

BIODIVERSITY ASSESSMENT AND MONITORING PROTOCOL IN THE CENTRAL FOREST SPINE LANDSCAPE

Editor

Chua, L. S. L.

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2021

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Foreword

National Policy on Biological Diversity (2016-2025), Target 8 has sets the national agenda and direction for the country to formulate and implement strategies for the conservation of important terrestrial ecological corridors. As foresight, the country had formulated the Central Forest Spine Master Plan (CFSMP) in 2010 and this implementation of this Plan is one of the several major initiatives that is crucial to the achievement of this Target. The main aim of this initiative is to address forest fragmentation by creating ecological corridors in Peninsular Malaysia.

This project "Improving Connectivity in the Central Forest Spine Landscape (IC-CFS)" supports the CFSMP initiative by strengthening the institutional and operational structures and piloting of sustainable forest landscape management plans in three tiger-priority landscapes, including exploring and diversifying funding resources for conservation.

This project is focused at three forest corridors namely CFS2 PL3 Panti Forest Reserve-Ulu Sedili Forest Reserve, Johor; CFS1 PL1 Sg. Yu Forest Reserve-Tanum Forest Reserve, Pahang and CFS1 PL2 Royal Belum State Park-Temengor Forest Reserve, Perak. As a component under the planning, compliance monitoring and enforcement sub-framework, a set of tools for monitoring biodiversity is needed to ensure changes that take place within the respective functional groups are systematically monitored.

A universal set of biodiversity monitoring tools would be valuable to capture data and understand ecological change that occurs in the rapid modification of forest landscapes of Malaysia. It is my sincere hope that these methodologies will be applied beyond the three targeted states of the IC-CFS project to encompass the remaining five states that form the CFS.

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Sincere thanks are due to the State Forest Department and Department of Wildlife and National Parks of the states of Perak, Pahang and Johor for granting permission to work in the forest areas under their jurisdiction. We are also grateful to the relevant District Forest Offices, Rangers' Offices and Wildlife District Offices who provided assistance and logistic support during field trips. We gratefully acknowledge the assistance provided by Forest Department of Peninsular Malaysia, as the project implementing agency.

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The protocol could not have materialised without the excellent and coordinated effort from the support staff of the Forest Biodiversity Division of FRIM, Wildlife Conservation Division of PERHILITAN (Headquarters), and PERHILITAN from the states of Perak, Pahang and Johor. The team from FRIM's Forest Biodiversity Division comprises staff members of the Flora, Conservation & Biodiversity Informatics, Entomology, and Zoology Units. Several interns from various universities also assisted in the field and sample processing.

We are grateful to the anonymous reviewers for their constructive comments and suggested improvements.

Executive Summary

The introduction chapter described the policies that relate to the Central Forest Spine (CFS) initiative in Peninsular Malaysia and the importance of the initiative. It also outlined the support provided by the Global Environment Facility and United Nations Development Programme to strengthen the CFS implementation through a joint project entitled "Improving Connectivity in the Central Forest Spine Landscape (IC-CFS)" which involves the states of Pahang, Perak and Johor. This monitoring protocol fulfils one of the three goals of the project. The purpose of this monitoring protocol and the eight taxon groups that were worked with were detailed in this chapter.

The chapter on sampling design described the protocols for selecting and establishing sample locations. The four main steps in selecting sample sites were: (1) Define the study area and its extent on a map; (2) Overlay a systematic grid of a specific size; (3) Select a predetermined number of sample locations from this grid based on a probability selection mechanism and a set of rejection criteria; and (4) Check these selected locations in the field against the rejection criteria.

The chapter on the inventory of forest taxa presented the methodology for sampling and observing specific genera, species, or subpopulation of each taxon at the sample plots described in Chapter 2. Information on the handling of specimens or sampled individuals and procedures for identification were described in detail in this chapter. This chapter is supported by a selected list of references.

This chapter on statistical analysis briefly described the statistical approaches to analyse the vast amount of data gathered. The choices of statistical approaches respond to three broad research questions: (1) Do the diversity of a taxon group differ between forest reserves and corridors? (2) How is diversity of a taxon group associated with the habitat conditions in the reserves and corridors? and (3) How are the diversity and habitat conditions related among different taxon groups.

Chapter 1.0

Introduction

1.0 Introduction
By Chua, L.S.L.

Policy 23 of the Second National Physical Plan (Anon 2010) states that "A Central Forest Spine (CFS) shall be established to form the backbone of the Environmentally Sensitive Area network". Measure 2 of the Policy further advocates that *Pelan Induk CFS perlu dilaksanakan untuk mewujudkan rangkaian ekologi bagi menghubungkan semula kompleks-kompleks hutan yang penting di Semenanjung Malaysia*. In line with this, a Central Forest Spine Master Plan (CFSMP) was prepared in 2009 (Anon 2009). The need for the CFSMP is timely in view of Malaysia's goal of conserving biodiversity and ecosystems during a period of rapid economic growth.

The four-major forest complexes encompassing the CFS in the Peninsular Malaysia are: (1) Titiwangsa–Bintang–Nakawan Ranges, (2) Taman Negara–Timur Range, (3) Southeast Pahang–Lake Chini–Lake Bera, and (4) Endau–Rompin–Kluang Wildlife Reserve. These four forest complexes are critical natural landscapes, which provide more than 90% of the water supply for the peninsula, hold more than 85% of the peninsula's carbon biomass, and provide critical habitat to diverse plant and animal species including the remaining populations of Malayan tigers, elephants, and other large mammals. For these reasons, the goal of CFSMP is to increase the integrity of the CFS through conserving and rehabilitating critical linkages between these forest complexes.

In support of this ambitious and extremely important national physical plan, a joint project funded by Global Environment Facility, United Nations Development Programme, and Government of Malaysia (GEF-UNDP-GoM) with the title "Improving Connectivity in the Central Forest Spine (IC-CFS)" was approved by the states of Pahang, Perak, and Johor in 2014. This project's overall goal is to improve habitat connectivity in the CFS and to ensure sustainable forest management in the identified landscapes with the following specific goals:

1. Strengthen institutional capacity of the federal and state governments, and other relevant agencies to implement the CFSMP and the National Tiger Conservation Action Plan in these landscapes so that connectivity between forest complexes can be enhanced and law enforcement against wildlife and forestry crime can be intensified,
2. Enhance current land management plans in the identified landscapes to ensure that biodiversity and ecosystem service values are accounted for and that all planned land uses are sustainable, and
3. Establish a sustainable financing mechanism for the conservation of the CFS in order to better mainstream biodiversity into national development plans.

To achieve the above goals, in particular #2, data and knowledge on biodiversity richness and their changes are required and must be acquired through a sufficient period of time in the identified landscapes. The biodiversity studies should take on a long-term perspective. Hence, an appropriate approach for data collection, monitoring, and evaluation needs to be developed.

This report provides inventory protocols for eight forest groups: ant, butterfly, dung beetle, herbaceous plants, herpetofauna, large mammals, small mammals, and trees. The intention of this report is to provide sufficient information for replicating the inventory protocol in the future, to serve as a permanent reference of survey methodology for any further scientific publication, and to build capacity of personnel of other agencies. The report is separated into two major sections. The first section is on sampling design, documenting the protocols for establishing sample locations at the three study sites. The second section documents the protocols for establishing observation units and methodologies for the eight forest groups. The inventory protocol described in this report is implemented in the three states targeted in the IC-CFS project. However, the CFSMP comprises eight states in the Peninsular Malaysia with the remaining states being Kelantan, Kedah, Negeri Sembilan, Selangor, and Terengganu.



Chapter 2.0

Sampling Design

2.0 Sampling Design
By Lam, T.Y., Yong, W.S.Y. & Siti-Fariezza, K.T.

This section documents the probability-based process of selecting sample locations to observe the eight forest groups on the ground and to collect relevant information for analyses. The steps described in detail are as follows. First, define a study area and its extent on a map. Next, lay out a systematic grid of a specific size over the map. Then, select a predetermined number of sample locations from this grid based on a probability selection mechanism and a set of rejection criteria. Lastly, check these selected locations in the field against the rejection criteria. Once determined, apply the protocol for collecting information for each forest group in Section 3. As an example, we describe the process of selecting sample locations for the Pahang Primary Link (PL) study area in detail. In addition, the final selected sample locations for the Johor Primary Link study area are available in Appendix A1.

2.1 Study Area and Extent

At first, agree on a study area and its extent between parties and delineate the area. The study area encompasses a corridor and one or more forest reserves. The choice of a corridor could be either a primary or a secondary linkage. The chosen forest reserves are in close proximity to the corridor so that it would reflect to a certain degree the conditions of the studied corridor prior to its current disturbed states. The forest reserves could be either a permanent forest reserve or a totally protected forest. The extent of the study area is decided based on factors such as logistics, finance, time, and human resources, but preferably, it is a few km^2 in area. Nonetheless, the study area can be expanded in the future when additional resources become available. Once the study area and its extent are confirmed, acquire a digital boundary map depicting the corridor and the forest reserves, and any other relevant maps to assist the selection of sample locations.

2.2 Systematic Grid

Overlay a systematic grid of 500 × 500 m on the boundary map covering the extent of the study area. To ascertain that the systematic grid is laid out with some degree of randomness, follow the procedures of Random Point Start (RPS, Iles 2003) method. For RPS, randomly select a starting point within the extent of the study area. This is done by generating a random x- and y-coordinate. As a matter of fact, the starting point could be selected from any location outside the study area if it is randomly selected. Once the random point is selected, expand the systematic grid from this point at an interval of 500 x 500 m. Orient the systematic grid such that an axis of the grid is parallel with the longest side of the corridor. Although the grid orientation is not randomized, its purpose is to maximize coverage of the grid over the corridor so that an adequate number of sample locations is available for selection. Furthermore, a corridor is generally smaller in area than a forest reserve; thus, this grid arrangement benefits sampling in the corridor.

2.3 Selection of Sample Locations

Given availability of resources in the GEF-UNDP-GoM IC-CFS project, 12 sample locations are selected within the study area. The decision of 12 sample locations is based on lengthy discussion during the project planning phase after critically evaluating available resources. However, if more resources such as financial and human capital are available, establishing more sample locations is recommended to increase the sample size, which is advantageous to statistical analysis. Of the 12 sample locations, 6 are in corridor, and 6 are in forest reserves. The sample locations are arranged in pairs resulting in a total of 6 pairs of sample locations. Each pair consists of a sample location in the corridor and another sample location in the forest reserves. Prior to selection of

sample locations, gather all available maps including forest reserve boundary, elevation, satellite images, road network, water body network, and infrastructures etc. These maps are essential for decision making during the selection process.

First, randomly select 6 sample locations in the corridor from all available grid points of the 500 × 500 m grid within the corridor. However, reject a randomly selected sample location if,

(1) it is on a paved primary forest road,
(2) it is in the middle of a water body including stream, river, pond, and lake,
(3) it is within 50 m from human-made infrastructures such as road and power transmission lines, and
(4) it is on a slope more than 45° due to operational difficulty.

The process continues until all 6 sample locations are selected in the corridor based on features from the available maps. Once selection completes, select 6 sample locations in the forest reserves on the same gridlines with the 6 selected sample locations in the corridor. The 6 sample locations in the forest reserves are at a distance ≥ 1 km away from the 6 sample locations in the corridor. While a distance of ≥ 1 km is desired, a certain degree of flexibility in the distance between a pair of sample locations is allowed to account for variable corridor sizes at different study areas. Check the 6 sample locations in the forest reserve against the rejection criteria. If rejected, increase the distance. The 12 sample locations are the preliminary list of sample locations to be checked on the ground. The remaining unselected grid points in the corridor and their corresponding points in the forest reserves are the first-level backups.

For the 12 preliminary sample locations, carry out field survey to determine their ground conditions. Check each pair of sample locations one by one. If one pair meets the above rejection criteria, reject it and replace it by a pair randomly selected from the first-level backups. Survey the selected backup pair on the ground for its suitability.

In the case that all first-level backups are exhausted without finding a replacement, subdivide the 500 × 500 m grid into 250 × 250 m grid. Grid points on the 250 × 250 m grid surrounding a rejected preliminary sample location in the corridor are the second-level backups. Randomly order these second-level backup grid points and identify their matching pairs in the forest reserve. Sequentially check the randomly ordered pairs on the maps and on the ground against the rejection criteria until an acceptable replacement pair is found.

The final output is a list of 6 pairs of sample locations with one in the corridor and one in the forest reserve. Thus, a total of 12 sample locations is finalized. Establish ground observation units and collect information on forest taxa on these locations.

2.4 Example: Pahang Primary Link

In Pahang, the study area consists of a corridor (a primary link) and its adjacent permanent forest reserve, the Tanum Permanent Reserve Forest (Tanum PRF), which is east of the corridor (Figure 2.1). A total of 12 sample locations are set up at the study area with 6 sample locations in the corridor and 6 in the Tanum PRF. Some major challenges during the selection process are: (1) proximity to manmade features such as major highway, railway, service and logging roads, and electric transmission network, (2) private land ownership, and (3) challenging terrain.

At first, select a random starting point (Figure 2.2). In this case, the random starting point is outside the study area. This is because the extent of the study area is specified to be larger than the actual study area. Nonetheless, it does not affect the probability mechanism of laying out the systematic grid. Secondly, expand a 500 × 500 m systematic grid out from the randomly selected starting point to cover the study area. Orient the 500 × 500 m systematic grid such that one of its axes is aligned with the major axis (longest length) of the corridor (Figure 2.2).

Fourteen grid points are found to be in the corridor (M13-M20, N15-N19, and O17; Figure 2.3a). Based on the satellite image overlaying the study area, we reject 6 grid points (M15, M17, N15, N17, N18, and N19) because they are less than 50 m from human-made features such as primary logging roads, highways, and power transmission lines. We reject grid point O17 because there is a disagreement as to whether this section of forests represents the conditions of the corridor. As a result, only 7 remaining grid points are available for selection. From them, randomly select 6 grid points in the corridor, and identify their corresponding grid points

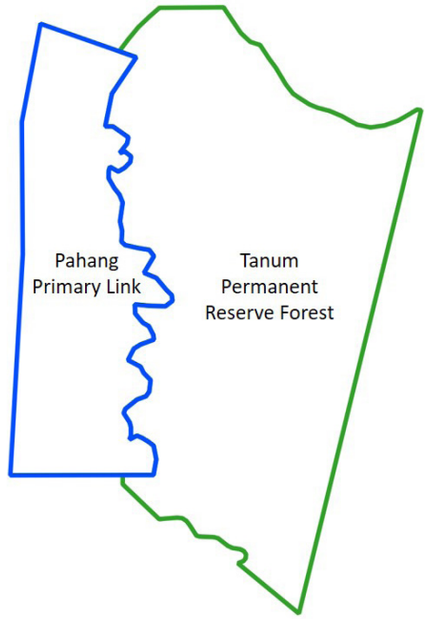


Figure 2.1 Study area in Pahang consists of a corridor (Pahang Primary Link) and a forest reserve (Tanum Permanent Reserve Forest).

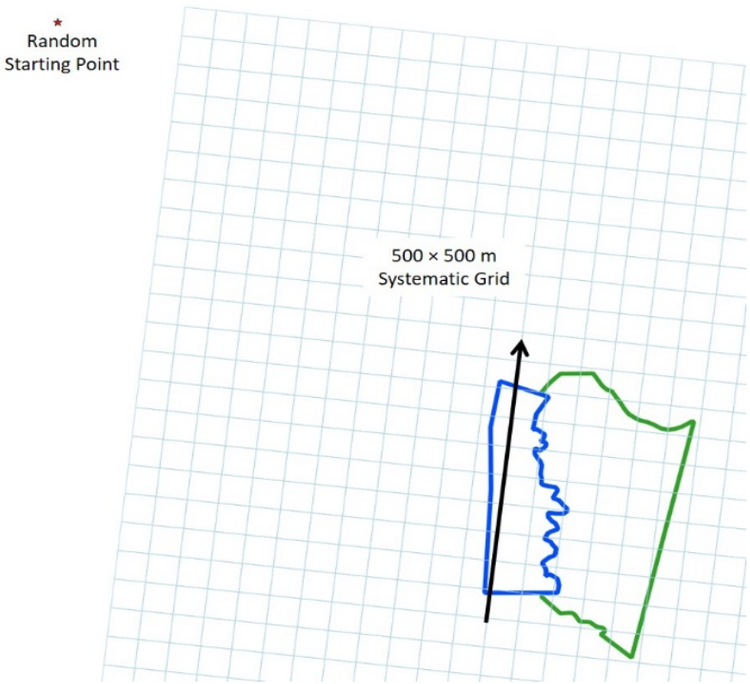


Figure 2.2 Random starting point and a 500 × 500 m systematic grid aligned so that one of its axes is aligned with the length of the corridor.

approximately 1.0-1.5 km away in the Tanum PRF (Figure 2.3b). The preliminary 6 pairs of corridor-forest reserve sample locations are:

(1) M13–O13
(2) M14–O14
(3) M16–O16
(4) M18–O18
(5) M19–O19
(6) M20–O20

Next, check the 6 pairs of sample locations in the field against the rejection criteria. From the ground survey, we reject three pairs M14–O14, M19–O19, and M20–O20. O14 is located on a slope of more than 40°. Both O19 and O20 are less than 50 m from human-made features of logging road and power transmission lines. Hence, three replacement pairs are needed.

Grid point N16 appears to be a suitable replacement for the sample location in the corridor. However, we reject this grid point because M16 and N16 are both on the same horizontal grid line. Given the small total sample size of 12, it is preferable to spatially spread the replacement sample locations across the corridor. Thus, a 250 × 250 m grid is established around grid points M14, M17, M19, and N17. The process of selecting a replacement grid point for each of M14, M17, M19, and N17 is based on the 250 × 250 m as described above. As an example, we describe the selection process for M19 (Figure 2.4). A total 5 grid points on the 250 × 250 m grid surrounding M19 are available for selection as its replacement: M18.5, MN18.5, MN19, MN19.5, and M19.5. First, randomly order these 5 grid points (the random order is illustrated as the number in the red circle in Figure 2.4). Survey them in the field against the rejection criteria. It is found in the field that the first ordered grid point MN19.5 is acceptable. Then, we check its corresponding grid point in the Tanum PRF in the field, and it is also found to be acceptable. Thus, the pair MN19.5–OP19.5 is selected as one of the three

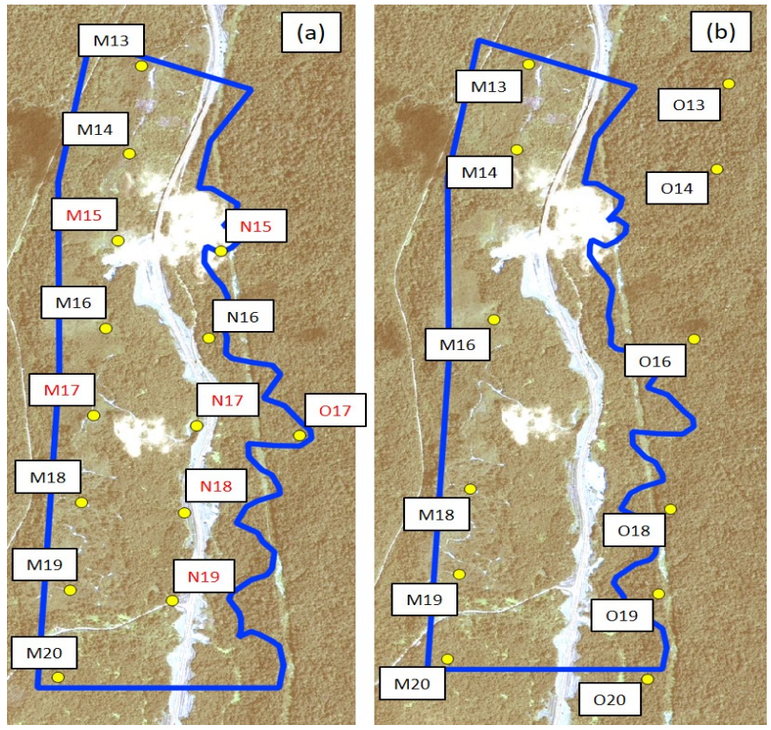


Figure 2.3 (a) A collection of grid points from the 500 × 500 m systematic grid inside the corridor overlaying with a satellite image with red letters being rejected grid points, and (b) preliminary randomly selected pairs of corridor-forest reserve sample locations.

