Abstract

While present in crime scenes and succession studies, little is known about the ecology and taxonomy of forensically important necrophagus beetles. Natural gradients such as mountains were used to determine how certain groups of animals react to changes in the environment. A survey of forensically important beetles was conducted along an altitudinal gradient on Gunung Ulu Kali using pitfall trapping. The corresponding diversity of blowflies was collected in the same site. Thirty three species of beetles were recorded, with the highest species richness found in the lowland forest at 200m. The beetle and fly communities above 1000m height had less diversity and with different species types than those below that altitude. Four species of Hybosoridae made up most of the collection in lowland forests, few specimens were found in transitional forests. Scarabaeid species could be divided into transitional forest dwellers and lowland forest dwellers. Only one species of Silphidae was collected, however this species displayed a wide altitudinal range. The high coefficient of variation in distribution on a microhabitat spatial scale suggests that aggregation in patches may facilitate species coexistance. The genera *Chrysomya, Lucilia* and *Phumosia* made up the majority of flies collected. *Chrysomya* spp. were mainly lowland and transitional forest species, while *Lucilia* were found in larger numbers in montane forests. The first of its kind on local species, a taxonomic key for common necrophagus beetle species was constructed based on the specimens collected and from previous forensic succession studies for use by forensic entomologists.

Abstrak

Walaupun dijumpai di dalam kes dan kajian forensik, tidak banyak diketahui tentang kumbang bangkai dari segi ekologi dan taksonomi. Gradien semula jadi seperti gunung telah digunakan untuk melihat kesan persekitaran terhadap kumpulan sesuatu haiwan. Tinjauan terhadap kumbang dan lalat bangkai yang berkepentingan forensik telah dijalankan dengan menggunakan perangkap 'pitfall' pada transek ketinggian Gunung Ulu Kali.Tiga puluh tiga spesies kumbang bangkai ditemui dengan kekayaan spesies yang paling tinggi di hutan tanah rendah pada 200m. Diversiti kommuniti kumbang dan lalat pada ketinggian yang lebih daripada 1000m adalah kurang daripada ketiggian yang lebiih rendah. Kebanyakkan kumbang di hutan tanah rendah adalah dari 4 spesies Hybosoridae. Hanya sedikit spesimen Hybosoridae dijumpai di hutan perantaraan. Famili Scarabaeidae boleh dibahagikan kepada kumpulan hutan tanah rendah dan hutan perantaraan. Hanya satu spesies Silphidae dijumpai, tetapi spesies ini menunujukan julat ketinggian yang luas. Pemalar perbezaan yang tinggi dalam mikrohabitat adalah akibat agregasi kumbang yang mungkin mengalakkan kewujudan bersama antara spesies. Kebanyakkan lalat yang ditemui adalah daripada genus *Chrysomya, Lucilia* dan *Phumosia*. Spesies *Chrysomya* mendiami hutan tanah rendah dan hutan peralihan, manakala spesies *Lucilia* dijumpai dengan bilangan yang banyak di hutan pergunungan. Kekunci taxonomi Kumbang bangkai yang kerap dijumpai dibina untuk kegunaan ahli entomologi forensik.

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List of symbols and abbreviations

cf. – Taxonomist shorthand indicating uncertainty.

UPGMA - Unweighted Pair Mean Analysis

UV - Ultra violet.

