 2a-b).

It was identified that large accretion happened at transects 1 and 2 between the months of July and September and between January and March. Deposition process could be due to seasonal monsoon and local longshore transport. The erosion process happened at all transects between September and November during the peak of the Southwest monsoon and continued to erode between November and January during the peak of the Northeast monsoon. Based on Yakof (2008), the UMS-ODEC Beach in September 2007 had a higher elevation compared to November 2007. This indicates that the beach experienced beach erosion within this time period.

Based on this one year observation, the impact of seagrass meadow at ODEC Beach is not clear. The beach profile of transect 1 (presence of seagrass) and transect 2 (absence of seagrass) is similar during both monsoons. Chen et al.(2007) reported that larger seagrass bed width in the direction of wave propagation would result in higher wave attenuation and less energy on the shoreline. However, the seagrass meadow located in transect 1 is too patchy or too small to attenuate the waves action. The seagrass canopy height is also too short to prevent the wave energy reaching the beach. Yang (2017) reported that the average canopy height of the seagrass meadow is estimated at 11.47 cm. The potential of seagrass beds to protect shorelines is probably influenced by the timing between wave events and seagrass growth. The greater the proportion of the water column that the seagrass canopy occupies, the more effective it is at reducing unidirectional water flows (Manca et al., 2012).

Beach Sediment Characteristics

Beach sediment characteristic at all transects are dominated by moderately sorted fine sand (FS) during the Southwest monsoon but are finer and more sorted in transects 2 and 3 during the Northeast monsoon (Table 3). The source of sediment supply, transporting medium and the energy conditions of the

depositing environment could influence the value of mean size sediments (Folk & Ward, 1957). Folk (1980) suggested that as the energy of the transporting medium decreases, the sediments deposited become finer. Lower current energy would produce better sediment sorting (Blott & Pye, 2001).

Skewness measures the asymmetry of the frequency distribution. The value of skewness at all transects in both the Southwest and Northeast monsoons show that the sediments are negatively skewed (Table 3). Friedman (1961) identified that the water turbulence caused by incoming waves and outgoing wash, characteristics of beach environments, winnow away the fines and skew the frequency curve to coarser sizes or negative sides. Negatively skewed curves could also indicate an erosion or non-deposition area, whereas positively skewed curves could indicate deposition and a mixture of both would indicate a region in a state of flux.

Kurtosis is the peakedness of the distribution and measures the ratio between the sorting in the tails and the centre. In UMS beach, it is mostly leptokurtic in both the Southwest and Northeast monsoons. The sediment characteristics of UMS-ODEC beach in September 2007 was fine sand similar to this study but sorting, skewness, kurtosis was different (Yakof, 2008).

The average values of mean size, standard deviation (sorting), skewness and kurtosis of sediments of the UMS-ODEC beach in September 2007 (Southwest monsoon) were fine sand, poorly sorted, extremely negative skewed, very leptokurtic respectively (Yakof, 2008). The sediment analysis was continued in November 2007 and the findings were almost similar with that of September 2007. These sediment characteristics may be influenced by the extreme events of tropical cyclones Mitag and Hagibis tails that occurred during that year.

Conclusion

Erosion and deposition of sediment is a continuous process which occurs along UMS-ODEC beach. The elevation of the beach profiling is higher during the Southwest monsoon than during the Northeast monsoon. This indicates that beach sediment was eroded during the Northeast monsoon. Similar beach profile formation at transects 1 and 2 where both transects have steeper beach at the high tide zone and a flattened beach from the mid to low tides zone. Findings show steeper and higher beach elevation at transect 3 in both monsoons. The three transects have similar sediment characteristics during the Northeast monsoon, where mean, sorting, skewness falls under fine sand, moderately

sorted and coarsely skewed, respectively. However only skewness of sediment has similar characteristics at all transects during the southwest monsoon. Based on this one year observation, there is no clear impact of the seagrass meadows as a natural coastal protection for the UMS-ODEC beach. The seagrass meadow condition is short leaves while the distribution and abundance are too patchy or too small compared to the size of Sepanggar Bay.

A combination of a natural conditions such as those present at Tg. Tarak Tarak and natural (islands) and man-made structures (UMS jetty and stone revetment near the jetty) could contribute to beach profile change and sediment characteristics variation of UMS-ODEC beach. Hydrodynamics within the Sepanggar Bay should be taken into account for future studies on the role of seagrass meadow for shoreline protection. Coastal modelling and simulation would help in detecting changes in water current, waves and shoreline in the coastal area, particularly at seagrass meadow sites.

Acknowledgements

We would like to thank the Ministry of Higher Education (MOHE) that had supported this study through Project FRG0424-SG-1/2015. We would also like to acknowledge and appreciate the staff of the Borneo Marine Research Institute that has supported the field trips and laboratory analysis. Appreciation is also given to the undergraduate Marine Science students (UMS) for their help and accompaniment throughout the sampling at UMS beach shoreline area.

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Journal of Tropical Biology and Conservation 15: 203–222, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Distribution and ethnomycological knowledge of wild edible mushrooms in Sabah (Northern Borneo), Malaysia

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Abstract

Ethnomycological knowledge is a combination of biological resources, cultural and human patterns, in particular collective traditional uses and the importance of fungi in daily life. Despite the large number of ethnic groups in Sabah, the native ethnomycological knowledge of wild edible mushrooms and poisonous mushrooms are poorly documented. This study attempted to document wild edible mushrooms and their ethnomycological uses and practices in the tropical rainforest of Sabah, Borneo. Opportunistic samplings and ethnomycological surveys were made within the indigenous communities of Sabah. Collectively, 50 respondents from four different ethnic communities i.e. Dusun, Kadazan, Orang Sungai, and Bisaya were interviewed. A total of 25 wild mushroom species were documented as edible mushroom for food, and five species for medicinal uses. The highest number of wild edible mushroom collected and reported were of the Pleurotaceae family (five species), followed by Polyporaceae family (three species) and Auriculariaceae family (three species). The results also showed that *Schizophyllum commune (Kulat Kodop), Volvariella volvacea (Kulat Sawit), Pleurotus* spp., (Cendawan Tiram) *Auricularia* spp., (*Kulat Korong*) and *Marasmiellus* species were mostly consumed by the indigenous people of Sabah as part of their daily diet. Local names, culinary, and the edibility types were distinct among the different local communities. Elderly indigenous people possess vast knowledge on uses of wild mushrooms compared to the younger generation. Women play an important role in wild mushroom collection and its edibility, uses and practices. The findings from this study showed that ethnomycological knowledge of wild mushroom in Sabah is still lacking and more attention is needed. A study on the ethnomycological aspect in Borneo is a necessity in creating awareness among the public on edible and poisonous mushrooms, and its culinary and medicinal properties.

Keywords: Ethnomycology, wild mushrooms, native, ethnic, Sabah, Borneo

Received 15 July 2018
Reviewed 30 July 2018
Accepted 01 August 2018
Published 15 October 2018

Introduction

Sabah is one of the states in Malaysia and is home to a large number of ethnic groups. Collectively, more than thirty indigenous ethnic groups are associated with different cultures (Ooi, 2004). Ethnic Kadazan-Dusun is the dominant indigenous groups in Sabah (Hans et al., 2008). In the past, they sustained themselves in the forest with wild food and cultivation work to secure their daily diet especially wild mushrooms (Christensen, 2002; Chang & Lee, 2004; Chang et al., 2005; Antons & Logan, 2017). Ethnomycological knowledge is generally held by the older generation across most ethnicities (Alonso-Aguilar et al., 2014). Hence, Sabah is a great platform to collect knowledge on wild mushrooms and their uses in scientific studies.

Wild mushrooms are the natural reservoir of many benefits which are very fundamental to many industrial applications i.e. agriculture, medicine and pharmacy. Countries like China, Japan, India and Thailand have broadly studied their native mushrooms as a sustainable practice and species conservation among local communities. The knowledge on identification of edible and inedible mushrooms is limited in terms of records and there is no systematic documentation available for Malaysian Borneo and Peninsular Malaysia (Chang & Lee, 2004; Abdullah & Rusea, 2009; Lee et al., 2009). There are a few ethnomycological studies of macrofungi in Peninsular Malaysia (Abdullah & Rusea, 2009; Lee et al., 2009), but very sparse information on wild mushroom in Sabah, Borneo (Corner, 1981; Pegler, 1997). Awareness efforts to conserve the genetic resources of wild mushrooms are not well nurtured among local communities in Sabah, Borneo. This dearth of information is probably due to the shortage of adequately trained mycologists or taxonomists (Hyde, 2003), and urbanization and deforestation (Lee et al., 2009). In addition, drastic reduction of endemic fungi population in this region may be happening due to deforestation, climate change and conversion of forests into oil palm plantations has raised trepidation among many local conservation biologists. Higher fungi or larger fungi (Basidiomycota) is not well explored in tropical forests and has always been overlooked compared to plants and animals. Thus, the main aims of this study were (i) to document the wild edible mushrooms in Sabah, and (ii) to determine ethnomycological knowledge (mainly uses and practices) from indigenous communities of Sabah.

Materials and Methods

Study area

Different forest types (lowland, highland, dipterocarp, primary and secondary forests) and local markets in Sabah were explored in this study. The average temperature at lowlands is 32 °C, and at highlands it is 21 °C. The mean annual rainfall is 250–350 mm, with the rainy season stretching from October to February. Mushroom (fruit bodies) samples were collected at Kinabalu Park, Kundansang (Ranau), Crocker Range Park (Sungoi Kiulu, Tambunan district), Tun Mustapha Park (Bambangan and Banggi islands, Kudat), Tawau Hills Park (Tawau), Kota Belud, Kota Marudu, and Lower Kinabatangan (Sandakan) (Figure 1). We also randomly surveyed the local markets (Kota Belud and Kota Marudu) for wild and domesticated mushroom collections throughout 2015-2017.

