

AN INSIGHT INTO THE DUNG BEETLE'S WORLD

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Dung beetles are a group of beetles that feed almost exclusively on dung. Most species of dung beetles are adapted to feeding on mammalian dung, typically harvesting undigested nutrients, small particles and bacteria found in the dung. Because of their high dependency on the availability of mammalian dung as their primary source of food and for breeding, the diversity and biomass of dung beetles are known to be closely associated with the presence of large mammals. Even though most species of dung beetles are not picky about the type of dung that they feed on, human dung has been known to be the most attractive bait for dung beetles (Whipple & Hoback 2012).

Some forest-adapted dung beetle species are sensitive to changes in their environment. Degraded forests tend to have fewer forest specialist species and the loss of mammalian species can also result in the loss of dung beetle species that have adapted to exploit the dung of those mammals. Their behaviour of burying and consuming dung helps to improve nutrient recycling and also soil structure which will indirectly improve forest health. These characteristics, along with ease of capture, make dung beetles an excellent focal taxon for environmental monitoring.

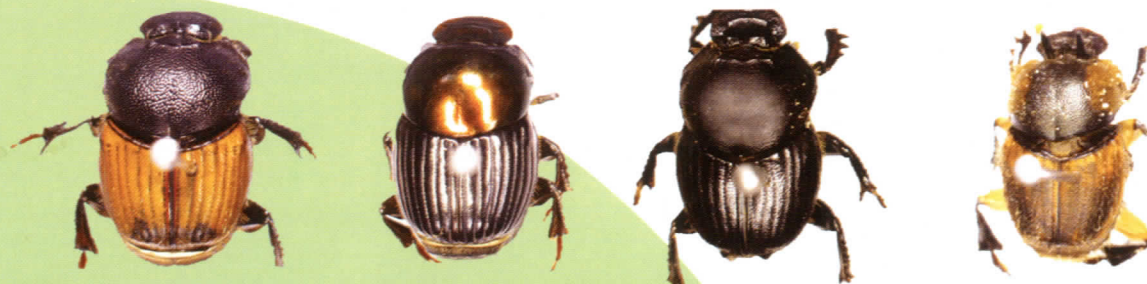
Dung beetles are divided into the subfamilies Scarabaeinae and Aphodinae of the family Scarabaeidae. Scarabaeinae, which consists of the true dung beetles,



A female *Copris ramosiceps* searching for food with its antennae out.

feed exclusively on dung, and is further divided into nine tribes based on morphological characteristics. One of the most diverse tribes of dung beetles is the tribe Onthophagini which consists of almost 2000 species of *Onthophagus*. On the other hand, Aphodinae are known as small dung beetles but not all species feed on dung. Non-dung feeding beetles are detritivores, saprophages, inquilines (living in other organisms' nests) with ants or termites, or predators.

Dung beetles are also categorised by guilds based on their breeding biology. The most primitive guild are the dwellers that build nests directly within dung or in shallow nests under dung. On the other hand, tunnelers have the derived behaviour of building deep tunnels



Onthophagus species with various colours:
from left *O. rorarius*, *O. rutilans*, *O. tsubakii* and *O. vulpes*.



Copris agnus (left) and *C. doriae* (right).



2 mm

The sole large male of *Copris gibbulus*, one among the rare species collected from Cherul FR, Terengganu. This large male has an impressive well-developed horn that is very large relative to its body size.

in the soil under dung which they fill with provisions of dung. These tunnels are usually guarded by a single male that fights off other males that try to enter the tunnel. Because of this, many species of tunnelers have evolved horns for combat. The remaining guild, namely the rollers, avoid competition for space under the dung by removing some dung, shaping it into a ball and moving it to a different location before burying it within a shallow nest. While the rollers do not have to fight in duels like tunnelers, they often have to defend their dung balls from scrums of other male beetles that try to steal them.