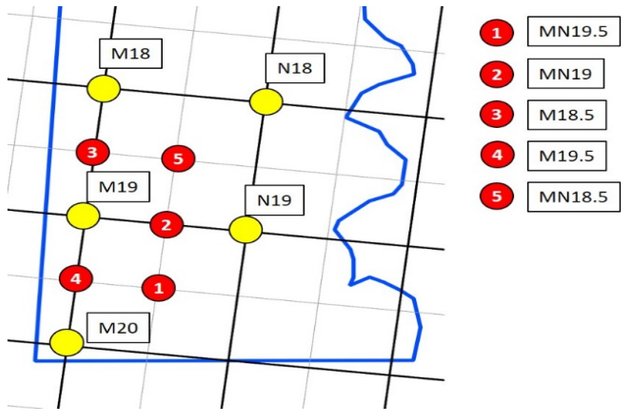


Figure 2.4 Selection process based on 250 × 250 m system grid surrounding a rejected grid point. Yellow circles depict grid points with their labels from the 500 × 500 m grid. Red circles depict grid points and their labels available for selection from the 250 × 250 m grid for grid point M19. The red circles are randomly ordered for field survey and the order is depicted by the number within the circle.

replacement pairs. Repeat this selection process for the other grid points (M14, M17, and N17) until two other replacement pairs are identified and confirmed in the field. The final 6 pairs of corridor-forest reserve sample locations selected for the Pahang Primary Link study area are shown in Figure 2.5.

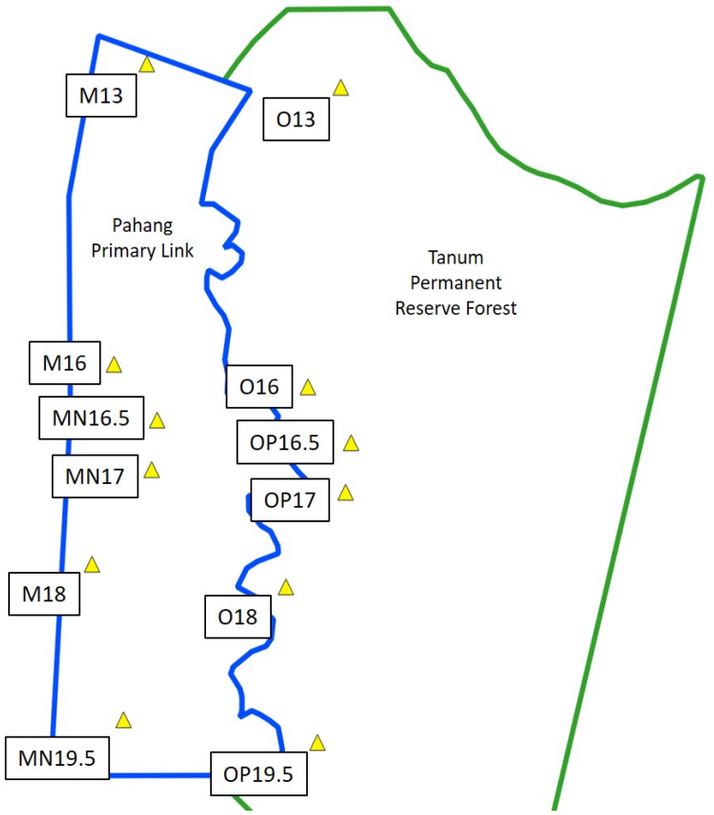
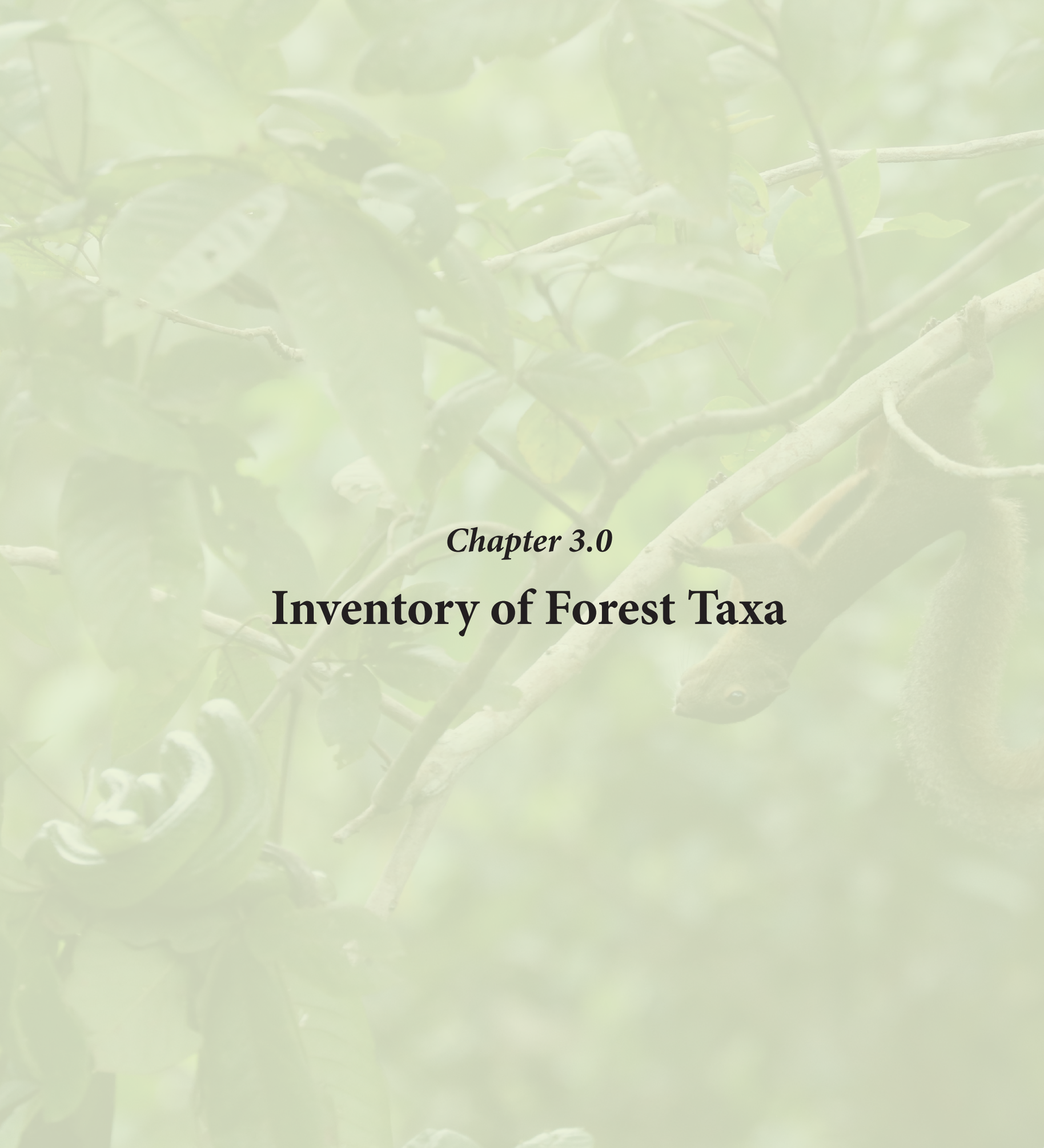


Figure 2.5 Final 6 pairs of corridor-forest reserve sample locations selected for Pahang Primary Link study area.



Chapter 3.0

Inventory of Forest Taxa

3.0 Inventory of Forest Taxa
By Lam, T.Y., Yong, W.S.Y. & Siti-Fariezza, K.T.

This section documents methodology for sampling and observing each forest taxon. Although methodology is specific to each forest taxon, an underlying commonality is that all forest taxa are sampled and observed at a sample location selected under a probabilistic mechanism as described in the previous section. For each taxon, we present a methodology to sample specific genera, species, or subpopulation of the taxon to make the sampling practical. The genera, species, or subpopulation sampled for each taxon are decided based on either taxonomy or habitat considerations. The field sampling methods and protocols for measuring each captured or sampled individual of a taxon are presented in detail below. Additionally, information on handling specimens of each sampled individual and procedures of identification are provided. Finally, a selected publication list is provided for future reference.

3.1 Sample Plot

At each selected sample location, establish a 20 × 20 m (0.04 ha) square plot with the sample location as its center (Figure 3.1.1). Orient the 0.04-ha plot so that the sides are aligned with the direction of the systematic grid established for each study site (Figure 3.1.1). Subdivide the plot further into four 10 × 10 m subplots (labeled as I-IV) for ease of recording during fieldwork. The labels are clockwise starting from the Northwest corner of the plot. Carry out assessment of all forest taxa either within the 0.04-ha plot or in the immediate area surrounding it.

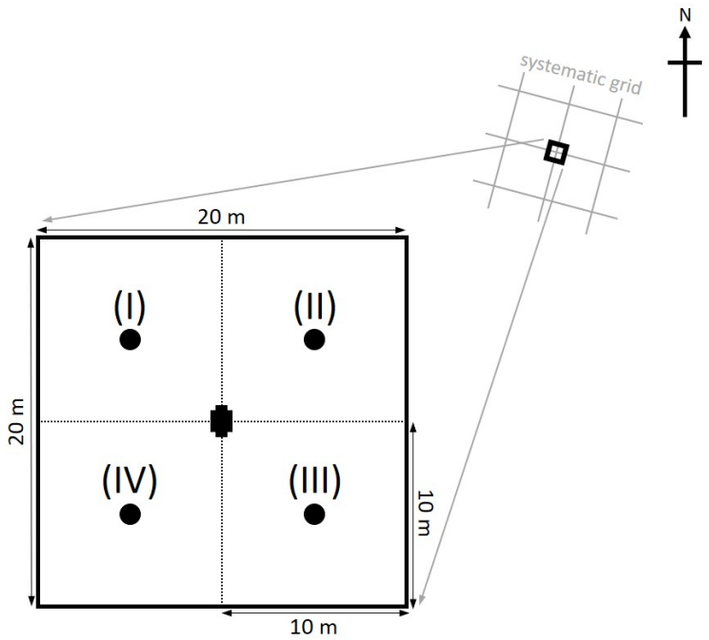


Figure 3.1.1 A 20 × 20 m (0.04 ha) plot centered at a sampling location (solid square). The plot is subdivided into four subplots (I-IV) with solid circles depicting subplot centers. The plot is aligned in the direction of a systematic grid.

3.2 Ant
By Nur-Zati, A.M.

Ants are insects classified under the order of Hymenoptera of the family Formicidae. So far, 21 subfamilies have been recorded worldwide, but five have been locally extirpated. These families consist of at least 300 genera and more than 15,000 species (Bolton et al. 2006). In Malaysia, there are 12 subfamilies consisting of 100 genera and approximately 1,000 species. In lowland Pasoh Forest Reserve in Negeri Sembilan, Peninsular Malaysia, Malsch (2000) recorded 427 ant species.

Ant communities are associated with landscape features and local forest conditions. On the landscape-level, Brühl et al. (2003) found that fragmentation of tropical forests had negative effects on leaf litter ant community in Sabah, Malaysia in terms of patch size, patch area, and patch richness. Ribas et al. (2003) found that relative patch richness was negatively associated with arboreal ant richness. Mitchell et al. (2002) found that historical land use and forest patch size affected ant communities.

At the forest-level, ant communities are correlated with several forest stand parameters. Mitchell et al. (2002) and Fayle et al. (2010) found that ant diversity was positively correlated with canopy closure, stand density, and stand volume. Brühl et al. (2003) found that ant communities were positively associated with horizontal heterogeneity of overstorey trees. Furthermore, ant communities closely track understorey tree, shrub, and ground vegetation cover (Brühl et al. 2003, Fayle et al. 2010). Warren II and Bradford (2012) found that ant diversity was positively associated with density, cover, and volume of coarse and fine woody debris. For some ant communities, density of ants and their nests are strongly related to litter biomass, soil type, and depth of soil organic matter (McGlynn et al. 2009). Lastly, diversity of ant communities is associated with aspect and topography (Sanders 2002, Vasconcelos et al. 2003).

3.2.1 Target Population

The inventory targets three ant communities: ground foraging, soil inhabitant, and arboreal ants. Ground foraging ants nest in tree trunks, tree canopies, and in the soil. Soil inhabitant ants only nest in the soil. Arboreal ants only nest in tree canopies. The three groups provide comprehensive understanding of how ant communities respond to a variety of forest disturbances.

3.2.2 Collection Methods

Set up a total of nine stations at a sample location (Figure 3.2.1). Locate one station at the plot center, four at the subplot centers, and four at the four corners of the 0.04-ha plot. Use four collection methods: leaf litter sifting, arboreal pitfall traps, ground pitfall traps, and baiting. Collect also ground vegetation conditions. Employ different collection methods at different stations as illustrated in Figure 3.2.1. Details of each collection method are as follows.

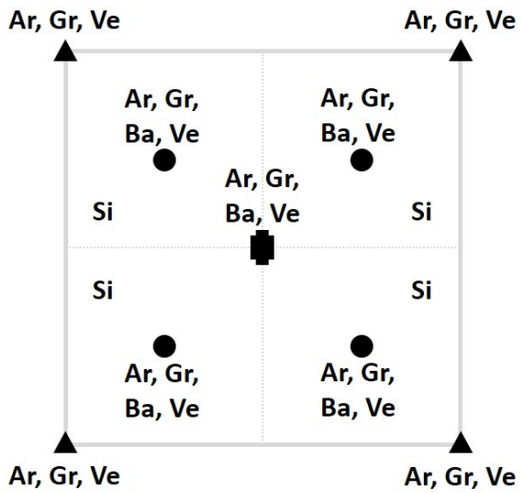


Figure 3.2.1 Ant collection at nine stations at a 0.04-ha plot. Five stations within the plot (solid circles and square), and four stations at the corners (solid triangle). Si = leaf litter shifting, Ar = arboreal pitfall trap, Gr = ground pitfall trap, Ba = baiting, and Ve = ground vegetation.

3.2.2.1 Leaf Litter Sifting

Leaf litter sifting targets ground foraging and soil inhabiting ants. Carry out leaf litter sifting at the four subplots with 15 minutes for each (Figure 3.2.1). Thus, complete the search of all ground surface in the 0.04-ha plot within 60 minutes. Sift in batches by collecting leaf litter up to 2 to 3 cm deep. Put the litter inside a coarse rectangular wire sieve placed on top of a 100 × 80 cm white paper sheet. The wire sieve measures 15 × 30 × 3 cm with a mesh size of 0.5 × 0.5 cm (Figure 3.2.2). Sift the litter for its content. Collect all ants found on the paper sheet using soft forceps and place them inside vials filled with 95% undenatured ethanol. The amount of ethanol in each vial fully covers all ant specimens. Label each vial by location, date, collector names, and reference number with fine head pigment ink pen.



Figure 3.2.2 Leaf litter sifting with a coarse rectangular plastic sieve over a 100 × 80 cm white sheet.

3.2.2.2 Arboreal Pitfall Trap

Arboreal pitfall trap targets ground foraging and arboreal ants. Set up one arboreal trap at each of the nine stations; thus, a total of nine arboreal traps (Figure 3.2.1). Seal a disposable plastic cup with a 5-cm opening with a lid (Figure 3.2.3). Tie it at 1.3 m above ground on a tree that is nearest to a ground pitfall trap and has dbh 5-20 cm. Made five 1 × 1 cm holes at 2 cm below the lid for ants to enter. Place two 1 × 1 cm square cottons moistened with honey solution (20% volume of honey/volume of water) and smeared with tuna flakes inside the cup at 3 cm above the base. Add about 20 ml water mixed with fragrance-free detergent solution with dilution rate of 1:100 into the cup. Operate the trap for 24 hours. Remove it after 24 hours. Decant water in the inside onto a cotton cloth (Figure 3.2.4). Collect ant specimens from the cloth and place them in vials filled with 95% undenatured ethanol. Label each vial by location, date, collector names, and reference number with fine head pigment ink pen.



Figure 3.2.3 An arboreal pitfall trap set up.

3.2.2.3 Ground Pitfall Trap

Ground pitfall trap targets ground foraging and soil inhabiting ants. Set up one ground pitfall trap at each of the nine stations; thus, a total of nine ground pitfall traps (Figure 3.2.1). Prepare a disposable plastic cup with a 5-cm opening (Figure 3.2.5). At a station, dug a hole the size of the plastic cup into the ground. Place the cup in it with the cup opening levelled with the ground surface. Place a coarse wire with a mesh size of 0.5 × 0.5 cm and a width of 2.5 cm around the cup opening. Its purpose is to prevent larger organisms from entering the trap. Place a 15 cm translucent plastic plate on top of the coarse wire covering the trap. Add about 20 ml water and fragrance-free detergent solution with dilution rate of 1:100 into the



Figure 3.2.4 Collection of ants from arboreal and ground pitfall trap on a cotton cloth. Ant specimens are placed into a vial.

cup. Operate the trap for 24 hours. Remove it after 24 hours. Decant water in the inside onto a cotton cloth (Figure 3.2.4). Collect ant specimens from the cloth and place them in vials filled with 95% undenatured ethanol. Label each vial by location, date, collector names, and reference number with fine head pigment ink pen.



Figure 3.2.5 A ground pitfall trap set up.

3.2.2.4 Baiting

Baiting targets ground foraging and soil inhabiting ants. Carry out baiting at five stations: one at the plot center and four at the subplot centers (Figure 3.2.1). Place a 15 cm translucent plastic plate at the station (Figure 3.2.6). Place a teaspoon of tuna flakes and a cotton ball moistened with honey solution (20% volume of honey/volume of water) on it. Leave the bait for 10 minutes. After which, record ant species found feeding on the bait over 10 seconds. Collect as many specimens of ant as possible using soft forceps and place them into vials containing 95% undenatured ethanol. Label each vial by location, date, collector names, and reference number with fine head ink pen. Repeat this process every 10-minute interval for a period of 30 minutes to observe change in communities.



Figure 3.2.6 Baiting station set up with tuna flakes and cotton moistened with honey solution.

3.2.2.5 Ground Vegetation Survey

Carry out ground vegetation survey at nine stations with 1 × 1 m square plots (Figure 3.2.7). Construct the 1 m^2 square plots using PVC pipes and lay it out next to the station. Measure litter depth (cm) using a ruler. Take photograph of ground condition in each 1 m^2 plots and analyse the photos in the laboratory for percent cover of litter, fine roots, bare rock, pebbles, bare soil, and dead wood (%). Take canopy images at approximately 1.3 m above ground for estimation of canopy covers (%).



Figure 3.2.7 The 1 m2 (1 × 1 m) square plot for ground vegetation survey.

3.2.3 Specimen Handling

Properly handle all vials containing ant specimens to avoid damage to specimens during transportation from field to laboratory (Figure 3.2.8). In the laboratory, pin representative of each species in each vial for identification. Carry out the pinning of specimen using stereomicroscope. At first, sort specimens in a vial to morphospecies based on visual morphology, and record number of individuals of each morphospecies. Choose and mount two representative specimens of each morphospecies. Label each mounted specimen with information recorded on the vial. Provide additional unique collector number for traceability (Figure 3.2.9a). Return the remaining specimens to the vial for future reference. Repeat this process for all vials.



Figure 3.2.8 Vials containing ant specimens from the field.

Pin ant specimens with double mounting method (Figure 3.2.9b). Pin a specimen to a triangle card using a resin-based glue. The triangle card measures 1.3 cm long on the sides and 0.3 cm wide at the base. Index card paper is preferred because of durability. The triangle card is made using a specialized point punch or fine pointed scissors. At first, place a triangle card on a pin, and place a small amount of white glue or clear fingernail polish on its tip. Use as little adhesive as possible to avoid excessive body part of a specimen covered in glue. Next, lay a specimen on its back at the edge of a block. Turn the pin upside down with the card facing the specimen. Press the tip of the card lightly against the right side of the specimen.

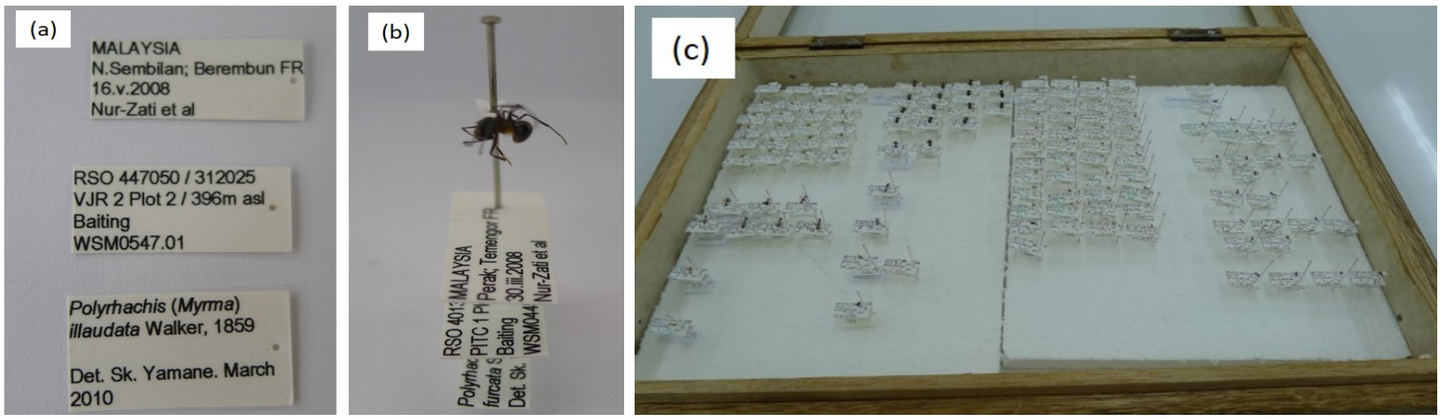


Figure 3.2.9 (a) Label of information for a specimen, (b) a mounted ant specimen, and (c) an insect box containing ant specimens and corresponding labels.