Chapter 1: Introduction

1.1 Current State of Forensic Entomology in Malaysia.

The pioneer forensic entomology studies conducted in Malaysia were observational or taxonomic in nature, Omar *et al.* (1994) conducted observations on the decomposition of monkey carcasses in the rubber plantations while Inder *et al.* (1974) and Kurahashi *et al.* (1997) recorded the taxonomy of Calliphorid species present in Malaysia. Forensic entomology was dormant for a period, until more studies modelled after Omar *et al.* (1994) were conducted in oil palm plantations (Heo *et al*, 2007; Azwandi & Ahmad, 2009). Results of these studies were similar, with most reporting a large dominance of *Chrysomya megacephala* adults and larva, with several other species of flies present in smaller numbers. This pattern persisted even in different carcass conditions such as the carcass being burned (Heo *et al.*, 2008b) or wrapped up (Azwandi *et al.*, 2011). The effects of different conditions (Rain, indoor conditions, forest cover, etc.) on the succession of carcasses was also explored (Heo *et al.*, 2008a, Azwandi *et al.*, 2011 ,Nazni *et al.*,2011). While weather proved to be a minor delay for oviposition, the change in habitat type does have an effect on the community of flies arriving on a carcass (Nazni *et al.*, 2011). It is likely that climate or habitat effects the distribution of some of these species, rain has been shown to slow down decomposition and delay the arrival of some fly species (Heo *et al.*, 2008a, Azwandi *et al.*, 2011), colder temperatures in highlands lead to an absence of some lowland dipteran species (Omar *et al.* 2007) and rainforests have a higher diversity of flies when compared to plantations (Nazni *et al.*,2011). Other invertebrates were also recorded aside from blowflies in some studies (Heo *et al.*, 2011)

Most experimental work has been conducted on the developmental biology of forensically important species, studies concerning Calliphorids indicate that temperature is positively correlated with the rate of larval development of local blowfly species such as *Chrysomya megacephala*, *Ch. rufifacies* and *Hypopygiopsis violacea*(Ahmad *et al.*, 2009; Chen el at., 2008). Flies of other families, such as *Synthesiomyia nudiseta* (Muscidae) show this trend as well (Kumara *et al.*, 2009b). Case studies indicate that only two species are regularly recovered from cadavers sent to various hospitals in the country, *Ch. megacephala* and *Ch. rufifacies* (Nor Afandy *et al.*, 2003; Lee *et al.*, 2004; Ahmad *et al.*, 2007; Ahmad *et al.*, 2008; Nazni *et al.*, 2008; Syamsa *et al.*, 2010, Kavitha, 2008). The use of these two species leads to relatively accurate estimations the time of death provided that the cadaver is not in a stage of advanced decay (Kavitha, 2008). Consequently, most studies tend to focus on these two key species and other species within this genus.

1.2 The Importance of the Present Study

However despite the aforementioned papers on dipterans, beetles are rarely mentioned in the literature and most descriptions are to family level. The few reports of beetles in the literature are: Lee *et al.* (2004) reported a single ‘beetle’ in 448 specimens received by the Institute of Medical Research from 1972-2002, unidentified Staphylinidae and Scarabaeidae were observed to visit a pig carcass in an oil palm plantation (Heo *et al.*, 2008a), a cadaver was discovered to be infested with *Dermestes ater* inside a house (Kumara *et al.*, 2009a), a ‘scarab beetle’ was found near a pig carcass in an oil palm plantation (Heo *et al.,* 2011), unidentified Staphylinids and Histerid beetles were collected from a monkey carcass in a secondary forest (Rumiza *et al.,* 2010) and several beetles from the families Scarabaeidae, Silphidae, Staphylinidae, Tenebrionidae, Lycidae and Lampyridae were collected in a forested area in Gombak (Nazni *et al.,* 2011). This neglect of beetles in forensic research is not unique to this region, as the amount of available references concerning dipterans is far more than that of coleopterans at the international level (Midgley *et al.*, 2009). Complicating the matter, studies concerning forensic coleopterology conducted in Europe (Dekeirsscheiter *et al*, 2011) should be interpreted with caution when applied to South East Asia, because of differences in species composition and community structure of necrophagus beetles (The low abundance and diversity of Silphidae in South East Asia being a key factor) (Hanski, 1983; Hanski, 1989; Sikes *et al.*, 2006, Růžička *et al.,* 2012). Due to the paucity of information, it is necessary to investigate the biodiversity of forensically important beetles in this region and to explore its potential in assisting forensic cases. Hence the present study was designed for the advancement in this field.