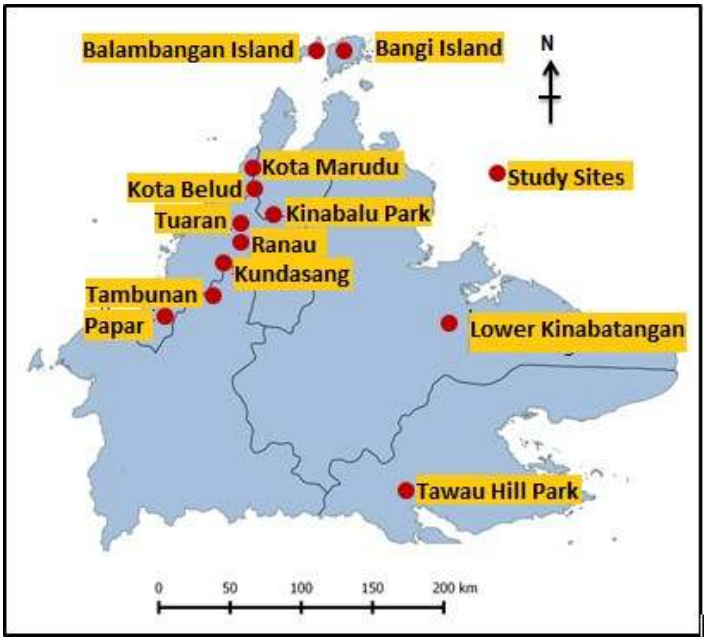

Figure 1. Map of Sabah showing the study sites.

Macrofungi collection

Sampling trips were conducted during the rainy season (August till December each year 2015-2017) (Figure 1). Opportunistic sampling of fruiting body was used for the sample collection of different types of substrates (i.e. soil, twigs, dead wood, living tree trunk). A Global Positioning System (GPS) device was used to record the points or coordinates at which the fruiting bodies were found.

Three to five fruiting bodies of macrofungi were collected for each sample. All collected samples were dried in a mushroom dryer and sealed in paper bags. All dried specimens were deposited in the Herbarium BORNEENSIS, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah (UMS). Each specimen was given a voucher number (i.e. BORH/F 0001).

Morphological identification

The collections were brought to the laboratory for identification and characterization using standard mycological keys and literature (Pegler, 1983). All macro-morphological characters were described based on fresh material, and documented by photographs. Simple descriptions of the habitat, substrate, physical morphology, and colouration of each specimen were noted. Photographs of the macro-morphological characters of all the specimens were also compiled. Thin sections were cut with a razor blade from dried specimens and mounted on slides with 5% KOH and Congo Red. The mounted slide was observed and measured using a compound microscope (Zeiss Axioskope 40). Colour designations were adapted from Kornerup and Wanscher (1978). Some mushrooms were indicated only as sp. by their taxonomic complexity, such as *Agaricus, Auricularia, Lignosus* and *Marasmiellus*.

Ethnomycological and local market survey

Kadazan, Dusun, Orang Sungai and Bisaya communities were randomly found for interview sessions related to ethnomycology of wild mushroom in Sabah. A questionnaire was used in the interview and the questions were specially designed for attaining information on ethnomycological knowledge of wild mushroom (edibility, uses, culinary, identification method). A sample size of 50 respondents was targeted with different ethnic groups (Kadazan, Dusun, Orang Sungai and Bisaya). Interviews with local people (mostly Dusun) were done. Two major groups of respondents were categorized; I) older generation (>45 years old) and II) young generation (<46 years old). Each interview session was aided with photographs of targeted fleshy mushrooms and dried mushroom samples. Vernacular names were also documented and species identifications were mainly based on the morphological characteristics. Local mushroom market surveys were also conducted in Kota Belud and Kota Marudu. The collected information was related to names in the local language (vernacular names) and dialect used among the villagers, and types of wild and domesticated mushrooms sold in local markets. All information was compiled from villagers and farmers or mushroom sellers in the market.

Results

In total, 50 indigenous respondents (27 females, 23 males) were interviewed (Table 2). The age of respondents ranged from 36 to 81 years old. Of the 50 respondents, 34% were aged >45 years, and 66% were <46 years old. From the entire sample, the Dusun people were the major indigenous group with 29 respondents (58%), while the rest were Kadazan (26%), Orang Sungai (12%) and Bisaya (4%). Two main components of ethnomycological knowledge were reported: distribution of wild edible mushrooms, and ethnomycological knowledge from indigenous people in Sabah.

Distribution of wild edible mushrooms at Sabah

The wild edible mushrooms consumed by the local populations within Sabah belong to two major classes of fungi, Ascomycota and Basidiomycota. A total of 12 families within Basidiomycota and two families within Ascomycota were recorded in this survey. A list of 25 wild edible mushrooms was documented for Sabah (Table 1). The highest number of wild edible mushroom species recorded was of the Pleurotaceae family (*Pleurotus pulmonarius, P. giganteus, P. tuberregium, P. djamor* var. *djamor,* and *P. djamor* var. *roseus)*. The second highest number of species recorded was from the Polyporaceae family (*Lentinus sajor-caju, L. squarrosulus,* and *Panus lecomtei*). All three species are white rot fungi with distant or crowded lamellae (as in Agaricales). Auriculariaceae family also comprises three species (*Auricularia polythrica, Auricularia auricular-judae* and *Auricularia sp.* 1) which are all edible.

Among 25 wild edible mushrooms, only five were reported for medicinal uses. These mushrooms are *Pleurotus tuber-regium, Auricularia* sp., *Xylaria* sp., *Lignosus* sp. and *Schizophyllum commune*. The rare species of *Termitomyces eurhizae* (Lyophyllaceae) and *Hygrocybe miniata* (Hygrophoraceae) from lowland forests was found in this study. An edible, *Calostoma insignis* (Calostomataceae) and poisonous *Agaricus pracelaresquamosus* were recorded for the first time at Serinsim lowland forest. Photographs of wild edible mushrooms found in Sabah is shown in Figure 2.

Table 1. List of wild edible mushrooms found at Sabah.
n.a: Not available; Local name varies for same or different species.

Family	Scientific name	Local name	Dusun names	Uses	Ecology
Polyporaceae	*Lentinus sajor-caju*	Ring mushroom	*Kulat Lengkugan/ Ungkugan*	Edible only when young	Saprophytic
	Lentinus squarrosulus	*Kulat Susu* (milky mushroom)	*n.a*	Edible	Saprophytic
	Panus lecomtei	*Kulat Kari-Kari* (Curry Mushroom)	*n.a*	Edible only when young	Saprophytic
	Lignosus sp.	*Cendawan susu harimau*	*n.a*	Edible (tuber part)	Saprophytic
Pleurotaceae	*Pleurotus giganteus*	*Kulat perut lembu*,	*Salimmatuwo*	Edible	Saprophytic
	Pleurotus tuber-regium	*Kulat Ubi* (potato mushroom)	*Dunsul*	Edible/ medicinal	Saprophytic
	Pleurotus djamor var. djamor	*Kulat tiram putih*	*Tombongongngong putih/ Tahang ngungut*	Edible	Saprophytic
	Pleurotus djamor var. roseus	*Kulat tiram merah*	*Tombongongngong merah/ Tahang ngungut*	Edible	Saprophytic
Auriculariaceae	*Auricularia polythrica*	*Kulat Telinga kera* (monkey ear)	*Korong*	Edible/medicinal	Saprophytic
	Auricularia sp. 1	*Kulat Telinga Gajah* (Elephant ear)	*Korong*	Edible	Saprophytic
	Auricularia auricular-judae	*Kulat Telinga*	*Korong*	Edible	Saprophytic
Agaricaceae	*Agaricus subrutilescens*	*n.a*	*n.a*	Edible	Ectomycorrhizal
	Agaricus sp. 1	*n.a*	*n.a*	Edible	Ectomycorrhizal
Marasmiaceae	*Marasmiellus sp.*	*Kulat sawit putih*	*n.a*	Edible	Saprophytic
	Lentinula edodes	*Kulat Jipun* (wild shitake)		Edible	Saprophytic
Tremellaceae	*Tremella fuciformis*	*Kulat Jeli putih* (white jelly)	*n.a*	Edible	Saprophytic
Hygrophoraceae	*Hygrocybe miniata*	*Kulat Topi*	*n.a*	Edible	Ectomycorrhizal

(Continued on next page)

Table 1. ***(continued)***

Family	Scientific name	Local name	Dusun names	Uses	Ecology
Pluteaceae	*Volvariella volvacea*	*Kulat sawit* (Paddy Straw mushroom)	*n.a*	Edible	Saprophytic
Schizophyllaceae	*Schizophyllum commune*	*Kulat Kodop*	*n.a*	Edible/ medicinal	Saprophytic
Lyophyllaceae	*Termitomyces eurrhizus*	*Kulat busut* (termite mushroom)	*Tamburong*	Edible	Symbiotic-Termites
Cantharellaceae	*Chanterellus cerinoalbus*	*n.a*	*n.a*	Edible	Ectomycorrhizal
Calostomataceae	*Calostoma insignis*	*Kulat Mata Babi* (Pig's eye mushroom)	*n.a*	Edible	Ectomycorrhizal
Sarcoscyphaceae	*Cookeina sulcipes*	*Kulat mangkuk* (plate mushroom)	*n.a*	Edible	Saprophytic
	Cookeina tricholoma	*Kulat Rambut* (Hairy mushroom)	*n.a*	Edible	Saprophytic
Sarcosomataceae	*Galiella rufa*	*Kulat Mata Rusa* (Deer's eye mushroom)		Edible	Saprophytic