Then, put the specimen into an oven for drying. One week of drying is sufficient for ants. Set temperature of the oven to 45°C. After drying, keep the specimen in an airtight insect box (Figure 3.2.9c). Prepare a label for each specimen containing collection details such as location, date, and name of collector. Place the label below the corresponding specimen. Place the insect box in an environment of 18-22°C and 40-60% relative humidity to minimize pest infestation. If such controlled environment is not available, place silica gel and moth balls inside the insect box. Side effects of using silica gel and moth balls include colour fading in specimens and specimen brittleness. Replenish silica gel and moth balls regularly. Store insect box in a clean environment and with minimum exposure to sunlight or artificial light.

3.2.4 Species Identification

Designate morphospecies of ant specimens with a microscope. Basic knowledge of ant morphology is essential. Identification of specimens to genus level should follow the classification in Bolton (1994) and Hashimoto et al. (2001). When possible, identify specimens to species level. Cross-referencing with specimens from FRIM Entomological Reference Collection (FRIM, Kepong) and other collection facilities is highly recommended.

3.2.5 Supplementary Materials

The following tables are provided in Appendix A2:
Table A2.1. List of equipment for sampling ant for one sample location.
Table A2.2. Field datasheet for baiting activity.
Table A2.3. Field datasheet for field collection of specimens.
Table A2.4. Field datasheet for ground vegetation survey.
Table A2.5. Datasheet for processing ant specimens in laboratory.

3.3 Butterfly
By Phon, C.-K.

Butterflies are classified under the order of Lepidoptera. They are also informally assigned to group Rhopalocera. There are 6 families of butterflies (Papilionidae, Pieridae, Nymphalidae, Riodinidae, Lycaenidae, and Hesperiidae) in Peninsular Malaysia, and at least 1031 species have been recorded in this region (Corbet and Pendlebury 1992).

Habitat requirements of butterflies are complex. Some male butterflies prefer river banks, while others prefer hill tops. Some species prefer to perch in forest clearings, while others restrict to forests with closed canopy. Diversity and abundance of butterflies are influenced by biotic factors. Many butterfly species in the tropics could potentially breed throughout the year, but reproduction has been found to closely link with availability of host plants. In Western Uganda, butterfly species richness and abundance peak for 2 to 3 months after new flushes of vegetation likely due to increase in host plants (Valtonen et al. 2013). Distribution of butterfly species is also likely linked to seasonality. Owen et al. (1972) found that abundance of species from Acraeidae family appeared to associate with seasonal changes of wet and dry seasons in Mount Aureol, Africa. Abundance and richness of fruit feeding butterflies are similarly affected in Brazil (Nobre et al. 2012). In Sabah, Malaysia, the diversity of some fruit feeding butterflies affected by wet and dry seasons in primary forest too (Hamer et al. 2005).

Butterfly richness and abundance have also been found to associate with landscape features and local forest features. At landscape-level, patch size (Crous et al. 2015), patch density (Flick et al. 2012), patch richness (Flick et al. 2012), elevation (Acharya and Vijayan 2015), and topography (Marín et al. 2015) are some of the associated features. In Sabah, Benedick et al. (2006) found that larger forest remnants supported higher butterfly richness and abundance. In Sierra De Javalambre, Central Spain, Sanchez-Rodriguez and Baz (1995) found that butterfly richness decreased with increasing elevation. Some of the forest parameters associated with butterfly richness and abundance are stand density (Milder et al. 2010), canopy closure (Barlow et al. 2007), shrub percent cover (Hamer et al. 2003), understorey tree cover (Barlow et al. 2007), and ground vegetation cover

(Hamer et al. 2003). In Sabah, butterfly assemblages strongly associate with gap such as Satyrinae and Morphinae subfamilies preferring shaded habitat (Hamer et al. 2003). However, common species prefer gaps with abundant light (Hamer et al. 2003). In some cases, butterfly diversity correlates with tree species composition (Crous et al. 2015). Lastly, Bobo et al. (2006) found that butterfly abundance and diversity were significantly affected by primary and secondary forests, agroforestry system, and annual cultures in southwestern Cameroon.

3.3.1 Target Population

The inventory targets all 6 families.

3.3.2 Collection Methods

Sample butterflies with active searches and collect related environmental data. Details of each collection method are as follows.

3.3.2.1 Active Collection

Establish four transects measuring 100 m each at a sampling location (Figure 3.3.1). Space the four transects 10 m apart so that they cover the area surrounding the 0.04-ha plot. Two of the four transects intersect the 0.04-ha plot at the subplot centers. Align the transects so that they are parallel to the 0.04-ha plot. Arrange the transects so that they start in the general direction of West to East.

Collect butterflies with one collector per transect. Start at one end of the transect and end at the opposite end. If there are less

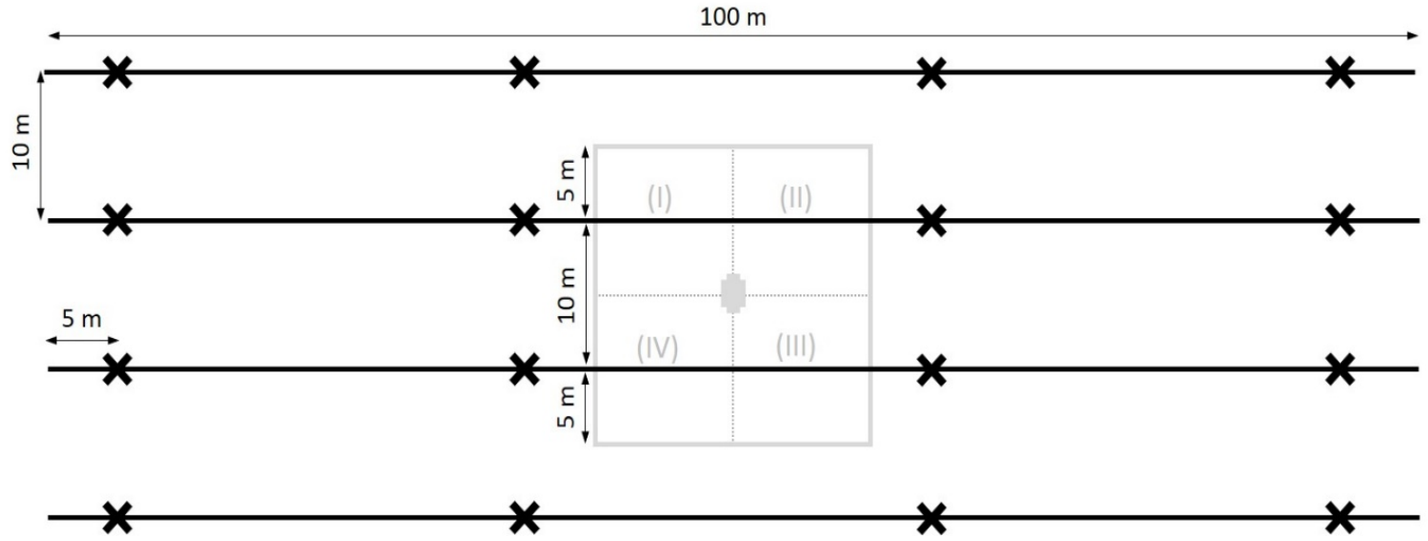


Figure 3.3.1 Four 100 m transects for active collection of butterfly species. Each transect measures 100 m and are placed at 10 m apart. A cross represents an environmental factor station. Four stations are established on each transect and are located at 5-, 35-, 65-, and 95-m marks.

than four collectors available for a sample location, start collecting at the next transect when complete the previous one. Search for butterfly within 5 m to the left and right of the transect. Spend all effort collecting any butterfly that flies into the transect area unless it disappears from view or flies at an unreachable height. Record starting and ending time for this effort. Conduct active collection between 11:00 and 15:00 under a considerably sunny without dark clouds. Record the weather conditions in the field sheets. Avoid sampling during windy and cloudy weather.



Figure 3.3.2 Butterfly collected using a butterfly net.

When spot a butterfly, collect it using a butterfly net (Figure 3.3.2). For specimen not identifiable in the field, once captured, kill it with a sharp pinch on the thorax by hand and place it in a glassine paper envelope (Figure 3.3.3a-b). Transfer the specimen into a waist pouch with compartments differentiating specimens collected from different transects and plots (Figure 3.3.3c). Place a few young leaves inside the pouch to avoid specimens from drying up. For identifiable specimen, carefully keep the specimen alive in envelope and release it once the sampling at a plot location is completed. Record the species and sexes in datasheet.



Figure 3.3.3 (a) Butterfly specimens kept in glassine paper envelope, (b) butterfly killed with a sharp pinch on the thorax by hand, and (c) butterfly specimens are placed into a waist pouch with compartments.

3.3.2.2 Environmental Data Collection

Record environmental data including air temperature, relative humidity, canopy openness, and light intensity during active search. Measure these environmental factors when complete butterfly collection. Take measurements at 5-, 35-, 65-, and 95-m marks along each transect; thus, a total of 16 environmental stations for a sample location (Figure 3.3.1). At each station, measure temperature and relative humidity with a Thermo Hygrometer, and measure light intensity with a lux meter. Take measurement at ground level. Read from the devices after they produce consistent values. This depends on a device response time, which could be one minute or longer. Response time is the time needed for a device to register a consistent value on its surrounding environment. Refer to user manuals for operation procedure. Take canopy images with a compact camera using same focal length

all the time. Point camera lens upwards at the canopy at 1.3 m above ground, and level the camera with the ground. Details of each environmental factor are in Table 3.3.1.

Table 3.3.1 Definition, description, and the measurement unit of environmental factors.

Parameter	Unit	Definition
Air temperature	°C	Air temperature at ground level
Relative humidity	%	Relative humidity at ground level
Light intensity	lux	Light intensity at ground level
Canopy openness	%	Percentage of canopy openness measured at 1.3 m from ground

3.3.3 Specimen Handling

Label every specimen in a glassine envelope at the end of a sampling day after returning from the field. Labels include locality, plot number, transect number, date of collection, and name of the collector. When available, add other information such as mating and feeding on nectars. Use pigment ink pen with fine head for labelling. Keep specimens in a plastic container lined with tissue papers and a small patch of moist tissue. Seal the plastic container inside a zip lock bag. Then, store it inside a polystyrene icebox filled with ice cube. Specimens may be kept in this manner for about one week with frequent replenishment of ice. Use a portable freezer instead when available.

Process specimens in the field when possible or in a laboratory after a sampling period. Use stainless steel insect pins to pin a butterfly specimen, which are available from any entomological suppliers (Figure 3.3.4a). Press the pin so that it goes through the thorax at an approximately 90° to the specimen body. Use pins sized 0 and 3, where size 0 is for a small specimen and size 3 for a large specimen. Pin specimen onto a polystyrene board or cork board with a groove in the middle (Figure 3.3.4b).

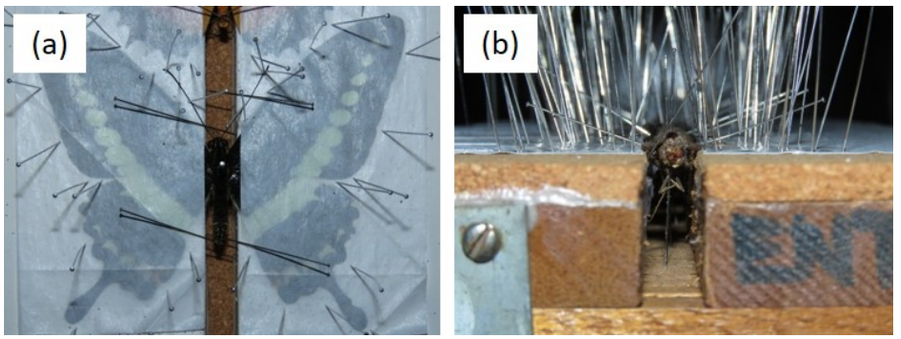


Figure 3.3.4 (a) A pinned specimen with a stainless steel insect pin on the thorax, and (b) groove in the middle of the pinning board.

Size of the groove depends on the size of the thorax. Spread wings with glassine paper and angle them with pins. Pin label with information such as locality, date, and collector next to the specimen. In field, keep pinned specimens in a zip lock bag with small amount of moth balls and silica gel to protect them from ants or other insect pests and fungus (Figure 3.3.5a). When transporting pinned specimens, take extra care to prevent damages to the specimens. Place boards in an airtight container or a custom-made box (Figure 3.3.5b). Secure boards with Velcro or other similar materials so that they do not dislocate during transportation. Place silica gel and moth balls securely inside the box to prevent damages from ants or other insect pests. They also help with partially drying the specimens. Place the boards into a larger box with padding (Figure 3.3.5c). Pad the box with pillow or cushion to minimize impact from vibration to the specimens during transportation.



Figure 3.3.5 (a) Boards containing pinned butterfly specimens stored in a zip lock bag with appropriate amount of silica gel and moth balls, (b) boards stacked and secured ready for transportation, and (c) board stored in a packaging box ready for transportation.

For drying in laboratory, place specimens in an oven. Small specimen requires about 1-week of drying, whereas large specimens require about 2 to 4 weeks. Set oven temperature to approximately 45 °C. After drying, keep specimens in an airtight insect box. Place label below each specimen (Figure 3.3.6a).

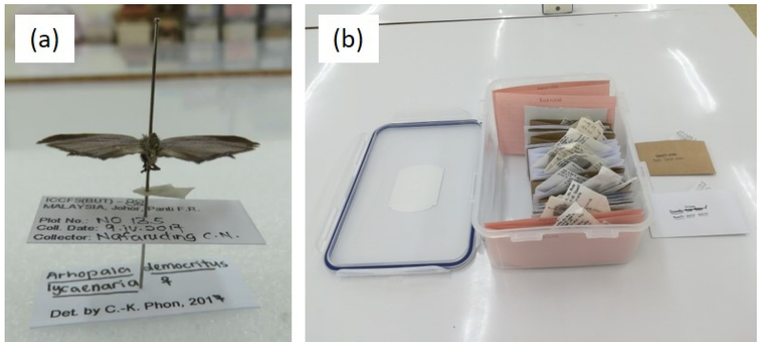


Figure 3.3.6 (a) Label of a pinned specimen, and (b) dried specimens in envelopes are kept in airtight container.

Store specimens in an environment around 18 to 22°C with relative humidity of 40 to 60% to minimize pest infestation. If this environment is not available, use silica gel and moth balls as alternatives. However, moth balls are carcinogenic and cause fading in specimen colours. Use appropriate quantity of silica gel to prevent specimens from becoming too brittle to handle in the future. Replenish both from time to time. Cleanliness of the surrounding is essential. Minimize exposure of specimens to sunlight or room light. When lacking capacity for setting specimens such as a lack of appropriate equipment, simply keep specimens in envelopes. However, it is important to thoroughly dry the specimens. Keep these specimens in fully sealed container with moth balls and silica gel to preserve them (Figure 3.3.6b).

3.3.4 Species Identification

Identify each butterfly specimen to species- or subspecies-level together with its gender. Identification follows that of Corbet and Pendlebury (1992), Eliot (2006), and Kirton (2020).

3.3.5 Supplementary Materials

The following tables are provided in Appendix A3:
Table A3.1. List of equipment for sampling butterfly for one sample location.
Table A3.2. Field datasheet for butterfly collection.
Table A3.3. Field datasheet for environmental data collection.
Table A3.4. Datasheet for summary of butterfly specimens collected in the field.

3.4 Dung Beetle
By Nur-Zati, A.M. & Wan-Zaki, W.M.

Dung beetles are classified under the family Scarabaeidae and subfamily Scarabaeinae. There are about 5000 species of dung beetles worldwide (Hanski and Camberfort 1991). Malaysia has recorded more than 150 species with almost 90 of them in Peninsular Malaysia. Dung beetles primarily feed, live, and breed in pads of mammal dung. Thus, they are found in high diversity and abundance in areas populated by mammals (Hanski and Camberfort 1991). For example, beetles of the genus *Caccobius* are known to prefer herbivore dung (Hanski and Camberfort 1991). This association allows indirectly assessment of mammal assemblages by trapping beetles. However, some dung beetle species are also known to feed on seeds and plants. Dung beetles have been increasingly used as bioindicator due to their sensitivity to changes in microclimatic disturbances, structures of vegetation, vegetation cover, soil components, and abundance of food resources (Davis et al. 2001, Andersen 2005).

Previous studies found that diversity and abundance of dung beetles are associated with landscape and forest characteristics. At the landscape-level, Nichols et al. (2007) found that diversity of dung beetles was negatively correlated to patch size, patch density, and patch richness. On the other hand, in Sulawesi, Indonesia, Shahabuddin et al. (2005) found that on the species level, abundance of the several commonly found species responds significantly to the habitat type. At the forest-level, changes in the forest canopy directly affect diversity of arboreal dung beetles (Davis and Sutton 1998). More broadly, habitat variations including vegetation types are important to determine dung beetle communities due to resource availability (Hanski and Camberfort 1991). Understorey conditions such as ground vegetation and shrub also influence dung beetle diversity (Louzada et al. 2010). Other factors associated with diversity of dung beetles include soil type, soil organic matter depth, elevation, and topography (Sowig 1995, Halffter and Arellano 2002, Escobar et al. 2005, Jay-Robert et al. 2008).

3.4.1 Target Population

The inventory targets dung beetles attracted to elephant dung. We select elephant dung because nearly all dung beetle species strictly rely on herbivore dung in their life cycles (Hanski and Camberfort 1991). Furthermore, it is largely available in most forests in Peninsular Malaysia. Doll et al. (2014) reported a total of 64 species of dung beetle collected in their study in Peninsular Malaysia using elephant dung as bait.

3.4.2 Collection Methods

Set up a total of nine stations at a sample location (Figure 3.4.1). Each station consists of a baited pitfall trap. Place one station at the plot center, four at the subplot centers, and four at the four corners of the 0.04-ha plot.

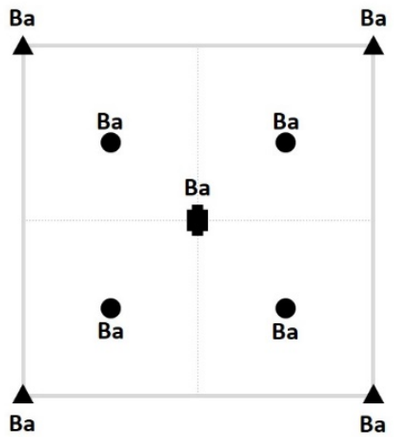


Figure 3.4.1 Dung beetle collection at nine stations at a 0.04-ha plot. Five stations within the plot (solid circles and square), and four stations at the corners (solid triangle). Ba = baiting pitfall trap.

3.4.2.1 Baited Pitfall Trap

Use elephant dung as bait for pitfall trapping dung beetles. It can attract beetles 200-300 m away. Collect elephant dung from local or national zoo. Obtain a special permit from the Department of Wildlife and National Parks (PERHILITAN) Peninsular Malaysia for handling the dung. Collect elephant dung from the same elephant whenever possible for homogeneity in composition and structure. Collect dung as close to the date of sampling as possible. Keep dung in airtight box to maintain moisture because dried out dung has less smell and affects sampling effort.



Figure 3.4.2 Two different views of a dung beetle baited pitfall trap. The trap consists of a hard plastic cup placed in the ground, a pocket containing elephant dung made from soft fiber mesh, a dry leaf for protection, and a 25 cm bamboo skewer for positioning the bait.

At each baiting station, dig a small hole. Place a plastic cup about 10 cm in height and 5 cm in diameter inside (Figure 3.4.2). Level the opening of the cup with the ground. Pour about 20 ml of killing solution into the plastic cup. Make the killing solution from a mixture of water and fragrance-free detergent diluted at 1:100. Insert a ball of elephant dung about the size of 50 cent coin into a triangle pocket made from soft fibre mesh. Attach the pocket to a dry leaf and hung it 3 cm above the middle of the cup with a 25 cm bamboo skewer. The dry leaf provides shade to the dung and prevents rainwater from getting into the cup. Check the trap 24 hours later. When checking, pour the contents of the cup onto a sieve. Place trapped dung beetles into 20 ml vials containing 70% ethanol using entomological forceps (Figure 3.4.3). Label each vial with location, date, collector names, and reference number. It is advisable to handle dung with gloves. When finish trapping, bring all dung out of the forests and properly dispose them to avoid introducing non-native plant seeds or foreign contaminants.