The beetle offspring go through complete metamorphosis before leaving the successfully constructed nest. During the whole process, dung beetles often display parental care, a behavioural trait that rarely occurs in insects. Typically, females will collect dung into several brood balls and lay a single

egg into each. The egg develops from a larva to a pupa and into an adult beetle, all within this controlled environment. During the development of the larvae, female dung beetles are known to seal themselves into the nest, cleaning and maintaining the dung balls until the young fully emerge. Due to the large amount of resources invested into raising their young, dung beetles typically produce only a small clutch of eggs at a time (approximately 1 to 45 eggs, depending on the species).

Collectively dung beetles are one of the most diverse groups of invertebrates in the world. To date, there are informal records of roughly 170 species of dung beetles recorded from the Malay Peninsula alone, while Borneo has an estimated 200 species (Davis, 2000). Many of these species are new to science and there is still a lot of taxonomic work that needs to be done to clear up the records and remove synonyms.



◀ *Sisyphus thoracicus* (roller dung beetle) attending elephant dung.

▼ *Sisyphus thoracicus* using its strong hind legs to roll the dung ball.





Paragymnopleurus maurus rolling a dung ball to the nest.

Among the genera most commonly found in Peninsular Malaysia are *Onthophagus* (Latreille, 1802) and *Copris* (Geoffroy, 1762). *Onthophagus* is the most dominant and abundant genus present in Peninsular Malaysia. It is also one of the most species-rich genera in the world, with over 2000 species discovered worldwide so far (Hanski & Cambefort, 1991), and more discoveries are expected. *Onthophagus* are usually small or medium sized beetles (7–12 mm), with colours ranging from black to brown to metallic greens and reds. *Copris* is a genus of dung beetles in the tribe Coprini which comprises more than 250 tunnelling species and has an almost worldwide distribution. To date, 19 species of *Copris* have been recorded in Malaysia (Doll *et al.*, 2014; Goh *et al.*, 2014). In contrast to *Onthophagus*, *Copris* tend to be larger (9–22 mm) and duller in colour, with colours limited to black or dark browns. Many rare or new species are found from these two genera every year.

An example of a very rare species is *Copris gibbulus* of which only 5 individuals have been found in Peninsular Malaysia in modern times. A single small male was collected from Kenyir, Terengganu (Huijbregts, J. collection), 3 individuals from the montane forest of Fraser's Hill (Goh T.G. collection) and a sole large male during the recent sampling at Cherul Forest Reserve (FR), Terengganu. Not much is known about the biology of this species as so few individuals have ever been caught. This rather small *Copris* (9–12 mm) is completely glossy black and both males and females have two short tooth-like protrusions on the pronotum. The large male from Cherul FR has an impressive well-developed horn that is very large (6 to 7 mm) relative to its body size, similar to the specimens collected from Java and Borneo (Huijbregts, J. collection). The collection of

the specimen from the lowland forest of Cherul FR is unique as previously this species had been found in upperhill and montane forest only. Thus, this raises an interesting question on their habitat preferences and their ecological needs.

In general, dung beetle diversity can provide an insight on the presence of mammals in the forest as well as levels of disturbance to forests. The presence of rare specialist species could provide a valuable insight on the niches available in forest habitats and the ecological values that they hold. Therefore, our understanding of dung beetle diversity and its potential as a bioindicator, especially for Peninsular Malaysia, should be further explored.