1.3 Objectives

1) Determine the diversity of necrophagus beetles on an altitudinal gradient.
2) Determine the corresponding diversity of blowflies on the same gradient.
3) To observe the response of blowflies to the presence of beetles on carrion patches.
4) Construct a taxonomic key for forensically important necrophagus beetles based on beetles collected from this study and previously available materials.

Chapter 2: Literature Review

2.1 Introduction to forensic entomology.

As previously mentioned, Amendt *et al.* (2010) defines forensic entomology as the use of insects and other arthropods in medico-legal investigations. While Forensic Entomology is frequently misunderstood to only concern investigations into murder cases, Catts and Goff (1992) divided it into three categories; urban, stored-product and medico-legal. Urban forensic entomology involves legal cases involving arthropods in dwellings, misuses of pesticides or house pests while stored-product forensic entomology is concerned mainly with litigation involving insect contamination in commercial products (Catts and Goff, 1992; Byrd and Castner, 2010; Gunn, 2009). The proper term for what the public understands as 'Forensic entomology' is more accurately termed 'Medico-legal entomology' (Smith, 1987 ; Gunn, 2009). There are several ways in which entomology is applied to provide medico-legal cases, the most common and popular of which is to provide evidence for murders or cases of suspicious death (Smith, 1987; Amendt *et al.*, 2004; Gunn, 2009). Insects are also useful as evidence in cases that concern neglect or animal welfare (Amendt *et al.*, 2004; Benecke, 2004; Gunn, 2009), wildlife poaching (Byrd and Castner, 2010) and determining the origins of illegal substances (Smith, 1987). Insects have also been shown to be useful in interpreting archeological information (Byrd and Castner, 2010; Lockwood *et al.* 1994)

2.2 Estimating the time of death.

The most common use of forensic entomology is to determine the Postmortem Interval (PMI), which is the time between the time of death and the time of discovery, using carrion flies (Smith, 1987). Other authors prefer to use the term EOD or estimated oviposition date, which is more accurate as there are factors that can delay the time of oviposition (Gunn, 2008). Determining the PMI or EOD is based on two major principles; the colonisation of the corpse by insects follows a reasonably predictable succession of species; and that developmental times for each stage of each species is also predictable. Both of these principles are greatly affected by the surrounding environment, particularly temperature (Gunn, 2008; Byrd and Castner 2010; Gennard, 2012; Amendt *et al.* 2009).

Thermal summation models calculate the PMI or EOD by comparing the developmental stage of the larva found on the body with the environmental temperature (Smith, 1986 ; Gunn, 2008). Normally, this is done by calculating the amount of time it takes for the larva to reach a certain state of development at a certain temperature. As the growth rate of maggots is dependent on the thermal input that they receive while developing, the accumulated thermal units such as Accumulated Degree Hours (ADH) or Accumulated degree days (ADD) that are required to reach the state of development in which the maggots are found can be calculated. These units are obtained from baseline studies that have been done in controlled laboratory conditions and studies into the accumulated thermal units that are required for the larvae to achieve a certain developmental stage (Smith, 1986; Gunn, 2008). Working back from the age of the maggots and using temperatures obtained from nearby weather stations (Sukontasan *et al.*, 2008; Smith, 1986), the PMI or EOD can be estimated. It should be noted that the temperature of the body is usually much warmer than the ambient air temperature due to heating from the sun or by concentrations of larvae known as a ‘maggot mass’, which can drive the temperature up to 30°C more than ambient air temperature (Smith, 1986;

Sukontasan *et al.*, 2008a). Amendt *et al.* (2007) outlines the proper protocol to be used when collecting and preserving forensic specimens to ensure standardisation.