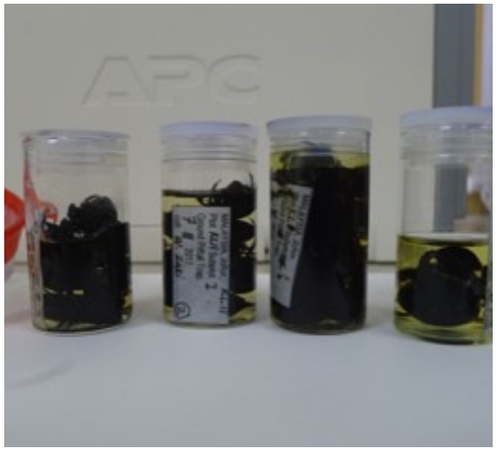


Figure 3.4.3 Vials containing dung beetle specimens with labels.

3.4.2.2 Ground Vegetation Survey

Carry out ground vegetation survey at nine stations with 1 × 1 m square plots (Figure 3.2.7). Construct the 1 m^2 square plots using PVC pipes and lay it out next to the station. Measure litter depth (cm) using a ruler. Take photograph of ground condition in each 1 m^2 plot and analyse the photos in the laboratory for percent cover of litter, fine roots, bare rock, pebbles, bare soil, and dead wood (%). Take canopy images at approximately 1.3 m above ground for estimation of canopy covers (%).

3.4.3 Specimen Handling

Store all specimens of dung beetle in vials containing 70% ethanol. Label vials with collection location, date, collector names, and reference number with fine head pigment ink pen (Figure 3.4.3). In laboratory, pin representatives of each species in each vial for identification. Carry out pinning of specimens using stereo microscope with magnification of 5 to 20 times. First, sort specimens in a vial to morphospecies based on visual morphology. Use characteristics of head, thorax, abdomen, femur, and tibia to sort and characterise morphospecies. Record number of individuals of each morphospecies. Choose and mount two representative specimens of each morphospecies. Label each mounted specimen with information recorded on the vial and provide an additional unique collector number for traceability (Figure 3.4.4). Return remaining specimens to the vial for future reference. Repeat this process for all vials.



Figure 3.4.4 A pinned dung beetle specimen with example labels attached to it.

Pin a beetle specimen with the direct mounting technique (Figure 3.4.4). First, clean a specimen with soft brush as thoroughly as possible because dirt could often be trapped on the specimen. Use a stainless steel insect pin to pin the specimen through right elytra of a specimen at 90° (Figure 3.4.5). Insect pins are available from any entomological supplier. Use pin sizes of 0 and 3 with size 0 for a small specimen and size 3 for a large specimen. Pin the specimen on a polystyrene board. Pin labels together with the specimen with labels placed below the specimen. Dry the mounted specimen in an oven at a temperature of 45°C for one to four weeks depending on the size of the specimen. Refer to Triplehorn and Johnson (2005) for further information on the mounting technique.



Figure 3.4.5 Dung beetle specimens pinned on the right elytra using insect pin.

Place pinned and dried specimens inside dust and pest-proof boxes (Figure 3.4.6). Store vials in airtight containers. The best environment for preserving the specimens is 18-22°C and 40-60% relative humidity. If such controlled environment is not available, place silica gel and mothballs inside the insect box. Side effects of using silica gel and mothballs are specimen brittleness and colour fading in specimens. Furthermore, they need to be replenished regularly. Store insect boxes in a clean environment with minimum exposure to sunlight or artificial light.



Figure 3.4.6 Pinned dung beetle specimens stored inside an insect box.

3.4.4 Species Identification

When possible, identify each specimen to the genus- or species-level. When not possible, group specimens into morphospecies. Identification of a specimen to genus-level follows Ochi et al. (1996). Identification to species-level follows Ochi et al. (2008) and Ochi et al. (2009). When possible, cross-reference with specimens from FRIM Entomological Reference Collection (FRIM, Kepong) or other reference collections.

3.4.5 Supplementary Materials

The following tables are provided in Appendix A4:
Table A4.1. List of equipment for sampling dung beetle for one sample location.
Table A4.2. Field datasheet for ground vegetation survey.
Table A4.3. Datasheet for specimen processing in laboratory.

3.5 Herbaceous Plant
By Sam, Y.Y. & Siti-Eryani, S.

The herbaceous plant community is an integral component of a forest ecosystem. However, its ecological significance in maintaining functions and structures of a forest is often overshadowed by overstorey trees. Diversity of herbaceous plants is similar to that of other forest strata, if not higher. In Peninsular Malaysia, about 2600 herbaceous plant species have been reported compared to 2830 tree species (Kiew 1991). Orchids is the largest flowering herbaceous plant group in Malaysia, and it constitutes about 850 species. The non-orchid herbaceous plants are classified into 93 families and 450 genera (Kiew 1991). Of the 93 families, 7 families make up 60% of the non-orchid plant groups, which are Gramineae (205 species), Gesneriaceae (200 species), Zingiberaceae (150 species), Rubiaceae (128 species), Araceae (120 species), and Acanthaceae (117 species) (Kiew 1991). In Sabah and Sarawak, the number of herbaceous plant species is unknown because there is no official documentation.

3.5.1 Target Population

This inventory targets 8 herbaceous plant groups (Figure 3.5.1): (1) grasses (e.g., *Digitaria* spp. and *Paspalum* spp.), (2) sedges (e.g., *Cyperus* spp. and *Fimbristylis* spp.), (3) ferns (e.g., *Adiantum* spp. and *Lindsaea* spp.), (4) herbaceous plants (e.g., *Codonoboea* spp. and *Homalomena* spp.), (5) small woody herbs (e.g., *Ophiorrhiza* spp.), (6) non-climbing palms (e.g., *Areca* spp. and *Licuala* spp.), (7) gingers (e.g., *Globba* spp. and *Zingiber* spp.), and (8) invasive herbaceous plant species (e.g., *Asystasia gangetica*). Small woody herbs are defined as plants with total height ≤ 1 m. Non-climbing palms and gingers are sampled regardless of their heights. Seedlings and saplings of tree and shrub species, and epiphytic, climbing, and twining herb species are excluded.

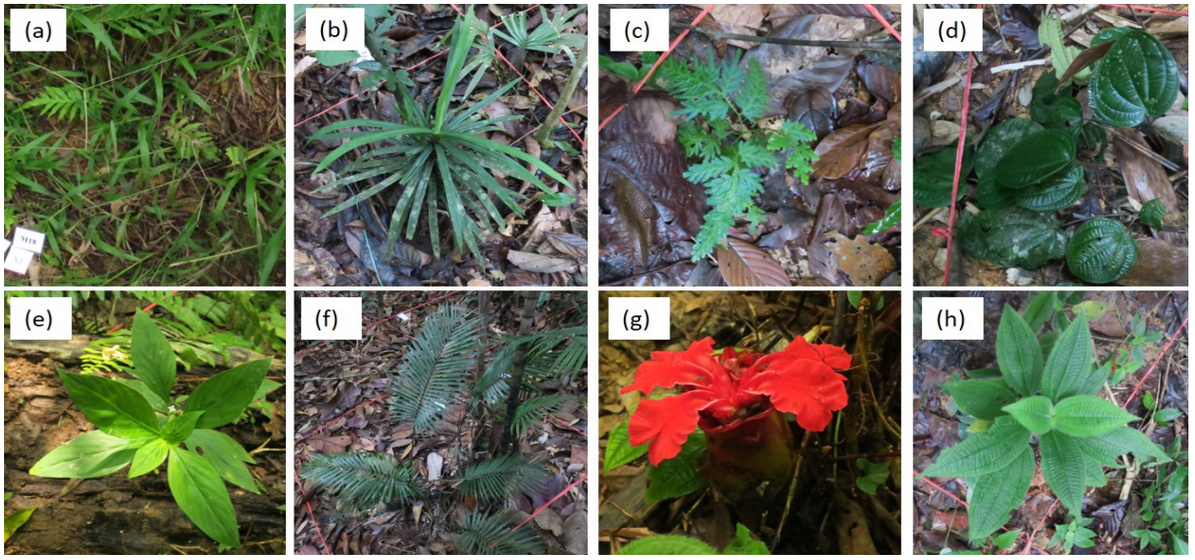


Figure 3.5.1 Example of (a) grass, (b) sedge, (c) fern, (d) herbaceous plant, (e) small woody herbs, (f) non-climbing palm, (g) ginger, and (h) invasive herbaceous plant.

3.5.2 Collection Methods

Establish four subplots measuring 5 × 10 m with the total sampling area of 0.02 ha (Figure 3.5.2). Place three subplots outside the 0.04-ha plot with one side of their boundaries touching the boundaries of the 0.04-ha plot. Place one subplot at the center of the 0.04-ha plot. Orient the four subplots as shown in Figure 3.5.2. The purposes for this configuration are to: (1) minimize disturbances to herbaceous plant layers from sampling activities within the 0.04-ha plot, and (2) capture maximum variation in distribution of local species due to local variation in microhabitat conditions. Survey herbaceous vegetation in each subplot by ocular estimation and photogrammetry approach.

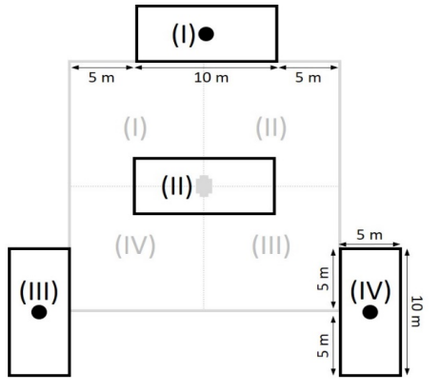


Figure 3.5.2 Four 5 × 10 m subplots for sampling of herbaceous plants. Three subplots are placed adjacent to the 0.04-ha plot, and one subplot is placed at the center. The subplots are aligned vertically (III, IV), and two subplots are aligned horizontally (I, II).

3.5.2.1 Ocular Estimation and Photogrammetry

Subdivide a 5 × 10 m subplot into 50 quadrats measuring 1 × 1 m (1 m^2) (Figure 3.5.3). Sequentially survey herbaceous vegetation in all 50 quadrats. Label each quadrat according to their positions in a subplot (Figure 3.5.4). If cannot lay out a quadrat on the ground due to obstructions such as tree stumps or plant clusters, further subdivide quadrat into smaller sub-quadrat of 0.5 × 0.5 m for survey.



Figure 3.5.3 A 5 × 10 m subplot is subdivided into 50 quadrats of 1 × 1 m (1 m2) using strings. Chopsticks are used to hold the strings during the delineation.

Start at a quadrat. At first, take a photograph of a quadrat as a permanent record for future reference and for estimating overall vegetation percent cover (Table 3.5.1). Hold the camera at 1.3 m above ground and directly above the center of the quadrat (Figure 3.5.5). Assign an identification number corresponding to the quadrat's label to the photograph (refer to Figure 3.5.4). Analyse the photograph of the quadrat in laboratory using Easy Leaf Area software to obtain overall vegetation percent cover.

Second, record all variables listed in Table 3.5.1. Estimate percent cover for the whole quadrat, for each herbaceous group, and for each species within a herbaceous group. Record density of each species within a herbaceous group with rules for counting individual defined in Table 3.5.1. Measure clump diameter for herbaceous groups that form clumps. Record presence/absence of each invasive species. Define a herbaceous plant rooted in a quadrat as belonging to that quadrat even if some parts of the plant are outside the subplot or cross over to other quadrats. As a result, density is based on where a plant is established. Percent cover of a herbaceous plant rooted in a quadrat includes parts of the plant outside the quadrat but within the subplot.

x1y5	x2y5	x3y5	x4y5	x5y5	x6y5	x7y5	x8y5	x9y5	x10y5
x1y4	x2y4	x3y4	x4y4	x5y4	x6y4	x7y4	x8y4	x9y4	x10y4
x1y3	x2y3	x3y3	x4y3	x5y3	x6y3	x7y3	x8y3	x9y3	x10y3
x1y2	x2y2	x3y2	x4y2	x5y2	x6y2	x7y2	x8y2	x9y2	x10y2
x1y1	x2y1	x3y1	x4y1	x5y1	x6y1	x7y1	x8y1	x9y1	x10y1

y (5 m)

x (10 m)

Figure 3.5.4 A 5 × 10 m subplot is subdivided into 50 quadrats of 1 × 1 m (1 m2). Quadrats are labelled according to their positions in the x- and y-direction.



Figure 3.5.5 A photographer taking photo at 1.3 m above ground and directly above the quadrat center.



Figure 3.5.6 Example of a clumping species.

Table 3.5.1 Variable measured at a quadrat.

Variable	Unit	Description	Rules
Overall percent cover	%	Overall ground vegetation cover in a quadrat	All vegetation including sapling and seedlings of trees ≤ 1 m height. Disregard overlapping plant cover. Estimated from photos.
Percent cover for a herbaceous group[1]	%	Vegetation cover of a herbaceous group in a quadrat	Grasses, sedges, ferns, herbaceous plants, small woody herbs (≤ 1 m), non-climbing palms (≤ 1 m), gingers (≤ 1 m), and invasive species. Visual estimation or cover measurement.
Percent cover for a species of a herbaceous group[1]	%	Vegetation cover of a species of a herbaceous group in a quadrat	Grasses, sedges, ferns, herbaceous plants, small woody herbs (< 1 m), non-climbing palms (< 1 m), gingers (< 1 m), and invasive species. Visual estimation or cover measurement for species or morphospecies.
Density of a species of a herbaceous group	count	Number of individuals of a species of a herbaceous group	Herbaceous plants, small woody herbs with solitary/branched stem (≤ 1 m), non-climbing palms (≤ 1 m), and gingers (≤ 1 m). Palms or gingers that are clumped are considered as one individual. Small clustering/ clumping plants such as grasses, sedges, and ferns are not included in the density count. Visual estimation for species or morphospecies.
Clump diameter	m	Width of a clump	Clumping species (non-climbing palms (> 1 m), gingers (> 1 m), and monocots (> 1 m) (Figure 3.5.6). Measurement carried out at subplot-level.
Frequency	1/0	Presence/absence of invasive species	Recorded at quadrat-level. Identify to species or morphospecies. Estimated from photos.

[1] Visual estimation is from 0% to 100% in 5% interval (i.e., 0%, 5%, 10%, 15%, …, 100%)

3.5.3 Specimen Handling

To avoid complication in species identification, separate protocols for handling specimens into field collection and laboratory processing. The field specimen collection procedures follow that of Bridson and Forman (1992). They are as follows:

1. Collect a representative individual (duplicates when necessary) or representative parts of a rare plant that show distinct morphological characters adequately. When available, collect flowering and fruiting specimens,
2. Record key habitat and field characters such as plant structure and colours of flower and/or fruits. They are useful for identification in the laboratory. Take photographs of specimens when possible,
3. Preserve fragile or soft specimens such as flowers in Copenhagen solution (10:1:8 mixture of Industrial Methylated Spirit, glycerol, and water) (Bridson and Forman 1992) (Figure 3.5.7a). Desiccate leaves collected from fertile specimens with silica gel to preserve their DNA materials,
4. Lay collected specimens between two or more sheets of newspapers. Pile up the specimens. Tie and compress all specimens between two wooden plant presses (Figure 3.5.7b). Drench the specimens in alcohol and store in air-tight plastic bags while waiting to be dried in the oven in the laboratory.

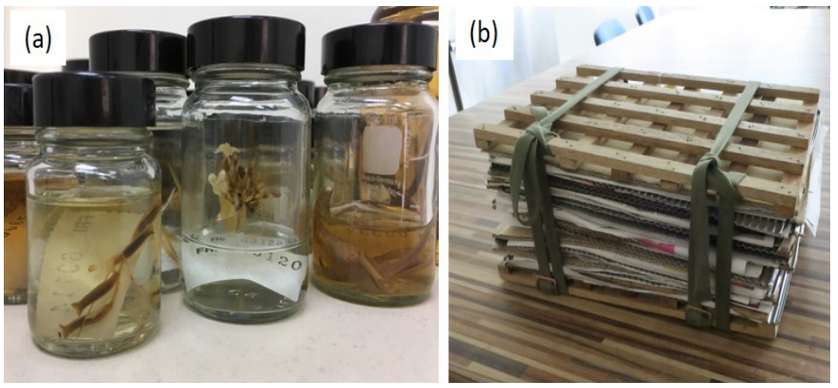


Figure 3.5.7 (a) Fragile or soft specimens such as flowers are preserved in Copenhagen solution, and (b) collected specimens compressed with newspaper and between two wooden plant presses.

Once specimens arrive in the laboratory, process the specimens as follows:

1. Place bulky specimens into oven maintained at 55-60°C for three or more days for drying,
2. Sort dried specimens accordingly. Mount them onto herbarium sheets. Properly label them with collection details such as name of collector, date of collection, location, habitat, plant description, and family and species name,
3. Pack specimens into air-tight plastic bags. Store them in a deep freezer maintained at -20°C for at least 72 hours for sterilization from disease and pests. Remove frozen specimen packs from the freezer. Thaw them to room temperature before opening to avoid damping of specimens from condensation,
4. Send sterilized specimens to herbarium for identification.

3.5.4 Species Identification

Identify a specimen in a laboratory. First, match a specimen to the closest family, genus, and species according to the flora accounts and reference specimens in the FRIM Herbarium. When available, extract information of previously collected specimens from the study areas from the herbarium database for comparison. Once successfully identify a specimen, enter specimen identity information and associated field notes into the database.

3.5.5 Supplementary Materials

The following tables are provided in Appendix A5:
Table A5.1. List of equipment for sampling herbaceous plant for one sample location.
Table A5.2. Field datasheet for herbaceous plant survey.

3.6 Herpetofauna
By Kaviarasu, M., Shahfiz, M.A. & Nor-Hazwani, A.R.

Herpetofauna encompasses amphibian and reptile species. Amphibians are grouped into three orders of Class Amphibia, i.e., Anura (frogs), Caudata (salamanders), and Gymnophiona (Caecilians). Reptiles are grouped into four orders of Class Reptilia, i.e., Crocodilia (crocodiles and alligators), Sphenodontia (tuataras), Squamata (lizards and snakes), and Testudines (turtles and tortoises). At present, more than 7600 amphibian species have been recorded (Frost 2017), and more than 10000 reptile species have been identified worldwide (Uetz 2017). In Malaysia, there are 276 species of amphibians (Frost 2017) and 480 species of reptiles (Uetz 2017). The amphibian species recorded in Malaysia are from the families Bufonidae, Megophryidae, Microphylidae, Dicroglossidae, Ranidae, and Rhacophoridae.

Presence of amphibians and reptiles is related to microhabitat conditions. For example, ranids are generally associated with rivers. Microphylids are mostly found in swampy areas. Reptiles species are associated with understorey density, temperature, canopy cover, leaf litter cover, and slope. Diversity and abundance of herpetofauna are associated with forest types and landscape characteristics. Loehle et al. (2005) found that herpetofauna richness differed remarkably between hardwood forests and mixed coniferous-hardwood forests. Pineda and Halffter (2004) and Pineda et al. (2005) found that diversity and abundance of amphibian species in shaded coffee plantations in Mexico were similar to neighbouring primary tropical forests. Inger (1980) found that number of lizard species in rubber plantations was comparably higher than in secondary tropical forests. At landscape level,

Nicolás Urbina-Cardona et al. (2006) found that diversity of herpetofauna correlated with surrounding land use classes, distances to nearest water body and protected areas, and patch richness in Los Tuxtlas Biosphere Reserve in Veracruz, Mexico. At the forest level, they also showed that diversity of amphibians was positively correlated with understorey density, canopy cover, altitude, and relative humidity (Nicolás Urbina-Cardona et al. 2006).

3.6.1 Target Population

This inventory targets all types of amphibians and reptiles.

3.6.2 Collection Methods

Inventory herpetofauna with two collection methods: pitfall traps and active search. Use pitfall traps for both diurnal and nocturnal species. Use active search to collect nocturnal species, and only do it when there is sufficient labour or the risk of injury to surveyor in the field is relatively low.

3.6.2.1 Pitfall Trapping

Pitfall trapping on a transect is one of the most widely used methods to collect herpetofauna species. The principal is to trap any ground crawling herpetofauna species into a container buried into the ground.

Establish four transects measuring 100 m each at a sample location (Figure 3.6.1). Space the four transects 10 m apart to cover areas surrounding the 0.04-ha plot. Arrange the transect so that they start in the general direction of West to East. Align the transects so that they are parallel to the orientation of the 0.04-ha plot as shown in Figure 3.6.1. Two of the transects intersect with the 0.04-ha plot at the subplot centers. At each transect, establish 10 pitfall traps spaced 10 m apart and place them as shown in Figure 3.6.1. As a result, there are 40 pitfall traps at a sample location. Out of which, 4 pitfall traps are inside the 0.04-ha plot and coincide with the subplot centers.