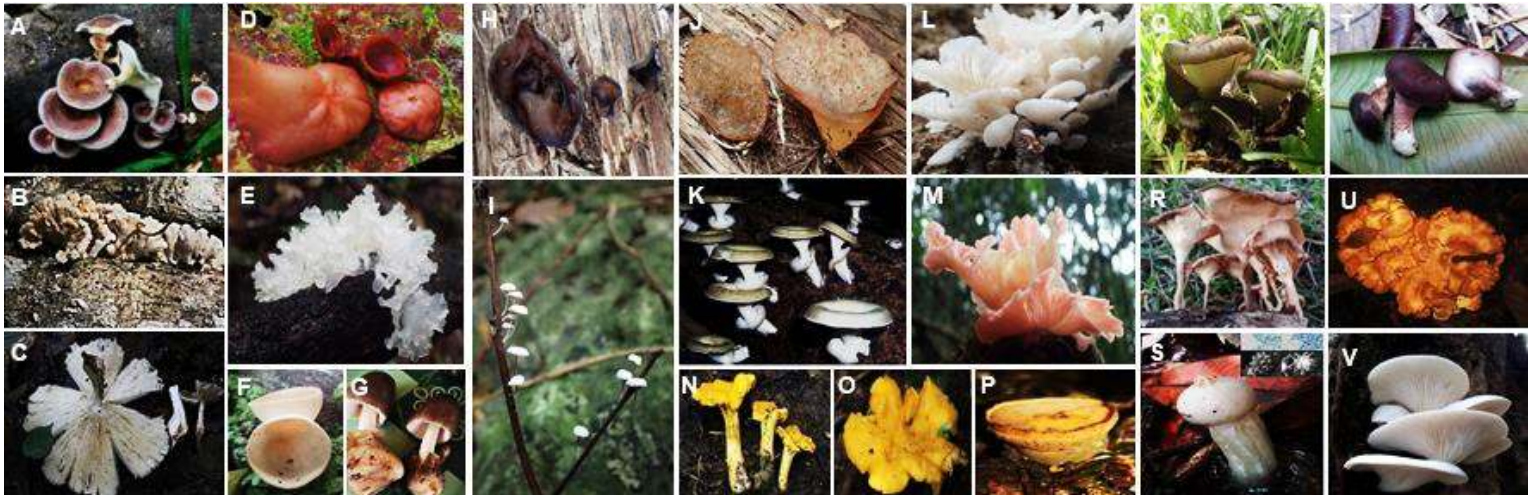

Figure 2. Wild edible mushrooms. A. *Panus lecomtei*. B. *Schizophyllum commune*. C. *Termitomyces eurrhizus*. D. *Auricularia sp*. E. *Tramella fuciformis*. F. *Cookeina sulcipe*. G. *Volvariella volvacea*. H. *Auricularia sp*. I. *Marasmiellus sp*. J. *Auricularia sp*. K. *Lentinus sajor-caju*. L. *Pleurotus djamor* var. *djamor*. M. *Pleurotus djamor* var. *roseus*. N-O. *Chanterellus cerinoalbus*. P. *Galiella rufa*. Q. *Pleurotus giganteus*. R. *Pleurotus tuber-regium*. S. *Calostoma insignis*. T. *Lentinula edodes (wild Borneo shitake)*. U. *Pleurotus tuber-regium*. V. *Pleurotus pulmonarius*.

Table 2. Indigenous communities and their ability in identification of wild mushrooms based on gender and age groups.

Community	No. of respondents	Age group		Gender		Ability to identify at least one species of wild mushroom			
		<46	>45	Male	Female	Male	Female	<46	>45
Dusun	29	11	18	12	17	11	14	11	14
Kadazan	13	5	8	7	6	5	6	4	7
Orang Sungai	6	1	5	3	3	2	3	1	4
Bisaya	2	0	2	1	1	1	1	0	2
Total	50	17	33	23	27	19	24	16	27

Ethnomycological knowledge from indigenous communities

A) Ethnolycological knowledge of wild mushrooms

The native communities of Sabah have been collecting wild mushrooms as part of their diet. Majority of the respondents (86%) were able to identify at least one species of wild mushroom, while 14% of the respondents indicated that they are not confident on the edibility of wild mushrooms (Table 2). Female respondents possess slightly more knowledge on wild mushrooms compared to males. A total of 89% female respondents knew at least one species and 82.6% native males knew about wild mushroom. The younger generation mostly knew the most common edible fungi, like *Schizophyllum commune*, which is locally known as '*Kodop*'. They were also able to recognise other edible mushrooms, such as *Dunsul* (*Pleurotus tuber-regium*), *Korong* (Jelly mushroom), *Kulat Tiram* (oyster mushroom). The knowledge on edible wild mushrooms seems to increase. The older generation (>45 years old) knew of more uses and had culinary use knowledge (81.8% of elderly respondents) compared to younger people. There are 94.1% young people (16 out of 17 young respondents) able to identify only one to two species of common wild mushrooms, but they have no idea on medicinal uses and culinary of wild edible mushroom. The information on culinary and wild mushrooms practices were mostly received from elderly people.

B) Wild mushroom market

Most of the local farmers are of Kadazan or Dusun origin. Both Malay and the local dialect (Dusun) are used by indigenous communities when speaking about mushrooms. The local farmers only grow and sell domesticated mushrooms such as *Pleurotus ostreatus* (Oyster mushroom, *Kulat Tiram Putih*), *P. pulmonarius* (*Kulat Tiram*), *Lentinula edodes* (*Kulat Jipun* or Shitake), *Auricularia auricular-judae* (wood ear, jelly fungi, *Kulat Telinga Kera* or in Dusun known as *Korong*) and *Tremella fuciformis* (jelly fungi) at the local market. All farmers mentioned that these cultivated mushroom substrate bags were supplied by the Rural Development Corporation (KPD), and that these are not native mushrooms (Figure 3A). However, *Schizophyllum commune* (*Kulat Kodop* in Dusun; in Peninsular Malaysia known as *Kulat Sisir*), *Volvariella volvaceae (Kulat Sawit), Marasimellus* species (*Kulat Sawit Putih*) and *Pleurotus djamor* var. *djamor* (*Kulat Tiram Putih*) which were sold at local market were from wild collections (Figure 3B-G). *Schizophyllum commune* is the only wild edible mushroom that is now popular and commonly seen at all local markets in Sabah.

The indigenous people in Kota Marudu and Kota Belud collect wild edible mushrooms near oil palm plantations. *Volvariella volvaceae* (straw mushroom), *Pleurotus djamor* (*Kulat Tiram*) and *Marasimellus* species (*Kulat Sawit Putih*)

were found on the empty fruit branches of palm kennel. Oil palm plantations are a preferred habitat for these three edible mushroom species (*Volvariella volvaceae, Pleurotus djamor*, and *Marasimellus* species). Both *Marasmiellus* sp. is collected together with *Pleurotus djamor* var. *djamor* (known as *Kulat Tiram*). *Termitomyces eurrhizus* (Termite-fungus, locally known as *Tamburong*) was also collected from oil palm plantations where most of the area was covered with termite mounts. The plantation workers collect and sell the wild *Termitomyces* occasionally during the rainy season.

Figure 3. Local fresh wild mushroom market. **A.** Commercial edible mushrooms at Kundasang. **B-D**. Wild edible *Volvariella volvacea*. **E.** *Pleurotus djamor djamor* and *Marasmiellus* sp. F-G. Wild mushrooms sold at Kota Marudu along the way to Serinsim by roadside.

C) Wild mushroom culinary and medicinal uses

The Dusun, Kadazan, Bisaya and Orang Sungai consume wild edible mushrooms as part of their daily diet. Indigenous people collect wild mushrooms for culinary purposes within their surroundings and in forest areas. Wild mushrooms are considered as popular culinary items for their flavour, taste and nutrition. There were four methods of culinary preparation using wild edible mushrooms: (1) stir-fry, (2) soup, (3) steam and (4) boil. *Linopot* is a traditional food of the Dusun which contains hill rice wrapped in banana leaves (Figure 4A). The mushrooms are usually prepared with vegetables, sambal, *Kijang* (deer) meat and wild ginger flowers (*Tuhau*) and ginger roots (Figure 4B). Several indigenous respondents from Kota Marudu reported that *Pleurotus djamor* (*Kulat Tiram*) has the best taste and aroma when combined with *Volvariella volvaceae* mushroom. According to the indigenous people, *Volvariella volvacea (Kulat Sawit)* is cooked together with young shoots of ferns with garlic and soy sauce.

Local Dusun have been using *Schizophyllum commune* (*Kulat Kodop*) together with *Tuhau* (a local endemic ginger: *Etlingera* species) to produce a floss called "*serunding*". The delicious floss contains dried *Schizophyllum commune* that is collected from wild forests. Indigenous communities also tend to deep fry *Kulat Kodop* and consume it with sambal sauce. The Dusun communities in Kundasang mostly use *Schizophyllum commune* (*Kulat Kodop*) in porridge mixed together with chicken, squirrel meat and white chillies. In addition, a local Dusun culinary called '*Tinamba Linopot'* is prepared using *S. commune* with chicken, beef meat and wrapped in banana leaves (Figure 4C-D). They also prepare wild mushrooms to serve with curry paste and potatoes and ginger flower during special events or festival (Figure 4E-F). Apart from this, another popular edible mushroom *Termitomyces eurrhizus* (*Kulat Temburung*) is usually prepared in soup and together with smoked squirrel meat. *Lentinus sajor-caju* (*Kulat Lengkugan*) is prepared as soup only when it is young as the taste will become pungent and hard to chew if it is too old. The collective information on culinary preparation using wild edible mushrooms varies among individuals but Dusun people tend to maintain the same ingredients. Thus, the traditional way of culinary preparation of wild mushroom is very scarce among younger generation.

From the findings, Dusun people reported that *Lignosus* sp. (not the same species as *L. rhinocerus*) is used for wound healing and cough treatment. *Xylaria sp.* is used for making a wrist band for health purposes. An elderly man in a village, Patrick (81 years old) mentioned that *Schizophyllum commune* (*Kulat Kodop*), *Auricularia* species (*Kulat Telinga Kera* and *Korong*) and *Pleurotus tuber-regium*

(*Kulat Ubi*) were the common wild edible mushrooms in their soups that are used to treat cold and fever.

Figure 4. Indigenous delicacies of wild edible mushrooms in Sabah. **A**. *Linopot* contains hill rice wrapped with banana leaf. **B**. *Pleurotus djamor djamor* fried with barking deer (*kijang*) meat and wild ginger flower (*Bunga Kantan*). **C-D**. *Schizophyllum commune* fried with vegetables and chicken. **E-F**. Wild mushrooms culinary serve at festival or on any auspicious day.

D) Identification criteria for wild mushrooms by indigenous people

The indigenous people of Sabah have different criteria in determining the edibility of wild mushrooms. Indigenous people reported that the ethnomycological knowledge of edible wild mushroom was transferred from the elderly to the younger generation. They were able to identify and classify the edible and inedible mushrooms based on native culture or vernacular names. Dusun people call the edible mushroom, '*Kawanit*' and poisonous mushroom, '*Akanen*'. Insects are the indicator for identification of edible and inedible wild mushrooms in the forest. A wild mushroom is considered edible if insects are found on the fruiting body of the mushroom. Mushroom colour is an important criterion for the identification of edible mushrooms. Dusun people reported that *Chanterellus cerinoalbus* is a poisonous mushroom due to its strong and bright yellow colour. In addition, the mushroom size is one of the identification criteria

to determine the edible mushroom. In this study, the huge size of *Pleurotus giganteus* (320 mm in diameter of pileus, larger than normal edible mushrooms) was considered as poisonous species by Dusun and Kadazan people.