2.3 Faunal Succession

Faunal succession is the other method to estimate the time between death and discovery, and it is based on the assumption that a corpse has a reasonably predictable succession of species (Gunn, 2008). Faunal succession is theoretically useful when the corpse is in a skeletonised or mummified stage, that is when blowfly larva are no longer on the corpse or several generations of blowfly have bred on the corpse (Gunn, 2008; Amendt *et al.* 2004; Gennard, 2012). In the succession of insects arriving at the scene, two main groups make up a majority of the entomofauna found on carcasses: Dipterans and Coleopterans (Amendt *et al.*,2004; Benecke, 2004). It is commonly believed that Dipterans tend to dominate the earlier stages of decomposition, while Coleopterans are more common in the advanced and dry stages (Wolff *et al.* 2001). In terms of Dipterans, Calliphorids, Sarcophagids and Muscids are most common in the earlier stages of decomposition, however in later stages such as the postdecay and dry stages are dominated by other more specialised families of flies.

As previously stated, it is commonly believed that beetles only play an important role in the decomposition of corpses in advanced decay. Documented roles of European/ North American beetles on carrion include Early Stage Necrophages (Silphids), Mid Stage Necrophages (Staphylinids), Late Stage Necrophages (Dermestids) and Predators

(Histerids, Silphids, Clerids and Staphylinids) (Smith, 1987). However, while beetles play a minor role in early stages of decomposition in temperate countries, it is not the case in tropical rainforests. The superabundance of Hybosoridae on fresh carrion in South East Asian rainforests is well documented among dung beetle ecologists (Hanski and Cambefort,1991), but there has been little knowledge exchange between ecologists and forensic entomologists. Additionally, Silphid diversity in South-East Asia is less than that of the Nearctic region. Most studies concerning the role of carrion beetles in decomposition mostly concern Silphids (Smith, 1987 Dekeirsscheiter *et al*, 2011). Consequently, there has been very little work on how beetles affect corpses in a tropical setting.

2.4 Ecology and Forensic Entomology

Succession is not as straightforward a process as some forensic entomologist would like to believe. Multiple factors play a role in influencing the pattern of succession such as rain (Mahat *et al.*, 2009; Gunn, 2008), land use (Omar *et al.*, 1994; Nazni *et al.*, 2007; Heo *et al.*, 2007; Heo *et al.*, 2008b; Azwandi and Ahmad, 2009; Heo *et al.*, 2011 Nazni *et al.*, 2011) and altitude (Omar *et al.*, 2003). These factors affect the composition of flies active at the time the carrion is exposed as well as the proportion of each species in the area (Gennard, 2012; Gunn, 2008).

Priority effects, which state that the first species to oviposit are likely to become dominant on the carrion, are believed to greatly affect the pattern of succession (Kneidel, 1983). Additionally competition and disturbance by mammalian scavengers also influence community composition (Kneidel, 1984). Most studies into fly ecology use smaller carrion due to the relative ease of replication, but this leads to problems when these mechanisms are applied to cases of larger carrion. As an example, the priority effect is more likely to play a larger role in smaller carrion in which there are less resources and higher rates of consumption. Action of scavengers is reduced in larger carrion as well (Kneidel, 1984). Larger carrion tends to have excess resources that cannot be totally exhausted by early comers. Biotic interactions increase the uncertainty involved in succession. Some fly species are able to utilise different resources which are available at different stages of decomposition to avoid being disadvantaged by later oviposition, for example some species such as *Ch. rufifacies* feed on other maggots as an alternative food source (Baumgartner, 1993) and *Ophyra* spp. appear at later stages to feed on the liquefied remains (Heo *et al.*, 2007). As flies are subject to additional competition, predation and parasitism by beetles and other organism, this is an important avenue to explore for a reasonable understanding of succession patterns in larger carrion. It is believed that dung beetles share similar ecological mechanisms with flies. However, dung is a relatively homogenous medium compared to carrion and dung beetles tend to be K-selected as opposed to r-selected carrion flies (Hanski and Cambefort, 1991). Such differences have not been explored. Current research into succession tends to not focus on ecological aspects and adopt a more case study approach (Michaud *et al.*, 2012). This approach produces results that cannot be generalised beyond the time and space of the study (Michaud *et al.*, 2012). The lack of a proper statistical framework means that most studies cannot be replicated or produce predictive models. Solow and Smith (2006) suggested the use of a Markov chain successional model to predict the probability of succession over time. The ecology of decomposition is a field that is still poorly understood and research into the basic science may have applications in applied forensic entomology.