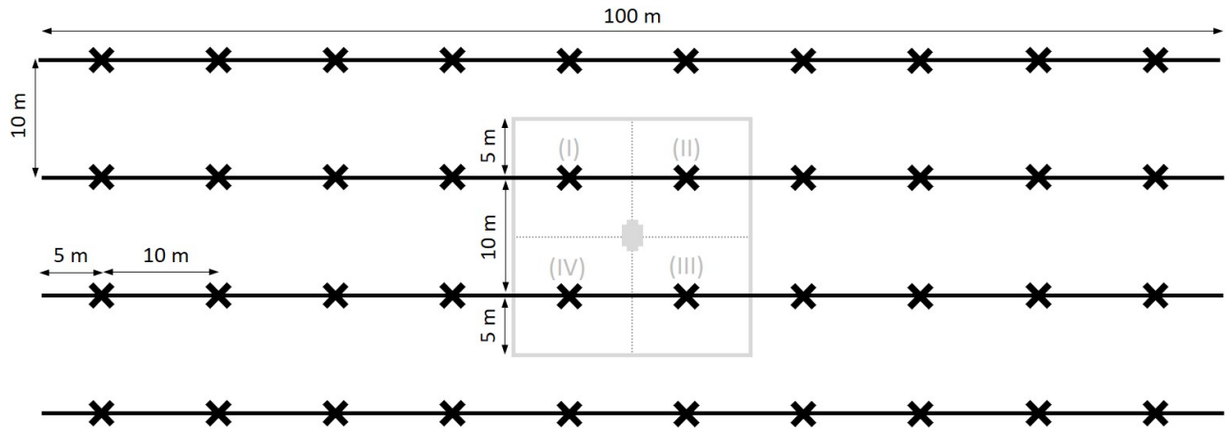


Figure 3.6.1 Four 100 m transects for pitfall trapping of herpetofauna species. Each transect measures 100 m and are placed at 10 m apart. A cross represents a pitfall trap. Pitfall traps are spaced at 10 m. Four traps are located inside the 0.04-ha plot and at the subplot centers.

Use an 18 L plastic bucket with lid as a pitfall trap (Figure 3.6.2a). Make four holes at the bottom of a bucket to allow draining of rainwater and prevent drowning of individuals (Figure 3.6.2b). Bury the bucket into the ground so that the lip of the bucket is level with the ground surface (Figure 3.6.2b). Seal all buckets with their lids when not collecting herpetofauna.

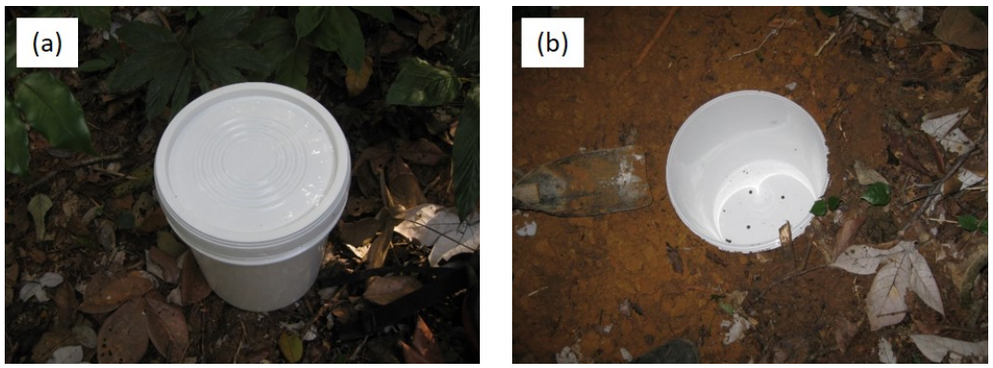


Figure 3.6.2 (a) A 18 L bucket with lid on served as a pitfall trap, and (b) bucket with four holes at the bottom and buried into ground.

Collect herpetofauna over a period of 6 days at a sample location. During the first day, remove lids of all buckets, and remove any dirt found inside. Check all buckets daily between the second and the sixth day for any trapped herpetofauna. If there is a trapped individual, carefully remove it and temporarily place it in a plastic bag before examination. Place each captured individual separately in a bag. If a trapped individual is a poisonous species such as snakes, remove it using snake hook and snake tong, and keep it in a thick cloth bag. For other trapped animals such as small mammals, hand the individuals over to the responsible researchers or release them into their habitats. At the end of the sixth day when collection is finished, clean the buckets and seal them with their lids.

When finish collecting at a pitfall trap, identify trapped individual to the species level with a field guide, and measure its morphology (Figure 3.6.3). For a small individual such as frog, use calliper for measurement. For a larger and longer individual such as snake, use measuring tape. Record all measurements in mm. For a reptile, measure snout-vent length (SVL) and tail length (TL) (Figure 3.6.3a). Define SVL as the length from nose to cloaca, and TL as the length from cloaca to end of tail. For an amphibian, measure only SVL (Figure 3.6.3b). After completing the measurement, photograph the individual, and identify and record any natural marking or old wound. Use a non-poisonous pen marker to mark the dorsal part of the individual with a specific mark. After this, release the individual back to the area nearest to the pitfall trap.

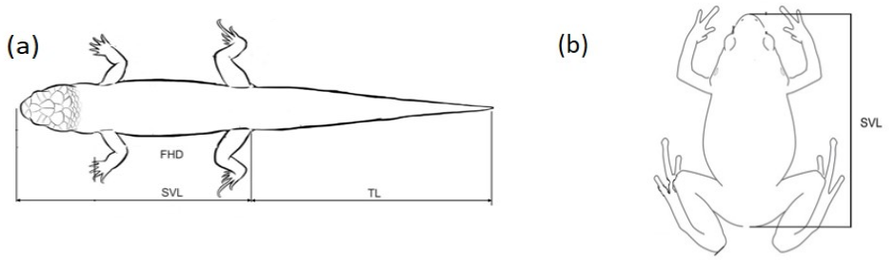


Figure 3.6.3 Measurement taken for (a) reptiles, and (b) amphibians. SVL is snout-vent length, and TL is tail length. All measurement is recorded in mm.

3.6.2.2 Active Search

Active search is optional. Conduct search when there is sufficient staff and sites are safe for conducting night search. Carry out the search in a group of minimum four persons, and between 20:00 and 23:00. Use flashlight to locate amphibians and reptiles foraging on the ground, beneath leaves, and resting on hanging branches. Place captured individual inside a holed plastic bag before bringing it back to camp for examination. Take measurements as above. Either release or keep the individual as a voucher specimen.

3.6.2.3 Microhabitat Survey

At a pitfall trap, collect information on leaf litter cover, leaf litter depth, and microhabitat variables related to water and substrate. Visually estimate leaf litter cover using a 1 × 1 m (1 m^2) square plot made from PVC pipes (Figure 3.6.4). Place the 1-m^2 plot right next to the pitfall trap on the right side and parallel to the transect. Align the center of the plot with the pitfall trap. Take a photograph at 1.0 m above ground using a camera. The photograph should include both the trap and 1-m^2 plot. Estimate leaf litter cover from the taken photograph. Measure depth of leaf litter at the center of the 1-m^2 plot using measuring tape. Measure other microhabitat variables related to water and substrate as described in Table 3.6.1.



Figure 3.6.4 A leaf litter survey plot of 1-m2.

Table 3.6.1 Microhabitat variables surveyed at each pitfall trap.

Variables	Unit	Definition	Description
Leaf litter cover	%	Percent cover of leaf litter at each pitfall trap	Visually estimate with a 1 × 1 m square plot. Take a photograph at 1.0 m above ground that includes both pitfall trap and the 1-m^2 plot.
Leaf litter depth	cm	Depth of leaf litter at each pitfall trap	Measure depth of leaf litter at the center of 1-m^2 plot.
Vegetation	-	Types of forest at a sample location	A category of primary forest, secondary forest, forest edge, agricultural land, and disturbed land.
Water	- / m	Types of water body at a sample location	A category of permanent stream, intermittent stream, permanent pond, temporary pond, and swamp. Record distance from water (m).
Substrate	-	Types of soil	A category of under soil, in/under leaf litter, on leaf litter, on bare soil, on log, on rock, under rock/log, in log, on grass blade, and in grass.
Vertical Position	-	The position of an amphibian or a reptile found vertically	A category of on leaf, on stem/branch, in tree hole, under tree bark, and on tree trunk.

3.6.3 Specimen Handling

Collect a representative voucher specimen of each species found at a sample location. The purpose of preparing a voucher specimen is for future reference, to prevent misidentification, and to address needs of other studies. Collect a minimum of three specimens of each species, preferably a male, a female, and a juvenile. An individual found dead in a pitfall trap may also be collected as a specimen.

The process of vouchering a specimen starts by injecting an individual with approximately 1 ml of Tricaine-S (methanesulfonate) to euthanize it. Once it dies, extract a sample of liver tissue for DNA analysis. Place the tissue in a vial containing 99% acetone solution. After that, inject 1 % of formalin solution into the specimen body to preserve it from decay. Once injected, mount the specimen in a container and cover it with tissue papers moistened with formalin solution. Leave the mounted specimen for minimum one day until it is fixed and dried. Once it is completely dry, wash the specimen with water to remove excess formalin. Transfer the specimen to a specimen jar filled with 70-75% of ethanol for long-term storage.

3.6.4 Species Identification

Identify species immediately in the field when removing an individual from a pitfall trap. For an amphibian, examine the individual for its general characteristics such as body size and shape, mouth, legs, and colorations or patterns of dorsal and ventral parts. Use reference books, local (www.amphibia.my) and international databases (Frost 2017) for aid identification. For a reptile, apply similar identification procedures, and use reference books by Das (2010) and online information such as Amphibians and Reptiles of Peninsular Malaysia (www.amphibia.my) and Frogs of Borneo (www.frogsofborneo.org) to aid identification. For a captured individual that is hard to identify, take photographs and consult experts.

3.6.5 Supplementary Materials

The following tables are provided in Appendix A6:
Table A6.1. List of equipment for sampling herpetofauna for one sample location.
Table A6.2. Field datasheet for recording capture of herpetofauna.
Table A6.3. Field dataset for microhabitat survey.

3.7 Large Mammal
By Tan, C.C., Meisery, A.A.H.A. & Fadlli, M.Y.

Large mammals play fundamental roles in many ecosystems by affecting species composition (Terborgh et al. 2001) and structures of surrounding vegetation (Skarpe and Hester 2008). According to the IUCN Red List, there are 5488 species, 412 subspecies, and 21 subpopulations of mammals in the world (IUCN 2017). However, only 263 species and subspecies belonging to 11 orders and 27 families are categorised as terrestrial large mammals (Morrison et al. 2007). According to Carbone et al. (1999) and Morrison et al. (2007), large mammals are mammals having a body weight of more than 20 kg. In Peninsular Malaysia, there are only 15 extant species of large mammal with the Javan Rhino (*Rhinoceros sondaicus*) being locally extinct (PERHILITAN 2017). The current IUCN (2017) status of large mammal species in Peninsular Malaysia is provided in Table A7.5.

Diversity of large mammals is associated with several landscape features, particularly patch size (Bowers and Matter 1997, Matthews et al. 2016, Ramesh et al. 2016). Ramesh et al. (2016) emphasised the need of large protected area in conserving large mammals in South Africa. Diversity also correlates with distance to the nearest protected area (Santini et al. 2015, Meyer et al. 2016), distance to settlement (Cardillo et al. 2005, D'Amico et al. 2015), distance to road (Clements 2013), and surrounding land use classes (Cardillo et al. 2005, Clements 2013). At the forest scale, diversity of large mammals correlates with habitat type (Rayan et al. 2012), elevation (Rayan et al. 2012), topography (Francis 2008, Rayan et al. 2012), and forest cover (Sampaio et al. 2010).

3.7.1 Target Population

This inventory targets the 15 extant species of large mammal in Peninsular Malaysia.

3.7.2 Collection Methods

Set up a camera trap station at the center of the 0.04-ha plot (Figure 3.7.1).

3.7.2.1 Camera Trap

A camera trap is a specialized wildlife monitoring equipment with a motion sensor or infrared sensor. It captures pictures or record videos when the sensor is triggered by any movement. The recommended camera trap model for this study is Reconyx HC500. It can capture images with 1/5th triggers speed but not video. Reconyx HC500 uses passive infrared sensor (PIR) and infrared LED flash to illuminate target area at night; thus, producing monochrome images at night. It comes with a security casing to prevent the camera from damage by animals or humans.

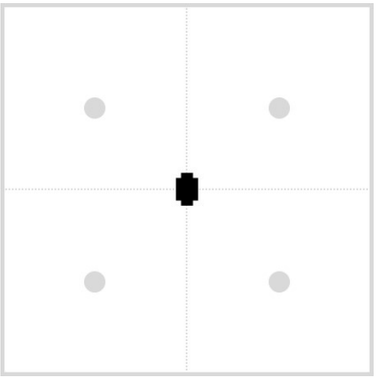


Figure 3.7.1 A camera trap is set up at the center of the 0.04-ha plot.

Prior to field set up, test each camera to ensure that it is immediately activated when detecting movement in the field. To do so, take test images in the laboratory. Once completing the test, inspect and clean all seals on the security casing to ensure that there is no leakage. Uniquely label each camera for identification purposes. The recommended camera settings are listed in Table 3.7.1.

Table 3.7.1 Settings of Reconyx HC500 camera trap.

Functions	Settings
Quickset	ADVANCED
Advanced Setup -> TRIGGER	TRIGGER
Motion sensor	ON
Sensitivity	HIGH
Pics per trigger	3
Picture interval	RAPIDFIRE
Quiet period	NO DELAY
Advanced Setup -> TIME LAPSE	TIME LAPSE
AM & PM period	OFF
Advanced setup	
Resolution	3.1 MP
Night mode	FAST SHUTTER
Date/Time	**refer to date/time displayed on GPS*
Show temperature	CELCIUS

At the center of the 0.04-ha plot, locate a straight tree closest to the plot center with a diameter of 15-20 cm. Once the camera trap location has been identified, clear the vegetation such as grass or over-hanging branches/ leaves which might affect the camera trap performance and quality of the photograph. Tightly secure the camera trap to the tree at about 35-40 cm above ground with a customized metal cable lock known as Phyton lock (Figure 3.7.2). Record the exact camera trap location with a handheld GPS unit. After the setup, record essential information of the camera trap accordingly as listed in Table A7.3.



Figure 3.7.2 A camera trap attached to a tree with a security casing.

Operate a camera trap for 60 days. Check the trap in the field after 30 days for any issue. Bring one or two cameras during the inspection to replace any damaged camera. Replace battery only when needed. If a camera trap is malfunctioned, record the information as listed in Table A7.4. If there is no issue, operate the camera for another 30 days. Replace memory card for each field trip to minimize the risk of losing camera trap data, then download all images for analysis in the laboratory. After 60 days, collect the camera from the field.

3.7.2.2 Image Analysis

Download all images from the memory card to a computer. Store them in a folder organised by locations. Backup a copy of the raw data on an external storage device to prevent data loss. Use the WWF-Malaysia camera trap database to store and process the images for statistical analyses if necessary. Store additional information recorded in the field such as GPS location, habitat type, locality type, elevation, and date and time of deploying and collecting camera trap in the same folder as the images. Store data collected from each image in the corresponding camera trap photo folder in the database (Figure 3.7.3). Once enter all relevant information into the database, the program generates the following parameters: total trap nights, total number of detections by species, naïve occupancy, activity patterns and classes, elevation by species, and relative abundance index (RAI). More information is found at: https://rimbaresearch.org/2018/06/08/updates-to-wwf-camera-trap-database/



Figure 3.7.3 An example of a camera trap image.

3.7.3 Species Identification

Catalogue images from a camera trap in a Microsoft Access camera trap database. Identify large mammals according to Francis (2008), Phillipps & Phillipps (2016) or consult an expert. Identify photographed small mammals when possible. Identify gender of large mammals with distinctive physical sex differences, but for others, identify gender when possible.

3.7.4 Supplementary Materials

The following tables are provided in Appendix A7:
Table A7.1. Orders and families of large mammal species with body weight more than 20 kg.
Table A7.2. List of equipment for sampling large mammal for one sample location.
Table A7.3. Field datasheet for camera trap.
Table A7.4. Field datasheet for damaged camera trap.
Table A7.5. Current status of large mammal species in Peninsular Malaysia.

3.8 Small Mammal

By Shahfiz, M.A., Nor-Hazwani, A.R. & Kaviarasu, M.

Small mammals are mammal species such as bats, rodents, civets and cats, whose adult weight does not exceed 5 kg. There are currently 320 mammal species from 131 genera and 37 families recorded in Malaysia (Wilson and Reeder 2005, Payne and Francis 2005, Davison and Zubaid 2007, Francis 2008). More than 70% of these are classified as small mammal species. Chiropterans represent the largest small mammal group consisting of 136 species from 8 families (Emballonuridae, Hipposideridae, Molossidae, Nycteriididae, Pteropodidae, Rhinolophidae, and Vespertilionidae). Rodentia is the second largest group with 86 species from 4 families (Hystricidae, Muridae, Sciuridae, and Spalacidae) (Medway 1983, Mohd Khan 1992, Corbet and Hill 1992, Wilson and Reeder 2005, Payne and Francis 2005, Davison and Zubaid 2007, Francis 2008).

Small mammals are known to play important ecological roles in forest ecosystems such as seed dispersal agents (e.g., civets) and seed predators (e.g., rats and mice). On the landscape-level, diversity and abundance of small mammals correlates with patch area, patch density, patch richness, distance to nearest water body, distance to nearest protected area, and distance to settlement and surrounding land use class (Harrison 1969, Bayne and Hobson 1998, Shanker and Sukumar 1998, Benítez-López et al. 2017). At the forest-level, their diversity and abundance correlate with stand density, stand volume, canopy closure, understory cover, coarse woody debris, and characteristics of nearby water body (Loman 1991, Converse et al. 2006, Dickman et al. 2011).

3.8.1 Target Population

This inventory targets non-volant small mammal species weighing below 5 kg at adult stage. They include 13 families: Erinaceidae (moonrat and gymnure), Herpestidae (mongooses), Hystricidae (porcupines), Manidae (pangolin), Muridae (rats and mice), Mustellidae (weasels), Ptilocercidae (pencil-tailed treeshrews), Sciuridae (squirrels), Soricidae (shrews), Spalacidae (bamboo rats), Talpidae (moles), Tupaiidae (treeshrews), and Viverridae (civets). Examples of non-volant small mammal species are: Long-tailed giant rat (*Leopoldamys sabanus*), Brown spiny rat (*Maxomys rajah*), Common treeshrew (*Tupaia glis*), and Plantain squirrel (*Callosciurus notatus*) (Figure 3.8.1).

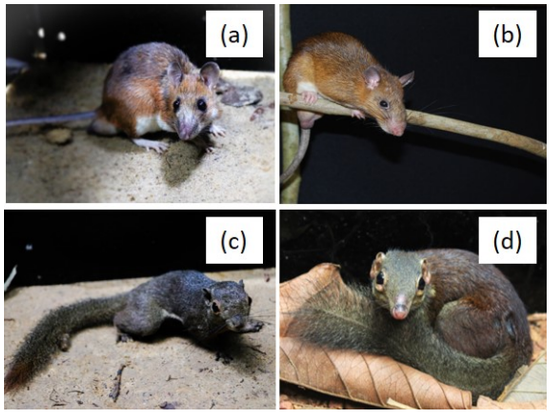


Figure 3.8.1 Examples of small mammals: (a) Brown spiny rat *(Maxomys rajah)*, (b) Long-tailed giant rat *(Leopoldamys sabanus)*, (c) Plantain squirrel *(Callosciurus notatus)*, and (d) Common treeshrew *(Tupaia glis)*.

3.8.2 Collection Methods

Live-trapping method is the most commonly used method to capture small mammals.

3.8.2.1 Live-Trapping Method

Establish four transects measuring 100 m each at a sample location (Figure 3.8.2). Space the four transects 10 m apart to cover areas surrounding the 0.04-ha plot. Arrange the transect so that they start in the general direction of West to East. Align the transects so that they are parallel to the orientation of the 0.04-ha plot as shown in Figure 3.8.2. Two of the transects intersect with the 0.04-ha plot at the subplot centers. At each transect, establish 10 traps spaced 10 m apart and place them as shown in Figure 3.8.2. As a result, there are 40 traps at a sample location. Out of which, 4 traps intersect the 0.04-ha plot boundaries and two traps are inside the plot.

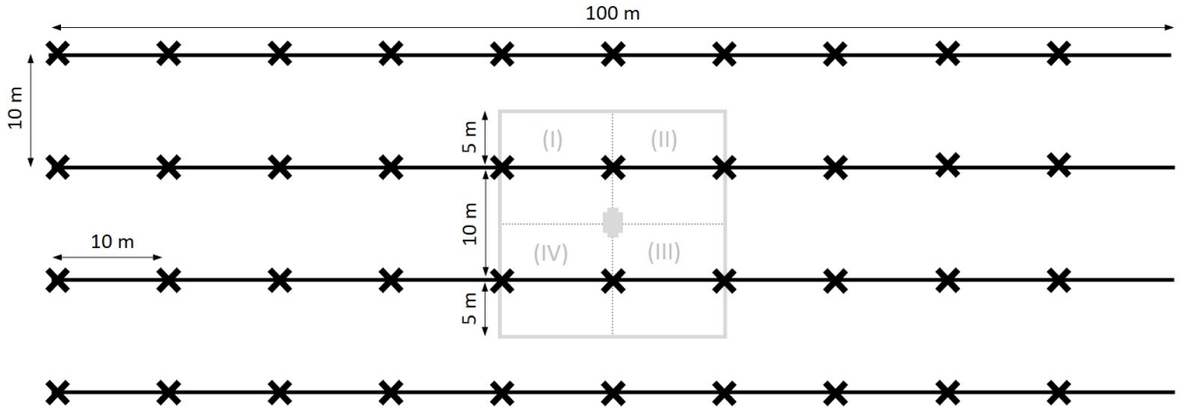


Figure 3.8.2 Four 100 m transects for trapping of small mammal species. Each transect measures 100 m and are placed at 10 m apart. A cross represents a trap, and traps are spaced 10 m apart.

