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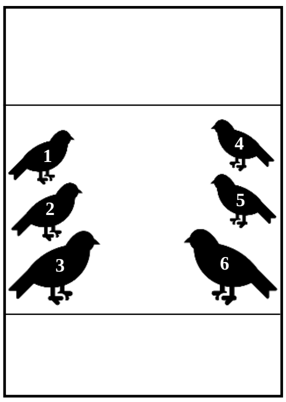

1: Dark-throated Oriole; **2**: Bornean Green Magpie; **3**: Temminck's Sunbird; **4**: White-crowned Shama; **5**: Chestnut-crested Yuhina; **6**: Bornean Treepie (Photos: Y.C. Lee & Alven Chang)
Drone images: Aerial view of the montane ecosystem in Bukit Hampuan Forest Reserve. (M.A.F. Suis)

Rivers are not complete barriers to the movement of tropical forest dung beetles

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Abstract: Understanding the impacts of natural and anthropogenic barriers to animal movement allows insights into how species assemblages form and diverge, and has implications for the effective design of protected areas and management of human-modified landscapes. Dung beetles are a functionally important taxon that can be sampled relatively easily with dung-baited traps. In this study, we used non-lethal mark-release-recapture (MRR) methods to test the ability of dung beetles to cross a large river (about 50 m width) in an area of Bornean primary lowland dipterocarp rainforest. We found that while the river appeared to be a barrier for the small and medium-bodied beetle species this was not the case for several of the large-bodied dung beetle species. Dung beetles moved distances of between 50–200 m in 24 hours. This study demonstrates the suitability of MRR methods for assessing movement of tropical forest dung beetles over natural barriers.

Keywords: Danum Valley Conservation Area, dung beetles, Mark Release Recapture, movement barriers, rivers

INTRODUCTION

Tropical rainforests in Southeast Asia are important ecosystems and biodiversity hotspots (Myers *et al.* 2000), containing large numbers of endemic vertebrate and plant species (Sodhi *et al.* 2004). However, these forests have been fragmented and degraded through logging (Bryan *et al.* 2013, Stibig *et al.* 2014) and conversion to oil palm plantations (Gaveau *et al.* 2014) with resulting losses of biodiversity and ecosystem functioning (Sodhi *et al.* 2004).

Barriers to movement and dispersal have been shown to drive ecological and evolutionary processes and can cause diversification of species (Ribas *et al.* 2012, Naka *et al.* 2012). Barriers can be anthropogenic, such as logging roads (Bryan *et al.* 2013), boundaries between rainforest and oil palm plantations (Scriven *et al.* 2017) or water reservoirs (Cros *et al.* 2020), or they can be natural, such as mountains (Sánchez-Montes *et al.* 2018) or glacial oscillations (Hewitt 1996). Such barriers might separate two habitat types, or they may form barriers to movement within an area of otherwise homogenous habitat. For example, the Congo River has been shown to reduce gene flow in some birds and their parasitic lice (Voelker *et al.* 2013), and genetic differentiation has been found in the dung beetle *Nanos binotatus* on either side of the Mangoro River in Madagascar (Knopp *et al.* 2011).

Small habitat fragments have been shown to lose biodiversity at a faster rate than larger fragments (Gibson *et al.* 2013). Therefore, the subdivision of forests by natural barriers such as rivers, and its potential effects on the movement of species, is important to consider when designing protected areas. It is important to study the effects that these natural barriers might have on the movement of species within and across habitat types. For vertebrates, the use of increasingly small Global Positioning System (GPS) tags allows for real-time tracking of movement on a fine spatial scale (Kays *et al.* 2015). For insects however, while some studies have made use of radar to track movement (Chapman *et al.* 2011), mark-release-recapture methods (MRR) are often used as they are relatively inexpensive (Hagler & Jackson 2001). Such studies can involve many thousands of sampled and marked individuals, requiring large study periods and high sampling effort (Molleman *et al.* 2007, Vlasanek *et al.* 2013). MRR of insects has been used in a variety of contexts, such as studying movement ranges (Nazni *et al.* 2005), measuring responses to habitat fragmentation (Slade *et al.* 2013), and estimating population sizes (Roslin *et al.* 2009). MRR methods are generally much harder in tropical forests compared to temperate regions due to highly dense vegetation and an abundance of rare species (Vlasanek *et al.* 2013). However, despite these challenges, MRR has been used in tropical ecosystems to study movement of Lepidoptera across habitat types (Lewis 2001, Scriven *et al.* 2017, Gray *et al.* 2019), examine butterfly lifespans (Molleman *et al.* 2007), and provide information on optimal dung beetle sampling strategies (Larsen & Forsyth 2005, da Silva & Hernandez 2015).

In this pilot study, we utilised non-lethal MRR of dung beetles (Coleoptera, Scarabaeidae, Scarabaeinae) to assess if they are able to move across a large river (about 50 m in width) in a relatively short time period (about 24 hours), in order to determine if it acted as a complete, or partial, barrier to movement. Dung beetles are an ideal study taxon for such a study as they have been successfully used in previous MRR experiments (*e.g.* Arellano *et al.* 2008, Viljanen 2009, da Silva & Hernandez 2015). This diverse group consists of about 6000 species across about 257 genera (Philips 2011) with a cosmopolitan distribution, being found on all continents except Antarctica (Hanski & Cambefort 1991, Davis *et al.* 2017). Dung beetles are primarily coprophagous and can be split into functional groups depending on behavioural traits such as how they utilise dung for reproduction and when they are active (Hanski & Cambefort 1991), and morphological traits, such as size (Raine *et al.* 2018). They can be inexpensively sampled with dung-baited pitfall traps (Nichols & Gardner 2011, Inward *et al.* 2011) and are often used as bioindicators of forest disturbance (Bicknell *et al.* 2014) and mammal abundance (Raine & Slade 2019). Dung beetle communities have been proven to be particularly useful in assessing the biodiversity retained in riparian forest buffers in oil palm plantations, and certain species are particularly associated with such habitats (Gray *et al.* 2014, Gray *et al.* 2016). In addition, dung beetles provide important ecosystem functions and services in tropical forests, such as secondary seed dispersal, nutrient recycling, and soil bioturbation (Nichols *et al.* 2008, Slade *et al.* 2011).

The aims of this study were to explore: A) the utility of non-lethal mark-release-recapture techniques for the study of movement of beetles across potential barriers in tropical rainforests; B) whether large (about 50 m) rivers act as natural barriers to the movement of a group of functionally important insects. We predicted that the river might act as a barrier to movement and predicted a greater proportion of marked beetles would be recaptured on the same side as initial trapping, rather than over the river. We also predicted that the river might act differently as a barrier for beetles of different body size classes, acting as more of a barrier for smaller beetles.

MATERIALS AND METHODS

Study site

Fieldwork was conducted in the Danum Valley Conservation Area (DVCA) in Sabah, Malaysian Borneo (4.58°N, 117°E). DVCA comprises 43,800 ha of lowland evergreen dipterocarp rainforest with the Segama river flowing through it. Average annual rainfall is 2,822 mm. Mean minimum and maximum temperature is 21.2 and 28.4°C, respectively (Marsh & Greer 1992).

Figure 1. A section of the Segama river showing how the river opened the forest canopy.

Dung beetle trapping was performed on a section of the Segama river near the Danum Valley Field Centre (DVFC). The river was classified as large as it was about 50 m from bank to bank when measured over the bridge at the DVFC, and the canopy did not cover it, creating an open environment across most of the river (Figure 1). The river is surrounded on both sides by primary rainforest, indicating that dung beetle communities of similar composition were likely to be found on each side of the river.

Mark-Release-Recapture

Dung beetles were collected using non-lethal human-dung-baited pitfall traps (Figure 2), made from a two-litre plastic bottle with a funnel constructed from the upper half of the bottle (Larsen & Forsyth 2005). Traps were buried with the edge flush to the soil and about 30 g bait of human dung wrapped in muslin cloth was supported over the trap opening. Traps were covered with a plastic plate to prevent flooding from rain. Crushed leaves were placed inside to provide more favourable conditions for the captured beetles.

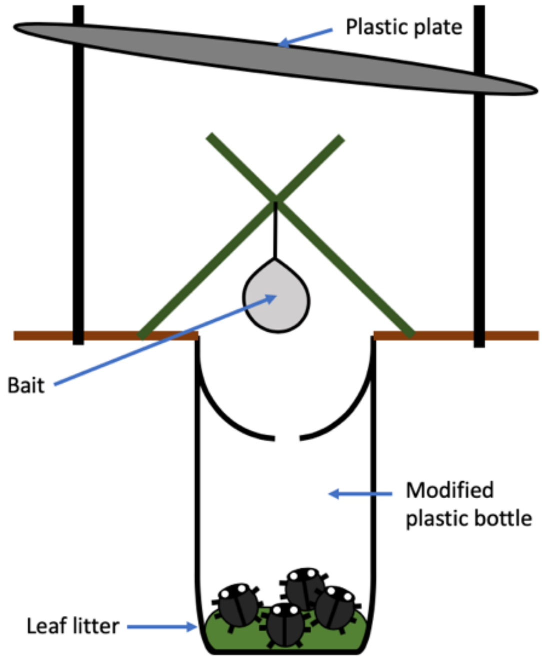

Figure 2. Diagram of the traps used in this study.

Initial Trapping and Marking

Fieldwork was performed during September in 2018. Fifteen traps were placed at approximately 25-metre intervals in parallel with the Segama river (Figure 3) approximately 10 m into the forest from the riverbank to avoid flooding of the traps in the event of heavy rain raising the water level (Points ‘A’: Figure 3). Traps were placed into the field around noon and were collected after 24 h and the traps removed.

Dung beetles were identified to species or morphospecies (see Appendix A for individuals identified to species) using photographic references and a dichotomous keys (Parrett *et al.*, unpublished) and assigned to functional groups (size and diel and nesting behaviour) following Slade *et al.* (2011). Beetles 5–8 mm in length were assigned as small, beetles 9–13 mm were assigned as medium, and beetles 14–30 mm were assigned as large. Individuals from each trap were separated by size. Individuals classified as large or medium were marked with a trap-specific combination of dots on their elytra using a silver Sharpie™ permanent marker (Figure 4) following Larsen & Forsyth (2005). Small dung beetles were marked with a single large dot on their elytra as it was not possible to fit trap-specific markings. All individuals from a trap were then transferred to containers lined with damp tissue paper prior to their release the next day.

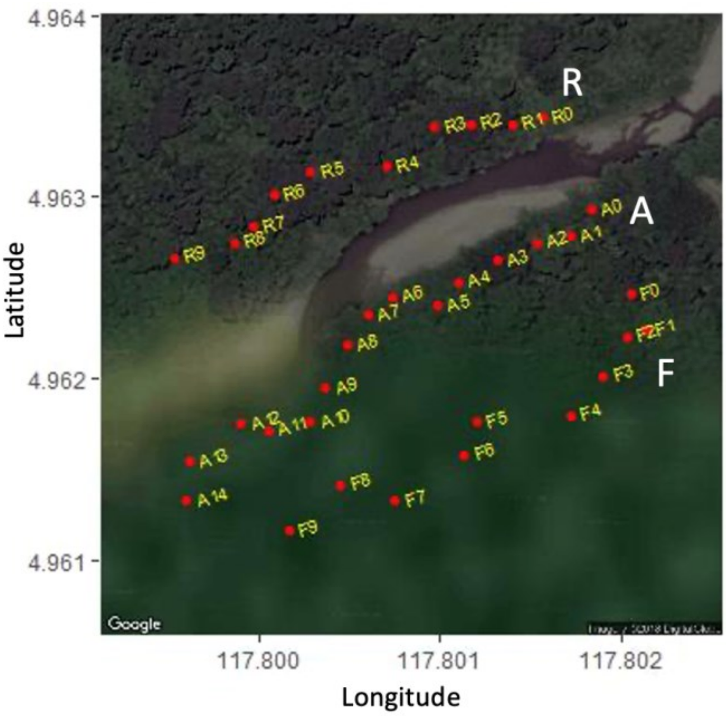

Figure 3. Satellite image (Source: Google Maps) of the Segama River, Danum Valley, Sabah. Trap locations are marked with red dots. A = initial capture and release points, R and F = recapture points on the river and forest side respectively.

Release and Recapture

Two new rows of ten traps baited with about 30 g of human dung, and each placed about 37.5 m apart, were placed into the field the following day. The trapping rows were placed equidistant (about 70 m) from the initial trap points. One row was placed about 70 m further into the forest from the original traps (Points ‘F’: Figure 3). The second row was placed across the river 10 m into the forest on the opposite bank to the original row of traps (Points ‘R’: Figure 3), equal to about 70 m from the original traps. The marked dung beetles were then released at their original capture points (Points A: Figure 3). Beetles were released by upturning the open container into the leaf litter.

The two rows of traps were checked after 24 h. Marked individuals were counted and identified to species or morphospecies (Appendix B). All dung beetles were released back into the forest at the end of the experiment.

Statistical analysis

The proportion of marked beetles recaptured on each side of the river was analysed with a one-tailed binomial test to see if movement differed from the null hypothesis of random movement (50% of recaptures either side of the river). In addition, recaptured beetles were separated into large beetles and medium / small beetles. One-tailed binomial tests were then performed for both groups to test for differences from random movement. Analysis was performed in R v3.6.1 (R Core Team 2020).

In addition, flight distances of large and medium beetles (with trap specific markings) were estimated from the grid coordinates of the traps using the package *Geodist* (Padgham *et al.* 2020) in R and GPS coordinates of trap locations.

RESULTS

In total, 497 dung beetles were captured, marked, and released (451 of these were identified to species, see Appendix A). During the recapture trapping, 960 dung beetles were captured, with 639 caught on the forest side and 321 on the river side. Of these, 37 were marked, representing a total recapture rate of about 7.5%. Dung beetles of all three size categories were recaptured (Appendix B) with recaptures predominantly consisting of large species with 26 large, 5 medium, and 6 small dung beetles recaptured. Both tunnellers and rollers were recaptured, with recaptured dung beetles being dominated by tunnellers (34) vs rollers (3).

Figure 4. Recaptured large dung beetles. Silver dot patterns on the elytra indicate which trap they were initially caught in.

Of the 26 recaptured large beetles, 23 retained their trap specific markings. For the 5 medium beetles, 4 retained their trap species markings (all were *Onthophagus mulleri*). Movement of many individuals was greater than 70 m. For the large beetles, the largest distance moved was 206.73 m, and the smallest 62.45 m, with a mean of 110.57 m ± 7.83 SE (Figure 5). For the medium beetles, the largest distance moved was 120.27 m, the smallest was 51.00 m, and the mean was 87.81 ± 15.68 m (Figure 5).

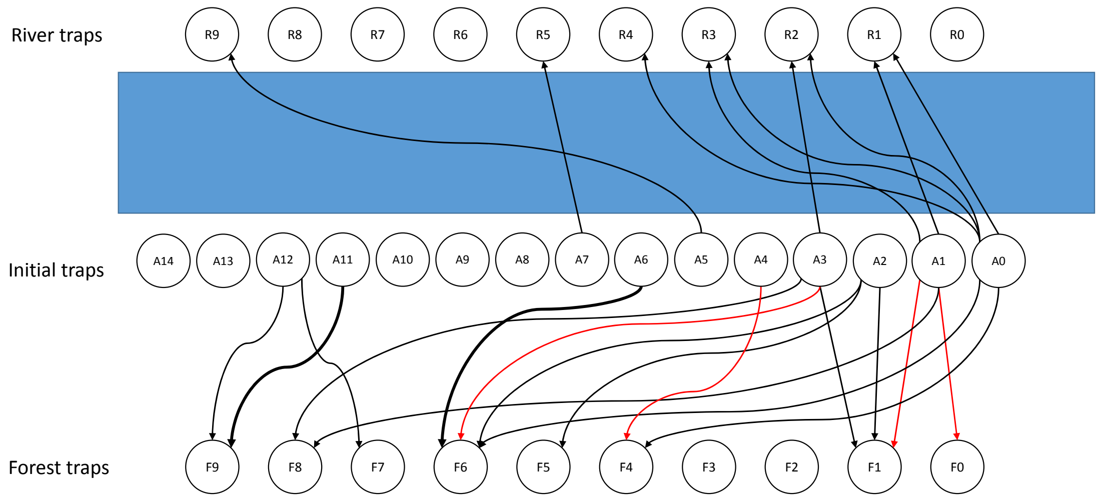

Figure 5. Movements of the 27 recaptured beetles with trap-specific markings. Thicker arrows represent the movement of two individual beetles. The movement of large beetles (14–30 mm) is represented by the black arrows while the movement of medium beetles (9–13 mm) is represented by the red arrows. The blue rectangle represents the river. Arrows are curved to aid in observation and do not represent actual movement routes.

Of the 37 recaptured beetles, 12 were caught over the river while 25 were caught on the forest side (Figure 6a) with the proportion of recaptures being significantly greater on the forest side compared to the river side (N = 37, expected proportion = 0.5, measured proportion = 0.68, 95% CI = 0.53–1.00, p = 0.024).

For the 26 large beetles, 16 were caught on the forest side and 10 were caught on the river side (Figure 6b) with the proportion of recaptures not significantly more on the forest side compared to the river side (N = 26, expected proportion = 0.5, measured proportion = 0.62, 95% CI = 0.44–1.00, p = 0.164). For the 11 medium and small beetles, nine were caught on the forest side and two were caught on the river side (Figure 6b) and the proportion of recaptures was significantly higher on the forest side compared to the river side (N = 11, expected proportion = 0.5, measured proportion = 0.82, 95% CI = 0.53–1.00, p = 0.033).

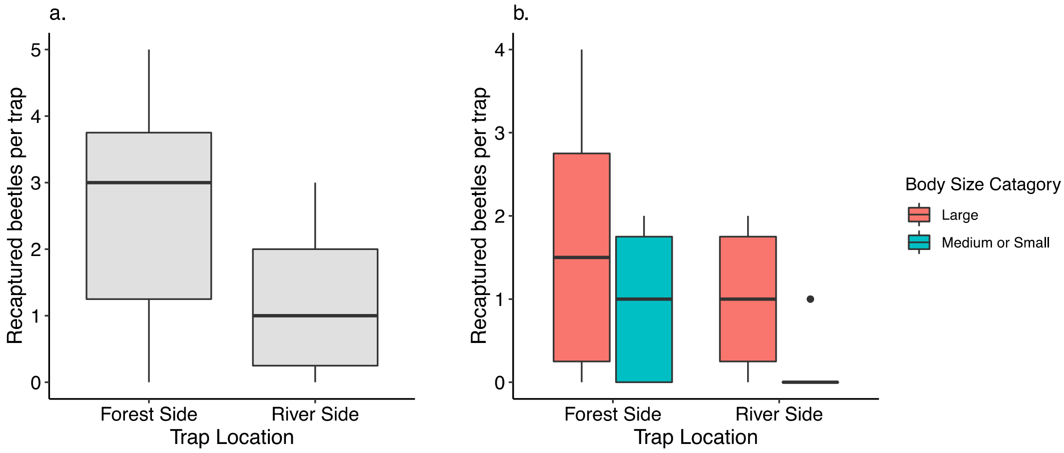

Figure 6. Box-and-whiskers plots showing (a) the number of recapture events per trap for the two sides of the river and (b) the number of recapture events per trap of large (14–30 mm) and medium (9–13 mm) or small (5–8 mm) beetles either side of the river.

DISCUSSION

Although, our study is preliminary, it suggests that large rivers (~50 m) do not form complete movement barriers to large tropical dung beetles, even over time periods as short as ~24 h, although they might act as partial barriers for small and medium dung beetles. The MRR technique used was an effective way to assess the movement of beetles from a range of size classes over short-time periods and resulted in a recapture rate of 7.5%.

Effectiveness of the methodology

MRR has been employed in a number of dung beetle studies; to answer a number of questions, such as movement between resources (Roslin 2000), dispersal capabilities of individual species (Viljanen 2009), population sizes of threatened species (Roslin *et al.* 2009, Cultid-Medina *et al.* 2015), and effective trapping methodology (Larsen & Forsyth 2005, da Silva & Hernández 2015). Here we have shown that MRR can be useful for studying dung beetle movement across river barriers in tropical rainforests. The materials required for trapping and identification were inexpensive and easy to set up, even for a small team of researchers, and this methodology could easily be upscaled for larger projects to test more specific hypotheses regarding the broad-scale impact of natural and anthropogenic barriers to dung beetle movement in different habitats.