Discussion

In Sabah, the basic ethnomycological knowledge is associated with age and gender. Ethnomycological knowledge is differently shared among the different age groups. Knowledge on edible mushrooms is usually transferred from the older generation to the younger generation. Indigenous people living in the rural forest mainly use natural resources to sustain their subsistence (Chang & Lee, 2004; Lee et al., 2009). Traditionally, older generations have vast knowledge on the uses of wild mushrooms. The younger generation only recognize the common edible wild mushrooms species. Currently, most of the younger generation have migrated to cities for job opportunities. Rural area have more forest coverage compared to urban centres. Previous studies have shown that depletion of ethnomycological knowledge may happen when people move to urban areas from rural villages (Boa, 2004; Tibuhwa, 2012).

A majority of elderly people shared that the depletion of wild mushroom distribution is tapering the traditional knowledge on wild mushrooms. Urbanization and land integration are two significant reasons that lead to the instant loss of their native knowledge from one generation to the next (Akpaja et al., 2003; Lee et al. 2009). Traditional knowledge among the indigenous people in Sabah was reported to be decreasing gradually due to change in land use for agriculture and human settlement. Due to the development of human settlements, many indigenous people are not been able to collect wild mushrooms within the surrounding areas.

Most of the culinary uses of wild mushrooms is mostly handled by women. Women tend to have slightly more knowledge on wild edible mushrooms compared to men. They are usually responsible in gathering forest resources, such as wild mushroom for culinary uses and practices in the family. In Sungoi Kiulu, Tambunan women are the most important mushroom collectors to sell mushrooms at the Donggonggon market. This finding concurs with reports from Tibuhwa (2013) and Teke et al. (2018).

In this study, different vernacular names were addressed to the same species by different ethnic groups. The identification methods on wild mushroom vary among the indigenous communities. Indigenous communities attribute the

names of the mushrooms based knowledge of elderly people. The Dusun community call *Pleurotus djamor* as "*Tombongongngong*" while others refer *Pleurotus djamor* with a different local name, "*Tahang ngungut*". The unity in species identification using vernacular names are not thoroughly the same and this needs to be further studied to avoid any poisonous species consumption by mistake.

The indigenous people from Kota Marudu misidentified *Marasmiellus* species as *Pleurotus djamor* var. *djamor*. They use the same local name for both species. Deadly toxic species, *Trogia venenata* which is also similar as *Pleurotus djamor,* are always mixed together in the same bag that was collected from oil palm plantations due to their Pleurotoid shape. The farmers are not able to distinguish the differences because of the same colour and pleurotoid shape. This was an important observation during the survey and we educated them that both species were not the same. Mushroom poisoning awareness should be delivered to locals so that they could be careful when collecting mushrooms. This will avoid any misidentification and mushroom poisoning cases in Sabah.

The presence of insect or flies, mushroom colour, smell and morphological characters were the main criteria in determining the edible groups. Similar results on these criteria in determining the edible mushrooms have been reported from other studies as well (Kinge et al., 2011; Alvarez et al., 2016). *Lentinula edodes* (Kulat Jipun, locally known as *Shiitake*), is another example of edible mushroom previously (in the early 80s) was not regarded as an edible mushroom until the Japanese market introduced the non-native strains in Malaysia. As for Sabah, local people assumed that when it contains hairy pileus it was not edible. However, in our study we obtained the wild strain of *L. edodes* (local species) with less colouration compared to the Japanese strain. The Dusun people were not aware that locally grown *L. edodes* are also found in Mount Kinabalu, Sabah. The hairy pileus in *L. edodes* suggests that hairy pileus is considered as a morphological criteria in determining whether the mushrooms are edible or poisonous. Thus, local identification based on the morphology is not sufficient enough to verify species level identification.

Based on our findings, it was affirmed that the natives of Sabah are very knowledgeable on wild edible mushrooms and the culinary uses for cooking and medicinal uses. *Schizophyllum commune, Volvariella volvacea, Pleurotus* species, *Auricularia* species and *Marasmiellus* species were mostly preferred by the indigenous people in Sabah. The culinary uses of various wild mushrooms varied between Kadazan, Dusun, Bisaya, and Orang Sungai communities. They

consumed wild mushroom as a main food and substitute it for meat in their meals. Most people consume mushrooms because of their flavour in replacing meat (Grangeia et al., 2011). Indigenous people imply that several wild mushrooms have a great flavour and taste, and high nutrition benefits. Among the wild edible mushrooms, *Pleurotus*, *Volvariella* and *Lentinus* mushrooms were mostly preferred by the indigenous communities in Sabah.

Regarding medicinal use, the Dusun people reported five wild mushrooms, *Schizophyllum commune* (*Kulat Kodop*), *Auricularia* species (*Kulat Telinga*), *Pleurotus tuber-regium* (*Kulat Ubi*), *Lignosus* sp. and *Xylaria* sp. as having high medicinal properties. The finding was similar to previous studies reported by Mirfar et al., (2014) as *Schizophyllum commune* contain antimicrobial properties against bacteria and fungi, and Sekara et al., (2015): *Auricularia* species have significant therapeutic properties to treat eye and throat infection. Several studies reported that *Pleurotus* species are valuable medicinal mushrooms (Lau et al., 2011; Phan et al., 2012; Wong et al., 2013). As an example, *Pleurotus djamor* var. *roseus* (*Kulat Tiram Merah*) is good in improving health of old people and children according to the Dusun community in this study. A study by Jegadeesh et al. (2014) showed *Pleurotus djamor* var. *roseus* is able to provide beneficial supplements for health. For *Lignosus* species, local people used the tuber part (sclerotia) for health purposes. *Lignosus* species has been recorded r as a high medicinal value mushroom in Lee et al. (2012).

The Dusun communities from Kundasang region tend to frequently use wild edible mushrooms collected from the mountains. It was noted that their families eat mushrooms throughout the year. The common collected wild mushrooms are *Kodop* (*Schizophyllum commune*), *Pleurotus djamor* (*Kulat Tiram Putih* and *Merah*), and *Auricularia auricular-judae* (*Kulat Telinga Kera*). This suggests the existence of a mycophilic culture in these regions which was similar to a study done by Akpaja et al. (2003).

In the Southeast Asia region, a diverse variety of wild mushroom have been found at local markets (Jones et al., 1994) and such a sight is rarely observed in Sabah. The documentation of tree associated mushrooms is well recorded in other regions compared to Borneo, particularly in Sabah, and most of the potential ectomycorrhizal mushrooms have not been studied well. Some of the ectomycorrhizal mushrooms, such as *Boletus* species are edible mushrooms. The dipterocarp trees are known for mutual relationship with ectomycorrhizal fungi (Peay et al., 2009). Borneo has been recorded for its rich diversity of Dipterocarpaceae trees (Slik et al., 2003) compared to Africa and America which

are dominated by the genus Monotoidae and Pakaraimea (Sasaki, 2008). Thus, extensive work on the cultural and economic potential of wild mushroom by researches needs to be highlighted.

Conclusion

This study has listed 25 wild edible mushroom species in Sabah. The traditional knowledge of wild edible mushroom was different among local communities (Kadazan, Dusun, Orang Sungai and Bisaya). Different vernacular names were addressed to similar species from different ethnic groups. The older generation are more knowledgeable in the identification, local names, and traditional uses of wild mushrooms in forest. The information of wild mushroom culinary and identification are mainly collected from women compared to men. Indigenous communities in Sabah use wild mushrooms in their daily diet, particularly *Schizophyllum commune, Volvariella volvacea, Pleurotus* species, *Auricularia* species and *Marasmiellus* species. This study reported that *Schizophyllum commune* (*Kulat Kodop*), *Auricularia* species (*Kulat Telinga*), *Pleurotus tuber-regium* (*Kulat Ubi*), *Lignosus* sp. (*Cendawan Susu Harimau*) and *Xylaria sp.* are valuable in health practices. There is a need to further their taxonomic fields for accurate identification, medicinal uses and cultivation basis of wild mushroom in Sabah. The findings of this study perhaps contribute to the knowledge of wild edible mushroom in Borneo and Malaysia. Ethnomycological knowledge has plenty of potential in terms of the conservation and preservation of our local culture and culinary practices related to wild mushrooms in order to encourage the public on the importance of wild strain mushrooms for both the environment and humans.

Acknowledgements

We would like to thank Sabah Parks and Kinabalu Park staff (Mr. Matsain Mohd Buang, Yabainus Juhailin, Rossiti Karim and Kalipin) for their kind assistance during sample collections. We thank Mr Joumin Rangkasan and Mr Yeong Kam Cheng for arranging the transportation to the study sites. The authors would like to thank Sabah Parks for providing a permit (Permit: TTS/IP/100-6/2 Jld, 4/51). This study was supported by the Universiti Malaysia Sabah Research Grant Scheme (SLB0119-STWN-2016) and UMS Great Grant (GUG0085-STWN-2/2016) to JSSS and FSF. Thanks to Whitley Wildlife Conservation Trust for providing partial funding to conduct the study.

Authors' contributions

FSF and JSSS did sample collection, traditional knowledge data collection, data analysis and interpretation, laboratory work and manuscript writing. FHS and JK contributed in the traditional knowledge data analysis.

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Journal of Tropical Biology and Conservation 15: 223–245, 2018 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Aquatic Insects and Water Quality Study at Kimanis River, Crocker Range National Park, Sabah, Malaysia

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Abstract

A survey on the aquatic insect communities was conducted in Kimanis River, Ulu Kimanis, Crocker Range Park (CRP), Sabah with the objectives to study (i) the composition of stream insect communities, (ii) stream water quality and (iii) the relationships between aquatic insects and freshwater quality parameters in Kimanis River, Ulu Kimanis, CRP. The sampling was carried out using surber net in October 2015 and January 2016. A total of 1,801 individuals from nine orders of 28 families were collected from Kimanis River. Trichopterans fauna was found to be the prominent taxa in this study. Shannon-Wiener Index of upstream strata was recorded slightly higher than downstream strata with H'= 1.97 and H'= 1.85 respectively. Water integrity of Kimanis River could be considered as excellent with minimal pollution. Both water quality parameters and biotic indices indicated that the aquatic insect population was affected by the water quality in their surroundings. This proved the use of aquatic insect communities as bioindicator for rapid assessment of water quality in CRP.