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DNA FORENSICS FOR TRACKING TIMBER TO SUPPORT BIODIVERSITY CONSERVATION AND SUSTAINABLE UTILISATION OF FOREST RESOURCES

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Illegal harvesting of forest resources poses a significant threat to the sustainability of forest ecosystems. In Malaysia, there are over 3,000 timber-size species, which belong to approximately 90 families. Of these, 408 species are known in the international market. Accurate timber species identification and the tracking of their geographical origins along the chain-of-custody are fundamental and necessary to combat illegal harvesting and associated trade. A range of scientific methods have been developed recently with potential application to forensic timber identification. These include visual identification methods (wood anatomy and dendrochronology), chemical methods (mass spectrometry, near infrared spectroscopy, stable isotopes and radiocarbon dating), and genetic methods (DNA barcoding, population genetics/phylogeography and DNA profiling) which rely only on inherent wood characteristics. These methods, unlike other product identification methods based on non-inherent features of wood such as paint markings, paper based certificates, physical barcoding tags and radio frequency identification (RFID) tags, do not require direct physical tagging of the timber product, but use the inherent wood characteristics as the method for identification. Consequently, certification using these methods is not affected by the problems associated with tagging, and cannot be falsified.

By using genetic methods, we at the Forest Research Institute Malaysia (FRIM) have developed DNA databases of six important tropical tree species for timber tracking. These

are *Neobalanocarpus heimii* (Chengal), *Gonystylus bancanus* (Ramin Melawis), *Koompassia malaccensis* (Kempas), *Shorea platyclados* (Meranti Bukit), *Aquilaria malaccensis* (Karas) and *Intsia palembanica* (Merbau). A total of 204 populations throughout Malaysia and 6,699 individual samples were used to establish the DNA databases of the six species (Figure 1). For each species, the DNA barcode was generated for taxonomic identity (i.e. from which genus or species the timber originates). Apart from that, the population identification database for each species using chloroplast DNA markers was developed to trace the source of a suspected timber up to population/regional level. In addition, the identification database for each species using short tandem repeat (STR) markers was developed. This enables the harvested logs to be matched against the original tree stumps with a high degree of accuracy.

For the application of these DNA databases, four standard operating procedures (SOPs) of DNA forensics for timber tracking were developed: Specimen Collection in the Field (SOP1); DNA Isolation and Purification from Cambium and Wood (SOP2); DNA Sequencing for Species and Population Identification (SOP3); and Short Tandem Repeat (STR) Genotyping for Population and Individual Identification (SOP4). The availability of DNA databases of these important tree species together with FRIM's SOP of DNA forensics for timber tracking enhances the capacity of Forest Department officials to curb the problem of illegal logging, indirectly