Our recapture rate of 7.5% is similar to those of other studies on dung beetles. Recapture rates have ranged from 12.2% (Roslin *et al.* 2009) to 19% (Roslin 2000) in Finland and 1.3% to 19% in Madagascar, depending on trap distances from release points (Viljanen 2009). In the tropics, recapture rates vary with recorded rates of 0% to 58.3% in Venezuela, depending on distance (Larsen & Forsyth 2005), to rates of 6.3% in Brazil (da Silva & Hernandez 2015), 18% in Colombia (Cultid-Medina *et al.* 2015), and 5% in Mexico (Arellano *et al.* 2008) although one large species studied in Colombia returned a very high recapture rate of 90.3% (Noriega & Acosta 2011).

While our recapture rates are similar to those found in the tropical forests of Brazil, they may have been underestimated. While silver pens or paints have been successfully used in several dung beetle MRR studies due to their ease of use and ability to identify recaptured individuals quickly in the field (*e.g.*, Larsen & Forsyth 2005, Arellano *et al.* 2008, Noriega & Acosta 2011), 3/26 large beetles and 1/5 medium recaptured beetles had their trap specific markings rubbed off or obscured. It may have been the case that other beetles completely lost their marks due to soil friction (Wuerges & Hernandez 2020). In addition, due to the size of the marking tip of the marker, trap-specific marking patterns were only possible for large and medium-bodied species. Other methods could be used in the future to allow more robust marking over longer time periods. For example, utilisation of elytral punctures (e.g. Unruh & Chauvin 1993, Roslin 2000, Roslin *et al.* 2009) can provide markings that cannot be rubbed off, although great care must be taken to avoid damaging the wings or abdomen. The technique has previously been used to study movement patterns of *Nanos viettei* showing its utility for dung beetles as small as 6 mm, and allowing unambiguous identification of recaptured individuals 12 months after being marked (Viljanen 2009). Alternatively, recently developed methods such as elytral scarification could be used (*e.g.*, Cultid-Medina *et al.* 2015), although little is known of whether such methods may affect survival of individuals in the field (Wuerges & Hernandez 2020). Whilst the approach used here may have led to some underestimation of movement, it allowed a very large number of beetles to be marked and released in a short period of time and allowed rapid identification of marked individuals without use of a microscope or harm to the beetle.

Pooling traps together was unavoidable in this pilot study due to the low total number of recapture events. However, a variety of species were recaptured representing both roller and tunneller species, both nocturnal and diurnal species, as well as small, medium, and large species (see Appendix B for a full list of recaptured species and functional group information). Upscaling the methodology to capture larger samples sizes, from additional sample sites, would allow the movements of different species, size categories, and functional groups across river barriers to be analysed individually. As a full complement of dung beetle functional groups is required to maximise ecosystem functioning in tropical forests (Slade *et al.* 2007), such work would provide valuable insights for conservation of species and ecosystem functioning.

Rivers as barriers to dung beetle movement in tropical forests

Our results show that the river did not form a complete barrier to dung beetle movement, agreeing with previous work in Madagascar that suggests that many dung beetle species ranges are not determined by river barriers (Knopp *et al.* 2011). Twelve of the 37 recaptured beetles were found to have crossed the river although all but two of the river-side recaptures were in the large body size category (Appendix B).

It appears that the river did act as a partial barrier to dung beetle movement of smaller species. Just over twice as many marked beetles were recaptured on the forest side compared to the river side but, dung beetles of different size classes differed in their ability to cross the river. For large beetles, the river did not appear to act as a significant barrier. However, the river did act as a significant barrier to movement when small and medium beetles were considered. The ability of large beetles to cross river barriers may be important for ecosystem functioning at the landscape level, as large tunnellers contribute disproportionately to dung burial (Slade *et al.* 2007).

Different dung beetle species in different habitats appear to show a wide range of movement and dispersal distances. Previous work on the small Madagascan dung beetle *Nanos viettei* revealed small movement distances, with the vast majority of individuals being recaptured in their 20×20 m patch of release (Viljanen 2009). Additionally, work on Aphodiine dung beetles in temperate pastures revealed that most recaptures occurred within the 45×20 m artificial pasture of release, although a small minority of individuals were recaptured about 1 km from their release point (Roslin 2000). On the other hand, *Canthon cyanellus* in Mexico was capable of moving between trapping sites separated by several hundred metres across a patchy forest / pasture landscape (Arellano *et al.* 2008). Similarly, a study of *Sulcophanaeus leander* populations on Amazonian riverine beaches revealed that some individuals moved between the two beaches studied, separated by about 500 m, over a 6-day sampling period (Noriega & Acosta 2011). Large dung beetles in the Andes have been recorded moving between 456–1,700 m over a 24–120 h period (Cultid-Medina *et al.* 2015). Finally, molecular work on *Nanos binotatus* revealed genetic differentiation of populations both along and across the Mangoro river in Madagascar, suggesting that wide rivers (250–400 m wide) can limit, but not totally prevent, gene flow (Knopp *et al.* 2011). Therefore, dung beetle movement rates seem to vary greatly across different habitats and species. It may be the case that the 24 h between the two sampling events did not leave enough time for the movement of small beetles. However, the presence of a small-bodied beetles recaptured on the river side suggests that dung beetles of this size class are capable of sustained flight of at least about 50 m to be able to cross the river. Future studies, performed over a longer time period, and with rivers of different sizes, would reveal if rivers are acting as movement barriers to small and medium sized beetles, or if they are able to cross rivers but do so at a lower rate than larger-bodied species, and if there are inter-specific differences within the size classes. These results also suggest that the frequently used standardised spacing of pitfall traps at 50 m (taken from a single species study in South America (Larsen & Forsyth 2005) is too small to ensure independence between traps. Based on our observations we suggest a minimum distance of 100 m between traps to ensure independence among traps for studies on dung beetle diversity in Southeast Asia.

Potential impacts of edge effects

An alternative explanation for the difference in beetle recaptures either side of the river may be due to trap location. Trapping on the recapture day took place both relatively deep in the forest (forest side traps; about 80 m from the river) or close to the river (river side traps; about 10 m from the river). Dung beetles are strongly affected by edge effects caused by human disturbance such as logging roads (Feer 2008, Edwards *et al.* 2017), logging gaps (Bitencourt *et al.* 2020), and agriculture (Barnes *et al.* 2014) and community turnover can occur over short distances with habitat change (Villada-Bedoya *et al.* 2017). In addition, edge effects from oil palm plantations can interact with abiotic conditions to influence dung beetle diversity in riparian buffers (Williamson *et al.* 2021). Previous sampling performed in the DVCA identified that some species appear to be riverine specialists, and decrease in abundance with increasing distance from rivers, whilst other species instead increase in abundance from the river to the forest interior (Davis *et al.* 2000). It may be the case that the river was not a barrier to movement of small and medium beetles but that, due to edge effects, individuals that crossed the river continued to fly deeper into the forest interior on the river side and were therefore not caught in the traps. By upscaling our methodology to include additional traps at various depths into the forest either side of the river, it may be possible to identify if edge effects were the cause of reduced recaptures on the river side, or whether the river did act as a partial barrier. In particular, such an approach would allow comparison of river permeability for riverine and forest specialist species (Davis *et al.* 2000), providing further insight into how barriers can differently affect different guilds of dung beetles, any interspecific differences within these groups, and the potential downstream effects on ecosystem functioning (Slade *et al.* 2007).

CONCLUSION

The importance of natural and anthropogenic barriers for insect movement in tropical forests is largely unknown. Our results, collected over just 48 hours, demonstrate that dung beetles are able to cross large rivers (about 50 m), and travel distances of over 100 m in 24 h. Our study also demonstrates the efficacy of short-term pitfall trapping and MRR to investigate tropical dung beetle movement. These results are important as little is known about the movement of Southeast Asian dung beetles. We suggest that dung beetle distributions in these forests are not constrained by the large rivers that flow through them, especially for large dung beetles (14–30 mm). Upscaling of this methodology may provide insights into guild-specific or size-specific permeability of river barriers to dung beetles.

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Appendix A. Dung beetle species captured and marked. See Slade *et al.* (2011) for classifications of body size, diel activity, and nesting strategy.

Species	**Body Size***	**Diel activity**	**Nesting strategy**	**Total Marked**
Catharsius dayacus	Large	Nocturnal	Tunneller	71
Catharsius renaudpauliani	Large	Nocturnal	Tunneller	49
Copris ramosiceps	Medium	Nocturnal	Tunneller	9
Copris sinicus	Medium	Nocturnal	Tunneller	5
Microcopris doriae	Medium	Nocturnal	Tunneller	9
Microcopris hidakai	Small	Nocturnal	Tunneller	104
Onthophagus mulleri	Medium	Diurnal	Tunneller	101
Onthophagus semiaureus	Medium	Diurnal	Tunneller	2
Paragymnopleurus maurus	Large	Diurnal	Roller	5
Paragymnopleurus sparsus	Large	Diurnal	Roller	21
Paragymnopleurus striatus	Large	Nocturnal	Roller	3
Proagoderus watanabei	Large	Diurnal	Tunneller	13
Sisyphus thoracicus	Small	Diurnal	Roller	58
Synapsis ritsemae	Large	Nocturnal	Tunneller / Roller	1

* Large = 14–30 mm, medium = 9–13 mm, & small = 5–8 mm

Appendix B. Species names, ecology, and numbers recaptured on each side of the river.

Species	**Body Size***	**Diel activity**	**Nesting strategy**	**Forest-side recaptures**	**River-side recaptures**	**Total recaptures**
Catharsius dayacus	Large	Nocturnal	Tunneller	7	6	13
Catharsius renaudpauliani	Large	Nocturnal	Tunneller	8	4	12
Microcopris hidakai	Small	Nocturnal	Tunneller	2	1	3
Onthophagus mulleri	Medium	Diurnal	Tunneller	4	0	4
Onthophagus sp.	Medium	Diurnal	Tunneller	0	1	1
Proagoderus watanabei	Large	Diurnal	Tunneller	1	0	1
Sisyphus thoracicus	Small	Diurnal	Roller	3	0	3
Total	-	-	-	**25**	**12**	**37**

* Large = 14–30 mm, medium = 9–13 mm, & small = 5–8 mm

Avifaunal survey of the lower montane forest of Bukit Hampuan in Ranau, Sabah, Malaysia

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Abstract. An avifaunal survey was carried out at Bukit Hampuan Forest Reserve in Ranau, Sabah, Malaysia. The MacKinnon List method was used to assess species diversity. The three-day survey recorded a total of 11 MacKinnon lists with 408 individuals detected. Seventy-one species from 33 families were recorded, with H=3.44 and E_H=0.57. True species richness was estimated (using SuperDuplicates® online calculator) to be approximately 71 species, with approximately 20 species not detected. The ten Bornean endemics detected were: Bornean Barbet, Bornean Flowerpecker, Bornean Leafbird, Bornean Swiftlet, Bornean Treepie, Chestnut-crested Yuhina, Crimson-headed Partridge, Dusky Munia, Mountain Barbet and the White-crowned Shama. Pycnonotidae (bulbuls) was the most speciose family with six species. Columbidae (pigeons and doves), Megalaimidae (barbets), Nectariniidae (sunbirds) and Timaliidae (babblers) each had five species. The Pellorneidae (jungle babblers) had four species. Zosterotidae (white-eyes), Pycnonotidae and Nectariniidae had the highest number of individuals detected with 53, 43 and 35, respectively. The five species with the highest relative abundance index were Chestnut-crested Yuhina (0.228), Yellow-vented Bulbul (0.081), Chestnut Munia (0.061), Little Spiderhunter (0.042) and Bornean Treepie (0.039). The majority of the species detected (60) were forest-dependent species. Of these, 40 species were strictly forest birds. Insectivores made up the most dominant dietary guild, i.e., a total of 39 species (from 19 families) with 33 species (from 15 families) being strict insectivores.

Keywords: avifaunal survey, MacKinnon List method, ultramafic soil, Bukit Hampuan Forest Reserve, Ranau district, feeding guilds

INTRODUCTION

Birds have evolved and diversified to a wide variety of habitats and foraging strategies (Naish 2014). They have intimate associations with their habitats, their prey, and some have established strong symbiotic relationships, such as flower-pollinators. Due to their conspicuous nature and relative ease of detection and study, they are among the best researched animals in forest ecosystems.

Recently the Sabah Forestry Department has begun rapid assessment of avifaunal communities to help it determine forest ecosystem health. This paper documents the outcome of a brief bird survey conducted during the Bukit Hampuan Forest Reserve (BHFR) Scientific Expedition, 7-12 September 2020. The expedition was organised by the Forest Research Centre, Sabah Forestry Department, under the auspices of the Heart of Borneo Initiative. The main objective of this survey was to provide a brief description of the bird species and ecology in the forest reserve to provide information for future forest management. Surveys using the MacKinnon List (ML) method (MacKinnon & Phillipps 1993) were conducted at three sites within the forest reserve.

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

Site description

Bukit Hampuan Forest Reserve (BHFR), a Class I Protection Forest Reserve gazetted in 2009, lies approximately within latitudes 5.983–6.029 N and longitudes 116.647–114.696 E, or about 3 km north of Ranau town. The reserve, with an area of approximately 1,300 ha, is located in the Ranau district and is administered by the Ranau District Forestry Office.

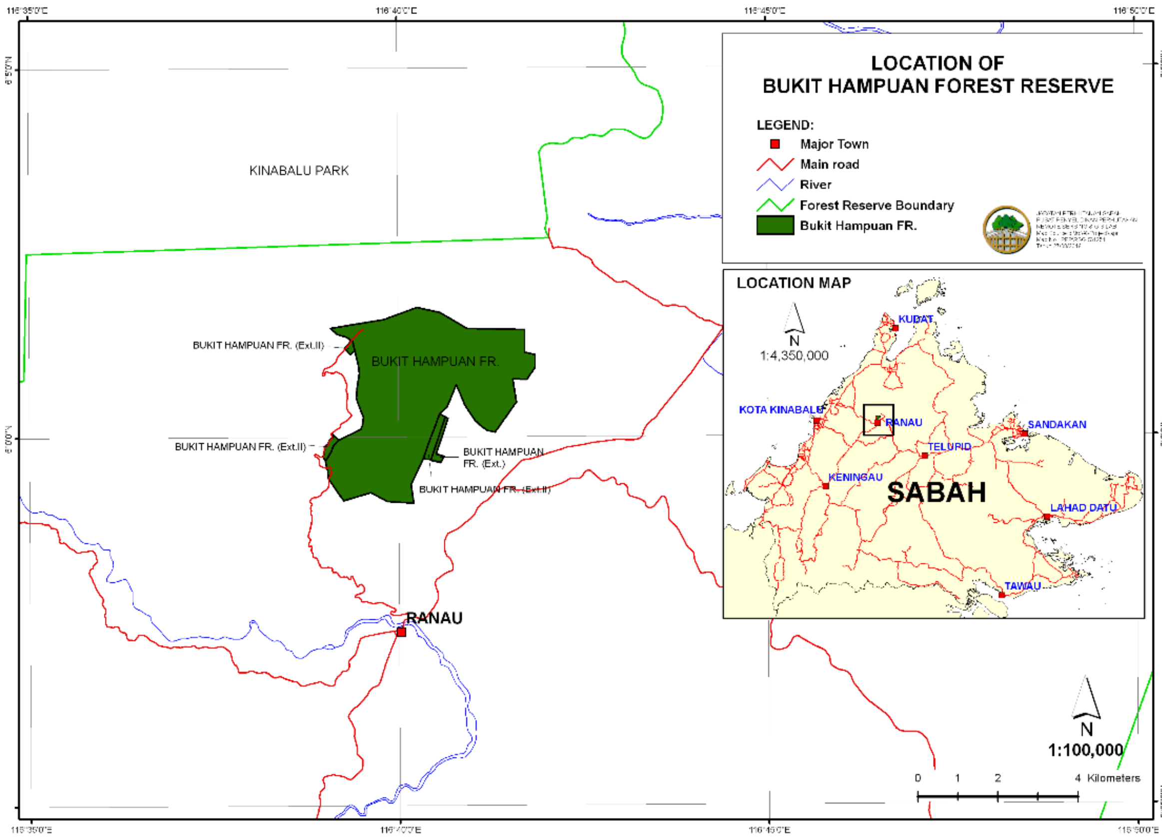

Figure 1. Location of Bukit Hampuan Forest Reserve.

Approximately 73.6% of the soils in the Bukit Hampuan are of the Bidu-bidu Association while the remainder are of the Pinosuk (16%), Malubok (10.3%) dan Dalit (1.5%) Associations. The natural vegetation consists of the upland and upper montane ultramafic forest types over the Bidu-bidu Soil Association, which are soils formed on parent materials derived from ultrabasic igneous rocks. Most of upland and lower montane ultramafic forest types were affected by severe fires, especially during the El Niño Southern Oscillation (ENSO) drought events. These areas are dominated by the highly invasive bracken fern, *Pteridium esculentum*, with occasional pioneer trees (*Trema* spp., *Macaranga* spp. and *Vitex pinnata*).

The lower montane ultramafic forest in the north was largely affected by the copper mining operations from 1975 to 1999. The resulting open pit quarry mine and its facilities (now abandoned) are located in the northwest corner of the reserve and at the southeast slope of Mt. Kinabalu. The two good representatives of lower montane forest over the Pinosuk Soil Association were in the southwest part of the reserve and in a narrow strip of intact vegetation west of the road (which also serves as an access road and boundary). Unfortunately, the latter was not within the reserve. The vegetation along the western boundary and in the northeast was similar to the degraded vegetation elsewhere in the reserve.

The main river systems of the reserve are Sg. Bambangan which flows south, Sg. Lohan which flows southeast to east, and Sg. Mamut which flows east.

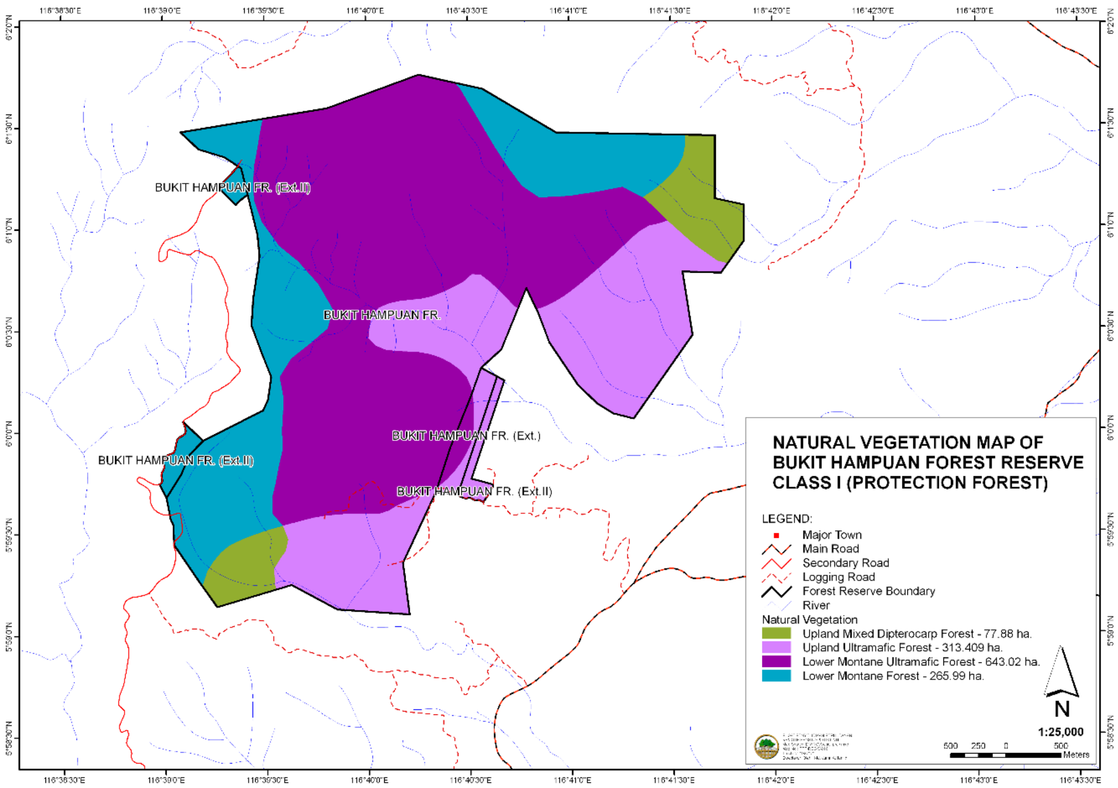

Figure 2. Natural vegetation map of Bukit Hampuan Forest Reserve.