Keywords: aquatic insect communities, trichopterans fauna, Shannon-Wiener Index, physico-chemical parameters, biotic indices, water integrity

Introduction

Aquatic macroinvertebrates are the key inhabitant of the freshwater ecosystem and serve an important role in keeping the ecosystem intact. Among the macroinvertebrates, insects are by far the most speciose and abundant macroinvertebrates established in freshwater ecosystems (Macadam & Stockan, 2015). Aquatic insects possess a vast array of morphological, physiological, and behavioural adaptations enabling inhabitation of virtually all bodies of water

Received 17 July 2018
Reviewed 07 August 2018
Accepted 24 August 2018
Published 15 October 2018

(Ward, 1992). Aquatic insect communities have the capacity to exploit most types of aquatic habitats and occur in a diverse group (Barman & Gupta, 2015). They are very wealthy inhabitants of freshwater environments that are in enormous number of broad distribution.

As one of the most widespread groups of organisms used to evaluate the health of the aquatic ecosystem (Rosenberg & Resh, 1993; Sharma & Rawat, 2009), benthic macroinvertebrates are ideal as bioindicator. Since macroinvertebrates constitute a heterogenous assemblage of animal phyla, consequently some members respond well to whatever stresses are placed upon them (Hellawell, 2012). Among macroinvertebrates, aquatic insects are often chosen for biomonitoring since aquatic insects are considered as good indicators of environmental condition. Aquatic insects are good indicators as they fulfil these few criteria: (i) abundant and sufficiently diverse in their habits and habitats; (ii) sensitive and predictable in their response to changes in environmental conditions, (iii) relatively easily sampled and identifiable to meaningful taxonomic resolutions, and (iv) bioaccumulate chemicals such that the pathways of toxins in the environment can be traced (Macadam & Stockan, 2015).

Since aquatic macroinvertebrates are feasible indicators of water quality, macroinvertebrates and water quality are interrelated (Sharma & Rawat, 2009). Therefore, the study of composition and structure of aquatic macroinvertebrates will be able to help in monitoring changes in water quality and the ecological integrity of streams and rivers (Arimoro & Ikomi, 2009). There is a considerable increase in the number of publications regarding biological monitoring using indicator species. This implies widespread and continuous growth in the use of indicator species in environmental monitoring and management (Siddig et al., 2015). In Malaysia, studies on aquatic insects and water quality have been carried out in Peninsular Malaysia (Che Salmah et al., 1999); Sarawak (Mercer *et al.*, 2014) and Sabah (Fikri et al., 2013; Harun *et al.*, 2015; Wong & Fikri, 2016; Shafie et al., 2017).

Crocker Range National Park (CRP) is located at the southern section of the Crocker Range in Northwest Borneo, Sabah, Malaysia (Zaini et al., 2012). CRP consists of about 139,919 ha, stretched from south of Kundasang in the north to Tenom in the south, approximately between latitudes 5^0 and 6^0 N and longitudes 115^0 and 119^0 E (Rahim et al., 2002). The CRP is bordered by the floodplain of the Pegalan/Padas River to the east and by the coastal plain of the west coast of Sabah (Rahim et al., 2002). CRP has been chosen as the study area since the site is less influenced by anthropogenic activities and hence the result obtained

from this study provides a benchmark towards the use of aquatic insect communities as biological indicator.

Numerous studies on aquatic ecosystems have been carried out in CRP, mostly focused on other freshwater organisms, especially anurans (eg. Ramlah et al., 2001; Rahim et al., 2002; Kueh et al., 2004; Das, 2006; Zaini et al., 2012). However, aquatic insect populations and water quality of the streams have received minimum amount of attention in CRP. The most recent studies regarding the aquatic insect communities and water quality of the CRP streams were by Long et al. (2002) and Manshoor & Fikri (2004) which were carried out in 2002.

Thus, in order to provide a more complete understanding towards the conservation effort of the aquatic ecosystems in CRP, it is critical to gain a better understanding on the health and the integrity of the aquatic ecosystem and the changes that have occurred throughout the years. The study of the capability of aquatic insect communities as bioindicators in freshwater streams is also important to improve understanding on the characteristics of aquatic habitats and to also monitor the water quality of freshwater which are needed to sustain the aquatic ecosystem in CRP, Sabah, Malaysia. As there is lack of a recent study in aquatic insect communities study in CRP, Sabah, this study was carried out to produce information on the aquatic insect communities as well as water quality and ecology health of Kimanis River, CRP. The objectives of the present study are therefore: (1) to study the diversity of stream insect communities; (2) to study the stream water quality and (3) to investigate the relationships between stream insect communities and water quality parameters in Kimanis River at Ulu Kimanis, Crocker Range National Park, Sabah, Malaysia.

Methodology

Study Area

Crocker Range Park (CRP) is located in the west coast of Sabah and stretches from Kundasang in the north to Tenom in the south. Ulu Kimanis River is the main river in the park, and consists of primary forest with lowland vegetation at 300 to 600 m asl (Nurhuda & Arman, 2002; Zaini et al., 2011). The average temperature is around 23 to 27°C (Zaini et al., 2011).

Sampling Campaign

Four stations were chosen from upstream and downstream of the Kimanis River respectively as indicated in Figure 1 and Table 1.

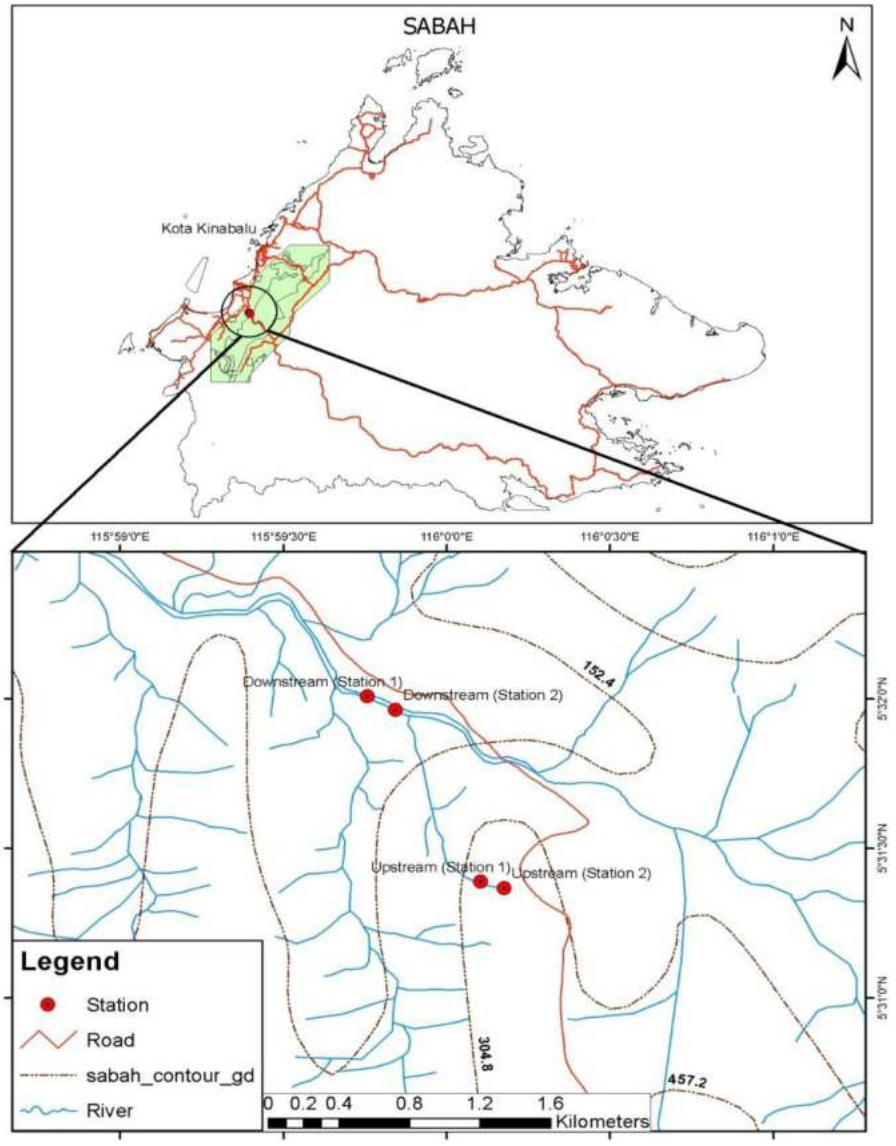


Figure 1. Map of Kimanis River, Crocker Range National Park, Sabah, Malaysia

Table 1. Site description of the sampling areas.

Sampling stations		Longitude	Latitude	Elevation	Habitat description	
					October 2015	January 2016
Upstream	Station 1	05^0 31' 44.6'' N	116^0 00' 13.9'' E	130 m	Moderate flowing and clear water, rocky bottom	Moderate flowing and clear water, rocky bottom
	Station 2	05^0 31' 39.9'' N	116^0 00' 17.9'' E	162 m	Moderate flowing and clear water, rocky bottom	Moderate flowing and clear water, rocky bottom
Downstream	Station 3	05^0 31' 91.9'' N	115^0 59' 74.8'' E	116 m	Swift flowing and milky water, sandy bottom, partially covered by forest canopy	Moderate flowing and clear water, sandy bottom, partially covered by forest canopy
	Station 4	05^0 31' 92.3'' N	115^0 59' 83.8'' E	122 m	Swift flowing and milky water, sandy bottom partially covered by forest canopy	Moderate flowing and clear water, sandy bottom, partially covered by forest canopy

Prior to sampling, the surrounding condition of the riparian zone was observed and recorded. Three substations, composed of different habitat types, were selected in each station. The selected sites were about 100 m from each other. Sampling was carried out in three different habitats, pools, riffles and runs for comparisons among the habitats of Kimanis River.

Aquatic Insects

Aquatic insects were collected over two sampling occasions between October 2015 and January 2016. Samplings for aquatic insects were done during the day using surber sampler, which is commonly used for quantitative sampling aquatic insects. Three substations, composed of three different habitat types, pool, riffle and run were sampled. The device is positioned with the opening facing upstream (Jalil & Mohamed, 2004) and the surroundings were agitated for 2 minutes. Big stones in swift-flowing water were hand-lifted and washed by rubbing on the rock surface to remove the aquatic insects into the net. Aquatic insects were identified to the family level using taxonomic keys of Yule and Yong (2004) and Merritt et al. (2008) and also preserved specimens from BORNEENSIS, ITBC in the laboratory. Four biotic indices specifically EPT Richness, Family Biotic Index (FBI), Biological Monitoring Work Party (BMWP) and Average Score Per Taxon (ASPT) were used to assess the water quality of Kimanis River at CRP. The values attained from the indices helped to determine the current status of

water integrity with the standard description for each range when compared with the standard range of scores.