Decomposition of animal remains is usually a result of the activities of necrophages, organisms that feed on decaying animal material (Hanski, 1983). Three orders of insect make up a majority of land based invertebrate necrophages, Coleoptera, Diptera and Hymenoptera (Smith, 1987). Invertebrate necrophages are often confined at one point of their life cycle to carrion, an island of high quality protein surrounded by a relatively nutrient poor environment (Hanski and Cambefort, 1991). It provides food, shelter, a place to meet and attract mates and a medium to rear young (Smith, 1987). As a limited resource that is exploited at an extremely high rate through scramble competition, carrion is rapidly exhausted; causing colonisation, succession, migration, extinction and collapse of the ecosystem to all happen within a relatively short and observable period (Hanski and Cambefort, 1991). The accelerated pace of carrion as an ecosystem has been used by ecologists to test and refine ecological models and theories (Hanski and Cambefort, 1991). Observations on the distribution of necrophagus flies and beetles on this scale shows how the community structures itself and how the patchiness of the habitat affects the distribution of species.

Mountains have been recognised as a type of ecological island with continuous boundaries instead of the discrete border between carrion and the surrounding environment (Wilson and MacArthur, 1967). Environmental factors such as temperature, pressure, humidity and precipitation gradually change as altitude increases (Whitmore, 1974). The response of vegetation (Whitmore, 1974), which results in a mosaic of forest types with different abiotic conditions, and insects to these changes is well documented (Hodkinson, 2005). The distribution of communities along a mountain shows how different community structures composed of species with similar evolutionary histories can arise from variation in environmental parameters.

Peninsular Malaysia has been separated from the islands of the Sunda Shelf since deglaciations during the Holocene period while maintaining a connection to the Asiatic mainland through the Isthmus of Kra (Sathiamurthy and Voris, 2006). The unique biogeographical history of this area can provide a 'control' for comparison when studying the effects of isolation on the diversity of necrophagus flies and beetles in the surrounding islands.

2.5 Diversity necrophagus beetles on altitudinal gradients.

In the tropical rainforests of South-East Asia, necrophagus beetles appear in large numbers to feed on carrion (Kuijten, 1981; Hanski, 1983). Ecological functions such as removal of carrion, control of fly populations, soil aeration and nutrient cycling are outcomes of the activities of these animals (Nichols *et al.*, 2008). Beetles from the families of Scarabaeidae, Hybosoridae and Silphidae are found to be common in the early stages of decomposition (Nazni *et al.*, 2011). These families are known to be sensitive to environmental factors and have been proposed as indicator species for conservation (Davis *et al.*, 2001; Lee *et al.*, 2009) and forensic entomology (Nazni et al, 2011). In terms of forensic importance, beetles are possibly useful as area indicators, that is indicators of the conditions that the corpse were previously exposed to, or Post-Mortem Interval (PMI) indicators, which is the time between the exposure of the corpse to the time of discovery (Gunn, 2008; Gennard, 2012). Knowledge of artifacts left by beetles is also important, as misinterpreations of bite and scratch marks left by beetles may lead to wrong conclusions. Benecke (2005) notes that the bites of silphids, histerids and clerids can resemble close or long range gunshot wounds. Ururahy-Rodrigues (2008) reported large carrion beetles in South America moving a human sized pig carcass by burrowing underneath it. Competition from beetles or predation by beetles on fly larvae may alter the pattern of faunal succession or affect the developmental rate of maggots.

Beetles are rarely mentioned in forensic Malaysian literature. However the lack of records does not mean that beetles are rarely recovered from forensic studies. Most forensic workers in the Malaysia kept a collection of beetles that were unidentified due to the lack of taxonomic expertise. This study attempts to plug the hole in taxonomic knowledge and to summarise available forensically important information. Apart from the family Silphidae (Sikes *et al.*, 2006; Ruzicka *et al.*, 2012; Peck 2001), basic data concerning development and ecology of the species belonging to other families treated here are still largely lacking.