Survey methods

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

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

Observation methods

Every observer had a pair of Nikon binoculars (8 x 42s). The reference field guide of choice was the 'Phillipps' *Field Guide to the Birds of Borneo*', *3rd Ed*., 2014. The latest taxonomic changes were determined from online sources and published papers. For example, the Brown Fulvetta was recently placed in the new family Alcippeidae (Cai *et al.* 2019), and the species has been treated accordingly in this paper. Audio identification was verified using pre-recorded bird songs.

The survey was conducted over four days (8th-11th September 2020), beginning at 6.30 am and ending after 4 hours. Unlike previous studies, surveys to detect nocturnal birds were not conducted due to weather conditions. The four surveys were conducted in all forest types close to or along existing roads.

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

Analyses

From the acquired data, basic diversity information was extracted, such as species richness, a diversity index (H), relative abundance (E_H), most common families, most speciose families, Bornean endemics, *etc*. A species accumulation curve was generated from the addition of those species not recorded on any of the previous lists to the total species number, which was then plotted as a function of the list number. To estimate true species richness of the area, we used the SuperDuplicates® online calculator developed by Chao *et* *al.* (2017), which requires only the total number of species observed and the number of species observed only once (uniques/singletons). The relative abundance indices of species observed were calculated. The most common families and species, and Bornean endemics, were also determined.

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

RESULTS AND DISCUSSIONS

Avifaunal Composition and Species Richness

The four survey days yielded 11 lists and 408 detected individuals, of which 263 (64.5%) individuals were detected by their calls/vocalisations. A total of 71 species belonging to 33 families were recorded (see Appendix I for the complete species list). The Shannon Diversity Index (H) value was 3.44 with Evenness Index (E_H) of 0.57. The survey also yielded ten species that were endemic to Borneo (Table 1). All species were categorised as Least Concern (LC) in The IUCN Red List of Threatened Species.

Table 1. Species endemic to Borneo and their respective categories in The IUCN Red List of Threatened Species. LC = least concern.

No.	Species	Category
1	Bornean Barbet	LC
2	Bornean Flowerpecker	LC
3	Bornean Leafbird	LC
4	Bornean Swiftlet	LC
5	Bornean Treepie	LC
6	Chestnut-crested Yuhina	LC
7	Crimson-headed Partridge	LC
8	Dusky Munia	LC
9	Mountain Barbet	LC
10	White-crowned Shama	LC

Table 2 lists species that are listed as NT (near threatened) and VU (vulnerable), respectively, in the IUCN Red List of Threatened Species. All species categorized as NT were also common lowland mixed dipterocarp forest species. The endemic Kinabalu Serpent Eagle and the Wreathed Hornbill listed as VU.

Table 2. Species listed as Near Threatened (NT) and Vulnerable (VU) in the IUCN Red List of Threatened Species.

No.	Species	Category	No.	Species	Category
1	Black-and-yellow Broadbill	NT	**7**	Red-throated Barbet	NT
2	Black-throated Babbler	NT	**8**	Rufous-crowned Babbler	NT
3	Brown Fulvetta	NT	**9**	Scaly-breasted Bulbul	NT
4	Crested Jay	NT	**10**	Sooty-capped Babbler	NT
5	Green Iora	NT	**11**	Kinabalu Serpent Eagle	VU
6	Maroon-breasted Philentoma	NT	**12**	Wreathed Hornbill	VU

Table 3 shows that Pycnonotidae (bulbuls) were the most speciose family surveyed. The second rank was shared by four families with five species each. Taken together, the 33 families in the survey had a mean number of species of 2.15 with a standard deviation of 1.54.

Table 3. Three most speciose families (with shared rankings).

Rank	Family	No. of species
1	Pycnonotidae	6
2	Nectariniidae	5
2	Columbidae	5
2	Megalaimidae	5
2	Timaliidae	5
3	Pellorneidae	4

As shown in Table 4, the Chestnut-crested Yuhina made the Zosteropidae to be the most commonly detected family with 93 individuals, i.e., 22.8% of all individuals detected. Unlike most other species, the yuhinas were detected visually as they flew in flocks of 20 to 30 individuals. Care was taken not to double-count the same flocks. With a total of 5 species, the Pycnonotidae (bulbuls) ranked second with 43 individuals detected (10.0%), of which 33 individuals were the very common Yellow-vented Bulbul. The Nectariniidae (sunbirds and spiderhunters) ranked third, with 46% of its individuals being the Little Spiderhunters. Temminck's Sunbird was also common in BHFR, with 12 individuals detected. Estrildidae (munias) ranked fourth with 25 individuals of the Chestnut Munia detected.

Table 4. Ten families with the highest percentage of individuals detected (note similar rankings).

Rank	Family	No. of individuals	% of individuals detected
1	Zosteropidae	93	22.8
2	Pycnonotidae	41	10.0
3	Nectariniidae	37	9.1
4	Estrildidae	27	6.6
5	Cisticolidae	21	5.1
6	Megalaimidae	19	4.7
6	Cettiidae	19	4.7
7	Columbidae	18	4.4
7	Corvidae	18	4.4
8	Timaliidae	15	3.7
8	Apodidae	15	3.7
9	Pellorneidae	14	3.4
10	Dicaeidae	11	2.7

Amongst the Cisticolidae, which was ranked fifth, the Red-headed Tailorbird was the most common species with nine individuals. Both Megalaimidae and Cettiidae were represented by 19 individuals, most of which were detected by their calls. For the Columbidae, the most common individual was the Little Cuckoo-dove with 10 individuals. The similarly ranked Corvidae was represented by 15 individuals of Bornean Treepie out of the 18 detected. For the eighth-ranked Timaliidae, most of the 15 individuals were detected by their calls close to streams. For the ninth-ranked Pellorneidae, the Brown Fulvetta was the most common species at 10 individuals. Amongst the Dicaeidae, the Orange-bellied Flowerpecker was the most common with 10 individuals detected, often flying past above the team.

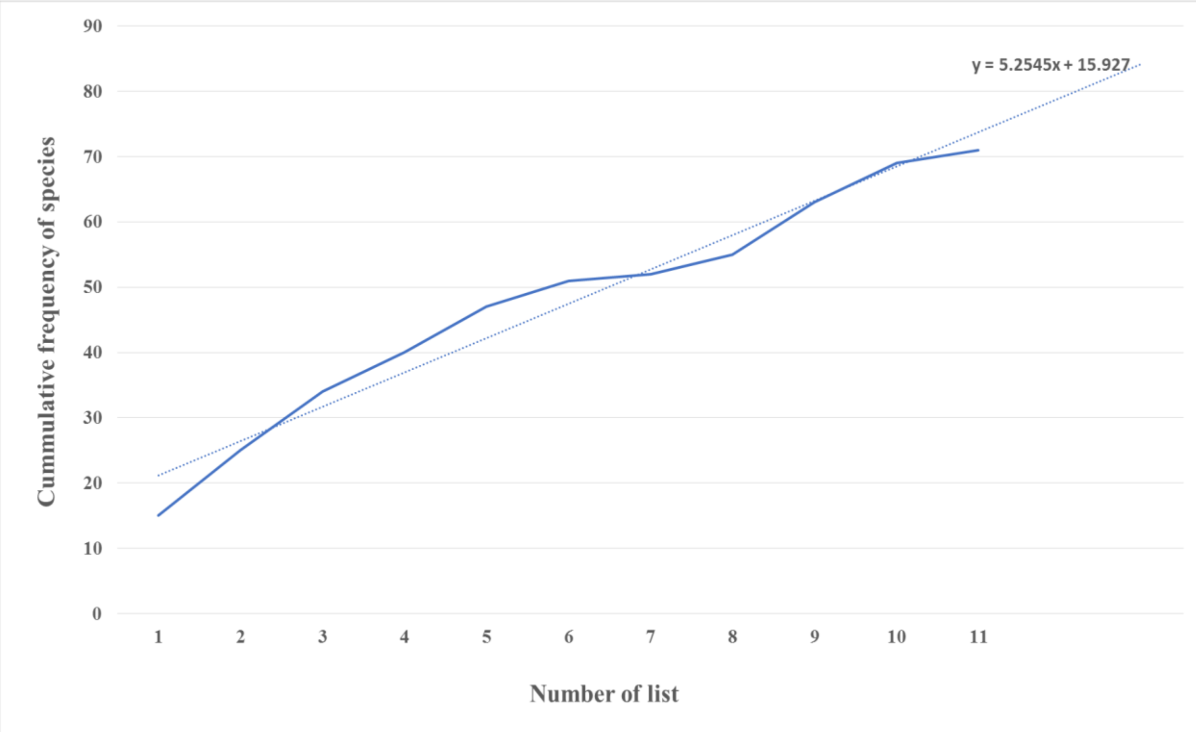

Figure 3. Species accumulation curve and linear regression line of bird species in BHFR.

As expected for the ML rapid assessment method, and with a 4-day duration of the survey, the species accumulation curve (Figure 1) had not achieved asymptote. To estimate the true species richness, the SuperDuplicates® online calculator was used (Chao *et al.* 2017). Only the total number of species detected and the number of singletons (species detected only once) were needed to input into the calculator. Table 5 is a summary of the results. It estimated Chao1 (species richness using abundance data) to be approximately 91 species, with an upper and lower threshold of approximately 108 and 81 species, respectively, in the 95% confidence interval. The number of doubletons was estimated to be about 11 while the actual number detected during the survey was 9 species. The calculator also estimated that approximately 20 species were undetected, *i.e.*, the survey managed to detect approximately 78.5% of the total species in the area. Based on the linear regression line in Figure 3, it estimated that another three lists, or an extra survey day, were needed to detect the estimated 91 species of birds.

Table 5. Results from SuperDuplicates®.

Estimated number of doubletons	Estimated species richness	Standard error	95% C.I. lower	95% C.I. upper	Number of undetected species	Undetected percentage (%)
10.56	90.5	6.47	81.35	107.74	19.5	21.54

Relative abundance index

During the survey, 145 individuals (35.5%) were detected aurally. Table 6 shows the five species with the highest relative abundance index. Individuals from these species represented about 45% of all the individuals detected. Apart from the Little Spiderhunter, these species were mostly detected in open or degraded vegetation. Chestnut-crested Yuhinas were easily detected due to their habit of flying high in flocks and by their highly vocal nature. Chestnut Munias had similar traits but they flew low in smaller-sized flocks at BHFR and were less conspicuous visually and aurally. Both the Little Spiderhunter and Bornean Treepie were always detected singly in intact forest and areas close to degraded vegetation.

Table 6. Top 5 species with the highest relative abundance index.

Rank	Species	Family	Total individuals	Relative abundance index
1	Chestnut-crested Yuhina	Apodidae	93	0.2279
2	Yellow-vented Bulbul	Pycnonotidae	33	0.0809
3	Chestnut Munia	Cisticolidae	25	0.0613
4	Little Spiderhunter	Psittaculidae	17	0.0417
5	Bornean Treepie	Aegithinidae	15	0.0368

Habitat types and feeding guilds

Species were categorised according to their preferred habitats (e.g., forests, forest edges, open areas) and feeding guilds (Figure 4). The majority of the species (60 species) were forest-dependent. Of these, 40 species from 21 families were strictly forest birds. The high number of forest-dependent species—and the low number of open area specialists—do not reflect the degraded nature of the forest at BHFR. However, this may be due to the fact that BHFR is adjacent to the larger Kinabalu Park (754 km^2), a World Heritage site.

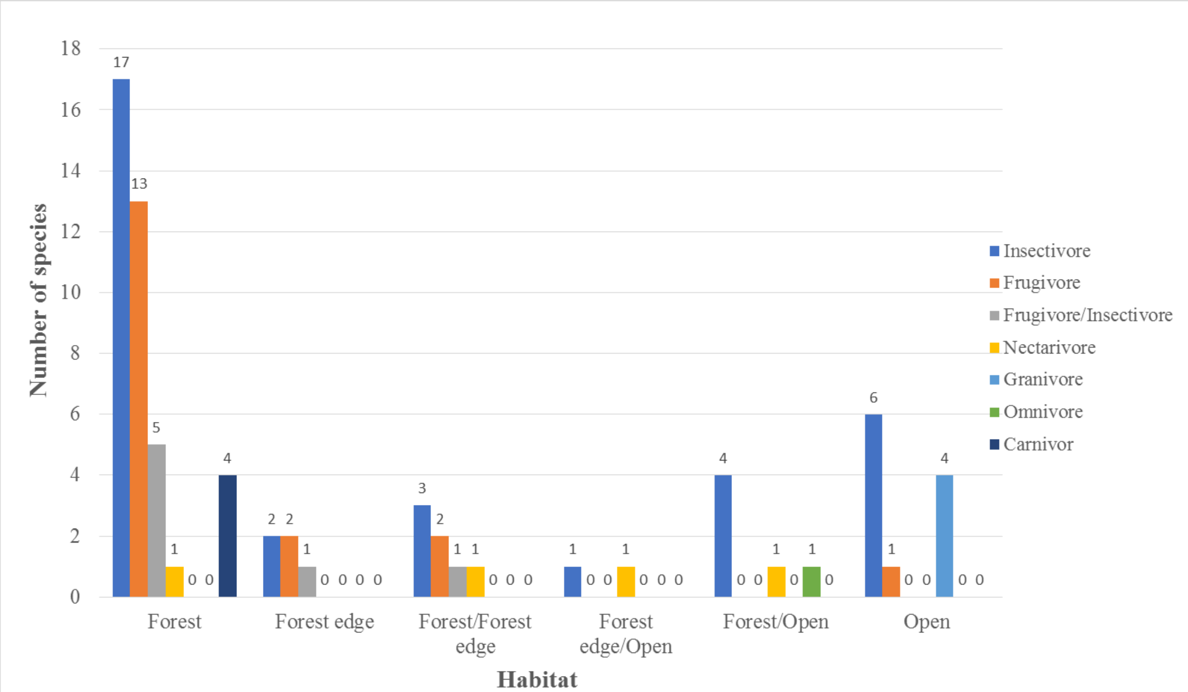

Figure 4. Number of species according to habitat types and feeding guilds in MFR.

As expected, insectivores made up the most abundant dietary guild, with a total of 39 species in 18 families. Of these, 33 species in 15 families were strict insectivores and the rest were mixed-diet insectivores. The second most dominant guild comprised frugivores, with 25 species, 7 of which were mixed-diet frugivorous species. The number of individuals that were strict frugivores was 113. In terms of individuals, both strict insectivores (215 individuals) and frugivores (113 individuals) made up 80.4% of the total individuals detected. The dominance of insectivorous and frugivorous birds in a forest would normally imply an abundance of insect pollinators and seed dispersers, which in turn reflect forest health. However, due the degraded nature of BHFR, this phenomenon may be due to the reserve's proximity to the larger Kinabalu Park.

All 4 species of nectarivores were from the spiderhunter/sunbird family, Nectariniidae. The four species of granivores were Zebra Dove (Columbidae), Chestnut and Dusky Munias (Estrildidae), and Eurasian Tree Sparrow (Passeridae). The sole, predominantly omnivorous species was the Hill Myna although it may consume more fruits/berries in forests.

CONCLUSION

The survey team managed to obtain preliminary insight into avian diversity and ecology in BHFR. Its diversity (71 species from 33 families) is representative of the surrounding natural forest types. However, the close proximity of Kinabalu Park, the World Heritage site approximately 754 km^2 in size, to BHFR may have had a positive influence on the species composition and ecology of the reserve's bird community. With some areas being rehabilitated by the Ranau District Forestry Office, it is hoped that these sites will be able to support a more diverse avian population in the coming years. With this in mind, BHFR deserves priority in the future as a site to compare the effectiveness of forest rehabilitation efforts in restoring ecosystem services.

ACKNOWLEDGEMENTS

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Appendix 1. List of bird species detected at Bukit Hampuan Forest Reserve.

No.	Common name	**Species**	Family
1	Black Eagle	*Ictinaetus malaiensis*	Accipitridae
2	Kinabalu Serpent Eagle	*Spilornis kinabaluensis**	Accipitridae
3	Oriental Honey Buzzard	*Pernis ptilorhynchus*	Accipitridae
4	Common Iora	*Aegithina tiphia*	Aegithinidae
5	Green Iora	*Aegithina tiphia*	Aegithinidae
6	Banded Kingfisher	*Lacedo pulchella*	Alcedinidae
7	Bornean Swiftlet	*Collocalia dodgei**	Apodidae
8	Plum-toed Swiftlet	*Collocalia affinis*	Apodidae
9	White-breasted Woodswallow	*Artamus leucorynchus*	Artamidae
10	Wreathed Hornbill	*Rhyticeros undulatus*	Bucerotidae
11	Mountain Tailorbird	*Phyllergates cucullatus*	Cettiidae
12	Sunda Bush-warbler	*Horornis vulcanius*	Cettiidae
13	Yellow-bellied Warbler	*Abroscopus superciliaris*	Cettiidae
14	Red-headed Tailorbird	*Orthotomus ruficeps*	Cisticolidae
15	Rufous-tailed Tailorbird	*Orthotomus sericeus*	Cisticolidae
16	Yellow-bellied Prinia	*Prinia flaviventris*	Cisticolidae
17	Emerald Dove	*Chalcophaps indica*	Columbidae
18	Green Imperial Pigeon	*Ducula aenea*	Columbidae
19	Little Cuckoo-Dove	*Macropygia ruficeps*	Columbidae
20	Mountain Imperial Pigeon	*Ducula badia*	Columbidae
21	Zebra Dove	*Geopelia striata*	Columbidae
22	Bornean Treepie	*Dendrocitta cinerascens**	Corvidae
23	Crested Jay	*Platylophus galericulatus*	Corvidae
24	Greater Coucal	*Centropus sinensis*	Cuculidae
25	Plaintive Cuckoo	*Cacomantis merulinus*	Cuculidae
26	Raffles's Malkoha	*Rhinortha chlorophaea*	Cuculidae
27	Bornean Flowerpecker	*Dicaeum monticolum**	Dicaeidae
28	Orange-bellied Flowerpecker	*Dicaeum trigonostigma*	Dicaeidae
29	Ashy Drongo	*Dicrurus leucophaeus*	Dicruridae
30	Chestnut Munia	*Lonchura atricapilla*	Estrildidae
31	Dusky Munia	*Lonchura fuscans**	Estrildidae
32	Black-and-yellow Broadbill	*Eurylaimus ochromalus*	Eurylaimidae
33	Barn Swallow	*Hirundo rustica*	Hirundinidae
34	Pacific Swallow	*Hirundo tahitica*	Hirundinidae
35	Bornean Leafbird	*Chloropsis kinabaluensis**	Irenidae
36	Chestnut-hooded Laughing-thrush	*Trochalopteron erythrocephalum*	Leiothrichidae
37	Blue-eared Barbet	*Psilopogon cyanotis*	Megalaimidae
38	Bornean Barbet	*Psilopogon eximius**	Megalaimidae
39	Gold-whiskered Barbet	*Psilopogon chrysopogon*	Megalaimidae
40	Mountain Barbet	*Psilopogon monticola**	Megalaimidae
41	Red-throated Barbet	*Psilopogon mystacophanos*	Megalaimidae
42	White-crowned Shama	*Copsychus stricklandii**	Muscicapidae
43	Little Spiderhunter	*Arachnothera longirostra*	Nectariniidae
44	Olive-backed Sunbird	*Nectarinia jugularis*	Nectariniidae
45	Purple-naped Sunbird	*Kurochkinegramma hypogrammicum*	Nectariniidae
46	Spectacled Spiderhunter	*Arachnothera flavigaster*	Nectariniidae
47	Temminck's Sunbird	*Aethopyga temminckii*	Nectariniidae
48	Eurasian Tree Sparrow	*Passer montanus*	Passeridae
49	Brown Fulvetta	*Alcippe brunneicauda*	Pellorneidae
50	Rufous-crowned Babbler	*Malacopteron magnum*	Pellorneidae
51	Scaly-crowned Babbler	*Malacopteron cinereum*	Pellorneidae
52	Sooty-capped Babbler	*Malacopteron affine*	Pellorneidae
53	Crimson-headed Partridge	*Haematortyx sanguiniceps**	Phasinidae
54	Maroon Woodpecker	*Blythipicus rubiginosus)*	Picidae
55	Blue-crowned Hanging Parrot	*Loriculus galgulus*	Psittaculidae
56	Black-headed Bulbul	*Brachypodius melanocephalos*	Pycnonotidae
575	Olive-winged Bulbul	*Pycnonotus plumosus*	Pycnonotidae
58	Red-eyed Bulbul	*Pycnonotus brunneus*	Pycnonotidae
59	Scaly-breasted Bulbul	*Ixodia squamata*	Pycnonotidae
60	Spectacled Bulbul	*Ixodia erythropthalmos*	Pycnonotidae
61	Yellow-vented Bulbul	*Pycnonotus goiavier*	Pycnonotidae
62	White-breasted Waterhen	*Amaurornis phoenicurus*	Rallidae
63	White-throated Fantail	*Rhipidura albicollis*	Rhipiduridae
64	Black-throated Babbler	*Stachyris nigricollis*	Timaliidae
65	Bold-stripped Tit-Babbler	*Mixornis bornensis*	Timaliidae
66	Chestnut-backed Scimitar-Babbler	*Pomatorhinus montanus*	Timaliidae
67	Chestnut-winged Babbler	*Cyanoderma erythropterum*	Timaliidae
68	Grey-throated Babbler	*Stachyris nigriceps*	Timaliidae
69	Maroon-breasted Philentoma	*Philentoma velata*	Vangidae
70	Blyth's Shrike-babbler	*Pteruthius aeralatus*	Vireonidae
71	Chestnut-crested Yuhina	*Staphida everetti*	Zosteropidae

*Bornean endemic

Appendix 2. Photographs of some interesting bird species of Bukit Hampuan Forest Reserve. All photographs courtesy of YC Lee.