Water Quality Parameters

Water samples were collected near the surface of the river and stored in 250 ml high-density polyethylene (HDPE) bottles. *In situ* parameters, pH, temperature (^{0}C), conductivity (µS/cm), dissolved oxygen (DO) (mg/l), salinity, total dissolved solids (TDS) (mg/l) and ammonia nitrogen were tested using YSI Professional Plus (ProPlus model 6026 S/N Y 5173), multi-parameter water quality instrument. YSI ProPlus must be fully submerged into the water to obtain accurate readings. YSI ProPlus was placed in the middle of the stream and permitted to stabilize before readings were taken (Harun et al., 2010). Three replicates of in-situ parameters were recorded at each station. Total suspended solids (TSS) was conducted following Gravimetric Method (Harun et al., 2015) and dissolved organic carbon (DOC) was analysed by using Shimadzu TOC-V-SCH analyzer with auto – sampler TOC-ASI-V. Samples were acidified with hydrochloric acid (HCl). The acidified samples (pH ~2) were sparged for 8 minutes at 75 or 100 ml/min with ultra-pure oxygen to remove all inorganic carbon from samples prior to measurement.

Data Analyses

Diversity of aquatic insects was computed using the Shannon-Wiener Diversity Index (H') and Evenness Index (E'). Sørensen's Quantitative Index (C_N) used to compare the diversity among two sites. The number of taxa (taxa richness) was calculated by counting the number of aquatic insect families found in the samples. Discriminant function analysis (DFA) is a multivariate statistical modelling and supervised pattern recognition technique and can be used to classify objects into exhaustive and mutually exclusive groups depends on set of independent variables (Gazzaz et al., 2012). DFA analysis in this study used statistical package SPSS to construct the graphs to identify the significance of certain water quality parameters in particular sites. Canonical correspondence analysis (CCA) is a multivariate method to elucidate the relationships between biological assemblages of species and their environment (Braak & Verdonschot, 1995). In this study, CCA is used to study the relationships between the aquatic insect communities and water quality parameters.

Results

Composition and Distribution of Aquatic Insects

Table 2. The list of aquatic insects distributed across downstream and upstream of Kimanis River, CRP.

Order	Family	Downstream			Upstream		
		Station 1	Station 2	Total	Station 1	Station 2	Total
Coleoptera	Elmidae	8	8	16	17	10	27
	Hydrophilidae	0	0	0	2	0	2
	Gyrinidae	0	0	0	10	0	10
	Psephenidae	2	0	2	5	6	11
Diptera	Tipulidae	4	1	5	4	9	13
	Chironomidae	15	1	16	3	0	3
	Stratiomyidae	0	0	0	2	0	2
Ephemeroptera	Baetidae	35	28	63	101	47	148
	Heptageniidae	22	16	38	42	58	100
	Leptophlebiidae	13	7	20	59	40	99
	Siphlonuridae	0	0	0	15	1	16
	Ephemerellidae	1	0	1	0	6	6
	Caenidae	1	2	3	2	2	4
	Potamanthidae	0	0	0	1	2	3
	Tricorytidae	0	0	0	2	0	2
Hemiptera	Mesoveliidae	0	0	0	0	2	2
	Gerridae	1	0	1	1	0	1
	Veliidae	0	0	0	1	0	1
Lepidoptera	Pyralidae	0	0	0	33	4	37
Megaloptera	Corydalidae	6	2	8	18	6	24
Odonata	Euphaeidae	3	0	3	2	1	3
	Gomphidae	1	0	1	0	0	0
Plecoptera	Perlidae	15	7	22	28	25	53
Trichoptera	Hydropscyhidae	110	92	202	272	309	581
	Philopotamidae	34	8	42	93	112	205
	Limnephilidae	0	0	0	2	0	2
	Polycentropodidae	0	0	0	1	1	2
	Phryganeidae	0	0	0	1	0	1
Grand Total		**271**	**172**	**443**	**717**	**641**	**1358**
Shannon-Wiener Index (H')				1.85			1.97
Evenness Index (H')				0.67			0.60

A total of 1,801 individuals of aquatic insects representing 28 families from nine orders were collected and identified along Kimanis River, Crocker Range Park, Ulu Kimanis, Sabah, Malaysia throughout the sampling during October 2015 and January 2016. The nine aquatic insects orders collected belong to Coleoptera (68 individuals; 3.78% of total abundance), Diptera (39 individuals; 2.17% of total abundance), Ephemeroptera (503 individuals; 27.93% of total abundance), Hemiptera (5 individuals; 0.28% of total abundance), Lepidoptera (37 individuals; 2.05% of total abundance), Megaloptera (32 individuals; 1.78% of total abundance), Odonata (7 individuals; 0.39% of total abundance), Plecoptera (75

individuals; 4.16% of total abundance) and Trichoptera (1035 individuals; 57.47% of total abundance) (Table 2). Hydropscyhidae yielded the highest in abundance in which it comprised 783 individuals, made up of almost half of the total collection of 43.48% out of 1,801 individuals followed by Philopotamidae which contributed 247 individuals or 13.71%.

Ephemeroptera, Plecoptera and Trichoptera (EPT) were significantly abundant especially at upstream stations in Kimanis River at CRP. Families Elmidae, Psephenidae, Chironomidae, Tipulidae, Baetidae, Caenidae, Ephemereliidae, Heptageniidae, Leptophlebiidae, Gerridae, Perlidae, Hydropscyhidae, Philopotamidae, Euphaeidae and Corydalidae were found at both upstream and downstream areas. Although family Chironomidae (Diptera) was present at both the upstream and downstream, the high individual count (16) was present in downstream strata. Shannon-Wiener Diversity Index (H') is higher in upstream with 1.97 and lower in downstream with 1.85. However, for Evenness Index (E'), the index is higher in downstream with 0.67 and lower in upstream with 0.60. Sørensen's Quantitative Index (C_N) between aquatic insect communities from upstream and downstream is 0.48 which indicates there is about 48% similarity in terms of species of aquatic insects between the downstream and upstream.

Water Quality Parameters

Biological Parameters

A total of 14 families of EPT were sampled in Kimanis River. Therefore, Kimanis River is categorized as having very good water quality. All the 14 families of EPT existed at upstream but only eight families were represented at the downstream. FBI values for downstream strata (3.88) and upstream strata (3.97) were both classified as very good. In addition, BMWP shows upstream has higher value (138) and is cleaner than downstream (96). BMWP value for Kimanis River is 152 indicating that the river is unpolluted and has not been impacted (unimpacted). ASPT for upstream strata and downstream strata are 6.90 and 6.86 respectively with the same description as good water quality and probably some organic pollution. Overall, Kimanis River has high water integrity and upstream strata of Kimanis River showed a better water condition in comparison to downstream strata (Table 3).

Table 3. Biotic Indices of Crocker Range Park.

Study Site/ Biotic Indices	Upstream		Downstream		Total	
	Value	Class	Value	Class	Value	Class
EPT Richness	14	Very Good Water Quality	8	Good Water Quality	14	Very Good Water Quality
FBI	3.97	Very Good	3.88	Very Good	3.71	Excellent
BMWP	138	Unpolluted, Unimpacted	96	Clean but slightly Impacted	152	Unpolluted, Unimpacted
ASPT	6.90	Good Water Quality, Some organic pollution probable	6.86	Good Water Quality, Some organic pollution probable	7.23	Good Water Quality, Some organic pollution probable

Water Quality Parameters

Table 4 summarises the water quality parameters in each sampling station. Overall, the value for each water quality parameter is higher in January 2016 in comparison to October 2015.

Table 4 Water quality parameters in each sampling stations.

Parameter	October 2015				January 2016			
	Upstream		Downstream		Upstream		Downstream	
	Station 1	Station 2	Station 3	Station 4	Station 1	Station 2	Station 3	Station 4
pH	6.51	6.95	6.50	6.93	6.76	6.62	7.75	7.42
Temperature (^{0}C)	24.57	25.67	24.60	25.37	25.30	25.63	26.77	25.23
Conductivity (µS/cm)	44.47	59.80	53.27	53.60	79.83	88.67	92.80	87.50
DO (mg/l)	7.45	7.66	7.16	7.30	7.94	7.84	8.97	7.39
Salinity	0.02	0.03	0.02	0.02	0.04	0.04	0.04	0.04
TSS (mg/l)	2.13	2.27	3.71	1.83	1.87	1.87	3.73	2.40
TDS (mg/l)	29.25	35.02	35.10	34.45	54.38	56.98	57.20	56.55
Ammonia Nitrogen (mg/l)	0.33	0.70	0.29	0.44	0.00	0.00	0.24	0.00
DOC (mg/l)	2.49	1.91	1.53	1.48	11.37	2.02	4.20	6.15

Table 5 explains the classification of water quality physico-chemical parameters for Kimanis River in accordance with the Interim National Water Quality Standards for Malaysia (INWQS).

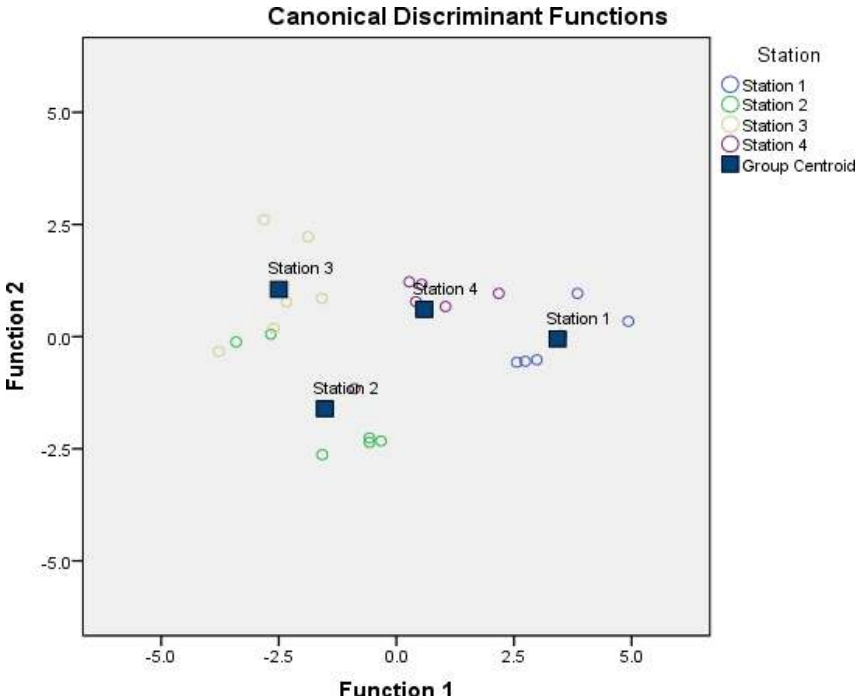
Table 5. Range of different water quality parameters and their classifications by INWQS of Kimanis River at CRP.