While Silphidae are the only beetles aside from Scarabaeidae that are known to exhibit parental behaviour, not all species within the family do so (Hanski and Cambefort, 1991; Dekeirsschieter, 2011). The behaviour of burying caracasses to use as medium for ovipostion and the raising of larvae is mostly limited to the burying beetles *Silpha* and *Nicrophorus* (Gennard, 2012; Dekeirsschieter, 2011). In these species, a mating pair will work together to bury small carcasses such as rodents to avoid competition with other necrophages such as flies (Gennard, 2012; Dekeirsschieter, 2011). The pair will then tend to their young until their offspring have pupated (Dekeirsschieter, 2011). Other Silphid species tend to be present on carcasses, but feed on the carcass itself or predate on the larvae of other insects (Gennard, 2012; Sikes *et al.*, 2006). There are no publications that focus on the ecology or behaviour of Silphidae in the region and most publications that mention Silphidae are about the taxonomy of separate generic groups (Sikes *et al.*, 2006; Peck, 2001; Růžička,*et al.* 2012). It has been noted that *Nicrophorus* species are often restricted to mountain habitats, with each island in the Archipelago having only one species (Hanski and Cambefort, 1991). This is in contrast to temperate regions where several species can coexist in a single location (Anderson, 1982). Almost nothing is known about the ecology of this family in this region.

The family Hybosoridae has about 200 species recorded worldwide (Hanski and Cambefort, 1991), this family can be differentiated from scarabaeids on the basis of the shape of the antennal club (Tripplehorn & Johnson, 2005). While Hybosoridae are relatively common and in some cases found in large numbers, nothing much is known about their entire life ecology (Kuijten, 1978; Kuijten 1981; Hanski and Cambefort, 1991). All that is known is that adults will appear on carrion at night to feed and mate. Breeding experiments have been conducted, (Kuijten, 1981) but there are no records of larvae from this family ever being described. In South East Asia, forests are regularly dominated by large numbers of *Phaeochroops* species (Hanski and Cambefort, 1991). They are believed to have strong interspecific interactions and different distributions due to the absence of other species occur, for example *Pc. silphoides* only occurs above 800m in Sumatra, while in Sarawak it is restricted to lowland forest (Hanski and Cambefort, 1991). The presence of a similar species in Sumatran lowland forests, *Pc. rattus*, is believed to cause this pattern of distribution. On the Malayan Peninsula several species, such as *Pc. rattus* and *Phaeochrous emarginatus* are considered to be common while *Pc. peninsularis* and *Pc. freenae* are relatively rare (Hanski and Cambefort, 1991). *Pc. freenae* was described through just a single female specimen (Kuijten, 1981).

Scarabaeidae, or more accurately the subfamily scarabaeinae, are commonly known as dung beetles and it is estimated that 1,000 to 2,000 species are present in the South East Asian region. Peninsular Malaysia is known to have a small proportion of endemic species (11%) and many species are shared with Sumatra, Borneo and Java (Hanski and Cambefort, 1991). However, recent taxonomic studies are describing more species that are unique to the Peninsula, so this previous estimation may be inaccurate. Dung beetles do not feed on undigested parts of dung, but filter out the bacteria living inside the dung (Hanski and Cambefort, 1991). As this method of feeding is poor in protein content, most dung beetles have to supplement their diet by feeding on carrion when the opportunity arises (Hanski and Cambefort, 1991). Dung beetles are divided into 4 categories, depending on their nesting habits: Dwellers, tunnelers, rollers and parasites. Dwellers live and oviposit inside the dung pat, tunnelers dig tunnels under the dung pat and deposit their eggs inside tunnels that have been filled with enough dung for the larvae to develop and rollers roll balls of dung that they transport a distance away from the dung pat, followed by construction of nests that are similar to tunnelers (Hanski and Cambefort, 1991). Parasitical beetles either display kleptoparasitic behaviour, exist as parasites within other organisms or parasitise colonies of ants or termites (Hanski and Cambefort, 1991). Currently there is little known about the ecology of Scarabaeids in South East Asia. A majority of the publications in recent years has been about the taxonomy of this large group, with a few large trapping studies being done on the use of these species as biological indicators. Most of the established knowledge of South East Asian dung beetles are based on studies conducted in Borneo by Hanski (1983) and Davis *et al.* (2001).