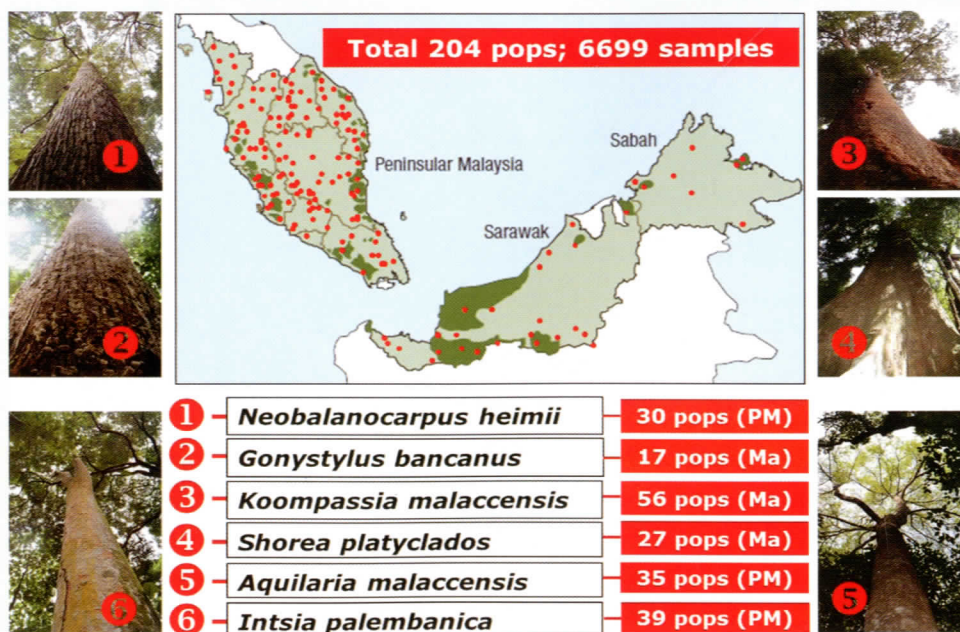


Figure 1: A total of 204 populations throughout Malaysia and 6,699 individual samples were used to establish the DNA profiling databases of *Neobalanocarpus heimii* (1), *Gonystylus bancanus* (2), *Koompassia malaccensis* (3), *Shorea platyclados* (4), *Aquilaria malaccensis* (5) and *Intsia palembanica* (6).

Suspected logs



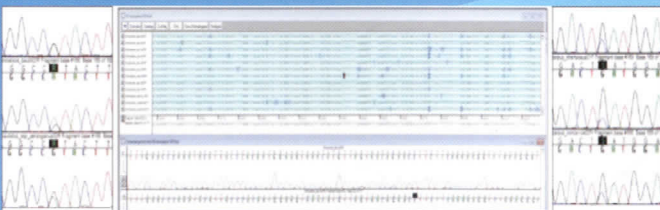
Specimen collection according to FRIM's SOP1



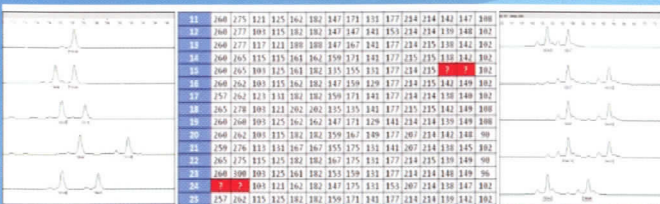
DNA isolation according to FRIM's SOP2



DNA sequencing according to FRIM's SOP3



STR genotyping according to FRIM's SOP4



FRIM's DNA barcoding and DNA profiling databases

contributing to the conservation and sustainable utilisation of forest resources in Malaysia. In fact, these databases have been used to assist Forest Department and Department of Wildlife and National Parks (PERHILITAN) personnel in the investigation of illegal logging activities (Figure 2). Besides, these databases have also been used to assist wood industry authorities in the authentication and identification of timber species. These databases can also be potentially used for timber certification to meet the international or consumer country regulations such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Canadian Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act, United States Lacey Act, European Union (EU) Forest Law Enforcement, Governance and Trade (FLEGT) initiative, as well as EU Timber Regulations, and Australian Illegal Logging Prohibition Act.

Glossary of terms

DNA barcoding – a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species.

DNA profiling – the analysis of a small amount of genetic material, which is unique per individual as is a fingerprint, as an aid to identification; also called genetic fingerprinting.

Mass spectrometry – an instrumental method for identifying the chemical constitution of a substance by means of the separation of gaseous ions according to their differing mass and charge.

Near infrared spectroscopy – a form of non-invasive imaging that applies near-infrared radiation (wavelengths 780 nm to 3,000 nm) to chemicals or biological subjects to measure differential absorption.

Phylogeography – the study of the historical processes that may be responsible for the contemporary geographic distributions of individuals. This is accomplished by considering the geographic distribution of individuals in light of genetics, particularly population genetics.

Population genetics – the study of the genetic composition of populations, including distributions and changes in genotype and phenotype frequency in response to the processes of natural selection, genetic drift, mutation and gene flow.

Radiocarbon dating – a technique for determining the age of organic materials, such as wood, based on their content of the radioisotope ¹⁴C acquired from the atmosphere when they formed part of a living plant.

Short tandem repeats – a short sequence of nucleotides (typically two to four) randomly distributed throughout the genome; also called microsatellite or simple sequence repeats (SSRs).

Figure 2: Utilisation of DNA databases and FRIM's Standard Operating Procedures of DNA forensics for timber tracking.