Parameter	October 2015	INWQS	January 2016	INWQS
pH	6.50-6.95	I	6.62-7.75	I
Temperature (^{0}C)	24.57-25.67	-	25.23-26.77	-
Conductivity (µS/cm)	44.47-59.80	I	79.83-92.80	I
DO (mg/l)	7.16-7.66	I	7.39-8.97	I
Salinity	0.02-0.03	I	0.04	I
TSS (mg/l)	1.83-3.71	I	1.87-3.73	I
TDS (mg/l)	29.25-35.10	I	54.38-57.20	I

Table 6 presents the factor structure coefficients from the discriminant analyses, while Figure 3 plots the first and second discriminant function from water quality physico-chemical parameters and sampling stations. The plot suggests that Station 1 and Station 4 are homogenous while Station 3 the most heterogeneous group. In Function 1, dissolved organic carbon (DOC), temperature and conductivity were dominant at sampling Station 1. Meanwhile total suspended solids (TSS), pH, salinity and ammonia nitrogen were prominent at sampling Station 3.

Table 6. Structure matrix from discriminant analyses for each stations and water quality parameters.

Variables	1	2
Temperature	-.202*	-.054
DOC	.133*	.078
Conductivity	-.103*	-.037
TSS	-.151	.315*
pH	-.114	.310*
Salinity	-.030	-.161*
Ammonia Nitrogen	-.087	-.141*
DO	-.083	.031
TDS	-.058	.001



Upstream: Station 1; Station 2; Downstream: Station 3; Station 4

Figure 3. Plots for discriminant functions for water quality parameters against stations

Influence of Water Quality Parameters on Abundance of Aquatic Insects

Canonical correspondence analysis (CCA) demonstrated that the total inertia in aquatic insect abundance had an eigenvalue of 0.3885; eigenvalues of the nine water quality parameters explained 51% of the total variance (TVE) (Table 7).

Table 7 Axis summary statistics of canonical correspondence analysis (CCA): eigenvalues, variance percentage, species-environment correlations for the first two axes and total inertia. Total variance ("inertia") in the species data: 0.3885

	Axis 1	Axis 2
Eigenvalue	0.123	0.075
Variance in species data		
% of variance explained	31.7	19.3
Cumulative % explained	31.7	51.0
Pearson Correlation, Spp-Envt*	0.847	0.888
Kendall(Rank) Corr., Spp-Envt	0.727	0.727

The order of aquatic insects are positively correlated with total suspended solids (TSS) (r=0.024) and dissolved organic carbon (DOC) (r=0.516) but negatively correlated with pH, temperature, conductivity, DO, salinity, TDS and ammonia nitrogen (Table 8).

Table 8. Intraset and interset correlations for nine physico-chemical parameters.

Variables	Intraset Correlations		Interset Correlations	
	Axis 1	Axis 2	Axis 1	Axis 2
pH	-0.069	0.286	-0.058	0.254
Temperature	-0.202	0.474	-0.171	0.421
Conductivity	-0.444	0.333	-0.376	0.296
DO	-0.201	0.074	-0.170	0.066
Salinity	-0.719	-0.133	-0.609	-0.118
TSS	0.024	0.354	0.020	0.314
TDS	-0.473	0.134	-0.401	0.119
Ammonia Nitrogen	-0.282	0.289	-0.239	0.256
DOC	0.516	0.558	0.437	0.496

Two environmental variables had high intra-set correlations, thus were more important in predicting community composition. Salinity strongly correlated with the first CCA axis while DOC correlated with the second CCA axis (Figure 4). The CCA output depicted that Coleoptera, Hemiptera, Ephemeroptera, Plecoptera and Diptera preferred high concentration of DOC.

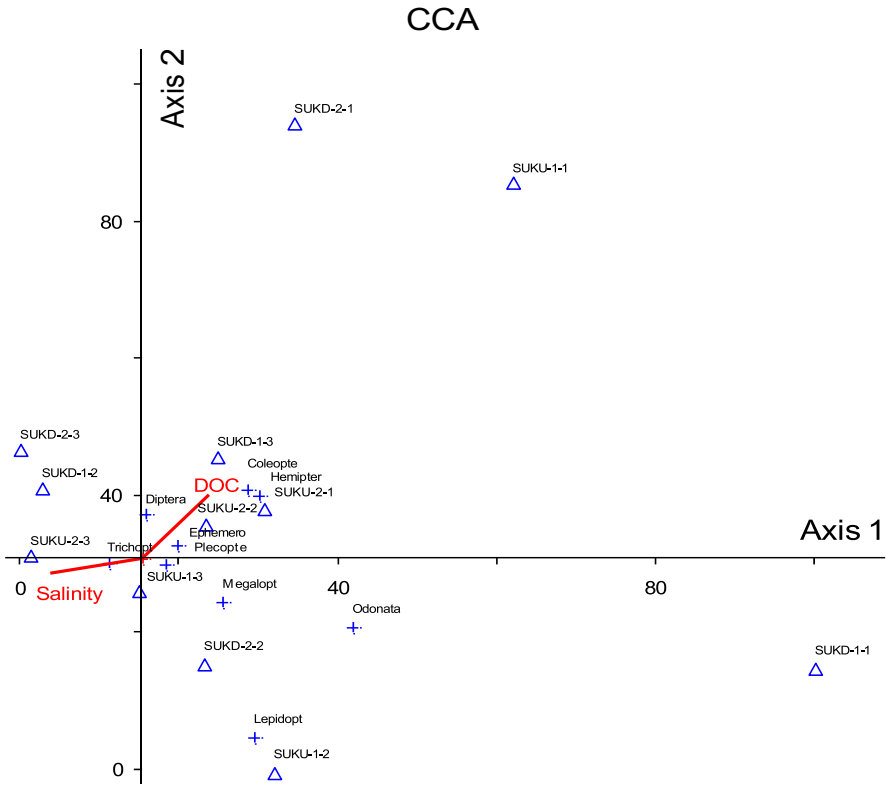


Figure 4. Canonical correspondence analysis (CCA) for the first two canonical axes of the aquatic insects (orders) and water quality parameters in Kimanis River.

Discussion

Composition and Distribution of Aquatic Insects

A total of 1,801 individuals of aquatic insects from nine orders and 28 families were collected from the sampling done at Kimanis River, Ulu Kimanis, CRP in October 2015 and January 2016. The orders are Coleoptera, Diptera, Ephemeroptera, Hemiptera, Lepidoptera, Megaloptera, Odonata, Plecoptera and Trichoptera. Ephemeroptera, Plecoptera and Trichoptera (EPT) communities composed of a large proportion of 89.56% of the total individuals sampled. Presence of high abundance of EPT communities marks high stream quality as EPT communities are prevalent in undisturbed streams (Che Salmah

et al., 1999) and exhibit low tolerance toward water pollutants (Keçi et al., 2012). Therefore, Kimanis River at CRP can be customarily considered clean.

Seven orders of aquatic insect species and hexapodan Collembola were encountered in the six rivers surveyed by Long et al. (2002) in 2001. Orders attained in a study done by Long *et al.* are similar with this study with the exception of Lepidoptera and Megaloptera. In addition, in this study, trichopteran hydropscyhids fauna was dominant but a study conducted by Long *et al.* showed dipteran chironomid fauna as prominent aquatic insect taxa. The difference in diversity and composition with this study was possibly due to the variation in the sampling method. Surber net was the only sampling technique utilized in this study while Long et al. (2002) took sediment samples and used plankton net with a mesh size of 100 µm. Moreover, Long et al. (2002) surveyed Sg. Mawau, Sg. Tandulu, Sg. Liawan, Sg, Ulu Senagang, Sg. Tikolud and Sg. Balayo but not Kimanis River. Stream insects possess ubiquitous nature in stream ecosystems, diverse behavioural, morphological and ecological traits, and are highly variable in community structure even between adjacent streams (Heino & Peckarsky, 2014). Hence, this might give an assumption that the composition of aquatic insect communities is distinctive between different rivers.

Trichoptera was the dominant taxa in aquatic insect population (57.47%) in Kimanis River at CRP in which the aquatic insects collected was dominated by family Hydropscyhidae (43.48%), followed by family Philopotamidae (13.71%). The large quantity of trichopterans may be associated with the presence of high food quality (Perterson, 1987; Harding, 1997; Prommi et al., 2014), stable water flow and stable substrata common in these habitats (Georgian & Thorp, 1992; Prommi et al., 2014). In addition, similarly low numbers and diversity of Plecoptera are reported in this study (Hamid & Rawi, 2011; Prommi & Payakka, 2015). The absence of high density of Plecoptera in Kimanis River is probably due to unfavourable conditions for their growth and reproduction as they prefer cooler, more northern latitudes (Sivec & Yule, 2004; Prommi & Payakka, 2015) while Kimanis River recorded relatively high surface water temperature.

In this study, significant differences were encountered for several abundance of orders of aquatic insect communities at different strata of Kimanis River. Family Chironomidae from order of Diptera was more abundant at the downstream of Kimanis River at CRP. A comparative example of distribution of Chironomidae has been attained by Prommi and Payakka (2015) in Mae Tao and Mae Ku watersheds, Northern Thailand. According to Yule (2004) and Wahizatul et al. (2011), family Chironomidae thrive in standing and slow-flowing streams and

muddy or sandy areas with high fine-sediment particles. Therefore, it can be argued that stations at the downstream with sandy bottom is more suitable for family Chironomidae to live compared to stations at the upstream which have a rocky bottom.