At a trap, set up a collapsible cage trap and use palm oil seeds as baits (Figure 3.8.3a-b). Operate the trap for 5 sampling nights. Check the trap between 10:00 and 14:00 on each day. Immediately transfer any small mammal caught in the trap to a cloth bag (Figure 3.8.3c). When secured, take out the small mammal from the cloth bag and anesthetize it to measure its body attributes.



Figure 3.8.3 (a) A collapsible cage trap, (b) oil palm seed used as bait for trapping small mammals, and (c) a Brown spiny rat *(Maxomys rajah)* caught inside a collapsible cage trap with a cloth bag beside the cage.

In addition to trapping, search for arboreal small mammal species and identify them with binoculars (recommended Minox 10x55). Record any faeces, scats, marks, dungs, and footprints found at a trap. Identify and record calls and nests that indicate their presences in the area.

3.8.2.2 Coarse Woody Debris

Sample downed coarse woody debris (CWD) with line intersect sampling (LIS) (Figure 3.8.4). CWD is an important habitat requirement for small mammals, which use it for shelter and breeding. Carry out LIS at the four transects. Sample a CWD that intersects with a transect when checking traps for captured small mammals. For the sampled CWD, measure and record diameters at two ends and length of the CWD. Define length of the CWD as a straight line connecting both ends of the CWD. Only sample and measure a CWD with a minimum of 5 cm in diameter at the smaller end. Sampling of CWD is a one-time effort and conduct it during the first day of trapping.



Figure 3.8.4 Coarse woody debris sampling with (a) measuring the length of a fallen log intercepted transect line, and (b) measuring the diameter of a fallen log.

3.8.3 Specimen Handling

For a captured individual, record 7 morphological features: head-body length (HB), tail length (T), hind foot length (Hf), ear length (E), weight (g), gender, and reproduction status whether the female is pregnant or lactating (Figure 3.8.5). Use ruler, callipers, and spring scale for measurement. Collect tissue samples for further research. Tag and photograph the individual. Lastly, release the individual as close to the trap location as possible.

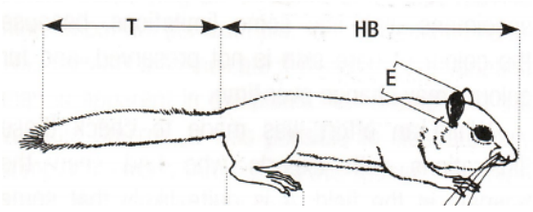


Figure 3.8.5 Measured attributes of a captured small mammal: head-body length (HB), tail length (T), hind foot length (Hf), and ear length (E). Photo depicted from Francis (2019).

3.8.4 Species Identification

Carry out species identification of captured small mammal in the field. Use Medway (1983), Mohd Khan (1992), and Francis (2008) as guides for species identification.

3.8.5 Supplementary Materials

The following tables are provided in Appendix A8:
Table A8.1. List of equipment for sampling small mammal for one sample location.
Table A8.2. Field datasheet for measurement of captured small mammals.
Table A8.3. Field datasheet for coarse woody debris sampled from Line Intersect Sampling.

3.9 Tree
By Chew, M.Y.

Trees are the most important biodiversity component in all forest ecosystems. The tropical rainforest in Malaysia harbours one of the most diverse tree floras in the world. Enumeration of tree species carried out at the long-term ecological plots in Lambir Hills National Park and Pasoh Forest Reserve, Malaysia has recorded 1173 species and 818 species, respectively (Lee et al. 2002, Davies et al. 2003). Euphorbiaceae appears as the most specious family at both sites. Other species rich families are Dipterocarpaceae, Lauraceae, Rubiaceae, Anonaceae, and Myrtaceae. In general, the flora of East Malaysia is richer than that of Peninsular Malaysia. For trees alone, it is estimated that both Sabah and Sarawak have about 44% more species than the Peninsular Malaysia.

Trees have been extensively studied for the relationship between species diversity and landscape- and forest-level variables. At the landscape-level, tree species richness and diversity correlate with patch area (Hernández-Ruedas et al. 2014), patch density (Cayuela et al. 2006), patch richness (Kumar et al. 2006), contiguity index (Saura and Carballal 2004), and surrounding land-use classes (Kessler et al. 2005). At the forest-level, tree species richness and diversity correlate with stand density (Majumdar et al. 2014), aspect (Ozcelik et al. 2008), topography (Liu et al. 2014), elevation (Toledo-Garibaldi and Williams-Linera 2014), soil type (Häger 2010), and ground water (Newbery and Proctor 1984).

Table 3.9.1 List of the 13 shrub species for sampling.

Species	Origin	Description
Cecropia peltata	Central–South America	Infests disturbed areas, watercourses, roadsides and abandoned land, steep slopes, forest gaps, and landslides.
Chromolaena odorata	America	Dominates ground layer of disturbed, open secondary habitats, wastelands, abandoned fields, grasslands, forestry plantations, trails, clearings, roadsides, and forest margins.
Clidemia hirta	Tropical America	Common weed at forest margins and shady areas.
Clidemia cf. *rubra*	Central–South America	Newly discovered in southern Johor.
Commersonia bartramia	Malaysia	Widespread native pioneer in lowland disturbed sites.
Leucaena leucocephala	Tropical America	Widespread in waste ground.
Melastoma malabathricum	Malaysia	Widespread native pioneer in secondary growth in lowlands and hills.
Mimosa diplotricha	Tropical America	Widespread weed in waste ground and plantation.
Mimosa pigra	Central–South America	Infests riverbanks, open floodplains, wetlands, marshy grassland, rice fields, immature oil palm plantations, fruit orchards, and roadsides.
Muntingia calabura	Tropical America	Common in waste ground, around towns, and villages.
Piper aduncum	Tropical America	Infests roadsides and clearings in lowlands and hills, and in waste ground.
Stachytarpheta cayennensis	Tropical America	Common in waste ground.
Solanum torvum	Central–South America	Dominates fairly-opened understory of degraded forests.

3.9.1 Target Population

This inventory targets tree and 13 shrub species. A tree is defined as it having diameter at breast height (dbh) ≥ 5 cm. The 13 shrub species with dbh < 5 cm and total height (ht) ≥ 1 m are: *Cecropia peltata*, *Chromolaena odorata*, *Clidemia hirta*, *Clidemia* cf. *rubra*, *Commersonia bartramia*, *Leucaena leucocephala*, *Melastoma malabathricum*, *Mimosa diplotricha*, *Mimosa pigra*, *Muntingia calabura*, *Piper aduncum*, *Solanum torvum*, and *Stachytarpheta cayennensis*. These shrub species are indicators of forest disturbance and most of them are invasive plants (Table 3.9.1).

3.9.2 Collection Methods

Establish a 20 × 20 m (0.04-ha) plot with its center at the sample location. Sample all trees with dbh ≥ 5 cm and the 13 shrub species in the 0.04-ha plot.

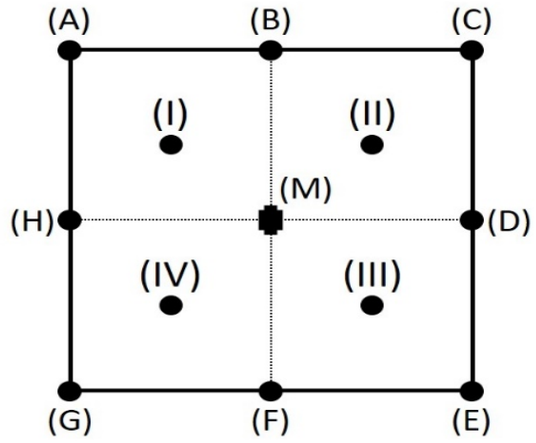


Figure 3.9.1 A 20 × 20 m (0.04-ha) plot centered at a sampling location (solid square) for sampling trees and shrub species. The plot is subdivided into four subplots (I-IV). Boundary of the plot is marked with eight points (A-H).

3.9.2.1 Sample Plot

Establish the 0.04-ha plot with a laser range finder for measuring distance and an angle encoder for finding appropriate azimuth. Record plot center geo-coordinates with a GPS unit. Establish 8 points on the plot boundaries with four at the corners and four at the middle between the corners. Mark the 8 points with 1.5 m PVC pipe. Spray the PVC pipe with orange paint and tie orange flagging tape on them for easy identification in the field. Label the PVC pipe clockwise from the Northwest corner with A to H (Figure 3.9.1). Mark plot center with red painted PVC pipe and tie an orange flagging tape on it. Subdivide the plot into four equal sized subplots using raffia strings. Label each subplot clockwise from the Northwest corner with Roman numerals (I, II, III, and IV) (Figure 3.9.1).

3.9.2.2 Tree Measurements

A tree is a sample tree if the center of its base or more than 50% of its basal area is inside the 0.04-ha plot. A tree is a border tree if the center of its base or 50% of its basal area is exactly on the boundaries of the 0.04-ha plot. First identify all border trees and tentatively give them numerical numbers starting from 1. Include odd-numbered border trees as sample tree, while exclude even-numbered border trees from sampling. Exclude dead trees from sampling. When finish identifying all tentative sample trees, start to tag them.

Tag sample trees starting from subplot I to subplot IV. In a subplot, identify trees clockwise from the boundary towards the subplot center. For trees on the boundary bordering two subplots, the rule is to consider a tree: (1) on the B-M boundary to be in subplot I, (2) on the D-M boundary to be in subplot II, (3) on the F-M boundary to be in subplot III, and (4) on the H-M boundary to be in subplot IV (Figure 3.9.1). Tag a sample tree with an aluminum tag facing towards the plot center. Each tag has two rows of code: the first row is the plot number, and the second row is the tree number. Plot number is a unique series of number assigned to a plot based on its location. Tree number always starts at 001 and runs continuously regardless of subplots or any other situations such as trees missed in the previous sampling session. However, for a tree that was sampled in the previous sampling session but is currently without a tag, cross-reference its species and diameter measurement to existing records. If confirmed, nail a replacement tag with correct labels on the tree. Give a recruit in a remeasurement a new tree number continuing from the last existing number.

The followings are special considerations:

(1) For a leaning, prostrate, or fallen trees that is still alive, if the center of its base is within the 0.04-ha plot or on the boundary but its height of measurement (HOM; defined below) is outside the plot, include the tree as a sampled tree,

(2) For a leaning, prostrate, or fallen tree that is still alive, if the center of its base is outside the 0.04-ha plot but its HOM is within the plot, exclude the tree from sampling,

(3) For a tree with the center of its base in subplot I but its HOM in subplot II, it is a sample tree for subplot I, and so forth.

For a tree with dbh ≥ 10 cm, nail the aluminum tag at 20 cm above its HOM (Figure 3.9.2). For a tree with dbh < 10 cm, use twist ties to tie the aluminum tag on it to minimize damage to the tree trunk (Figure 3.9.2). For a forked tree or a tree with coppices, tag each stem with dbh ≥ 5 cm with a unique number consisting of tree number plus an alphabet starting from a (e.g., 001a, 001b, …). For a tree with buttresses or stilt roots that are > 80 cm in diameter and its HOM at a height > 1.3 m, nail the aluminum tag at about 1.5 m above ground for ease of checking.



Figure 3.9.2 Tag is nailed on sampled trees with dbh ≥ 10 cm while twist ties are used to hold the tag for trees with dbh < 10 cm.

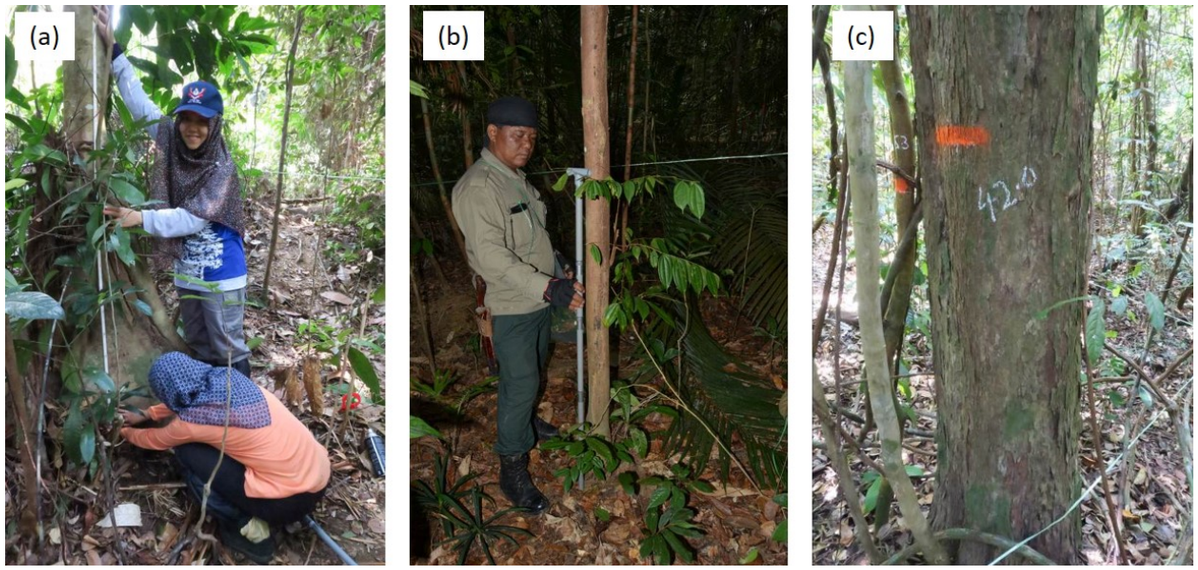
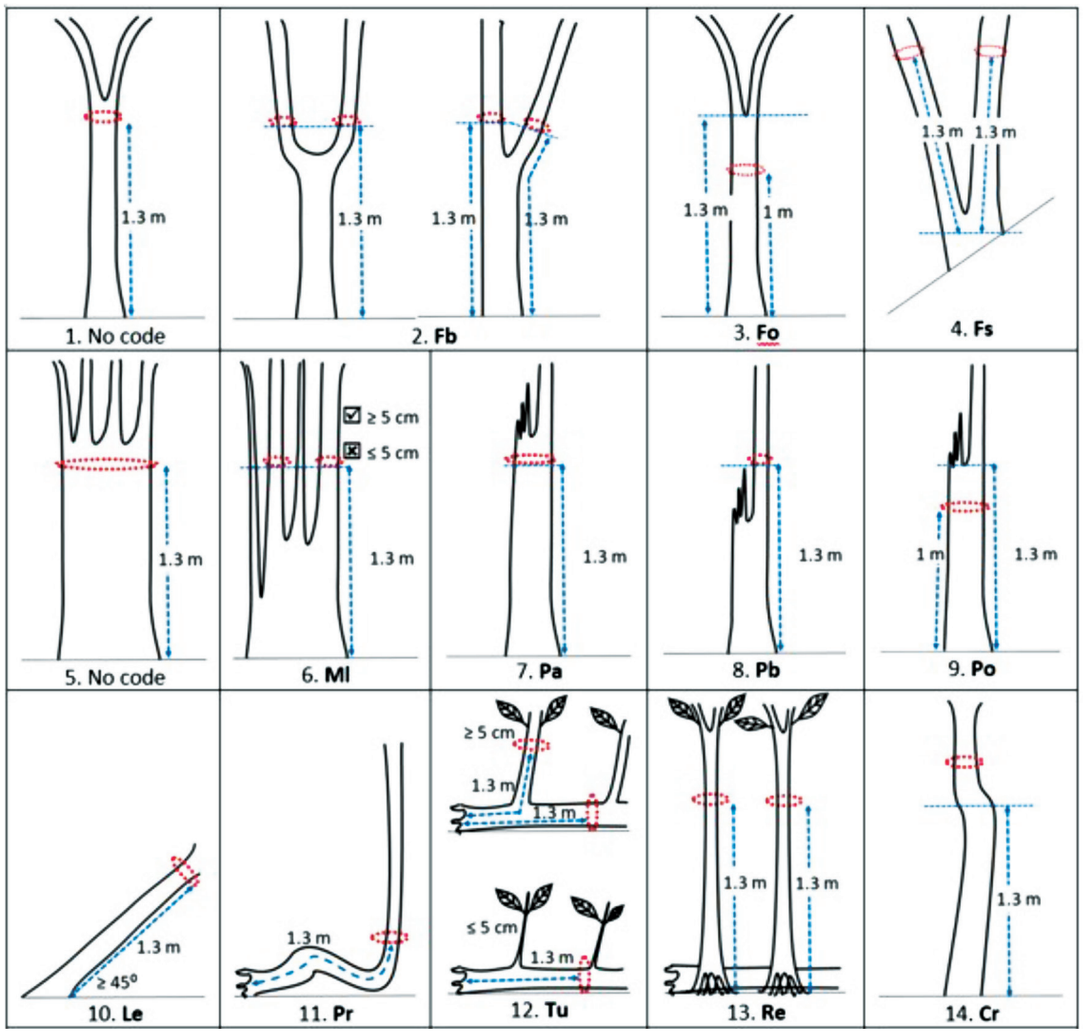
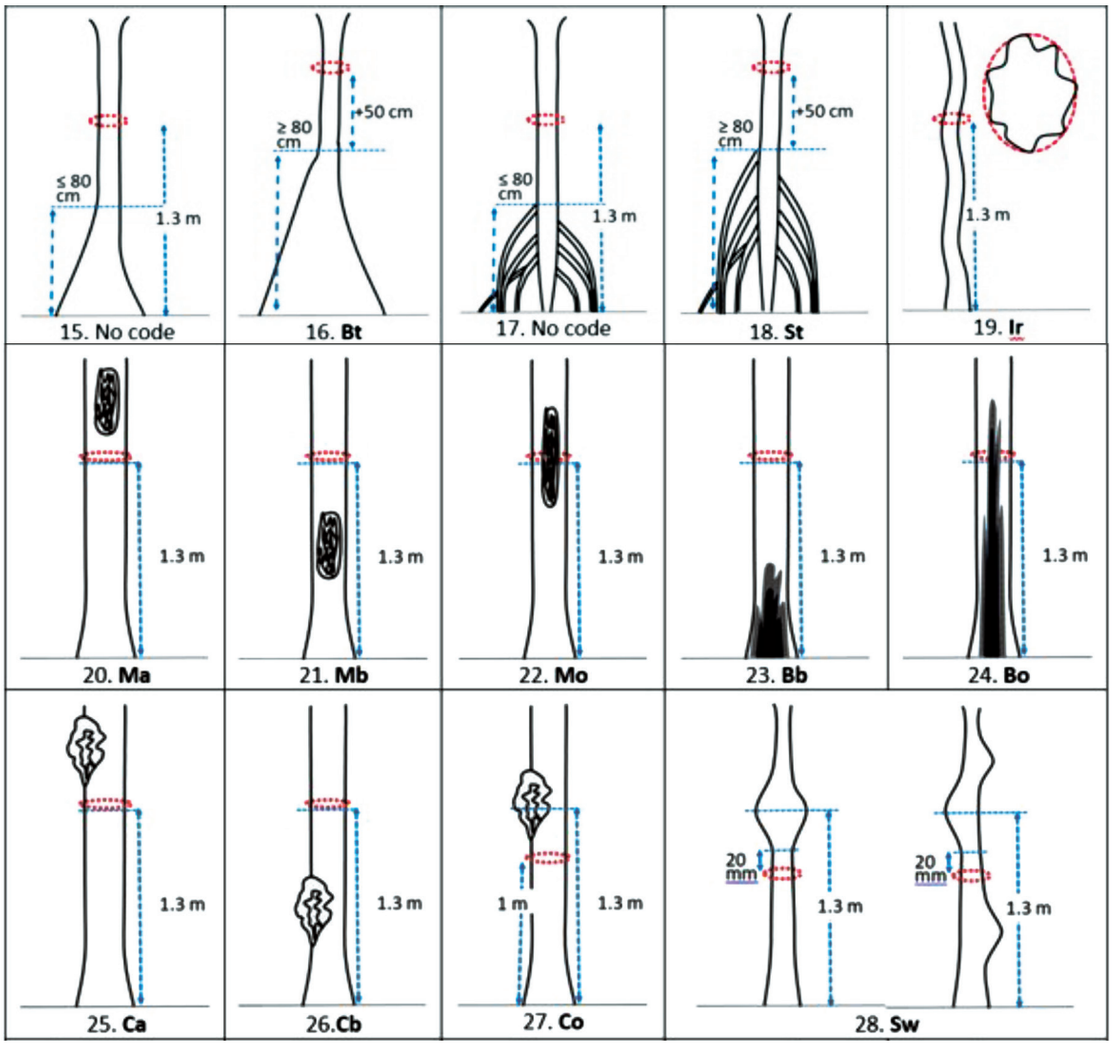


Figure 3.9.3 (a) Locating height of measurement (HOM) for a sample tree with buttress (height of buttress + 50 cm), (b) marking the level of dbh with a 1.3 m T-jointed standard PVC pole, and (c) a 5-10 cm red strip is sprayed onto each sample tree to mark the HOM.