Ashy Drongo


Chestnut-crested Yuhina*


Bornean Hooded Laughingthrush*


Dark-throated Oriole


Little Cuckoo-dove


White-crowned Shama*


Black-headed Bulbul


Temminck's Sunbird

*Bornean endemic

Germination of Merbau seeds (*Intsia palembanica* Miq.)

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Abstract. Seed germination without specific treatments proved to be costly and inefficient for Merbau planters. This study investigates the optimum germination technique for Merbau with the following objectives: (i) to determine a cost-effective and rapid scarification techniques to break seed dormancy; (ii) to investigate seed weight on germination rate; and (iii) to investigate seed germinability post long-term storage. For the first objective, it was observed that a combination of mechanical and soaking techniques such as chipping the seed coat and soaking in water for overnight was the best and most effective method in breaking seed dormancy. The Improvement Rate (IR) was estimated to be 8.5 times better in terms of Germinated Seed Percentage (GSP), along with an 86% Mean Germination Time (MGT) enhancement and a 6.6 increase in Mean Germination Rate (MGR). For the second objective, the group of Very Light seeds was the fastest MGT and highest MGR, but heavier seeds have higher GSP compared to smaller seeds. However, no significant differences (p = 0.924) or significant correlation (p = 0.132) were observed between difference of seed weight and germinability. For the third objective, a floatation test was conducted on seeds that have been stored for 14 months. We observed that seeds that float may not necessarily be non-viable, as more than half of the *floaters* germinated and showed no significant difference from the *sinkers* in terms of seedling development, such as mean height, number of leaves and leaf area. However, it was interesting to note that seedlings developed from heavier seeds perform better in terms of diameter size (r^2 = 0.294, p = 0.013) and height (r^2 = 0.214, p = 0.04).

Keywords: Merbau, scarification, seed dormancy, seed viability, seed weight

INTRODUCTION

Merbau or *Instia palembanica* was declared as Malaysia's national tree in 2019. This species has the reputation of producing high-end luxury wood and is regarded to be a highly valued timber throughout Southeast Asia. The species is recorded as widespread, ranging from India, Thailand, Sumatra, Malaysia, Singapore, and Borneo (Whitmore 1972). It prefers a rainfall of more than 2000 mm per year and is sometimes found growing gregariously and occasionally can be a dominant species on some sites. The mature trees are large; they can reach 145 cm in DBH and 55 m in height. The bole is slightly sinuous, the buttresses are steep, and the crown is wide and dome-shaped, with big ascending limbs (Appanah & Weinland 1993). The sapwood of Merbau is pale white to yellowish color and can be differentiated from its heartwood, which is yellow-brown but can turn to dark brown when exposed (Beaman 2000). One of the prominent characteristics of Merbau is the wood density. The timber is classified as heavy hardwood with an air-dry density of 850 – 900 kg/m3 and considered very durable when exposed to natural elements (Burgess 1956 & Wong 1982).

The wood is very attractive with its growth rings and deep color, making it highly prized as interior finishing or furniture wood. Currently, this species has not been widely planted, in terms of threat status it has been assessed as Near Threatened as the threat degree varies from one country to another, depending on the local demand.

Despite its slow growth, Merbau has been used in planting trials due to its dormant seeds (Appanah & Weinland 1993). Seed dormancy is a plant's survival mechanism in order to tolerate adverse environmental conditions in which seed germination is delayed until the surrounding factors are favorable (Mousavi *et al.* 2011, Lacerda *et al.* 2004, Li & Foley 1997). The pivotal role of seed dormancy in forest ecology is to ensure the continuation of the species by geographic spread, a key factor that influences natural population dynamics (Bewley & Black 2014). Seed dormancy is a desirable trait in some crop plants, such as winter cereal to prevent germination before harvesting and ensuring the seed quality remains intact (Larsen & Eriksen 2002 & Koornneef *et al.* 2002). However, it can pose a real challenge in tree plantation or restoration due to the seed physiological delay that will make them become susceptible to seed predators and pathogens, decrease in viability and non-uniform germination (Turner *et al.* 2013 & Kildisheva *et al.* 2020). Therefore, assisted germination to break the seed dormancy is essential to eliminate the physiological limitations, especially in the case of Merbau.

The seed of Merbau has the ability to withstand moisture content that is less than 10% without losing seed viability for more than three years (Sasaki & Ng 1981). The pods are large and leathery and the seeds are released when the leathery pod dehisce. Normally one pod contains up to 4 to 6 seeds (Plate 1.A). The 2-layer hard-coated seed contains two large cotyledons which are attached to a plumule and radical. The outer seed coat is impermeable to water and scarification is needed to break the seed dormancy. Suggested treatments of breaking seed dormancy are few, those which are available were of treatments performed on *Intsia bijuga* (Colebr.) *Kuntze.* (Wulandari *et al.* 2015 & Prosea 2017), however there is a need to obtain more information if we need to try out the methods and achieve optimum germination for *Instia palembanica*.

The study aimed to develop a protocol for the germination of Merbau with the following objectives: (i) to determine cost-effective and rapid scarification techniques to break seed dormancy; (ii) to determine if seed germinability is influenced by seed weight; and (iii) to investigate seed viability after long term storage.

MATERIALS & METHODS

Seed collection

Seed collection was conducted from a mature tree in 2016 and located at the delta of Sg. Papah (bordering a river) of Kampung Muruk within the district of Ranau, Sabah (Figure 1). The studied tree is located at an altitude of 380 m above sea level (Plate 1.B). The diameter at breast height (DBH) and height of the tree were recorded to be 94 cm and 39 m respectively. Seeds were collected using net trapping, in which a nursery shading polynet was installed 1 m from the ground covering a 10 m radius of the mature tree (Plate 1.C). The installation of the net trap was from October 2016 to November 2016. Collection of seeds was done on a weekly basis and stored in a gunny sack before being transferred to the nursery at the Forest Research Centre, Sepilok. Herbarium specimen was collected for this tree (Plate

1.D). A total of 749 seeds were successfully collected in this research. Seeds were then randomly selected and assigned to three different experimental studies.

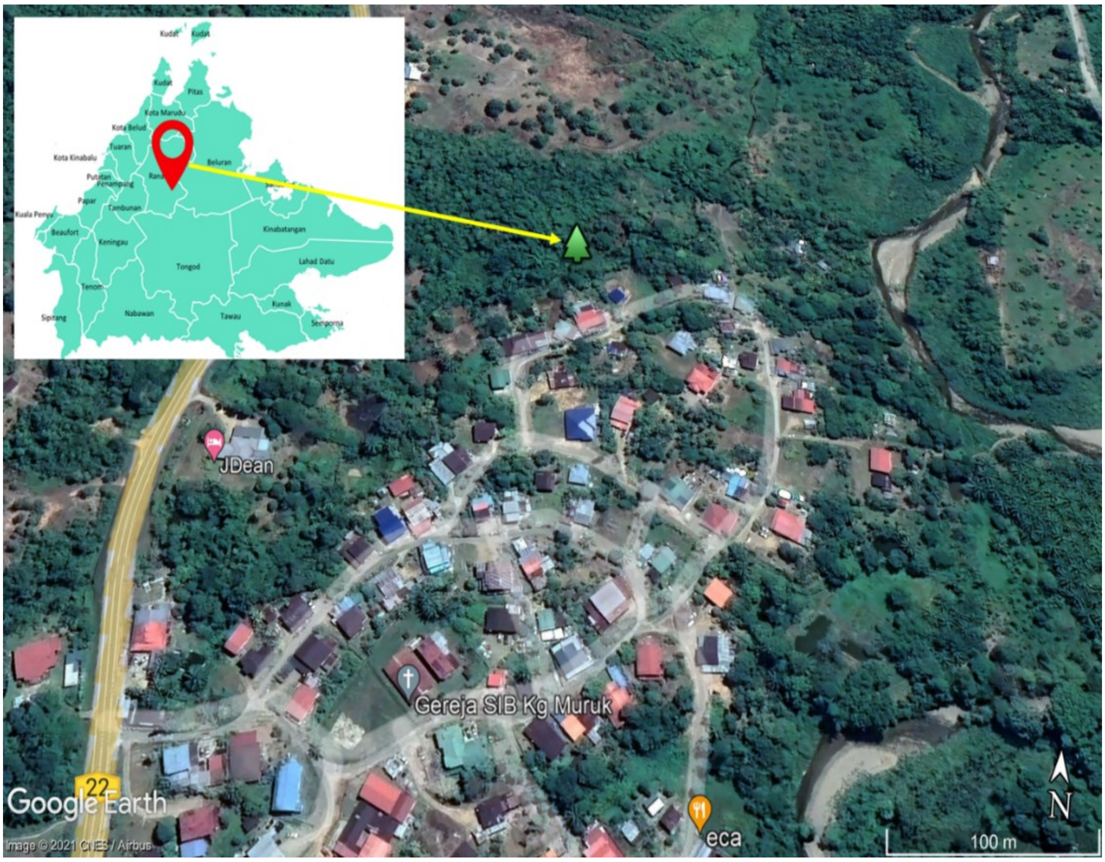

Figure 1. Location of the tree (Source: Google Earth 2020).

Protocol development for optimum germination

i. Seed dormancy breaking

A total of 19 treatments including a control, were applied using five different scarification techniques: mechanical, chemical, heating, soaking and a combination of mechanical and soaking.

Under mechanical treatment, two techniques were applied: first by nicking around the seed edge using a secateur (Plate 2.A) whereas the second was using an electric drill to puncture the seed coat (Plate 2.B). The time required to process the seeds were recorded to assess efficiency of the techniques. Four types of chemicals were used for chemical scarification: Sulphuric acid (SO_4), Hydrochloric acid (HCI), Ethanol (EtOH) and Sodium hypochlorite (NaOCl). Seeds were soaked in the chemicals using either absolute or 70% concentration at different time exposure (Plate 2.C). Scarification through heating was done by using a laboratory water bath that was set at 60°C with different time exposure. For the soaking technique, the seeds were soaked in water and placed at room temperature. As for the combination techniques, the seeds were subjected to the two different scarifications techniques and later on soaked in water.

Each of the treatments consists of 25 Merbau seeds that were randomly assigned. After the treatment, the seeds were sown in a germination bed containing sterile river sand inside a misting room (Plate 2.D). The misting room is under 70% shade with a mist spray system that is set for three (10 minutes) scheduled misting per day. Germination data was recorded on a weekly basis for up to 29 weeks. Seeds were counted as germinated when radicle and plumule emerged (Plate 2.E) and calculation of germination parameters was performed using the R package of GerminaR (Pompelli *et al.* 2019). Germinated seed percentage (GSP), mean germination rate (MGR) and mean germination time (MGT) were calculated according to the method proposed by Labouriau (1983).

ii. Seed germinability by seed weight

A subsample of 100 randomized seeds was weighed and grouped into four classes of Light (F1), Intermediate (F2), Heavy (F3) and Very Light (F4) categories (Appendix 1; Plate 3.A). For this experiment, all seeds were untreated and germinated using the same media and condition applied in the seed dormancy breaking section (Plate 3.B). Data on germination was recorded on a weekly basis up to 29 weeks and Germinated seed percentage (GSP), mean germination rate (MGR) and mean germination time (MGT) were calculated.

iii. Seed viability after storage

A total of 139 seeds were kept at room temperature for 14 months after experiment section (i) and (ii) were conducted. Seeds were later subjected to a floatation test to distinguish viable and non-viable seeds. The assumption was seeds that float may lack viable embryos or nutrient stores, making them less dense compared to seeds that sink in water. In some cases, viable seeds may also float due to the presence of air pockets beneath the seed (Pieters 1895). Thus, to avoid bias, seeds that float or sink were weighed individually prior to germination test. A combined scarification technique using both mechanical and soaking was conducted (Plate 3.C). Data on germination were recorded after 14 days (D_{14}) to estimate germination percentage. Germinated seedlings were then transferred into a perforated polythene bag of 36 x 45 cm with a medium of garden soil and sawdust (ratio 3:1) and placed under shade in the main nursery using 70% shade (Plate 3.D). Manual watering of the polybag seedlings was conducted once a day.

Assessment of seedling growth and mortality was carried out on day-50, (T_{50}) according to Farooq *et al.* (2006); a method that calculates data by time to reach 50% germination. Measurements of the height (cm), seedling diameter (mm), number of leaves and leaf area (cm^2) were determined using ten randomly selected seedlings under each group for statistical analysis. Diameter of the seedling was measured using a digital caliper, while leaf area was measured using an android application, Leaf-IT (Schrader *et al.* 2017).

Data analysis

For the breaking of seed dormancy, one-way ANOVA was performed using GerminaQuant (Lozano-Isla *et al.* 2019) followed by Tukey's test ($p < 0.05$) to determine differences of scarifications applied over growth parameters. Subsequently, rates of improvement of six scarification classes were pooled from their assigned treatments and the index was measured by dividing the final means for each germination parameter over control treatment (base value) and multiplying by 100 (Simple Index Number 2008).

In the study of seed weight influence on germinability a Normality test (Shapiro-Wilk test) was performed and non-parametric covariance analysis (ANCOVA) was used to determine the differences between seed mass (F1 = Light, F2 = intermediate, F3 = heavy, F4 = very light) and germinated seed percentage (GSP). The relationship between classes of seed weight and germinability (using binary data, 1=germinated, 0=no germination) was analyzed using Spearman Rank correlation.

As for the determination of seed viability after long storage, t-test was used to compare the difference between two groups (*floaters* & *sinkers*) over growth parameters. Also, a simple linear regression analysis was performed to estimate the relationship between seed weight (independent variable) and each of the growth parameters. Data from pooled groups (*floaters* & *sinkers*) were used to predict the regression equation (Appendix 2), with the assumption of randomized seed selection and without flotation test. All statistical analysis was performed using PAST 3.22 program (Hammer *et al.* 2001), Sigmaplot ver. 14 (Systat software inc, 2017).

RESULTS

Seed dormancy breaking

The results are given in Table 1. A total of 475 seeds were assigned to 19 treatments (including one control) with five different scarification techniques for breaking the seed dormancy. The best treatment was recorded for *T17* (a combined technique using mechanical and soaking), which gave the highest germination rate (MGR = 0.40) and the fastest germination time (MGT = 2.478), in which the recorded germinated seed percentage was 92%. This was followed by treatment *T1* where the mechanical scarification treatment recorded a gemination of 100%, however it has a slower germination time (MGT = 5) and lower germination rate (MGR = 0.20). The other treatment, *T18 (*drilled & soaked in water overnight) has shown to have a high seed germination (GSP = 60%) with MGT = 3.87 and MGR = 0.26. As for the other treatments, chemical scarification was observed to have high MGR and fast MGT but very poor GSP (*T9 & T4*), whereas both soaking (*T*11, *T*12) and heating (*T*13-*T*16) produced the lowest GSP and MGR, also much slower in MGT. The control treatment recorded 8% germination with the lowest MGT and MGR recorded. It has to be pointed out that one of the treatments in the heating technique (*T13)* did not yield any germination data.

Analysis of growth parameters between classes of scarification (Figure 2) shows that combination of mechanical and soaking provided good results for all growth parameters, followed by mechanical scarification. The lowest GSP was recorded in chemical scarification, while the slowest MGT was recorded in soaking. Statistical analysis by one-way ANOVA (Table 2) shows that there were significant differences observed among class of scarifications for GSP ($F_{4,13}$ = 3.875, p = 0.028) and MGR ($F_{4,12}$ = 3.502, p = 0.041), but no significant difference observed for MGT ($F_{4,12}$ = 1.947, p = 0.167). Post hoc comparison by Tukey's test indicated the mean score for chemical scarification and combined techniques of mechanical and soaking were significantly different from the rest of growth parameters for GSP, P<0.05 (Figure 2; Table 3). However, there were no significant differences in the mean score for growth parameters of MGT and MGR (Figure 2; Table 3).

Rates of improvement were calculated using an index of scarification classes over control treatment (Table 4). By taking the best scarification method (combined techniques of mechanical and soaking) as an example, the improvement rate in GSP was estimated to be

8.5 times better, an increase of 86% faster in germination time (MGT) and 6.6 times in germination rate (MGR), over the untreated Merbau seeds (control). This shows that unassisted, Merbau seeds could take a long time in breaking dormancy.

Table 1. Germination of *Intsia palembanica* seeds subjected to treatments and scarifications.

T	T applied / T duration	GSP (%)	MGT	MGR
T1	Rounded edge chipping (3 minutes per seed) [a]	100	5	0.20
T2	Drilled seed (<10 secs per seed) [a]	8	3	0.33
T3	Soaked in 70% SO_4 acid / 4 hours [b]	16	5.25	0.19
T4	Soaked in 70% SO_4 acid / 8 hours [b]	32	4.25	0.24
T5	Soaked in 70% SO_4 acid / overnight [b]	16	7.75	0.13
T6	Soaked in 70% HCl acid / 4 hours [b]	8	21.5	0.05
T7	Soaked in 70% HCl acid / 8 hours [b]	28	19.86	0.05
T8	Soaked in 70% HCl acid / overnight [b]	12	14	0.07
T9	Soaked in absolute EtOH / overnight [b]	16	3	0.33
T10	Soaked in absolute NaOCl / overnight [b]	12	17.67	0.06
T11	Soaked in water / overnight [c]	28	15.14	0.07
T12	Soaked in water / 72 hours [c]	28	18.14	0.06
T13	Boiled & leave at room temperature [d]	0	Na	Na
T14	Soaked in water bath at 60°C / 4 hours [d]	24	15	0.07
T15	Soaked in water bath at 60°C / 8 hours [d]	28	15.14	0.07
T16	Soaked in water bath at 60°C / overnight [d]	32	6.88	0.15
T17	Rounded edge chipping + soaked in water / overnight [e]	92	2.48	0.40
T18	Drilled + soaked in water / overnight [e]	60	3.87	0.26
T19	Control [f]	8	23	0.04

Note: T = Treatment, Class of scarification: [a] mechanical, [b] chemical, [c] soaking, [d] heating, [e] mechanical + soaking, and [f] untreated seeds with normal germination on bed. GSP = germinated seed percentage, MGT = Mean germination time (by week), MGR = mean germination rate, Na = not available.