On the other hand, family Pyralidae was found abundant in the upstream strata but are not sampled from the downstream strata. Pyralidae larvae thrive in rapid streams on the surface of submerged rocks which provide protection from the current by a case consisting of an irregular sheet of silk cemented around most of its periphery to the rock (Lavery & Costa, 1973). Hence, it is suggested that the rocky bottom at the upstream of Kimanis River serves as a better habitat for Pyralidae as compared to sandy bottom at the downstream.

Shannon-Wiener Index (H') of aquatic insect communities in Kimanis River shows that the diversity of aquatic insects of upstream is slightly higher than downstream with 1.97 and 1.85 respectively. The differences between diversity indices among the stratum are not apparent probably due to the short distance between the sampling sites. The diversity and evenness indices were basically higher at upper stream and decreased at downstream (Salman et al., 2011; Mohd Rasdi et al., 2012). However, in this study, downstream has slightly more evenness with the index value of 0.67 than in the upstream with an index value of 0.60. The lower evenness of upstream could possibly be due to the occurrence of high abundance of trichopterans at the upstream which caused unbalanced distribution of aquatic insects at upstream. This study recorded 48% similarity in terms of species of aquatic insects between downstream and upstream based on the Sørensen's Quantitative Index (C_N). The composition of aquatic insect population that thrive in downstream and upstream of the Kimanis River was similar and this was probably due to less variation of water quality parameters between downstream and upstream areas.

Biotic Indices

Biotic indices values for upstream and downstream strata of Kimanis River do not vary significantly. EPT richness was calculated based on the number of families of EPT communities found in the upstream and downstream respectively. The overall EPT richness of Kimanis River at CRP is 14. From the study, Kimanis River can be assumed as a non-impacted stream as the EPT richness value exceed 10, which is the cut-off value to be qualified as a non-impacted stream (Fikri, 2004). Family biotic index obtained at downstream strata and upstream strata are 3.97 and 3.88 respectively, both indicating very good water quality. Biological Monitoring Work Party (BMWP) of Kimanis River depicts that the river

is unpolluted and unimpacted. Pollution intolerant families have high BMWP scores, while pollution tolerant families have low scores (Barman & Gupta, 2015). Presence of immense abundance of pollution intolerant families such as Heptageniidae, Leptophlebiidae and Perlidae in Kimanis River enable the river to be classified as unpolluted. Meanwhile, the Average Score Per Taxon (ASPT) that represents the average tolerance score of all taxa within the community (Barman & Gupta, 2015) was measured for both downstream and upstream strata and show that they were both considered as having good water quality.

Water Quality Physico-Chemical Parameters

The water quality physico-chemical parameters readings did not show much variation between October 2015 and January 2016, implying that the level of stream disturbance in Kimanis River at CRP was not serious. Concentration of dissolved organic carbon (DOC) is a general description of the dissolved organic matter (DOM). DOC concentration in this study varied between 1.48 mg/l to 11.37 mg/l. DOC was the most important water quality parameter in predicting the order of aquatic insect community. Aquatic insect communities in the upstream particularly families Coleoptera, Hemiptera, Ephemeroptera, Plecoptera and Diptera positively correlated with DOC concentration. Harun *et al*. (2015) reported correlation between hemipterans and DOC at the Lower Kinabatangan River Catchment, Sabah and proposed Hemiptera as indicators of elevated (present or past) nutrient levels in the stream systems. From this study, CCA results support the use of Hemiptera as nutrient level indicators. The correlation is possibly due to more abundance of preys (food) at locations with increased nutrient concentrations (Maul et al., 2004; Harun et al., 2015).

Total dissolved solids (TDS) in January 2016 documented almost two-fold of the values recorded in October 2015. The range of TDS in October 2015 is between 29.25 mg/l and 35.10 mg/l while TDS in January 2016 ranged between 54.38 mg/l and 57.20 mg/l. A similar pattern is observed for the recorded conductivity. The conductivity documented in October ranged from 44.47 µS/cm to 59.80 µS/cm but increased significantly in January, recording 79.83 µS/cm to 92.80 µS/cm. The large variation in TDS and conductivity between sampling periods is possibly due to rainy days in the October sampling which influence the TDS and conductivity values. The rainy period may alter conductivity substantially as rain water has lower conductivity due to lack of minerals (Mahazar *et al.*, 2013). Apart from this, the increment of TDS and conductivity could also be attributed to weathering intensity during the wet season (Makwe & Chup, 2013) which January is known for here.

Total suspended solids (TSS) is usually due to the introduction of external factors carried by runoff rain waters which cause the increment in concentration of this parameter (Jonnalagadda & Mhere, 2001; Rossi et al., 2005; Suratman *et al.*, 2006; Suratman et al., 2015). Low TSS was recorded in Kimanis River suggesting that the river is undisturbed and unimpacted by human activities. On the other hand, ammonia nitrogen ranged from 0.29 mg/l to 0.44 mg/l in October 2015 and 0.00 mg/l to 0.24 mg/l in January 2016.

The surface temperature ranged from 24.57 ^{0}C to 25.67 ^{0}C in October and 25.23 ^{0}C to 26.77 ^{0}C in January. The rise in water temperature probably due to low flow conditions and strong sunshine occurred in January 2016. Temperature impacts both the chemical and biological characteristics of surface water (Prommi & Payakka, 2015). As temperature is one of the major factors determining the distribution of Hydropsychidae (Kimura et al., 2008; Prommi & Payakka, 2015), in the present study, a slight temperature rise in Kimanis River from October to January has caused drastic increase in the abundance of Hydropsychidae species. This implies that higher water temperatures favour the density and diversity of Hydropsychidae.

The surface water dissolved oxygen (DO) concentrations ranged from 7.16 mg/ to 8.97 mg/l. Meanwhile, salinity is relatively constant in Kimanis River, Ulu Kimanis, CRP. As salinity affects dissolved oxygen solubility, the constant salinity enables relatively stable dissolved oxygen concentration present in Kimanis River. High dissolved oxygen content recorded implies that condition of Kimanis River is suitable for the establishment of aquatic insect communities. With regard to the pH, this varied between 6.50 and 7.75. Among the water quality parameters, pH would be the most stable parameter with small differences and also most stable for every 3 months with no drastic changes (Mahazar et al., 2013). However, a polluted river usually would have unstable pH rather than stay in a durable form (Mahazar et al., 2013). As the pH value stayed relatively stable in this study, this can therefore infer that Kimanis River is unpolluted and unimpacted.

Regarding the Interim National Water Quality Standards for Malaysia (INWQS) classification, the water quality of the Kimanis River (except ammonia nitrogen) were categorized as Class I stream. Class I stream functions as conservation of natural water supply with no water treatment required. Class I is defined as very clean and treatment is not required at this stage, except by disinfection or boiling (Harun et al., 2010).

Conclusion

Diversity index was basically higher at upstream and decreased at downstream followed the same trend as that of the river water quality. The water quality parameters readings did not indicate much variation between October and January. Hence, it infers that the stream condition in Kimanis River at CRP was relatively stable. The water quality in Kimanis River was classified in Class I for most of the water quality parameters, which is consistent with the assessment made by the biotic indices. This implies that the aquatic insect communities are useful as a faster and cheaper way for rapid assessment of stream water quality. In view of the above, more research would provide a better representation of the aquatic insect communities of Ulu Kimanis, Crocker Range Park. This will help in representing the species richness and composition available in the streams of CRP. Meanwhile, current species checklist could be further extended with more research conducted.

Acknowledgements

The authors wish to thank the Institute for Tropical Biology and Conservation of Universiti Malaysia Sabah (UMS) and Sabah Parks for the facilities and logistics provided during the Crocker Range Scientific Expedition 2015. The research is partially contributed by UMS grant GUG0148/2017. Thanks also to Mr Bertus and Mr Farhan for their assistance during sampling.

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Appendix

Table 9. The list of aquatic insects distributed across downstream and upstream of Kimanis River, Ulu Kimanis, CRP.

Order	Family	Downstream			Upstream		
		Station 1	Station 2	Total	Station 1	Station 2	Total
Coleoptera	Elmidae	8	8	16	17	10	27
	Psephenidae	2	-	2	5	6	11
	Gyrinidae	-	-	-	10	-	10
	Hydrophilidae	-	-	-	2	-	2
	Total	**10**	**8**	**18**	**34**	**16**	**50**
Diptera	Tipulidae	4	1	5	4	9	13
	Chironomidae	15	1	16	3	-	3
	Stratiomyidae	-	-	-	2	-	2
	Total	**19**	**2**	**21**	**9**	**9**	**18**
Ephemeroptera	Baetidae	35	28	63	101	47	148
	Heptageniidae	22	16	38	42	58	100
	Leptophlebiidae	13	7	20	59	40	99
	Siphlonuridae	-	-	-	15	1	16
	Ephemerellidae	1	-	1	-	6	6
	Caenidae	1	2	3	2	2	4
	Potamanthidae	-	-	-	1	2	3
	Tricorytidae	-	-	-	2	-	2
	Total	**72**	**53**	**125**	**222**	**156**	**378**
Hemiptera	Mesoveliidae	-	-	-	-	2	2
	Gerridae	1	-	1	1	-	1
	Veliidae	-	-	-	1	-	1
	Total	**1**	**0**	**1**	**2**	**2**	**4**
Lepidoptera	Pyralidae	-	-	-	33	4	37
	Total	**0**	**0**	**0**	**33**	**4**	**37**
Megaloptera	Corydalidae	-	-	-	18	6	24
	Total	**6**	**2**	**8**	**18**	**6**	**24**
Odonata	Euphaeidae	3	-	3	2	1	3
	Gomphidae	1	-	1	-	-	-
	Total	**4**	**-**	**4**	**2**	**1**	**3**
Plecoptera	Perlidae	15	7	22	28	25	53
	Total	**15**	**7**	**22**	**28**	**25**	**53**
Trichoptera	Hydropscyhidae	110	92	202	272	309	581
	Philopotamidae	34	8	42	93	112	205
	Limnephilidae	-	-	-	2	-	2
	Polycentropodidae	-	-	-	1	1	2
	Phryganeidae	-	-	-	1	-	1
	Total	**144**	**100**	**244**	**369**	**422**	**791**
Grand Total		**271**	**172**	**443**	**717**	**641**	**1358**

Instructions for Authors

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Journal of Tropical Biology and Conservation

Vol. 15 http://jurcon.ums.edu.my/ojums/index.php/jtbc **2018**

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Jalan UMS, 88400 Kota Kinabalu, Sabah, MALAYSIA
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E-ISSN 2550 - 1909

ISSN 1823 - 3902