When finish tagging, measure a sample tree for dbh. Record its stem code and identify it to species-level. Height of measurement (HOM) is the height at which dbh is measured. For a regular tree, HOM is 1.3 m above ground. For a tree with buttresses or stilt roots that are ≤ 80 cm, HOM is 1.3 m above ground. For a tree with buttresses or stilt roots that are > 80 cm, HOM is 50 cm above the height of the buttress (Figure 3.9.3a). For a forked tree or a tree with coppices, measure each stem with dbh ≥ 5 cm with the protocol specified in Figure 3.9.4. For other special conditions, refer to Figure 3.9.4 for HOM definitions and approaches to measuring dbh. After identifying HOM of a tree, mark the sample tree with a long line of paint about 5-10 cm long and 2 cm thick at HOM facing the plot center (Figure 3.9.3c). Spray the long line onto the bark with a bright red and weatherproof oil paint using a T-jointed standard PVC pole for levelling (Figure 3.9.3b). Refer to Table 3.9.2 for further details on tree measurement including stem codes.




≤ 80 cm
1.3 m
15. No code
≥ 80 cm
+50 cm
16. Bt
≤ 80 cm
1.3 m
17. No code
≥ 80 cm
+50 cm
18. St
1.3 m
19. Ir
1.3 m
20. Ma
1.3 m
21. Mb
1.3 m
22. Mo
1.3 m
23. Bb
1.3 m
24. Bo
1.3 m
25. Ca
1.3 m
26.Cb
1 m
1.3 m
27. Co
20 mm
1.3 m
20 mm
1.3 m
28. Sw

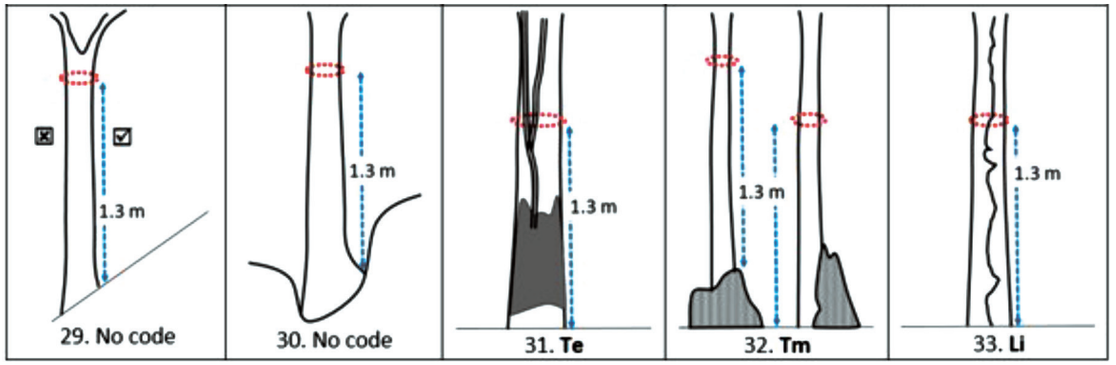


Figure 3.9.4 Protocol for measuring dbh of each sample tree. The tree codes are as follows: forked below 1.3 m (Fb), forked at 1.3 m (Fo), forked tree on slope (Fs), multiple stems due to coppice (Ml), broken above 1.3 m (Pa), broken below 1.3 m (Pb), broken at 1.3 m (Po), leaning tree (>45°) (Le), trunk leaning near 90° (Pr), fallen tree (Tu), prostrate stem re-sprouted (Re), crooked at 1.3 m (Cr), large buttressed tree (Bt), stilt-rooted tree (St), irregular trunk (Ir), mechanical damage above 1.3 m (Ma), mechanical damage below 1.3 m (Mb), mechanical damage at 1.3 m (Mo), burnt below 1.3 m (Bb), burnt at 1.3 m (Bo), canker above 1.3 m (Ca), canker below 1.3 m (Cb), canker at 1.3 m (Co), swelling at 1.3 m (Sw), termite damage (Te), tree on termite mound (Tm), and lightning damage (Li) (Details refer to Table 3.9.2).

Table 3.9.2 Description of stem codes to be assigned to each sampled tree.

No.	Description of stem condition	Stem Code
1.	**Forked above 1.3 m** Diameter is measured at 1.3 m from the base.	-
2.	**Forked below 1.3 m** Diameter of each branch ≥ 5 cm is measured at 1.3 m from the base.	**Fb**
3.	**Forked at 1.3 m** Diameter is measured at 1.0 m from the base.	**Fo**
4.	**Forked tree on slope** Diameter is measured at 1.3 m as indicated.	**Fs**
5.	**Stem with coppices above 1.3 m** Diameter is measured at 1.3 m from the base.	-
6.	**Multiple coppices below 1.3 m** Diameter is measured at 1.3 m from the base for each stem ≥ 5 cm.	**Ml**
7.	**Broken above 1.3 m** Diameter is measured at 1.3 m from the base.	**Pa**
8.	**Broken below 1.3 m** Diameter is measured at 1.3 m from the base.	**Pb**
9.	**Broken at 1.3 m** Diameter is measured at 1.0 m from the base.	**Po**
10.	**Leaning tree (> 45°)** Diameter is measured at 1.3 m from the base, following curve if necessary.	**Le**

No.	Description of stem condition	Stem Code
11.	**Near prostrate tree** Diameter is taken at 1.3 m from the base of the trunk following the curve.	**Pr**
12.	**Fallen tree, alive, branch ≥ 5 cm not rooted** Any branch ≥ 5 cm within 1.3 m from base of stem that is not rooted is measured; any branch beyond 1.3 m is not measured.	**Tu**
13.	**Prostrate stem with rooted new sprouts ≥ 5 cm** Diameter is measured at 1.3 m from where it roots.	**Re**
14.	**Crooked at 1.3 m** Diameter is measured 20 mm above affected point.	**Cr**
15.	**Buttress ≤ 80 cm** Diameter is measured at 1.3 m from the base if highest buttress is ≤ 80 cm.	**-**
16.	**Large buttress ≥ 80 cm** Diameter is measured at 50 cm above buttress if highest buttress is ≥ 80 cm.	**Bt**
17.	**Stilt-root ≤ 80 cm** Diameter is measured at 1.3 m from the base if highest stilt root is ≤ 80 cm.	**-**
18.	**Large stilt-root ≥ 80 cm** Diameter is measured at 50 cm above stilt root if highest stilt root is ≥ 80 cm.	**St**
19.	**Trunk irregular at all heights** Diameter is measured at outer perimeter at 1.3 m from the base.	**Ir**
20.	**Large mechanical damage above 1.3 m** Diameter is measured at 1.3 m from the base.	**Ma**
21.	**Large mechanical damage below 1.3 m** Diameter is measured at 1.3 m from the base.	**Mb**
22.	**Large mechanical damage at 1.3 m** Diameter is measured at 1.3 m from the base.	**Mo**
23.	**Burnt below 1.3 m** Diameter is measured at 1.3 m from the base.	**Bb**
24.	**Burnt at 1.3 m** Diameter is measured at 1.3 m from the base.	**Bo**
25.	**Large canker above 1.3 m** Diameter is measured at 1.3 m from the base.	**Ca**
26.	**Large canker below 1.3 m** Diameter is measured at 1.3 m from the base.	**Cb**
27.	**Large canker at 1.3 m** Diameter is measured at 1 m from the base.	**Co**
28.	**Trunk has swelling or constriction at 1.3 m** Diameter is measured 20 mm below the lowest point.	**Sw**
29.	**Tree on slope** Diameter is measured at 1.3 m from the base on the uphill side.	**-**
30.	**Tree in depression** Diameter is measured at 1.3 m from the base at the highest ground level.	**-**

No.	Description of stem condition	Stem Code
31.	**Termite** Diameter is measured at 1.3 m from the base.	**Te**
32.	**Termite mount** Diameter is measured at 1.3 m from the base of stem on termite mount.	**Tm**
33.	**Lighting damage** Diameter is measured at 1.3 m from the base.	**Li**

3.9.2.3 Shrub Measurement

For the 13 shrub species listed in Table 3.9.1, record their presence and absence in each subplot with 1 indicating presence and 0 indicating absence. *Cecropia peltata*, *Commersonia bartramia*, *Leucaena leucocephala*, *Muntingia calabura*, and *Piper aduncum* are shrubby small trees with dbh of mature individuals ≤ 5 cm. *Melastoma malabathricum*, *Mimosa diplotricha*, *Mimosa pigra*, and *Solanum torvum* are woody shrubs with dbh of mature individuals ≤ 5 cm. *Chromolaena odorata*, *Clidemia hirta*, *Clidemia* cf. *rubra*, and *Stachytarpheta cayennensis* are tall shrubby herb with dbh of mature individuals ≤ 5 cm but height sometimes ≥ 1.3 m.

3.9.3 Specimen Handling

3.9.3.1 Field Specimen Collection

Collect specimens of sampled trees to facilitate species identification. Collect specimens only for the first encountered sampled tree of every tree species. Avoid collecting specimens for other individuals of a tree species if species of these individuals can be confidently confirmed in the field. Otherwise, collect specimens for other individuals of a questionable tree species in the field.



Figure 3.9.5 (a) Collecting leaf specimens with long-poled cutter, and (b) shooting down leaf specimens from tall trees with a catapult.

Follow procedures in Bridson and Forman (1992) when collecting specimen in the field:

(1) Collect twigs have length ≥ 30 cm and adequately show arrangement of leaf and key characters such as flowering and fruiting parts when available. For a tree with low and reachable branches, cut twigs with long-poled cutter (Figure 3.9.5a). For a tree with branches beyond reach, use a catapult to obtain leaf samples and use a pair of binoculars to confirm leaf arrangements (Figure 3.9.5b),

(2) Record key habitat and field characters including bark, crown, branching, and colours of flower and/or fruits in the field. Take photographs of the specimens whenever possible,

(3) Tag each specimen with the same number as on the aluminum tag of the sample tree and record date of collection (Figure 3.9.6),

(4) Preserve fragile and soft specimens such as flowers in Copenhagen solution (10:1:8 mixture of Industrial Methylated Spirit, glycerol, and water),
(5) Press collected specimens between two or more sheets of newspapers. Pile up the pressed specimens and tie them between two wooden plant presses for compression. Drench pre-compressed piles of specimens awaiting oven-drying in alcohol and store them in large, air-tight plastic bags.



Figure 3.9.6 (a) Each specimen is tagged with the same number as the aluminum tag on the sampled tree, and (b) an example twig and leaf specimen obtained for species identification.

3.9.3.2 Herbarium Processing

When specimens are at laboratory or herbarium, process the specimens as follows:

(1) Place specimens into an oven set at 50°C for a minimum of 3 days for drying. Bulkier specimens require longer drying time,
(2) Properly label dried specimens with relevant information,
(3) Pack specimens into air-tight plastic bags. Freeze them in a deep freezer set at -15°C for at least 24 hours to sterilize them from bugs and other pests,
(4) Remove frozen specimen packs from the freezer and thaw them to room temperature before opening to avoid damping to the specimens from condensations,
(5) Send sterilised specimens to a herbarium for identification.

3.9.4 Species Identification

Take prepared specimens to a herbarium and compare them to reference specimens for validating their species. The process of species identification is as follows:

(1) Match specimens to the closest family, genus, and species according to flora accounts and reference specimens in the FRIM Herbarium,
(2) Sort unidentified specimens into morphospecies and give each specimen a unique ID (UNK001, UNK002, …). Keep these labels until identification can be made when better materials are available later,
(3) When available, extract information of previously collected specimens from the study area where specimens are collected from the Botanical Research and Herbarium Management System (BRAHMS) database for comparison,
(4) Enter specimen identity information and associated field notes into the Botanical Research and Herbarium Management System (BRAHMS) database.

3.9.5 Supplementary Materials

The following tables are provided in Appendix A9:
Table A9.1. List of equipment for sampling tree and shrub for one sample location.
Table A9.2. Field datasheet for recording sample tree information.
Table A9.3. Field datasheet for recording presence and absence of shrub species.



Chapter 4.0

Statistical Data Analysis

4.0 Statistical Data Analysis
By Lam, T.Y.

This section briefly discusses statistical approaches to analyse data collected in this document. Given the extensive amount of information collected, this section by no means documents the full extent of the possibilities that the data could be analysed. The choice of statistical analysis approach depends on the research question or direction to be addressed. Without being specific, a diversity index refers to a quantitative indicator reflecting diversity of a taxa group at a location. It could be richness (count of species), Shannon index, Simpson index, and etc. Three broad research questions are: (1) Are diversity of a taxa group differed between forest reserves and corridors? (2) How is diversity of a taxa group associated with habitat conditions in forest reserves and corridors? and (3) How are diversity and habitat conditions related among different taxa groups?

For the first research question, use paired two sampled parametric or non-parametric hypothesis test methods such as Student's *t*-test or Wilcoxon signed-rank test. For each pair of forest reserve and corridor sample locations, calculate the difference in the diversity index. Test these differences whether they are significantly different from zero. When include data from two or more primary links in the analysis, possibly modify the hypothesis test methods for random effects.

For the second research question, use linear regression methods for each taxa group. The diversity index is the response variable, and the habitat conditions are the explanatory variables. When include data from two or more primary links in the analysis, treat primary links as random effects by using mixed effects models. Use variable selection methods such as leaps and bounds algorithm to identify explanatory variables that are significantly associated with the diversity index. Lastly, if the response variable is a count variable such as species richness, using Generalized Linear Model (GLM) or its variant Generalized Linear Mixed Model (GLMM) assuming Poisson link is more appropriate.

For the third research question, multivariate analysis approaches are appropriate. Some of the common and well-known multivariate analysis approaches are classification and discrimination analyses and Multidimensional Scaling Method. These are non-parametric statistical models that could relate diversity of multiple taxa groups to habitat conditions. For parametric approach, multivariate regression models and Structural Equation Modelling are the options.

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Appendix

Appendix

Appendix A1. Johor Primary Link Study Area

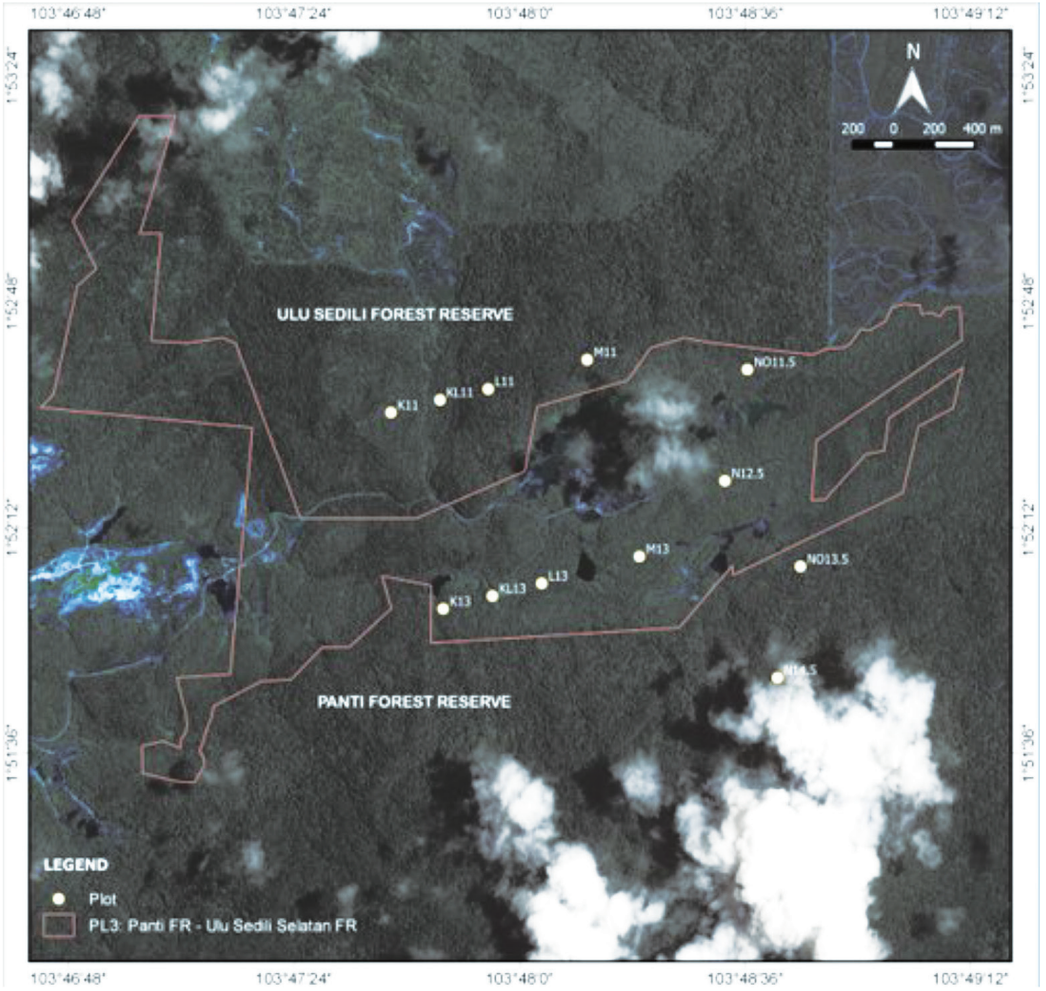


Figure A1.1 Final 6 pairs of corridor-forest reserve sample locations selected for Johor Primary Link study area.

Appendix A2. Ant

Table A2.1 List of equipment for sampling ant for one sample location.

Item	Quantity
Ground Pitfall Trap	
Hand spade	1
Plastic cup	9
Bottle filled with detergent (dilution rate 1:100)	1
Vials containing 95% undenatured ethanol	9
Vials labels	9
Soft forceps	1
Wire sieve	1
Fine head pigment ink pen	1
Arboreal Pitfall Trap	9
Plastic cup with holes on the top	9
Vinyl coated twist wire	1
Bottle filled with detergent (dilution rate 1:100)	1
Tuna and honey solution	9
Face cotton	9
Vials containing 95% undenatured ethanol	9
Vials labels	1
Soft forceps	1
Wire sieve	1
Fine head pigment ink pen	9
Leaf Litter Sifting	1
White sheet with plastic protective cover	1
Plastic sieve (mesh size 15 x 30 x 3 sm)	1
Hand gloves	1
Soft forceps	4
Vials containing 95% undenatured ethanol	4
Vials labels	1
Fine head pigment ink pen	
Baiting	4
Transparent plastic plate	9
Face cotton	-
Tuna and honey solution	12
Vials containing 95% undenatured ethanol	9
Vials labels	1
Soft forceps	1
Wire sieve	1
Baiting datasheet	1
Fine head pigment ink pen	

Table A2.2 Field datasheet for baiting activity.

ANTS BAITING DATASHEET

Location: **Recorded by:**

No	Vial no	Plot no	Subplot	Baiting time	Morphological characteristic	No of ants seen	No of ants collected	Specimens no

Verified by:

Table A2.3 Field datasheet for field collection of specimens.

ON SITE COLLECTION RECORDS DATASHEET

State: _____

Recorded by: _____ **Date:** _____

No	Vial no	site	Plot	Subplot	Sampling point	Sampling method*	Collector	Sampling date

*Sampling method: Arboreal Pitfall Trap (APT), Ground Pitfall Trap (GPT), Baiting (BT), and Leaf Litter Sifting (LLS)

Verified by:

Table A2.4 Field datasheet for ground vegetation survey.

MICROHABITAT DATA

Site: _____ **Recorder:** _____

Plot no: _____ **Date Recorded :**_____

Subplot	Canopy Cover (%)		Lower vegetation cover (%)						Rain volume (ml)
	Percentage	Picture no	Litter	Bare Soil	Pebbles	Fine Roots	Grass	Picture no	
I									
II									
III									
IV									

Verified by:

Table A2.5 Datasheet for processing ant specimens in laboratory.

SPECIMENS PROCESSING DATASHEET

Site: _____ **Personnel Processing:** _____

Compartment: _____ **Date Processed:** _____

Page no: _____

Vial No	Specimens no	Site	Site category	Plot	Point	Sampling Method*	Date			Collector	Species Name	No of individual	Number of pinned specimen
							dd	mm	yyyy				

*Sampling method: Arboreal Pitfall Trap (APT), Ground Pitfall Trap (GPT), Baiting (BT), and Leaf Litter Sifting (LLS)

Verified by:

Appendix A3. Butterfly

Table A3.1 List of equipment for sampling butterfly for one sample location.