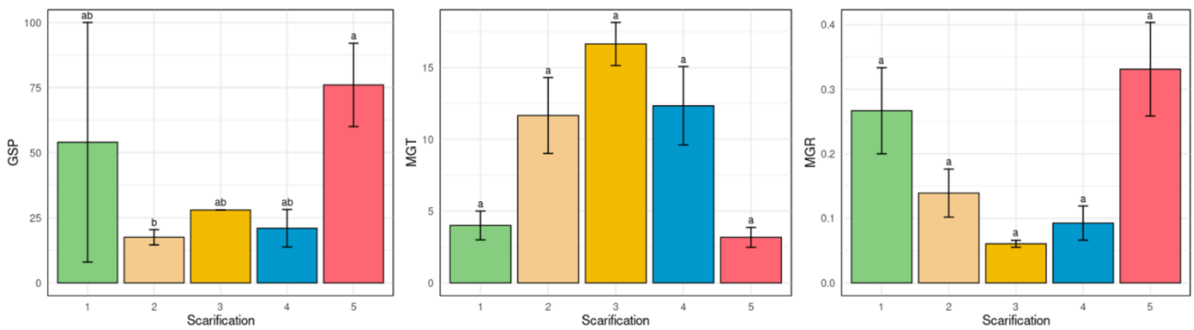

Figure 2. Bar graphs with growth parameters according to scarification classes. Scarifications were denoted by numbers which refer to 1= mechanical, 2=chemical, 3=soaking, 4=heating and 5= mechanical + soaking. Different letters indicate significant differences between classes of scarification (*P* < 0.05). Error bars indicate standard error.

Table 2. One-way ANOVA on growth parameters.

Source of variance	D.f	Sum of squares	Mean squares	F-value	*p*
1. GSP	4	6966	1741.5	3.875	0.0276 *
Residuals	13	5842	449.4		
Total	17	12808	2190.9		
2. MGT	4	288.2	72.06	1.947	0.167

Residuals	12	444.1	37.01		
Total	16	732.3	109.07		
3. MGR	4	0.1181	0.029527	3.502	0.0408 *
Residuals	12	0.1012	0.008431		
Total	16	0.2193	0.037958		

Note: *Significant at $p \leq 0.05$; GSP = germinated seed percentage (%), MGT = mean germination time (week), MGR = mean germination rate.

Table 3. Tukey's test on class of scarification.

Class of scarification	GSP	MGT	MGR
1. Mechanical	54 ± 46^{ab}	4 ± 1^{a}	0.2667 ± 0.07^{a}
2. Chemical	17.5 ± 2.92^{b}	11.659 ± 2.65^{a}	0.1391 ± 0.04^{a}
3. Soaking	28 ± 0^{ab}	16.643 ± 1.5^{a}	0.0606 ± 0.01^{a}
4. Heating	21 ± 7.19^{ab}	12.339 ± 2.73^{a}	0.0927 ± 0.03^{a}
5. Mechanical + soaking	76 ± 16^{a}	3.172 ± 0.69^{a}	0.3311 ± 0.07^{a}

Note: means followed by the same letter within each growth parameter do not differ significantly using Tukey's test; $p < 0.05$. Data are mean ±Standard Error (SE). GSP = germinated seed percentage (%), MGT = mean germination time (week), MGR = mean germination rate.

ii. Seed weight and germinability

A total of 100 seeds were randomly selected and individually weighted. Seed mass ranges from 3.10 g to 11.65 g and is classified into four classes of Light (F1), Intermediate (F2), Heavy (F3) and Very Light (F4). The weight range for each class was set according to Table 5. Seed weight in four classes were tested for normal distribution (Shapiro-Wilk test, $p = 0.009$). Heavy seeds (F3) recorded the highest germination percentage of 28%, followed by F1 and F4 with 16% germination (Table 5; Figure 3). F2 constituted the lowest germination percentage of 8%. The fastest germination time recorded for F4 (week 12). The slowest germination time recorded was shared by F1 and F2 (week 23). The best mean germination rate was recorded for F4 (MGR = 0.08), followed by F3 (MGR = 0.06). F1 and F2 recorded a similar rate of MGR = 0.04. There were no significant differences observed between the seed weight class and germination percentage (Table 6; $F_{(3,92)} = 0.159$, $p = 0.924$). No significant correlation observed between seed weight and germinability using Spearman Rank correlation ($p = 0.132$).

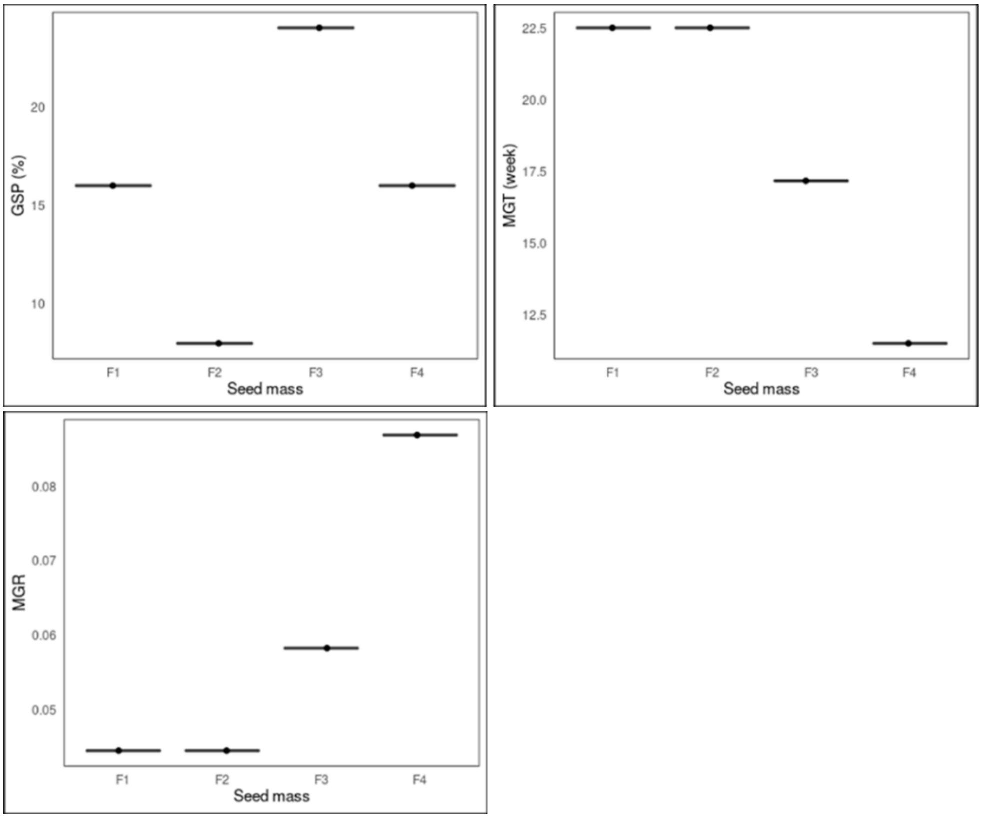

Figure 3. Bar-Line graphs with growth parameters according to seed mass. Seed mass referred to different groups as described F1= Light, F2=Intermediate, F3=Heavy and F4=Very Light.

Table 5. Seed mass and growth parameters.

Seed mass	RSW	MW±SE	GSP (%)	MGT	MGR
Light (F1)	6.36 - 7.36g	6.97±0.05	16	23	0.04
Intermediate (F2)	7.37 - 9.94g	8.46±0.13	8	23	0.04
Heavy (F3)	9.95 - 11.41g	10.55±0.86	28	17	0.06
Very Light (F4)	< 6.36g	5.44±0.18	16	12	0.08

Note: RSW = Range of seed weight, MW= Mean of weight, SE=Standard error, GSP = germinated seed percentage, MGT = Mean germination time (by week), MGR = mean germination rate.

Table 6. Analysis of variance (ANCOVA) between seed mass and GSP.

Source of variance	D.f	Sum of squares	Mean squares	F-value	*p*
Group x Germination	3	0.186	0.0621	0.159	0.924
Residual	92	35.956	0.391		
Total	99	393.162	3.971		

iii. Seed viability after long storage

In the floatation test, 109 seeds were *floaters*, while 30 seeds were *sinkers*. Germination percentage recorded for sinkers was 73.3% compared to floaters (53.2%); Table 7). Five dead seedlings were recorded for *floaters* but no seedling mortality was recorded for *sinkers* during germination. The distribution data for each seedling growth parameters were normally distributed (Shapiro-wilk test). Significant differences between the groups were observed for diameter ($P > 0.022$), but no significant differences were observed with the other parameters for height, number of leaves and leaf area. Regression analysis showed that seed weight had a linear positive relationship with seedling diameter and height (Figure 4). Significant regression equations (Table 8) were found for diameter ($r^2 = 0.294$, $p = 0.013$) and height ($r^2 = 0.214$, $p = 0.04$). Thus, heavier seeds strongly influence the seedling development for diameter and height.

Table 7. Initial seeds characteristic and growth parameters between two groups. Data was recorded on day-50 of germination.

Parameters	*floaters*	*sinkers*
a) Seeds, *n*	109	30
b) Germination percentage, (D_{14})	53.2	73.3
c) Seedling mortality, *n*	5	0
d) Percentage contribution to seed lot	38.1	15.8
Mean of growth parameters (n=10)		
i. Diameter (mm)	4.641±0.22*	5.341±0.17*
ii. Height (cm)	31.75±2.86	36.67±1.43
iii. No. of leaves	12.8±1.09	14.6±1.64
iv. Leaf area (cm^2)	33.24±3.51	37.34±2.80

Note: * Significant at $p < 0.05$; (D_{14}) = Data recorded after 14 days germination.

Table 8. Regression equation (Y=A+BX) between initial seed weight with growth parameters. Data for seed weight from *sinkers* and *floaters* were pooled ($n = 20$) for linear regression analysis.

Variables	Regression equation	*r*	r^2	*p-value*
i. Diameter	Y=3.9374+0.13807x	0.542	0.294	0.01353*
ii. Height	Y=24.787+1.2348x	0.462	0.214	0.04025*
iii. No. of leaves	Y=11.685+0.26405x	0.167	0.0277	0.48282
iv. Leaf area	Y=27.127+1.0696x	0.29602	0.0876	0.20507

Note: *Significant at $p < 0.05$.

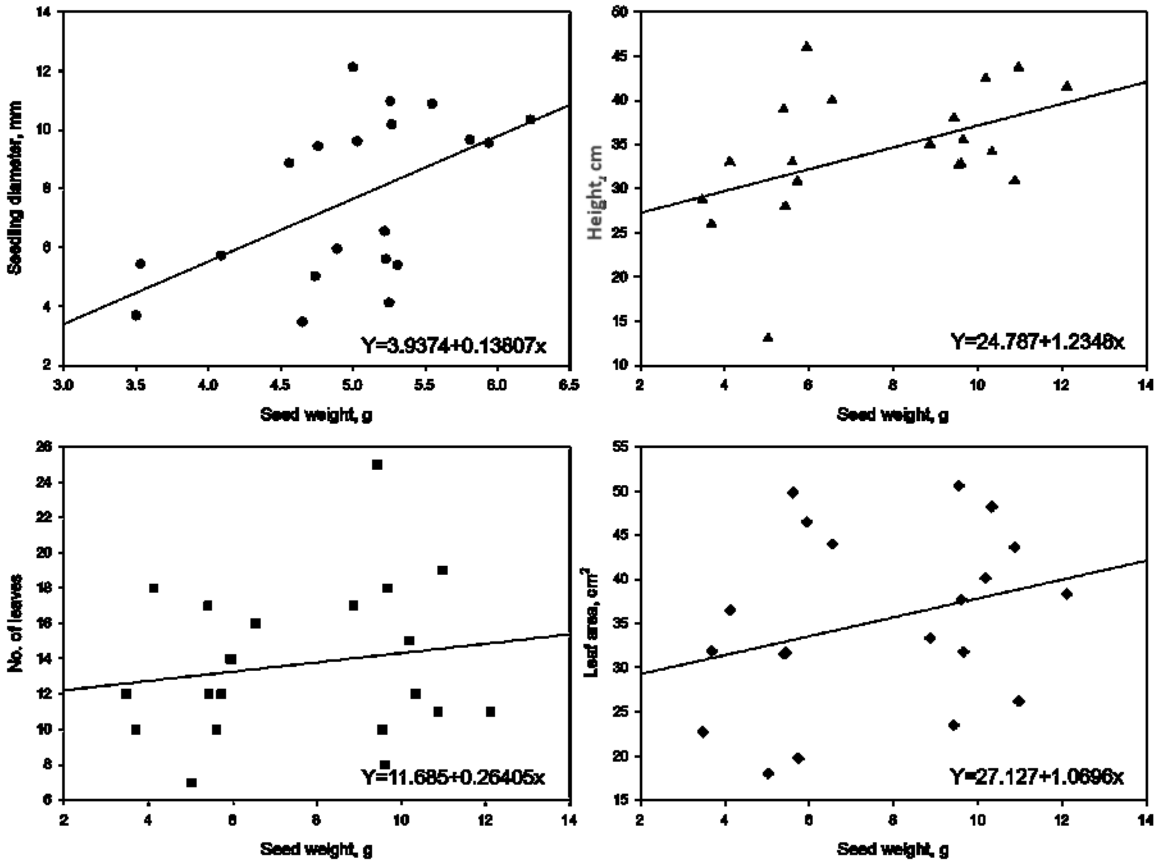

Figure 4. Scatter plots showing the relationship between seed weight with four growth parameters of the seedling development on day-50.

DISCUSSION

Breaking of seed dormancy

Scarification on *Intsia palembanica* seeds using mechanical treatment requires effort and time, as recorded for *T1.* The average time to process a single seed was about 3 minutes by chipping around the seed edge using a secateur. Besides, the seed had a waxy surface which made it difficult to clip down as it slips most of the time. Thus, another treatment (*T2*) using an electric drill was introduced to reduce the time process but resulted high germination failure (*T2*, mortality = 82%). We observed that puncturing the center of the seed caused internal injury and easily promote fungal infection (Plate 2.F). Other treatments using chemical and heating scarifications did not yield the desired results in terms of germination rate. High mortality rates were recorded for both treatments (chemical = 83%; heating = 79%) due to prolonged exposure to chemicals at high concentration and continuous heating in the water bath. This might have caused injuries to the seed embryo and resulted in seed mortality, despite having an improved MGT compared to soaking technique (MGT; Figure 2). The safety in handling corrosive chemicals is also a concern for operators, even though there were reported successes using chemical scarification (H_2SO_4) and heating technique to break down seed dormancy in Merbau (Sasaki 1980, Yuniarti 1994 & Prosea 2017). The key to success using such techniques is finding the ideal treatment time but difficulty in germination uniformity may still be an issue. Therefore, seed mass classification is required in order to perform these techniques, as different seed weight has might have different seed coat thickness.

The technique of mechanical and soaking, *T17*, provided rapid and optimum seeds germination. By soaking the seeds overnight, water will infiltrate the gap created on the seed coat, eventually the imbibition process will lead to the rupture of the seed coat, in turn resulting in the germination of the seeds. Another recommended option was using *T1,* as it provides 100% GSP. Both treatments were feasible applications in terms of rapid germination success and safe work practices.

Seed weight and germinability

Seed mass is often associated with the seed coat thickness where one or more layers of impermeable palisade layers of lignified cells are present (Corner 1976). Under natural conditions (untreated seed), we observed that the weight of the seeds did not impede germination. However, it seems that smaller seeds have greater permeability due to thinner seed coat and this enables them to germinate faster (Dolan 1984, Souza & Fagundes 2014), which was reflected in the result for F4 seeds. They recorded the fastest MGT and highest MGR compared to heavier seeds. In principle, the heavier seeds have a decrease in seed relative surface (volume ratio) that might result in a lower relative ability to absorb water and to initiate germination (Fowler & Bianchetti 2000; Souza & Fagundes 2014). Nevertheless, the heavy seeds in this experiment have shown a high germination percentage compared to the lighter seeds, as projected in F3 vs F4. Similar result was reported in *Intsia palembanica* where heavier seeds produced better germination percentage after mechanical treatment was applied (Wulandari *et al.* 2015). This could be due to the fact that larger seeds have greater nutrient reserve for energy conversion to stimulate germination (Flint & Palmblad 1978). It was observed that the seedlings from heavier seeds tend to need more time to germinate, possibly due to the drive to produce quality seedlings and with higher competitive ability (Leishman 2001, Souza & Fagundes 2014). In terms of mean germination time (MGT), there were no significant differences among the seed weight groups over germinability. This was in line with the previous study in *Intsia bijuga,* where no significant differences were observed between seed weight and germination variables (Hanson *et al.* 2005).

Seed viability after storage

The orthodox Merbau seed with exogenous dormancy is able to survive for more than three years, as seeds are tolerant of low moisture content of less than 10%. It is estimated that seed dormancy for mature Merbau seeds may last up to two years in the natural environment (Prosea 2017). Thus, seed viability was investigated after 14 months in storage. In principle, flotation tests are related to specific gravity in seed testing, as seed viability is mainly controlled by enzymes and hormones. Therefore, seeds that float on water were assumed to be nonviable due to immature or unfilled seeds that tend to have lower gravity. However, seeds that float may not necessarily be nonviable in all cases, as shown in this study. More than half of the *floaters* germinated and about 38% of this group contributed to the seed lot. Long term seed storage has obviously reduced the seed moisture content resulting in the seeds to become much lighter in weight, especially with the smaller seeds. Despite that, substantial numbers of the *floaters* germinated and showed no significant differences with *sinkers,* in terms of mean height, number of leaves and leaf area. However, seedlings from heavier seeds produce significant results in terms of diameter size and height, based on regression analysis. The result was in agreement with Souza & Fagundes (2014), where larger seeds of *Copaifera langsdorffii* (Fabaceae) produced more vigorous seedlings compared to the smaller seeds. Floatation tests may not be necessary to be carried out for Merbau seeds after a long storage, but it is crucial to select viable seeds that favor seedling quality.

Limitation of this study

Although this study has yielded some important findings, we are aware of the limitation. The seeds were collected from a single tree. Thus, more seed samples from other reproductive trees are needed to increase data reliability of seedling phenotypic in future. In addition, it is recommended to conduct field testing to observe the actual growth parameters in each experimental section for more conclusive findings.

CONCLUSIONS

Finding the best protocol for rapid and cost-efficient optimum germination is important, especially when it comes to cost management in plantation establishment. Therefore, this study provides insight on working with limited seed resources of highly prized *Intsia palembanica*. Seed germination without specific treatments proved to be costly and inefficient for Merbau planters. Suggestion to apply a combination of mechanical and soaking techniques by chipping the seed coat and soaking in water overnight, is prerequisite to break Merbau seed dormancy, subsequently to achieve rapid and optimum germination. Besides, selection of seed mass for planting material may favor a better all-rounder of seedling quality. This study proves that selection of heavier seeds produced better growth performance in diameter and height. Besides, it is also important to understand the variability of seed mass after a long storage as seeds become lighter and less viable. Selecting seeds for restoration programs may not be necessary, if the purpose is to upscale seedling production and utilize seeds resources that are available. The floatation test conducted found seeds that float may still have more than 50 percent of germinability. Thus, proper planning in managing limited seed resources for ex-situ restoration is more sensible. Otherwise, selection of heavier/viable seeds are a good option for tree plantation as it strongly influences the development of seedling in performance and quality.

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Table 4. Scarification classes over control.

Scarification classes	GSP			MGT			MGR		
	Pooled	Index	IR (%)	Pooled	Index	IR (%)	Pooled	Index	IR (%)
1) Mechanical	54	675.0	575.0	4	17.4	82.6	0.2667	613.1	513.1
2) Chemical	17.5	218.8	118.8	11.659	50.7	49.3	0.1391	319.8	219.8
3) Soaking	28	350.0	250.0	16.643	72.4	27.6	0.0606	139.3	39.3
4) Heating	21	262.5	162.5	12.339	53.6	46.4	0.0927	213.1	113.1
5) Mechanical + soaking	76	950.0	850.0	3.172	13.8	86.2	0.3311	761.1	661.1
* Untreated (control)	8	100		23	100		0.0435	100	

Note: GSP = germinated seed percentage, MGT = Mean germination time (by week), MGR = mean germination rate;
Pooled = Data from pooled groups (*floaters* & *sinkers*)
Index = index was measured by dividing the final means for each germination parameter over control treatment (base value) and multiplying by 100 (Simple Index Number 2008)
IR (%) = Rate of improvement over base value * control.