Species identification and tracking into original stumps with random match probabilities



Provide direct forensic evidence to prove that the logs were illegally harvested

STINKHORN FUNGI – A FOOD SOURCE FOR BUTTERFLIES?

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Our first encounter with a stinkhorn fungus was at Jeram Tebrau in Negeri Sembilan, on a sunny day on 16th April 2015, at around 11.00 a.m., while we were conducting a survey on the distribution of the Rajah Brooke's Birdwing butterfly. We were walking along a forest path near a stream, when we suddenly detected a very bad odour, like that of carrion. We followed our noses and found this beautiful fungus growing among *Commelina*-like plants. The fungus had an off-white bell-shaped cap on a stalk with a yellowish lacy skirt-like structure hanging beneath the cap, reaching almost to the forest floor. The fungus was about 15–20 cm high, and the cap, which had a circular opening in the middle, was covered with a slimy, brown fluid. The fungus was later identified as the stinkhorn, *Phallus luteus* belonging to the family Phallaceae. This fungus has been recorded from the grounds of the Forest Research Institute Malaysia (FRIM) (Lee, 2017) and is also known to occur elsewhere in Peninsular Malaysia (Lee, pers. comm.).

What surprised us was the presence of several butterflies feeding on the slimy fluid on the fungus cap: a Lesser Cruiser (*Vindula dejone erotella*) and three Straight Line Mapwings (*Cyrestis nivea nivalis*). Surprisingly, the butterflies were not bothered by our presence and continued feeding greedily on the slimy fluid on the fungus cap. We endured the unpleasant smell and took out our compact camera to record our observations. During this time, the butterflies flew away but came back shortly to continue their meal. We also found *Drosophila* fruit flies and other flies dining together with the butterflies. Some flies were on the cap while many were on the lacy skirt-like indusium.

Other species of butterflies have been reported to be attracted to stinkhorn fungi. The Banded Swallowtail (*Papilio demolion demolion*) and Malay Viscount (*Tanaecia pelea pelea*) have been found feeding on the Basket Stinkhorn (*Phallus indusiatus*) in MacRitchie Forest, Singapore (Bird Ecology Study Group, 2013). Magnificent males of *Morpho amathonte* and *M. peleides* have also been found feeding on decayed stinkhorn fungi in Costa Rica (Young, 2017). Smaller butterflies such as *Prosotas* (most likely species *dubiosa*) were observed to feed on *Dictyophora multicolor* in Queensland (Australian National Botanic Gardens, 2013). At present, we know that there are at least seven butterfly species that feed on stinkhorn fungi. Males of some of these butterfly species are known to settle



and take up nutrients from wet mud on forest paths or from carrion. They imbibe nutrients such as sodium and nitrogen (Beck *et al.*, 1999), phosphorus (Lai-Fook, 1991), and several others. The males transfer these nutrients to the females through their spermatophores during copulation. The function of phosphorus in butterflies is yet to be discovered, but sodium and nitrogen are known to be important for reproductive success (e.g. important in egg production).

We do not know why the butterflies were attracted to the stinkhorn fungus; it could be due to the carrion-like smell or perhaps they actually benefitted from the nutrients in the slimy fluid. The properties of the slimy fluid are not well studied. The volatile compounds of another species of stinkhorn fungus, that is, *P. impudicus* were studied by Pudil and co-workers (2014), and they found that the strong and foul odour was contributed by dimethyl oligosulfides. Stinkhorn fungi do not produce air-borne spores but the spores are borne in the slimy fluid which serves as a medium for spore dissemination. The fungus uses the rotting odour to attract mycophagous insects so that these insects consume the slimy fluid and egest the spores as they are unable to digest the spores effectively (Tuno, 1998). However, the stingless bees, *Trigona crassipes* and *T. fulviventris* were reported to be able to digest stinkhorn fungi spores (Oliveira & Morato,



2000). While butterflies may benefit from the nutrients in the slimy fluid, do they also consume the spores or just disperse them, or do they do both? The answer remains unknown and is worth further study.