Item	Quantity
Field Collection	
Butterfly net	1 to 4 *
Waist porch to keep specimens	1 to 4 *
Fine head pigment ink pen	1 to 4 *
Soft forceps	1 to 4 *
Writing board	2
Thermohygrometer	1
Lux meter	1
Camera	1
PVC pole with 1.3 m length	1
Butterfly envelopes in small, medium and large sizes	Enough for sampling
Datasheet	1 set for one plot
Tissue paper	1 packet
Specimen Processing at Field	
Lining tissue	2 to 3 sheets for one container
Plastic container (to keep specimens in icebox)	2 to 3
Zip lock bags	2 to 3
Ice box	1
Ice cube	Enough to fill up the ice box
Specimen Pinning at Field	
Pinning boards	8
Stainless steel insect pins	1 box for size “0” and “3”
Glassine paper	32 strips (depends to the collection)
Large zip lock bags	3 to 4
Moth balls	3 to 4
Silica gel	1 bottle of 1 kg
Fine mesh cloth pockets to keep silica gel and moth balls	3 to 4
Carrying boxes	2 to 4 (depends to the design)
Padding peanuts	Enough to fill up the carrying boxes

*depending on the number of collectors available.

Table A3.2 Field datasheet for butterfly collection.

State:	**Date: .....**
Plot:	**Collector Name: .....**
Sampling Time (Start – End):	**Transect: .....**
General description on transect:	
General description on weather:	

Species/Genus sighted	Collected successfully? (Yes/No)	If not collected, why? Disappear / Too high

Table A3.3 Field datasheet for environmental data collection.

State	Date	Time	Transect	Points	Temperature	RH	Light intensity	Canopy image no.

Table A3.4 Datasheet for summary of butterfly specimens collected in the field.

State	Plot no.	Transect no.	Date (DDMMYY)	Time start	Time end	Collector	Specimen code (Code to identify each specimen	Family	Subfamily	Tribe	Genus	Species & subspecies	Full Name	Gender (Female/ Male)

Appendix A4. Dung Beetle

Table A4.1 List of equipment for sampling dung beetle for one sample location.

No.	Item	Quantity
1.	Garden trowel	1
2.	Plastic cup	9
3.	Dry leaf	9
4.	Elephant dung ball	9
5.	Soft fiber mesh	9
6.	Bamboo skewer	9
7.	Sifter	1
8.	Entomology forceps (soft forceps)	1
9.	Vials 20 ml (filled with 70% ethanol approximately 10ml)	9
10.	Fragrance free detergent water	200ml

Table A4.2 Field datasheet for ground vegetation survey.

Plot No:							**Date:**		
Subplot	**Canopy Cover**		**Lower Floor Cover (%)**						**Rain (Yes/No)**
	%	**Pic. No**	**Litter**	**Bare Soil**	**Pebbles**	**Roots**	**Vegetation**	**Pic. No**	
i									
ii									
iii									
iv									

Table A4.3 Datasheet for specimen processing in laboratory.

SPECIMENS PROCESSING DATASHEET

Site:_____ Compartment: _____ Plot/Transect:_____

Personnel Processing: _____ Date Processed _____

Page no: _____

Vial No	Specimens no	Site	Site category	Plot	Point	Date			Collector	Species Name	No of individual	Number of pinned specimen
						dd	mm	yyyy				

Verified by:

Appendix A5. Herbaceous Plant

Table A5.1 List of equipment for sampling herbaceous plant for one sample location.

No	Equipment	Quantity
1.	Measuring tape (30 m)	2
2.	Measuring tape (5 m)	2
3.	Camera	1
4.	Clipboard	2
5.	Raffia string (9mm wide)(Red)	2
6.	GPS	1
7.	Clinometer	1
8.	Quadrate size 50 x 50 cm made from PVC pole	1
9.	External hard disk	2
10.	Micro SD card	3
11.	Melamine chopsticks	100
12.	Labels to indicate subplot number	1 set
13.	Cardboard	20
14.	Corrugated zinc	10
15.	Plant press	3 set
16.	Tag/label for tagging voucher	50
17.	Methylated spirit	1 L
18.	Fixative/preservative, e.g., 70 % ethanol, Copenhagen mixture	250 ml
19.	News paper	50 sheets
20.	Collection book	1
21.	Note book	2
22.	Laptop	1
23.	Plastic bag (big) for collecting vouchers	2
24.	Plastic bag (small) for collecting flowering materials	10
25.	Silica gel for DNA sample	2 bottle
26.	Tea bag to store DNA samples	1 pack

Table A5.2 Field datasheet for herbaceous plant survey.

State: _____ **Plot:** _____ **Subplot:** _____ **Date:** _____

Species	H (cm)	D1 (cm)	D2 (cm)	Image no	Notes

H=Height; D1=Vertical width; D2=Horizontal width

Appendix A6. Herpetofauna

Table A6.1 List of equipment for sampling herpetofauna for one sample location.

No.	Equipment	Purpose	Quantity	Remarks
1.	Snake hook	Handling poisonous species	2	
2.	Snake tong	Handling poisonous species	2	
3.	Plastic bags	To temporarily store all captured individuals	100 pcs	
4.	Cloth bag	To temporarily store poisonous individuals e.g., snakes	10	
5.	Caliper	To measure morphology of frogs or small sized individuals	2	
6.	Measuring tape	To measure morphology long sized individuals such as snakes and monitor lizards	2	
7.	Digital camera	To photographs captured species	1	
8.	Gloves	To handle specimens	1 box	Gloves are used throughout specimen measurement and DNA collection; to avoid any contamination
9.	Head lamp or flash light	To aid in searching herpetofauna during active search at night	4-5	
10.	18L bucket with lid	To catch ground crawling herpetofauna	40 buckets per plot (320 for one state)	
11.	Solution Tricaine-S (methanesulfonate)	To euthanize capture individual for voucher specimen	500ml	
12.	Solution formalin (1-2 %)	To voucher specimen fixation	2 liters	
13.	Vial or capped tubes	For DNA tissue collection	100 tube	

Table A6.2 Field datasheet for recording capture of herpetofauna.

Examples of Filling Up the Datasheet
Data Form for Herpetofauna
Zoology Unit
FRIM

Location: Panti FR **Manner Caught: Pitfall** **GPS:**
Habitat: Secondary **Time:** **Weather: Hot/Rainy/Cloudy**

Date	Tag No	Trap No	Species	Sex	TL (mm)	SVL (mm)	Tagging	Remarks
16/08/2017		L1 20M	*Hylarana labialis*	M/F	-	46		Released
16/08/2017		L4 90M	*Aphoniotis fusca*	F	107	89		Voucher: HZF00453

Table A6.3 Field dataset for microhabitat survey.

MICROHABITAT	SPECIES CODE	*HZF00477*	*HZF00478*										
VEGETATION	Primary Forest												
	Secondary	*√*	*√*										
	Edges												
	Agriculture												
	Disturbed												
WATER	Permanent Stream	*√*	*√*										
	Intermittent Stream												
	Permanent Pond												
	Temporary Pond												
	Swamp												
	Distance from water	*2m*	*10m*										
SUBSTRATE	Under Soil												
	In/Under Leaf litter												
	On Leaf Litter	*√*											
	On Bare Soil												
	On Log												
	On Rock												
	Under Rock/Log												
	In Log		*√*										
	On Grass Blade												
	In Grass												
VERTICAL POSITION	On Leaf	*√*											
	On Stem/Branch												
	In Tree Hole												
	Under Tree Bark		*√*										
	On Tree Trunk												

Appendix A7. Large Mammal

Table A7.1 Orders and families of large mammal species with body weight more than 20 kg.

Order	Family	Number of species
Artiodactyla	Antilocapridae	1
	Bovidae	76
	Camelidae	3
	Cervidae	40
	Giraffidae	2
	Suidae	14
	Tayassuidae	3
Carnivora	Canidae	7
	Felidae	9
	Hyaenidae	3
	Ursidae	7
Cingulata	Dasypodidae	1
Dasyuromorphia	Thylacinidae	1
Diprotodontia	Macropodidae	12
	Vombatidae	3
Perissodactyla	Equidae	7
	Rhinocerotidae	5
	Tapiridae	4
Pholidota	Manidae	1
Pilosa	Myrmecophagidae	1
Primates	Cercopithecidae	13
	Hominidae	4
	Pongidae	2
Probosccidea	Elephantidae	2
Rodentia	Caviidae	1
	Hystricidae	2
Tubulidentata	Orycteropodidae	1
	Total	225

* Note: Total number of species in Peninsular Malaysia which does not include subspecies

Table A7.2 List of equipment for sampling large mammal for one sample location.

No.	Item	Quantity
1.	CT form or note book	Variable
2.	Pen	2
3.	Marker pen	1
4.	Clip board (optional)	1
5.	Ruler (small 15 cm type)	2
6.	Spirit level	2
7.	Line spirit level	2
8.	Measuring tape (> 5 meter)	1
9.	Compass	1
10.	Silica gel in small plastic pack	Variable
11.	Bungee cord	Variable
12.	Pad lock	Variable
13.	Pen knife	1
14.	Duck tape (2 inch roll)	1
15.	Cleaning cloth or tissue (small piece)	Variable
16.	Memory SD card and back up	Variable
17.	CT and back up CT	Variable
18.	Cable lock	Variable
19.	Cable lock key	Variable
20.	Batteries for CT	Variable
21.	Back up batteries for CT	1 set
22.	Digital camera + batteries	2 sets
23.	Back up digital camera batteries	1
24.	GPS + extra AA batteries	2 sets
25.	Topographic map of the area	Variable
26.	Umbrella	1
27.	A large bag (better quality)	1
28.	Machete	Variable

Table A7.3 Field datasheet for camera trap.

Program:							**Site:**					
No	**Date**	**Time**	**Group**	**Camera Id**	**Key Id**	**Coordinate - x**	**Coordinate - y**	**Altitude (m)**	**Habitat Type**	**Topography**	**Trail Type**	**Canopy**	**Flood Risk**

Table A7.4 Field datasheet for damaged camera trap.

Camera Trap Damage Form				
No.	**Camera Trap Serial Number**	**Date Purchased**	**Location of Installation**	**Note (Type of Damage, Summary of Damage)**

Verified by

.....

Table A7.5 Current status of large mammal species in Peninsular Malaysia.

No.	Common Name	Scientific Name	Red list[1]	IUCN[2]
1	Javan rhinoceros	*Rhinoceros sondaicus*	EX	CR
2	Banteng	*Bos javanicus*	CR	EN
3	Sumatran rhinoceros	*Dicerorhinus sumatrensis*	CR	CR
4	Malayan tiger	*Panthera tigris*	CR	CR
5	Leopard	*Panthera pardus*	EN	VU
6	Malayan sun bear	*Helarctos malayanus*	VU	EN
7	Asian elephant	*Elephas maximus*	VU	EN
8	Sambar deer	*Rusa unicolor*	EN	VU
9	Gaur	*Bos gaurus*	EN	VU
10	Dhole	*Cuon alpinus*	NT	EN
11	Clouded leopard	*Neofelis nebulosa*	NT	VU
12	Malayan tapir	*Tapirus indicus*	EN	EN
13	Bearded pig	*Sus barbatus*	DD	VU
14	Barking Deer	*Muntiacus muntjac*	NT	LC
15	Southern serow	*Capricornis sumatraensis*	EN	VU
16	Wild boar	*Sus scrofa*	LC	LC

[1]Red List of Mammals for Peninsular Malaysia (PERHILITAN 2017), [2] The IUCN Red List of Threatened Species (IUCN 2017). Ex: Extinct; CR: Critically Endangered; EN: Endangered; VU: Vulnerable; NT: Near Threatened; DD: Data Deficient.

Appendix A8. Small Mammal

Table A8.1 List of equipment for sampling small mammal for one sample location.

No.	Item	Quantity
1.	Collapsible cage traps	40
2.	Ruler	2
3.	Caliper	2
4.	Spring scale	2
5.	Cloth bag	10
6.	Tag	20
7.	Glove	2
8.	Animal studio for photograph	1
9.	Binoculars	2
10.	Datasheet	1
11.	Pencil	3
12.	Handheld GPS	1
13.	Camera	1
14.	Reference books	2

Table A8.2 Field datasheet for measurement of captured small mammals.

SMALL MAMMAL DATASHEET

Site: Johor / Perak / Pahang
Plot: **Collected by:**

Date	Bait	Trap no	Tag no	Species	Sex	HB (cm)	TL (cm)	Ear (cm)	Hindfoot (cm)	Weight (g)	Notes

Key: Head-Body Length (HB), Tail Length (TL), Hind Foot Length (Hf), Ear Length (E) and live weight (g)

Table A8.3 Field datasheet for coarse woody debris sampled from line intersect sampling.

WOODY DEBRIS COARSE MEASUREMENT

Site: Johor / Perak / Pahang
Plot: **Collected by:**

Date	Line	Diameter Start (m)	Diameter End (m)	Length (m)	Notes

Appendix A9. Tree

Table A9.1 List of equipment for sampling tree and shrub for one sample location.

Item	Quantity
Sample Plot Setup	
LTI Truangle	1
LTI Impulse 200LR	1
Garmin GPS 64s	1
Suunto Tandem	1
Motorola Walkie Talkie GP328	3
Canon Powershot SX50 HS	1
Francis Barker Prismatic Compass	2
Trimble Data Controller TCS 2	1
Monopod (2m)	4
Monopod (3m)	1
Bipod	1
Survey Reflector	3
10 m steel measuring tape	1
20 m fiber glass measuring tape	1
PVC pipes class 7 (Size: 1.5m x 15mm, 1.0m x 15mm, and 0.5m x 15 mm)	102
Flagging tape (Orange fluorescent and pink)	3
White and Red stripe marking tape (Caution tape)	2
Spray paint (Colour: Fluorescent orange, fluorescent pink, green, yellow and red)	3
Tree Measurement	
Raffia string 20(+10) m	6
Measuring tape (50m)	1
Lensatic compass	1
Plot layout plan laminated	2
1.3-m T-jointed standard PVC pole	1
Chalk	8
Fiber glass diameter tape	1
Spray paint (Red)	2
DBH marking bar mould (Corrugated plastic cardboard)	2
Aluminum tag with plot name and tree number engraved	100
Aluminum tag blank	
Twist tie	100
Stainless steel concrete nail	100
Hammer	1
Engraving puncher set A-Z, 0-9	1

Item	Quantity
Tool waist bag	1
Clipboard	1
Tree survey form	10
Waterproof note book	1
Stem code illustration reference laminated	2
Stem code description table laminated	2
Pencil	2
Eraser	2
Haglof Vertex V	2
Specimen Collection	
Long-pole cutter	1
Catapult	1
Catapult rubber	1
Fishing thread 200 m on small plastic reel	1
Nylon rope 100 m on large plastic reel	1
Lead weight	3
Angled stones (as catapult bullet)	200
Secateurs (to trim specimens)	1
Machete (for bark slash when necessary)	1
Protective helmet and eye piece set	2
Specimen tag	100
Binocular	1
Camera	1
Battery set (spare)	1

Table A9.2 Field datasheet for recording sample tree information.

Tree (dbh ≥ 5 cm) survey form Plot:_____ Date:_____(YY/MM/DD) Recorder:_____Team members:_____							
Quad No.	**Tree No.**	**Stem No.**	**Species**	**Dbh (cm)**	**Stem Code**	**HOM (m)**	**ID Notes**

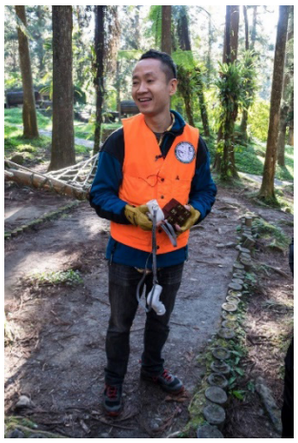
Table A9.3 Field datasheet for recording presence and absence of shrub species.

Shrub (dbh ≤ 5 cm, H ≥ 1.3 m) survey form Plot: _____ Date:_____(YY/MM/DD)					
Note: absence = 0 presence = 1					
Species	**Q I**	**Q II**	**Q III**	**Q IV**	**Notes**
Cecropia peltata					
Chromolaena odorata					
Clidemia hirta					
Clidemia cf. *rubra*					
Commersonia bartramia					
Leucaena leucocephala					
Melastoma malabathricum					
Mimosa diplotricha					
Mimosa pigra					
Muntingia calabura					
Piper aduncum					
Solanum torvum					
Stachytarpheta cayennensis					

About Authors



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Dr. Sam Yen Yen is Senior Forest Botanist at the Kepong Herbarium, FRIM. She has a PhD degree in plant taxonomy. Since joining FRIM in 1999, she has carried out botanical explorations to various parts of Malaysia, particularly in Peninsular Malaysia. Her research involves plant documentation, taxonomy, molecular systematics and conservation with a special interest on wild gingers.



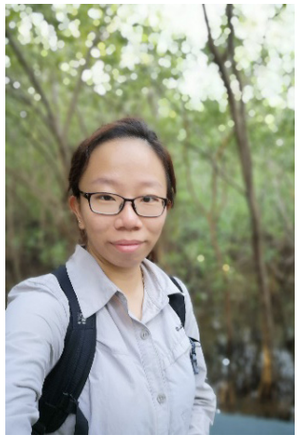
Dr. Phon Chooi-Khim is a entomologist in FRIM. She completed her PhD in Ecology and Biodiversity at the University of Malaya. Her PhD research was on the taxonomy, behaviour and ecology of the Rajah Brooke's Birdwing in Peninsular Malaysia. She is also involved in several studies on butterfly diversity, impact of highways on butterfly dispersal, butterfly composition in different period of regeneration forests and conservation. Besides being active in the field, she is also curating the butterflies' collection in FRIM Entomological Reference Collection.



Nur-Zati Akma Mustafa is an Entomologist in Entomology Branch FRIM. She is a MSc holder from University Kebangsaan Malaysia in the field of Zoology (Entomology). Her research focuses on ecology and biodiversity of ants in Malaysia. Her interest is on using ants as bioindicator for forest health and disturbance. She has also been working on dung beetle ecology in several projects.



Kaviarasu Munian is a research officer of Zoology Branch under Forest Biodiversity Division, FRIM since the year 2008. His first bachelor degree was on biology science from University Putra Malaysia and completed his Master of Science in genetics and genomics from University Malaya. Currently he is pursuing his doctorate on environmental DNA (eDNA) and Next Generation Sequencing of freshwater vertebrates in University Tun Hussein Onn Malaysia (UTHM). The scope of research includes on taxonomy, ecology and genetic of vertebrates especially on group of herpetofaunal and ichthyofaunal in Peninsular Malaysia. He has led a couple of research projects and published several peer-reviewed journals, international and national proceedings, articles, and other forms of publications related to biodiversity and conservation.



Tan Cheng Cheng is a nature conservationist and wildlife enthusiast. She works as a Project Assistant for UNDP/GEF-GoM Project: Improving Connectivity in the Central Forest Spine Landscape. She completed her BSc in Animal Science and Wildlife Management from the University of Queensland. She then pursued her MSc in Biodiversity and Conservation from the Universiti Malaysia Terengganu, where she conducted camera trapping study to investigate the impact of hydroelectric dam on wildlife. She previously served as a Research Officer for the Department of Wildlife and National Parks Peninsular Malaysia, focusing on mammal research projects at designated wildlife corridors. She is selected as ASEAN Youth Biodiversity Leader 2019 for her work in wildlife conservation field.



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Ahmad Meisery bin Abd Hakim Amir was a research officer in the Department of Wildlife and National Park from 2016 until 2020. He has been involved in wildlife surveys for the Improving Connectivity in the Central Forest Spine (IC-CFS) and 1st National Tiger Survey (1st NTS) programme. He graduated with a Bachelor Degree in Forestry Science from Universiti Putra Malaysia (UPM) in 2013. His interest is on big mammals especially Tiger and Malayan Tapir. He is currently working as an Assistant Project Manager in the Forestry Management Division, Ministry of Energy and Natural Resources (KeTSA).

BIODIVERSITY ASSESSMENT AND MONITORING PROTOCOL IN THE CENTRAL FOREST SPINE LANSCAPE

The **Biodiversity Assessment and Monitoring Protocol in the Central Forest Spine Landscape** provides a set of specific inventory protocols for eight taxonomic groups: ants, butterflies, dung beetles, large mammals, small mammals, herpetofauna, herbaceous plants and trees. These groups were selected based on their sensitivity toward environmental changes. These protocols were implemented in the states of Perak, Pahang and Johor under the IC-CFS project. The aim of this publication is to provide guidance and reference for survey methodologies and to build capacity of field personnel in other agencies. This publication fulfils one of the objective under the IC-CFS project which aims to enhance current land management plans in the identified landscapes to ensure the integration of biodiversity and ecosystem service values and that all planned land use activities are sustainable.

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