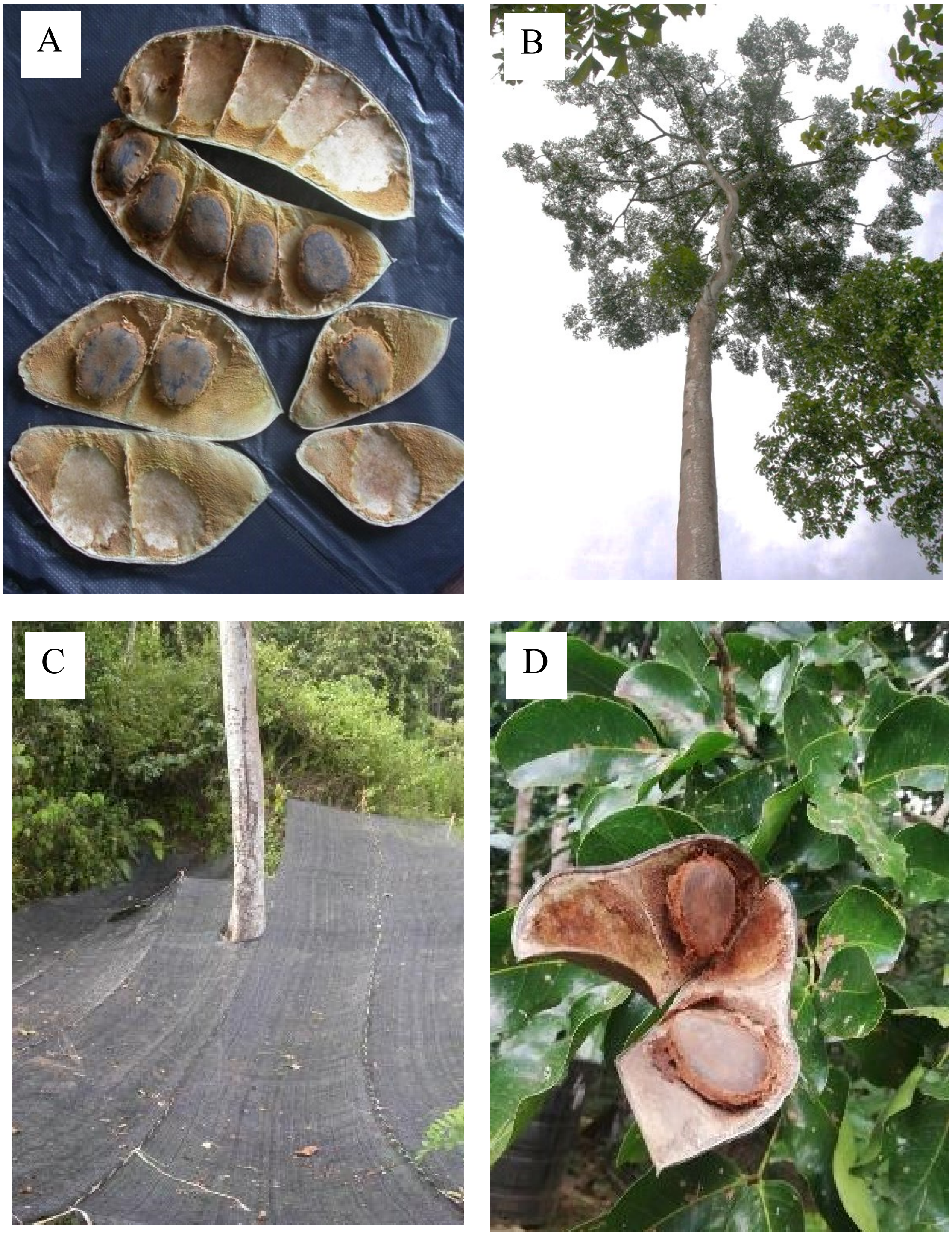

Plate 1. A. Seed pod of *Intsia palembanica*. B. *Intsia palembanica* tree. C. Installed polyethene shading net to capture fallen seeds. D. Specimen voucher for herbarium record.

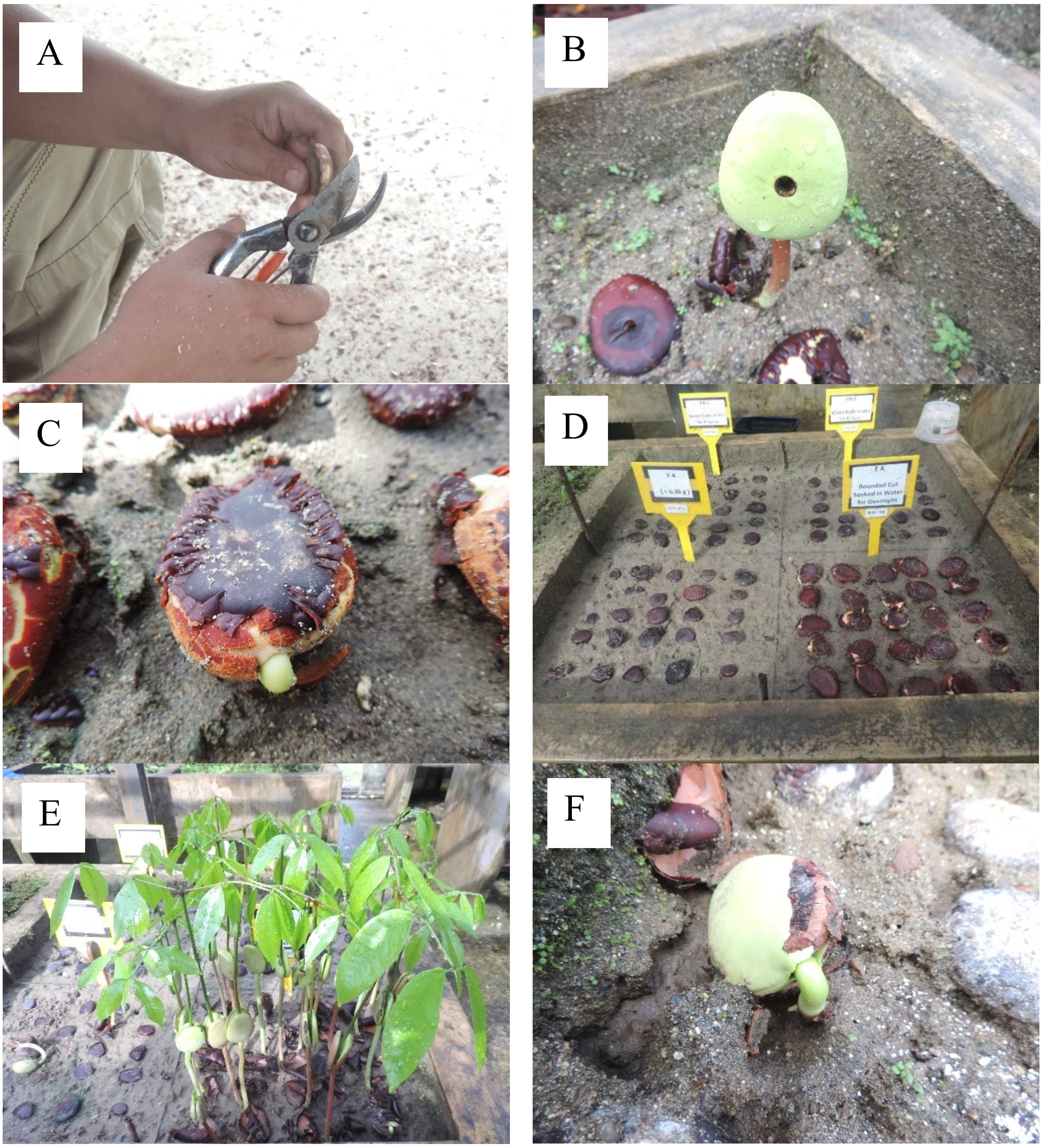

Plate 2. A. *T1* of chipping around the seed edge. B. *T2* of drilled seed. C. Chemical scarification (70% SO_4 acid/8 hours). D. Treated seeds at germination bed. E. Germinated seeds by combination techniques of *T17*. F. Germinated seed infected by fungus.

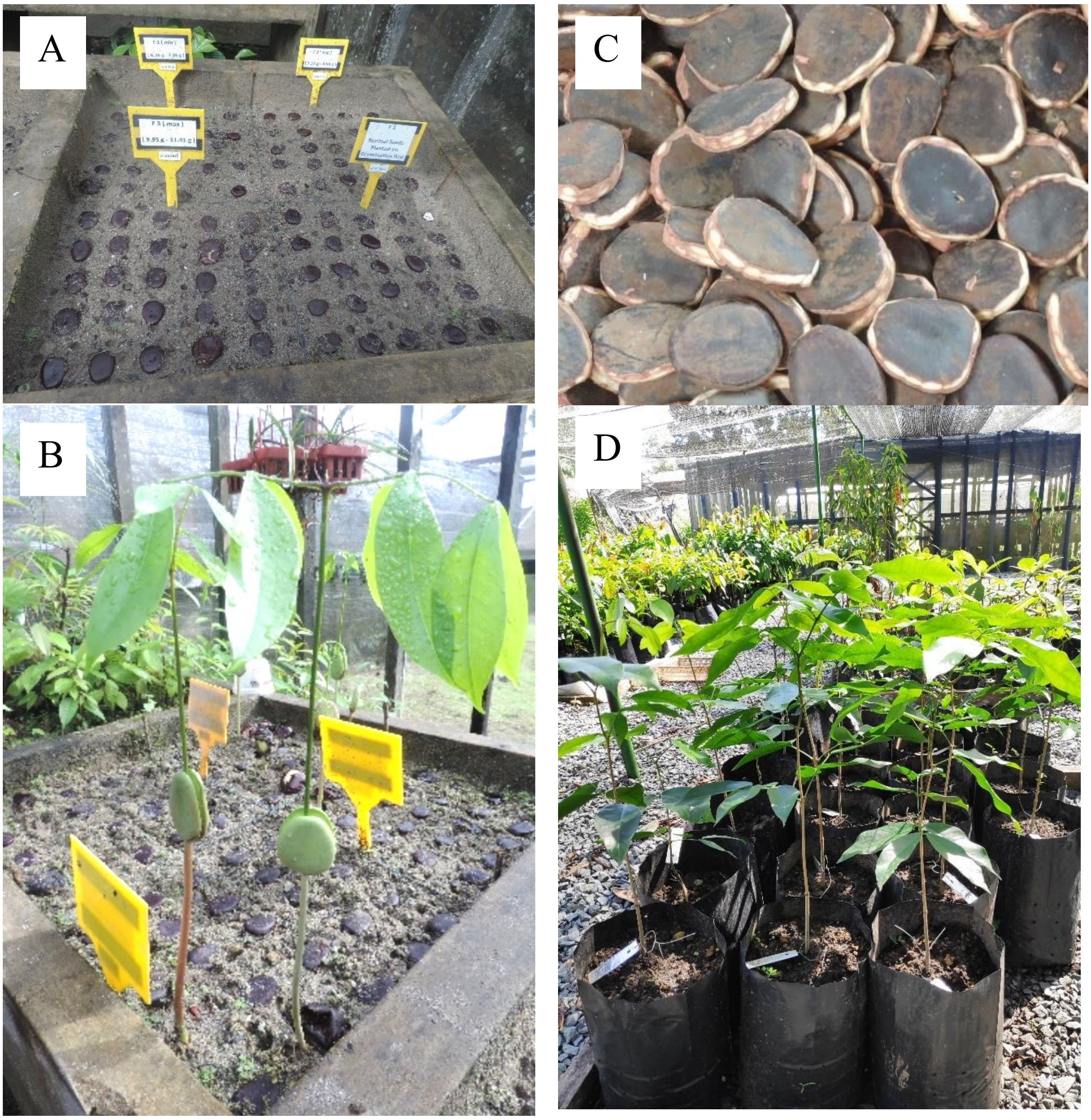

Plate 3. A. Testing seed viability of the four weight classes. B. Seedlings in seed weight classes. C. Treated seeds of *sinkers* and *floaters*. D. Seedlings from *sinkers* and *floaters*.

Appendix 1. Groups of seed weight to determine germination rate.

No.	F1	F2	F3	F4
1	7.14	8.1	9.98	5.77
2	7.21	8.61	10.16	6.25
3	6.99	9.1	10.26	6.01
4	7.2	7.96	10.35	5.78
5	6.93	9.06	10.75	6.11
6	6.92	7.95	10.43	3.1
7	6.61	9.47	10.75	6.04
8	6.7	9.03	11.65	4.53
9	7.24	7.44	11.47	6.22
10	6.77	8.4	10.68	5.75
11	7.22	8.12	10.54	6.07
12	6.91	8.92	10.01	6.28
13	7.18	8.06	10.22	6.34
14	7.02	8.61	10.5	4.39
15	6.51	7.69	10.23	4.64
16	6.56	8.07	10.5	5.72
17	6.95	9.55	9.99	5.01
18	7.32	9.55	10.1	5.75
19	7.05	8.73	11.09	6.15
20	7.08	7.72	10.32	5.76
21	7.04	8.15	10.64	5.51
22	6.92	7.56	10.98	6.31
23	7.15	7.58	10.69	4.72
24	7.26	9.35	10.51	3.29
25	6.36	8.72	10.91	4.48
Min	6.97	8.46	10.55	5.44
median	7.02	8.40	10.50	5.76
std dev	0.26	0.66	0.43	0.92
std err	0.05	0.13	0.09	0.18
%CV	3.69	7.86	4.10	16.98

Note: Min = minimum, std dev = standard deviation, std err = standard error, %CV = percentage of coefficient of variation.

Appendix 2. Seed viability influence on the seedling characteristic (variables).

Group	*Ind var.*	*var1*	*var2*	*var3*	*var4*
	weight, g	diameter, mm	height, cm	No. of leaves	leaf area, cm2
floaters	3.69	3.5	26	10	31.912
floaters	5.44	3.53	28	12	31.702
floaters	5.03	4.74	13	7	17.998
floaters	6.55	5.22	40	16	44.005
floaters	5.4	5.31	39	17	31.522
floaters	4.12	5.25	33	18	36.493
floaters	5.61	5.23	33	10	49.825
floaters	3.47	4.65	28.7	12	22.689
floaters	5.95	4.89	46	14	46.515
floaters	5.73	4.09	30.8	12	19.709
sinkers	9.66	5.81	35.5	18	31.8
sinkers	10.34	6.23	34.2	12	48.19
sinkers	10.88	5.55	30.9	11	43.632
sinkers	9.61	5.03	32.8	8	37.69
sinkers	9.55	5.94	32.6	10	50.603
sinkers	12.12	5	41.5	11	38.354
sinkers	10.97	5.26	43.7	19	26.186
sinkers	10.19	5.27	42.5	15	40.147
sinkers	9.44	4.76	38	25	23.461
sinkers	8.87	4.56	35	17	33.351
Min	7.63	4.99	34.21	13.70	35.29
median	7.71	5.13	33.60	12.00	34.92
std dev	2.77	0.71	7.40	4.39	10.00
std err	0.62	0.16	1.65	0.98	2.24
% CV	36.29	14.13	21.63	32.05	28.35

Note: Min = minimum, std dev = standard deviation, std err = standard error, %CV = percentage of coefficient of variation.

A note on mammal browsing within a forest plantation research site in Lungmanis Forest Reserve, Sabah

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In 1974, the first commercial forest plantation project was initiated by the Sabah State Government following the decline of natural forest cover (Pinso & Vun 2000). Thenceforth, the forest plantation industry within the state has grown rapidly (FAO 2002). This industry from 2015–2019 has successfully established at least 342,759 ha of forest plantation area with some parts were allocated for research (SFD 2019). Most of the local studies within these plantations emphasized tree improvements, phenological observations, plant diseases and insect pests (SFD 2019). However, much is not known about mammal disturbances within these forest plantation sites in Sabah.

This research note aims to disseminate information about mammal browsing in Kolapis A; a tree plantation research site located in Lungmanis Forest Reserve, Beluran, Sabah. A study was undertaken after a report emerged of mammal disturbances in areas with planted tree seedlings. A rapid field survey was conducted from 8th to 16th December 2020; to identify what was causing various types of damages to the planted seedlings. Eight wildlife cameras (Bushnell Trophy Cam HD Aggressor) were deployed and placed strategically at the study site for seven trap nights. Additionally, a day transect survey was also conducted. The camera detection rate is defined as the percentage of the number of independent photographs of a species over total trap nights.

Tree species planted in Kolapis A include *Diospyros* sp., *Dryobalanops beccariana*, *Eurycoma longifolia*, *Eusideroxylon zwageri*, *Durio* sp., *Aquilaria malaccensis*, *Parashorea tomentella*, *Acacia mangium*, *Octomeles* sp., *Eucalyptus pellita*, *Shorea leprosula*, *Neolamarckia cadamba*, *Terminalia copelandii* and *Albizia moluccana*. The areas in the vicinity of the study site consist of a secondary forest, a disturbed lowland mixed dipterocarp forest, and oil palm estates.

A total of 14 mammal species were recorded by both survey methods (Table 1). Of the total species recorded, six are globally recognised as threatened, namely the Sunda pangolin (Critically Endangered), sambar deer (Vulnerable), bearded pig (Vulnerable), Western tarsier (Vulnerable), pig-tailed macaque (Vulnerable), and long-tailed macaque (Vulnerable). The pig-tailed macaque is relatively common in Kolapis A and has the highest camera detection rate (Table 1). The present study identified 12 out of the 14 species observed in Kolapis A as pest mammals of the planted seedlings (Table 1).

Typical browsing damage is the breaking of main shoots and branches of planted tree seedlings. These damages were mainly perpetrated by sambar deers along with the macaques but to a lesser extent by the Malay civets and mousedeers (Table 1; Plates 1 & 3). More alarming findings, the sambar deer, macaques, and plain treeshrews were known to cause a fatal wound to seedlings by debarking its main stem and browsing on the palatable parts of its inner bark; which contains photosynthates that are translocated from leaves to other parts of the plant via the secondary phloem (Ryan & Asao 2014; Plate 2). This means that nitrogen-fixing plants such as those from the Fabaceae tree species are more attractive because they are known to accumulate and store proteins in their secondary phloem and xylem parenchyma (Hao & Wu 1993). In other plantation sites in Sabah, treeshrews have also been reported to have stripped the barks of *Eucalyptus pellita* stems (Chung *et al*. 2015). As it been reported, the population of plain treeshrews are quite high in forest plantation sites with dense understoreys (Stuebing & Gasis 1989).

A different group of mammals were involved in foraging for earthworms and indirectly, caused root damages to planted tree seedlings (Plate 4). The most severe damage is caused by the bearded pig, as it rummages forest soils to feed on earthworms (Phillips & Phillips, 2018). This study also noticed that the affected soils were those that were recently treated with fertilizers. The supply of fertilizers for the planted trees increased its growth and may indirectly also increase the production of dead organic material which is the primary food for earthworms (Syers & Springett 1984). The presence of earthworms could have attracted the bearded pigs which led to severe disturbances to the soil and roots of planted trees. Other negligible root damages were caused by short-tailed mongoose, porcupines, Sunda pangolin and Sunda stink-badger. It is worth noting that the severity of damages caused by each mammal pests varied. Based on our observation, macaques, sambar deers and bearded pigs caused the most severe browsing damages to planted trees while Sunda pangolins and porcupines often inflicted minimal damages to the plants.

The findings of this study indicate that mammal browsing in Kolapis A is a serious issue because even within the barb-wired fence, the newly planted trees could be disturbed by mammal pests. Trees impacted by mammal browsing are prone to diseases because their wounded tissues may serve as a point of entry for pathogens (e.g. *Ceratocystis* spp.); which could subsequently lead to high mortality rates of planted trees (Lee 2018). Much cost, time and effort were invested for a seedling to grow hence, a mammal pest control plan is urgently needed to address this issue.

If such plans involve culling of mammal pests in order to reduce the population and pressure, effective collaboration with Sabah Wildlife Department may be required. It is important to note that the extinction risks of each pest reported herein ranged from Least Concern to Critically Endangered. Therefore, culling may not be a possible solution for all. Furthermore, a majority of them are treated as Totally Protected Animals (Schedule 1) and Protected Animals (Schedule 2) in the Sabah Wildlife Conservation Enactment 1997 (2017 Amendment). However, there were some exemptions given; due to economic risks and threats to the sustainability of their agricultural operation, Sawit Kinabalu Sdn. Bhd. was given the approval to cull bearded pigs following the standard operating procedures stipulated in the Sabah Wildlife Conservation Enactment 1997 (2017 Amendment) (Charis Saliun pers. comm.). The Sabah Wildlife Department is also known to be able to translocate animals such as crocodiles and elephants to other suitable habitats in a bid to control human-animal conflict. This can also be a secondary solution to the problem for mammals that are threatened or protected.