Acknowledgements:

We thank Yao Tze Leong for botanical identification and Dr. Lee Su See for fungus identification. We also thank Dr. Laurence G. Kirton and Veronica Khoo for contributing to improvements in the manuscript.

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Above left : A Lesser Cruiser and three Straight Line Mapwings feeding on the fluid on the fungus cap.

Above : The Lesser Cruiser is sharing his meal with *Drosophila* (arrow) and other flies.

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NEW PLANT RECORD

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Ridleyandra flammea (Ridl.) A. Weber (Gesneriaceae)

Beitr. Biol. Pflanzen. 70 (1998, '1997') 248. **Basionym:** *Didissandra flammea*, Ridl., J. Linn. Soc. 32 (1896) 503. Type: Thailand, Narathiwat Province, Toh Moh, Legeh gold mines, 1893, *Machado s.n.*, cultivated in Botanic Gardens, Singapore (SING).

Ridleyandra flammea (Ridl.) A. Weber (Gesneriaceae) appears in Ridley's 1923 *Flora of the Malay Peninsula* but it was in fact collected in southern Thailand from the Legeh gold mines at Toh Moh in Narathiwat Province. Ridley described the species in 1896 from plants growing in the Botanic Gardens, Singapore. It was thought to be endemic in the Narathiwat Province until W.K. Fletcher and D.M. Baylis observed it in bloom in lowland rain forest at approximately 400 m asl 10 km south of the Thai border and a thirteen-minute walk east of Kampung Sepor in Royal Belum State Park, Perak. The discovery prompted this notation: "But here, these flowers, gorgeous fuzzy orange-yellow tube-like flowers – like a lipstick plant – held on by a yellow base and fronted by red lips. This is something new, perhaps a gesneriad." (Baylis, 2006). In spite of several expeditions, this species has not been encountered anywhere else in the Royal Belum State Park.

It is a striking and extremely beautiful species by a combination of its rosette habit, dark glossy green bullate leaves that contrast with the large orange-yellow trumpet-shaped flowers, 4-6 cm long with deep red lobes. Ridley's name 'flammea' (meaning flame-coloured in Latin) is completely appropriate for these fiery orange-yellow flowers. Until *R.*

Above left : *Ridleyandra flammea* (Ridl.) A. Weber (Gesneriaceae)
This is the first record of this very beautiful Thai species in Malaysia, where it was discovered in the Royal Belum State Park. Photograph: Fletcher & Baylis.

Above : *Spatholirion ornata* Ridl. (Commelinaceae)
First described from the Legeh gold mines in southern Thailand, this rare species has since been discovered from two limestone karst hills in Kelantan and once from Terengganu. Photograph: A.R. Rafidah.

flammea was discovered, all 21 species of *Ridleyandra* in Peninsular Malaysia were endemic and, although all of them have beautiful, large trumpet-shaped flowers, none of them are orange-yellow with red lobes. Indeed, most are purple with or without white lines, the best known being *R. morganii* (Franch.) A. Weber that is common in Cameron Highlands. Only two have lemon-yellow flowers (*R. castaneifolia* (Ridl.) A. Weber and *R. quercifolia* (Ridl.) A. Weber) and just one has deep red flowers (*R. iminii* Siti-Munirah).

Plants had been sent from the Legeh gold mines by Alfred Dent Machado, who in his varied career had worked as a policeman, miner, planter, and for a year or two in the Botanic Gardens, Singapore. He obviously had a good eye for plants as he also sent live plants of *Spatholirion ornata* Ridl. (Commelinaceae) that Ridley described as a new genus in 1896. Machado was commemorated by Ridley naming the large fan palm, *Borassodendron machadonis* (Ridl.) Becc., for him. He had supplied the fruits from Kamunting, Perak, which were grown in the Botanic Gardens, Singapore where the palm can still be seen today.

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