Accordingly, a collaborative effort between the Sabah Wildlife Department and Sabah Forestry Department can be helpful and necessary to circumvent the issue of mammal browsing in forest plantation research plots. These research plots are important departmental assets and also essential demonstration sites for developing tree plantation model to support the state wood industry in pursuing sustainable production of timber resources.

The findings of this study are still in the initial stage and far from providing a complete understanding of the impacts of mammal browsing on planted trees or to the plantation activities in Kolapis A. Further studies on effective pest management plan in tree plantations to reduce the potential damages caused by mammal pests without affecting their population and conservation status are required.

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Table 1. Summary of recorded mammals and the damages they inflicted onto tree saplings in Kolapis A, Sabah.

Family	Scientific name	Vernacular name	Type of damage inflicted	Camera detection rate (%)	IUCN	Sabah WCE
Cercopithecidae	*Macaca nemestrina*	Pig-tailed macaque	BSB, BB & DMS	194	VU	Schedule 2
Cercopithecidae	*Macaca fascicularis*	Long-tailed macaque	BSB, BB & DMS	-	VU	Schedule 2
Cervidae	*Tragulus kanchil*	Lesser mousedeer	BSB	16	LC	Schedule 3
Cervidae	*Rusa unicolor*	Sambar deer	BSB, BB & DMS	30	VU	Schedule 3
Felidae	*Prionailurus bengalensis*	Leopard cat	-	1	LC	Schedule 2
Herpestidae	*Herpestes brachyurus*	Short-tailed mongoose	RT	-	NT	Schedule 2
Hystricidae	*Hystrix crassispinis*	Thick-spined porcupine	RT	7	LC	Schedule 2
Hystricidae	*Trichys fasciculata*	Long-tailed porcupine	RT	8	LC	Schedule 2
Manidae	*Manis javanica*	Sunda pangolin	RT	-	CR	Schedule 1
Mephitidae	*Mydaus javanensis*	Sunda stink-badger	RT	3	LC	Schedule 2
Suidae	*Sus barbatus*	Bearded pig	BS & RT	12	VU	Schedule 3
Tarsiidae	*Cephalopachus bancanus*	Western tarsier	-	1	VU	Schedule 2
Tupaiidae	*Tupaia longipes*	Plain treeshrew	DMS	14	LC	Not listed
Viverridae	*Viverra tangalunga*	Malay civet	BSB & BB	1	LC	Schedule 2

Note: Potential damage inflicted to tree saplings, BSB – broken main shoot; BB – broken branches; BS – broken main stem; DMS – debarked main stem; RT – root trampling. IUCN Red List of Threatened Species, CR – critically endangered, EN – endangered, VU – vulnerable, NT – near threatened, LC – least of concern. Sabah Wildlife Conservation Enactment (WCE) 1997 (2017 Amendment), Schedule 1 – totally protected animals, Schedule 2 – protected animals, Schedule 3 – game animals.

Plate 1. A group of pig-tailed macaques (*Macaca nemestrina*) and two individuals (circled in red) found standing on stacking pole along the planting line in Kolapis A, Sabah.

Plate 2. Damaged tree trunks of a Laran (*Neolamarckia cadamba*) in Kolapis A, Sabah.

Plate 3. Snapped shoots of the Talisai Paya (*Terminalia copelandii*) sapling.

Plate 4. Disturbed soil around a Talisai Paya (*Terminalia copelandii*) sapling.

Improving rooting efficiency through Multiple Rootstock Technology – A preliminary study in *Neolamarckia cadamba*

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Abstract. Multiple Rootstock Technology (MRT) is a concept of growing a plant with two or more rootstock from the same species. This technique is widely adopted in fruit trees such as durian, mango, rambutan and others to speed up the propagation or reproduction of desired varieties with the purpose to decrease mortality, promote growth uniformity and increase plant life span. In this study, we applied MRT to eleven Laran (*Neolamarckia cadamba*) seedlings that were raised in the nursery. The aim was to enhance the seedling's rooting efficiency using a threefold rootstocks method and a list of growth parameters (height, diameter, number of leaves & leaf area) was assessed. Parameters such as diameter (166.13%), leaf area (84.42%) and number of leaves (49.29%) showed a positive increase, whereas seedlings height (-12.35%) did not show any positive results. The results presented are preliminary and an extensive study is recommended.

Keywords: Multiple Rootstock Technology, threefold rootstocks, *Neolamarckia cadamba,* tree plantation

INTRODUCTION

The root system is the basic foundation of plants. The structure of a tree root is made up of a primary root, which is used for anchorage, while lateral roots are for the intake of water, nutrients and oxygen for respiration (Macdonald & Stevens 2019). Plant growth is dependent on its root development, the degree of development influences the time frame needed to reach the fruiting stage. Therefore, if a plant has a well-developed and extensive root system, its growth will be greatly accelerated. Through the years, various propagation practices were introduced to enhance the production and precocity of desired plants, at the same time to improve their nutrient uptake efficiency. One of the popular propagation practices is called Multiple Rootstock Technology (Dizon 2010).

Multiple Rootstock Technology (MRT), otherwise known as multi-root systems or multi-rooting architecture is a concept of growing a plant with two or more rootstocks from the same species at the intermediate stage of seedling development. The MRT is not a new technique, in fact it is an improvisation of grafting techniques that have been adopted centuries ago. Techniques related to rootstock grafting such as inarching (Asadullah & Khan 1960), bridge grafting (Samad *et al.* 1999), nurse grafting (Fan *et al.* 2008) and interstock grafting (Barascu *et al*. 2016) have been around for quite a while, nonetheless, MRT is considered superior over the usual single rootstock systems due to its impact on plant development, which sees an enhanced production of 300 percent (Epino 2004). On top of that, propagation of the desired variety is speed with a decrease in plant mortality, coupled with growth uniformity and increased life span, thereby enhancing and prolonging the productivity life span (Dizon 2010).

This technique is widely adopted in fruit trees such as durian, mango and rambutan for greater yield production. It has never been applied to forest plantations or other timber species due to the slow growth timber trees. However, when compared, the durian trees, which is a Light Hardwood has an air dried density of 420-865 kg/m3 (Wong & Lim 1990), which is comparable to Red Shorea that has an air dried density of 415-885 kg/m3 (Choo & Lim 1982). Therefore, it is interesting to see how plantation species would respond to the application of MRT.

In forest plantation establishment, one of the emphasis is the minimization of planting stress. Stress incurred during planting will affect plants' roots system establishment, root–soil contact, and root hydraulic conductivity. If the root system is disrupted, it will affect the plants' ability to supply water to transpiring leaves, maintenance of water balance and survivability (Grossnickle 2005). Stress is not the only factor affecting plant mortality in plantations, other factors such as environmental conditions, seedling quality and health at the time of planting also play a role in the success of seedling establishment (Rietveld 1989; Burdett 1990). Therefore, the seedling's response to its new environment and health of its root system plays an important role in its survival in the field.

The utilization of MRT to improve plantation seedlings root system efficiency is relatively new and an innovative idea to be considered in tree plantation. In this study, a MRT was applied to Laran seedlings (*Neolamarckia cadamba*). A threefold rootstocks method was used to enhance the rooting system. We expect the corresponding growth parameters will improve by 30 percent when compared to seedlings in the control setting. The objectives of this study were:

1. To determine the feasibility of using MRT using threefold rootstocks.
2. To assess growth parameters between treated and control groups.
3. To determine the improvement rates of treated seedlings over the control seedlings.

MATERIALS AND METHODS

Experimental site

This research was conducted from November 2020 to February 2021 in the Forest Research Centre nursery, Sepilok. During the experimental period, the reported mean temperature and rainfall for the site was 27°C and 452 mm, respectively (Figures 1 & 2). The highest mean precipitation was recorded in January 2021 with 723.3 mm, marking the peak of the rainy season during the study period. Temperature and rainfall data were obtained from *www.worldweatheronline.com.*

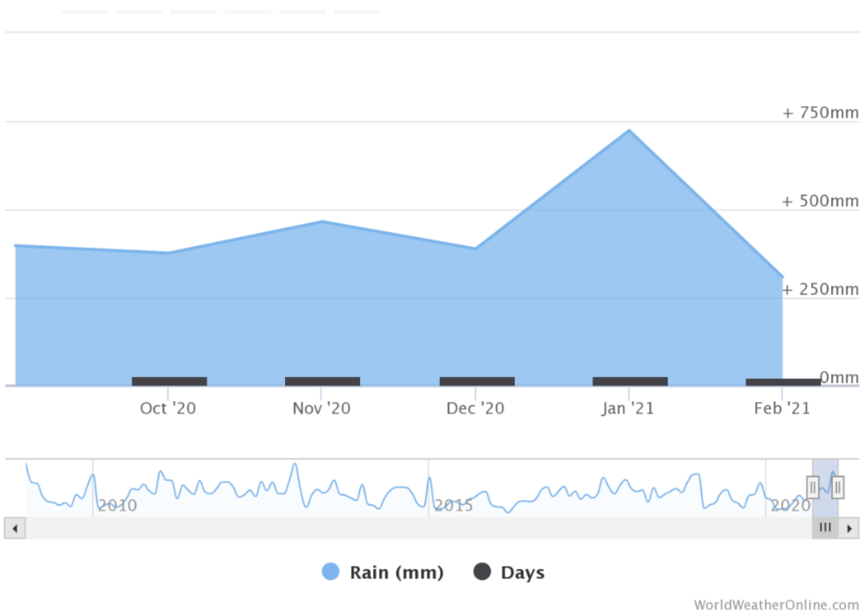

Figure 1. Average rainfall (mm) for Sandakan (Oct 2020 – Feb 2021).

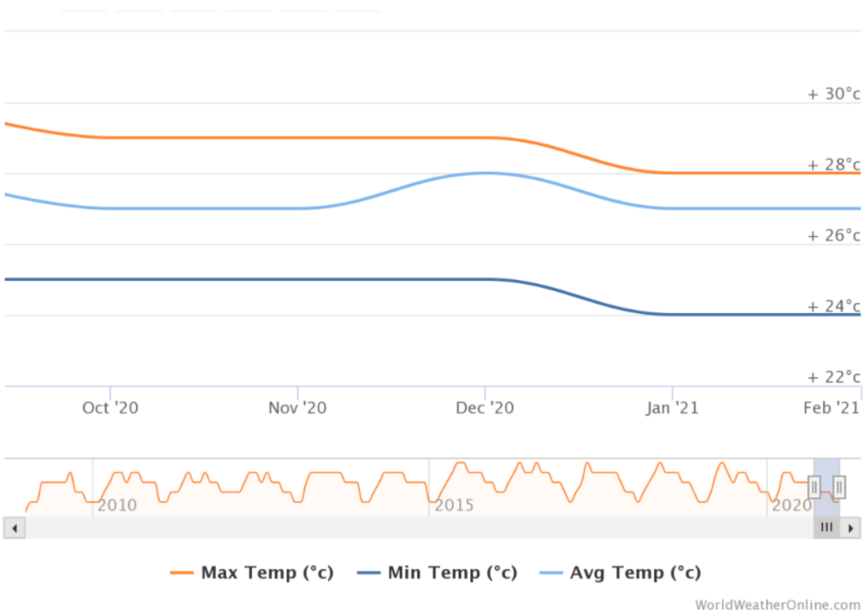

Figure 2. Average temperature for Sandakan (Oct 2020 – Feb 2021).

Planting materials

Eleven *Neolamarckia cadamba* seedlings of about a year old were selected as host plants, while another twenty-two seedlings (height > 50 cm) aged 6-months were used as rootstock intermediators. All seedlings were propagated from seeds collected from mother trees located in Lungmanis FR. and Bukit Piton FR. For comparison, another 11 seedlings from the same seedling lot were selected as control.

Grafting method using threefold rootstocks

In the experiment, both control and the seedling host with its pair of rootstock intermediators were planted in a perforated polythene bag of 36 x 45 cm containing garden soil and sawdust (ratio 3:1). To perform the MRT using threefold rootstocks, an inch of the host seedling bark at approximately 30 cm from the media base was peeled off to expose the cambium. The pair of the rootstock intermediators were then aligned to the seedling host's cambium and were sliced at a 30-degree slanting angle. The sliced rootstocks were then firmly fitted to the cambium host by tightly wrapping the joint-sections using parafilm to prevent fungal infection during the fusion process. The fusion site generally recovers in three weeks, however this process can be affected by climatic parameters, such as temperature and precipitation. Both treated and controlled seedlings were kept under shade. Monitoring is carried out to ensure the joint sections are constantly dry to ensure fusion success. Successful fusion between segments is indicated by the green colour inner stem, after small abrasion was applied to the surface of rootstock intermediators. This MRT with threefold rootstocks process can be found in Plate 1.

Data collection

Assessment of the growth parameters were done with digital calliper, measuring tape and cutter on day 1 and day 90. The dual assessments were performed to determine differences in growth parameters, between treated and control seedlings in which height, stem diameter and leaf area were measured. The latter was measured with the condition that if it was not defoliated. The leaf area was estimated on Day 90, using an android application, Leaf-IT (Schrader *et al.* 2017).

Statistical analysis

Box plots with 95% confidence interval (notches) and percentile graph were used to present the means of growth parameters between treated and control seedlings. Normality tests using Shapiro-Wilk and Anderson Darling were performed before differences in mean for growth parameters between groups were tested using T-test and Kolm-Smirnov. To enable statistical calculation, data on the number of leaves were transformed (data = n/100) due to negative mean differences of Day 90 and Day 1. All statistical analysis was performed using PAST 3.22 program (Hammer *et al.* 2001) and Sigmaplot version 14 (Systat software inc, 2017). Rate of improvement for each parameter were measured by dividing the final means of treated over control (base value) seedlings and multiplying by 100 (Simple Index Number 2008).

RESULTS AND DISCUSSION

Percentage of successful grafting

The success rate of treated seedling using threefold rootstocks changes over time. During the first and second month (Day 30 – 60), all the treated seedlings were observed to be 100% successful without broken or rotten rootstock intermediators to its seedling host. In the following month (Day 90), two seedling hosts were recorded to have one rotten rootstock and two rotten rootstocks. Based on the growth parameters (Day 90) data, none of these seedling hosts showed deviation from the other seedlings within the group. The final percentage of successful MRT grafting was calculated on Day 120 and it was a 45% success rate, in which the fusion site is still in good intact condition between that of rootstocks and its seedling host. The remaining 55% of the seedling hosts were having either one or two rootstocks that were not functional in improving the rooting system.

Figure 3. A seedling host with two functional rootstocks after 120 days.

Range of seedling performance up to 90 days

The seedling performance for both treated and control from Day 1 to 90 is presented in Table 1. The height of treated seedlings ranged from 103.81 cm to 114.14 cm, while a much lower height range than the control seedlings (117.89 cm to 129.67 cm). The diameter for treated seedlings ranged from 8.85 mm to 11.85 mm, while a lower range was recorded for control seedlings (9.03 mm to 10.15 mm). A significant drop in the range of the number of leaves of the control seedlings (8.18 -5.09) was detected in comparison to the treated seedlings whose proportionality ranged from 6.73 to 7.55. Unhealthy and defoliated leaves were the reason for the difference. This corresponded to the percentile graph on leaf area over the control seedling (Figure 4) which shows marginal differences in leaf sizes between the groups. The bigger the leaf area measured in size, the healthier the seedling performed. The comparison of the growth parameters is illustrated by Box-plot with notches in Figure 5.

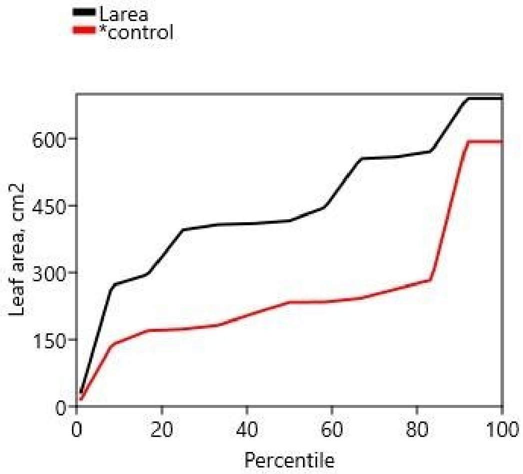

Figure 4. Percentile graph of leaf area for treated seedlings (black line) and control (red line).

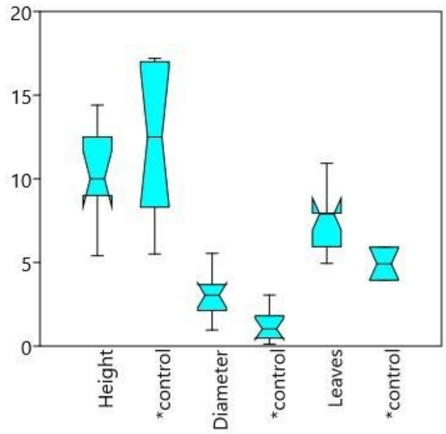

Figure 5. Box-plot with notches of growth parameters over control. *

Comparing seedlings performance (treated vs. control)

Statistical analysis using t-test shows significant difference between the groups was observed in diameter but not in height (height, t = 0.975, p = 0.34; diameter, t = 3.7219, p = 0.0013; Table 2.B), whereas the non-parametric statistical analysis using Kolm-Smirnov shows significant difference between the groups were observed in leaves number and leaf area (Leaves, D = 0.909, p = 0.001; Leaf area, D = 0.8182, p = 0.001; Table 2.B). The results showed that the technique applied to treated seedling was significant compared to the control seedling. Of all the measured growth parameters, only the seedling height showed no differences after using MRT through threefold rootstocks.

Improvement rate over control

Based on Table 2.C, the highest improvement rate was recorded for seedling diameter with 166.13%, followed by leaf area and leaf number with 84.42% and 49.29%, respectively. However, negative improvement rate was recorded for seedling height, (Height = -12.35%). The pooled index of growth parameters recorded an increase of 79% in improvement rate over the control seedlings.

ISSUES AND RECOMMENDATION

Success rate in MRT

The success rate of MRT during the first two months showed positive interaction between the host plant and its rootstock intermediators. The major challenge in setting up the MRT seedlings was the heavy precipitation experienced during the months of December 2020 to January 2021 (Figure 1). The prolonged wet condition could be the probable cause that resulted in the continual dampness observed at the joint-sections between the host plant and rootstocks intermediators. Moisture that is trapped within the fusion area could induce microbial activity and subsequently result in the decay of the rootstocks (Plate 2). A few of the seedling hosts recorded either one or two rotten rootstocks during the third month (Day 90), however it did not stunt the seedling hosts' overall growth performance.

We observed that the treated seedlings with threefold rootstocks grew vigorously in the beginning with clear positive impact on the growth parameters. This could be the result of rapid root system development. In contrast, there was no significant difference observed on the host plant in which the fused rootstocks had deteriorated (Day 90 to Day 120). To be specific, MRT with threefold rootstocks is basically a system for improving nutrient uptake efficiency, as well as to stimulate host plant growth as demonstrated by the results obtained on growth parameters of the treated *Neolamarckia cadamba*.

Another advantage of this system is the enhancement of the seedlings' sturdiness to withstand environmental pressure such as windy condition. Most of the tall seedlings from the control group were observed to have gyrating issues due to the small and thin stem (Plate 3). With the application of threefold rootstocks, seedlings become sturdier by the tripod anchorage and larger stem. The plantation programme can consider planting seedlings with the threefold rootstocks system in order to mitigate gyrating issues and to lessen seedling stress experienced in the field. All the seedlings that were used in this study were planted out at Gum Gum FR at a spacing of 6 m x 6 m (Plate 4). Pending on the results obtained from the MRT seedlings planting trial, there is a possibility to de-escalate the usage of chemical fertilizer commonly practiced in tree plantations. The excessive application of chemical fertilizer may cause environmental pollution, which in turn will have an impact on human health (Savci 2012).

The study is a preliminary attempt of the MRT technique. For future research, there is a need to expand the study sample size to ensure data reliability and, to continue a field testing to observe the overall improvement rate over control in a single rotation.

CONCLUSIONS

Based on the results of this preliminary study, we conclude that the application of MRT with threefold rootstocks can enhance the growth parameters of the seedlings. Based on the pooled index of growth parameters (Table 2.C), the growth parameters surpassed the expected 30% improvement rate, with a recorded increase of 79% improvement rate. Further extensive study on MRT and its application on other plantation species will shed more light on the feasibility of applying this technology in the plantation field.

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Table 1. Recorded growth parameters for treated (S1, S2) and control (C1, C2) on Day 1 and Day 90. * Recorded data on Day 90. Std dev = standard deviation.

Seedling no.	**Height, cm**				**Diameter, mm**				**No. of leaves**				***Leaf area, cm^2***	
	Day1	Day90	Day1	Day90	Day1	Day90	Day1	Day90	Day1	Day90	Day1	Day90	Day90	Day90
	S1	S2	C1	C2	S1	S2	C1	C2	S1	S2	C1	C2	S2	C2
1	125	137.5	95	102	8.91	14.45	9.75	10	5	10	8	5	690.058	138.178
2	105.8	115.2	99	104.5	6.62	11.5	7.77	9.58	6	7	6	4	271.104	242.5
3	107	114.6	117	129.5	9.88	12.01	9.12	9.65	6	6	9	6	295.379	169.997
4	102.6	112.5	143	160	10.92	13.96	8.12	8.73	10	8	8	5	572.03	182.324
5	125.2	135.4	109	122.4	7.82	11.45	8.73	10.62	10	8	8	5	416.36	263.206
6	94	107.8	119.2	127.5	8.93	10.24	7.88	8.94	6	8	8	4	395.823	233.354
7	54	64	111.2	119.8	7.65	9.84	8.03	9.57	6	6	6	4	407.932	233.969
8	103.6	115	108.4	121.3	8.6	12.28	9.58	10.06	5	8	10	6	555.345	283.45
9	100.1	109.1	134	151.2	8.87	9.83	10.5	11.53	6	6	10	5	559.259	208.514
10	98	103.4	129	139.2	10.24	12.48	9.69	9.81	6	5	9	6	445.81	173.42
11	126.6	141	132	149	8.94	12.26	10.11	13.16	8	11	8	6	410.282	592.78
mean	103.81	114.14	117.89	129.67	8.85	11.85	9.03	10.15	6.73	7.55	8.18	5.09	456.31	247.43
std dev	19.23	20.04	14.49	17.77	1.16	1.45	0.93	1.20	1.71	1.72	1.27	0.79	120.02	116.92

Table 2. Data analysis on growth parameters, statistical analysis between groups and improvement rates over control.
Note = * data transformed to n / 100, ** Recorded data on Day 90; [a] normal [b] not normal (Normality test using *Shapiro-wilk,* W & *Anderson Darling*, A)

A. Growth parameters	Treated		Control	
	Day_1	Day_90	Day_1	Day_90
Mean of height, cm	103.81 (19.23)	114.14 (20.04)	117.89 (14.49)	129.67 (17.77)
Mean of diameter, mm	8.85 (1.16)	11.85 (1.45)	9.03 (0.93)	10.15 (1.20)
*Mean of leaves number, n	6.73 (1.71)	7.55 (1.72)	8.18 (1.27)	5.09 (0.79)

B. Differences in mean of parameters between group, Day 90 - Day 1	Treated	Control	[a] T-test	[b] Kolm-Smirnov
Height, cm	10.33 (2.63)	11.78 (4.19)	t = 0.975, p = 0.341	
Diameter, mm	2.99 (1.41)	1.12 (0.88)	t = 3.7219, p = 0.0013	
* Leaves number, n	7.48 (1.80)	5.01 (0.82)		D = 0.909, p = 0.001
** Leaf area, cm^2	456.31 (120.02)	247.43 (116.92)		D = 0.8182, p = 0.001

C. Improvement rate over control seedlings	Treated / Control	Index number	Improvement rate
Height, cm	0.88	88	-12.3%
Diameter, mm	2.66	266	166.1%
Leaves number, *n*	1.49	149	49.3%
Leaf area, cm^2	1.84	184	84.4%
Pooled index of growth parameters	1.79	179	79%

Plate 1. Multiple Rootstock Technology with three rootstocks (1-8).

Plate 2. A seedling host observed with one rotten rootstock (*left*) while another host plant observed with two malfunctioned rootstock intermediators (*right*).

Plate 3. Treated (*left*) and control (*right*) seedlings after 90 days.

Plate 4. Planting out experimental seedlings of *Neolamarckia cadamba* in Gum Gum FR. (01/07/2021).

Plate 5. Best performer of treated *Neolamarckia cadamba* seedling (GL15) (*left*) after 19 weeks planted in Gum Gum FR, measuring 2.0 cm DBH and 2.77 m in height. Fusion of MRT on treated seedling (GL15) remained intact (*right*).
(Pictures by Eldy M. Adland on 12/11/2021).

Insect defoliators on dipterocarp seedlings in a forest rehabilitation site in Lentang Forest Reserve, Pahang

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In Peninsular Malaysia, Dipterocarpaceae forms the most dominant stands from the lowland to the hill dipterocarp forests and, to a lesser extent, in the upper dipterocarp forests (Symington, 1943). Dipterocarps are highly sought-after timber and the increasing timber harvesting has led to the depletion of these resources in the natural forests. Forest restoration and rehabilitation efforts are therefore important for the recovery of forest resources, biological diversity and ecosystem functions in a degraded forest.

Lentang Forest Reserve, at an elevation of 500–600 m above sea level, is one of the sites selected for the "Restoration, Reclamation and Rehabilitation of Degraded Forests" project undertaken by the Forestry Department of Peninsular Malaysia. The rehabilitated area comprises 100 ha of a mixture of high value dipterocarp species, such as Meranti Tembaga (*Shorea leprosula*), Meranti Bukit (*S. platyclados*), Meranti Sarang Punai (*S. parvifolia*), Meranti Rambai Daun (*S. acuminata*), Balau (*Shorea* spp.) and Keruing (*Dipterocarpus* spp.) (Figure 1).

A total of 120 saplings in four planting blocks were sampled randomly in March 2020 in this survey of defoliators. Moth larvae collected from the leaves of the seedlings were reared in the laboratory until the emergence of the adult moths for identification. Five species of moths belonging to two families were recorded (Table 1). All are new records, based on Robinson *et al.* (2010) and through internet search. The low occurrence of the insect defoliators could be associated with the diversity and interactions between the plants, insect pests and natural enemies, including seasonality, thus more research in these aspects is needed. Given the low level of infestation, no control is necessary. Some notes on the moth species are given below.

Table 1. Moth species collected from the dipterocarp saplings in Lentang Forest Reserve. The specimens were deposited in the FRIM Entomological Reference Collection.

Family	Species	Host plant	No. of individuals collected
Lymantriidae	*Lymantria marginalis*	*Shorea leprosula*	1
	Orgyia osseata	*Shorea leprosula*	1
	Ilema nr. *baruna*	*Shorea leprosula*	1
	Arctornis sp.	*Shorea leprosula*	1
Limacodidae	*Thosea vetusta*	*Dipterocarpus* sp.	2

NOTES ON THE MOTH SPECIES

Lymantria marginalis

The hairy larva of *Lymantria marginalis* has a mottled, brownish orange head and body with a dorsal whitish patch near the centre of its body, and prominent blue circles on each abdominal segment (Figure 2). When the larva was about to pupate, it constructed flimsy silky webs on the surface of the leaves. Clumps of spiny hairs were incorporated on the pupal skin to provide protection to the otherwise exposed pupa (Figure 3). The pupal stage lasted 7 days and the emerged male adult has whitish wings, in which its forewings are marked with black spots and light wavy bands (Figure 4). Other host plants that have been reported were *Eucalyptus* (Holloway, 1999) and, *S. macrophylla* and *Mangifera indica* (Robinson *et al.* 2010).

Orgyia osseata

The larva of *Orgyia osseata* has a reddish head and dull body covered with pinkish spiny setae, and four yellow dorsal brushes with a black patch (Figure 5). During pre-pupation, the larva shed its larval hairs to construct a cocoon for its pupal stage that lasted for 6 days (Figure 6). The female moth has a stout body and is dull brown with black markings on its forewings (Figure 7). *Orgyia osseata* also feeds on *S. parvifolia* and *S. symingtonii*, and a wide range of host plants that include ornamental plants and agricultural crops (Robinson *et al.* 2010, Chung *et al.* 2014).

Ilema nr. *baruna*

The larva of *Ilema* nr. *baruna* shows striking display of colours as it matures (Figures 8–9). Its black head is protected by dense hair pencils at the sides and white setae anterior to its head. At the posterior end of its body, there are tufts of yellow setae and a fan-like hair pencil. Four orange tufts of hairs arose on the first four abdominal segments and are flanked by four pairs of black and white setae. Its body is also covered with long setae with white tips (Figure 10). The larva pupated among several folded leaves and pupation lasted for 8 days before the adult moth emerged. The female moth has a stout body and green forewings marked with dark, wavy lines (Figure 11).

***Arctornis* sp.**

The *Arctornis* larva has an orange head and a brownish body speckled with white and yellowish spots (Figure 12). Four tufts of bushy setae can be observed on each of the first two abdominal segments and two parallel white lines on the mid-dorsal of its abdomen. There are also multiple setae at the top and sides of its body. The pupa of *Arctornis* resembled a butterfly chrysalis, as it was hanging on the underside of a leaf, supported only by silk threads (Figure 13). The adult moth emerged 4 days later (Figure 14) and laid 62 unfertilised eggs in the rearing box (Figure 15).

Thosea vetusta

The nettle larva of *Thosea vetusta* has a gelatin-like body with its head concealed beneath its body (Figure 16). The green body of the young larva is well camouflaged against the big leaf of a keruing seedling where the larva was resting on. It has a white band with blue borders along the mid-dorsal of its body and two prominent orange spots at the centre of the band. The larva is protected by spiny setae radiating from the sides of its body. The adult moth could not be obtained as rearing of the larvae in the laboratory were unsuccessful. This species has been recorded on agricultural crops, such as *Camellia sinensis*, *Elaeis guineensis*, *Cocos nucifera* and *Theobroma cacao* (Robinson *et al.* 2010), as well as forestry species, such as *Neolamarckia cadamba* (Chung *et al.* 2009) and *Avicennia alba* (Chung & Tangah 2013).

ACKNOWLEDGEMENTS

We would like to thank Dr. Arthur Chung for the confirmation of moth species and foresters from the Bentong District Forestry Office for their assistance. This survey was supported by the Forestry Department Peninsular Malaysia through the Restoration, Rehabilitation and Reclamation of Degraded Forests and Forest Health Surveillance Projects under the 11th Malaysia Plan.

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Figure 1. Saplings in one of the planting blocks in Lentang Forest Reserve. Weeds along the planting strips were removed.

Figure 2. Larva of *Lymantria marginalis* measuring about 1.8 cm.

Figure 3. The pupa of *Lymantria marginalis* is covered with setae and flimsy silk webs that hold the pieces of leaves together.

Figure 4. A male specimen of *Lymantria marginalis* with a wingspan of 3.1 cm.

Figure 5. The larva of *Orgyia osseata* has a pale body with pinkish setae and four yellow dorsal brushes, a typical characteristic of a tussock moth larva in the tribe Orgyiini.

Figure 6. The pupa of *Orgyia osseata* enclosed within a cocoon.

Figure 7. The adult female of *Orgyia osseata* with a wingspan of 4 cm.

Figure 8. A young *Ilema* larva about 1 cm in length collected from a *S. leprosula* seedling.

Figure 9. A mature *Ilema* larva about 5 cm in length with striking colouration and long setae.

Figure 10. Close-up of the fine barbs at the end of each seta.

Figure 11. An adult female of *Ilema* nr. *baruna* with a wingspan of 4.8 cm.

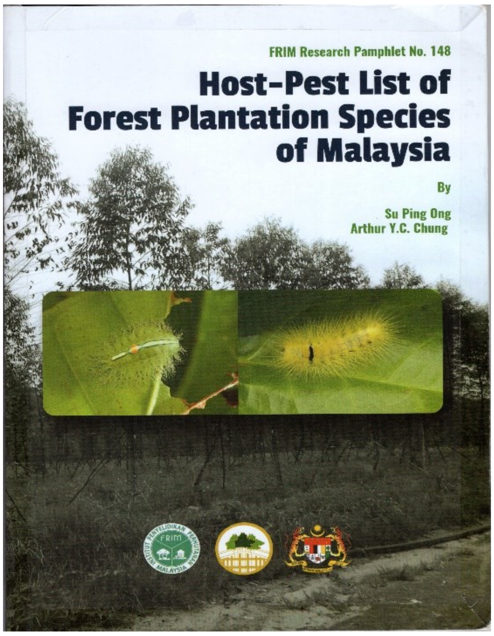
Figure 12. The mature larva of *Arctornis* sp. feeding on *S. leprosula* leaves.

Figure 13. The pupa is suspended by silk threads on the underside of a leaf.

Figure 14. An adult female has translucent wings with greenish tinge and dark triangular spots at the margins of the forewings. Its wingspan measures 2.8 cm.

Figure 15. The eggs of *Arctornis* sp. are yellow with a concave surface.

Figure 16. A young larva of *Thosea vetusta.*



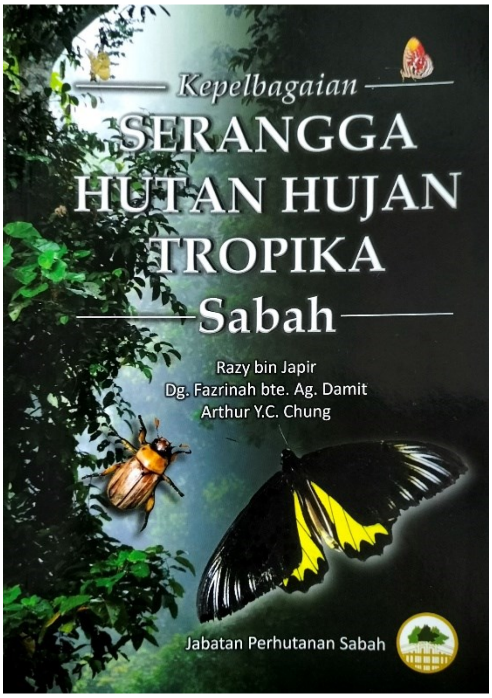
Host-Pest List of Forest Plantation Species of Malaysia. FRIM Research Pamphlet No. 148. By Su Ping Ong and Arthur Y. C. Chung. Published by Forest Research Institute Malaysia, Sabah Forestry Department & Ministry of Energy and Natural Resources, 2020. Pp. 74. ISBN 978-967-2149-88-0.

Reviewed by V. S. Guanih

Since the initiation of the forest plantation scheme in 2003, apart from investment and the provision of soft loans, research and development have been conducted on the different aspects of forest plantation. The latter is essential due to the initial high cost involved in the establishment of plantations; one such aspect is the identification and documentation of known pests and diseases that can greatly affect plantation productivity. This book covers a total of 12 genera of commercial forest plantation trees namely rubber trees, Acacia, Teak, Mahogany, Binuang and a few others. Of these, 19 species that are popular species in forest plantations have been highlighted and their common insect pests are listed. Information on types of damage on each species in relation with pests is listed and some pictures were provided to show damages by pests. It is a useful guide for plantation owners to be aware and find solutions to either prevent or control the damage.

The only limitation of this book is lack of illustrations and pictures showing tree damages and pests. It would be helpful if the book had included information on how to control or measure these attacks.

Nevertheless, the latest Host-Pest List of Forest Plantation Species of Malaysia is very suitable and useful for the different stages of the plantation industry: research, establishment, management, processing and export. This publication is recommended for plantation managers, timber traders, international border guard officers, plant biosecurity and quarantine officers, forestry staff, researchers and students.



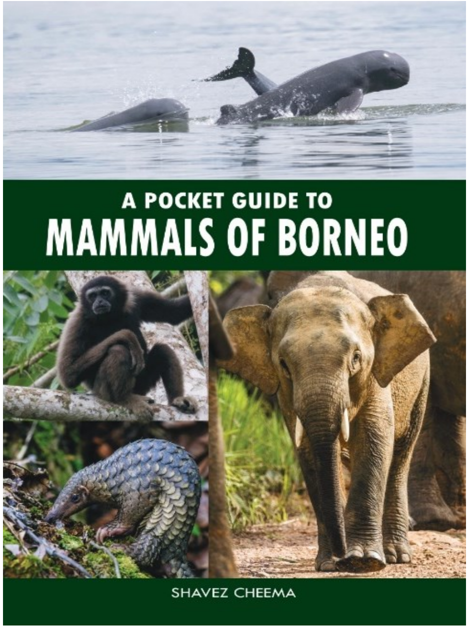
Kepelbagaian Serangga Hutan Hujan Tropika Sabah by Razy bin Japir, Dg. Fazrinah bte. Awg Damit & Arthur Y.C. Chung. Published by Sabah Forestry Department, Sabah, 2021. Pp. 116. ISBN 978-967-0180-23-6.

Reviewed by Jesselyn binti Kijin

This book provides an overview of insects in Sabah and it can enhance environmental awareness on the importance of forest conservation and rehabilitation. The book begins with a brief description of insect anatomy and its habitat. The importance of insects is also elaborated in the book, emphasizing on the benefits of insects, such as insects as pollinators and decomposers. The book is informative as the authors have gathered information for at least 80 species from 15 groups of insects. Each species has its common name and scientific name listed, and the authors provided interesting facts which make it easier to remember a particular insect's species name and characteristics. The authors has also included attractive pictures in each insect description that were captured during their fieldwork, particularly during insect surveys under the Heart of Borneo Initiative. In the last chapter of the book, the authors explain the common threats of insects and conservation efforts are also exemplified to give readers information on how insects are conserved and protected.

I highly recommend the book to anyone especially to those who are interested in insects. The book is written in Bahasa Melayu and using layman terms, hence, it is suitable for public reading, especially for students from primary to secondary schools. For those who want direct access to the book, a digital copy is available for free and it can be downloaded from Research Gate.

Personally, I am very intrigued by the way the authors presented the information. I believe the book can stimulate an individual's interest in entomology. Although not all species are included, I am certain that the book is able to provide readers basic information on insects in the rainforests of Sabah. It can also be used by tourist guides as they can simply refer to this easy-to-read book and help promote the beauty of insects in Sabah. In research, the book can be a quick reference for researchers and conservationists.



A Pocket Guide to Mammals of Borneo by Shavez Cheema. Edited by Allister Latan. Published by Onestop Borneo Wildlife Tours & Travel Sdn. Bhd., 2020. Pp 79. ISBN 978-967-18407-0-2.

Reviewed by Viviannye Paul

This photographic guide covers over 185 out of more than 240 mammal species in Borneo. The readers will be captivated with the wild cat and civet species, 63 bats and 48 rodent species as well as marine mammals in a pictorial form. Each mammal is beautifully captured where a tick box consisting of the species name, location and ecology of the illustrated mammal is provided to ease reader in identifying the animal. The author has also highlighted several recommended sites for mammal watching in Borneo.

I find the guide book enjoyable to browse through, particularly the photographs of mammals featured as they remind me of some of my most memorable encounters with animals in the forest, for instance, the pygmy elephant, bearded pig and sambar deer. Seeing most of Borneo's mammals together in one publication is truly astounding. It allows me to discover numerous species that I have never even heard of before, like the five species of dolphins from the family Delphinidae and abundance of little-known scarce species like a finless porpoise (*Neophocaena phocaenoides*). I am also fascinated to learn that there have been quite a few splits in some well-known groups, especially Rodentia, such as the flying and ground squirrels, rats, mouses and porcupines. The checklist also reminds me of the scarce and secretive mammals that I have sadly failed to see despite spending significant time in their habitats, such as slow loris and tarsier. Reading this book has reignited my interest in these animals, which I have always wanted to see.

There are two minor comments on this book. Firstly, the tick box containing description of family and species name, location and ecology of each mammal should be put on the photos of the mammal, instead of putting it separately on the following page. This will ease readers to learn about the mammal right away without the trouble of flipping the next page for its information. Secondly, the *IUCN Red List Conservation Status* for each mammal can be highlighted to show how threatened the species is.

This book is ideal for people with an interest in mammals. It is a must-have for anyone who enjoys reading about animals. This handy pocket guide is most suitable for scientists, mammals-watchers, photographers, students and nature-lovers when they are in the field.

"Animals have heart that feel, eyes that see, and families to care for, just like you and me." ~ Anthony Douglas William

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