Davis *et al.* (2001) conducted extensive sampling in the Danum Valley Region of Sabah, surveying primary, plantation, riverine and logged forests. In total, 86 species were recorded in Borneo after examining more than 35,279 specimens. He demonstrated that some species could be categorised as ‘edge specific’, such as *Catharsius molussus, Paragymnopleurus sparsus, Microcopris reflexus,* and *Sisyphus thoracicus*. These species had a larger tolerance for human disturbance due to their adapted to harsher edge environments and therefore argues for the use of indicator species as opposed to forming conclusions based on raw species richness information as an indication of ecosystem health.

Previous studies into necrophagus beetle diversity along altitudinal gradients have been limited to the islands in the Indonesian achipelago (Hanski and Niemela, 1990; Kahono and Sitiadi, 2007) and Borneo (Hanski, 1983). The general trend shown is that Scarabaeid species tend to dominate in lowland areas, while highland areas tend to hold a larger diversity of Silphid species. Hanski (1983) and Hanski and Niemela

(1990) conducted studies on the community structure of dung beetles on an elevational gradient in Sarawak and Sulawesi respectively. Both studies used similar methodology and were baited using fish and animal carcasses. The number of species collected in the lowland forests were similar in both studies, but in Sulawesi the number did not decrease with altitude as it did in Sarawak. Clear zonation of necrophagus beetle families were observed in both sites, with some species being limited to either highland or lowland forests. It is believed that the area of mountains played a role in the number of species that could be supported at the altitude as larger mountain areas would have a higher carrying capacity for more species (Hanski and Cambefort, 1991). Kahono and Setiadi (2007) conducted the only study of its kind in Gede Pangrango National Park located in Western Java. This study mainly used human dung as a bait, and therefore is not directly comparable with the methodology used by Hanski (1987) and Hanski and Niemela (1990). While this study focused mainly on Scarabaeid species, it reported that larger scarabaeids tended to be limited to lower elevations (500-1000m) while higher elevations were dominated by smaller *Onthophagus* species.

2.6 Diversity of Blowflies on altitudinal gradients.

Blowflies (O: Diptera, F: Calliphoridae) are characterised by the short bodies, large, reddish eyes and metallic coloration (Senior-White *et al.*, 1950, Tripplehorn & Johnson, 2005). These flies are named after the clutches of hundreds of eggs that they lay, which are also known as 'Blows' (Smith, 1987; Byrd & Castner, 2009, Gennard, 2012). This strongly r-selected reproductive strategy allows them to rapidly dominate the rotting material that typically makes up their larval habitat (Smith, 1987; Byrd &

Castner, 2009, Gennard, 2012). Like most flies, blowflies are incredibly diverse with ‘several thousand’ being the number of species listed by Bickel (1982). Most species of blowflies do not interact with humans and tend to be very specific to habitat types (Smith, 1987, Senior-White *et al.*, 1950; Kurahashi *et al.*, 1997). However, several genera have adapted to breed in human waste and are now pest species (Byrd & Castner, 2009, Olsen, 1998, Macleod and Donnelly, 1956). These blowflies are vectors of fly-borne diseases such as dysentery and other bacterial infections (Olsen, 1998). Others such as *Lucilia sericata* harm livestock through acts of parasitism such as myiasis, which is when blowfly maggots bore into living tissue (Macleod and Donnelly, 1956). While there are many negative effects are a result of interactions between humans and blowflies, this family is both important in terms of the ecological services that it provides and in its economic and medical use. Blowflies carry out numerous ecological functions, such as the break down and decomposition of rotting material (Smith, 1987), nutrient cycling through the act of feeding during the larval stage (Nichols *et al.*, 2008) and pollination for fruit trees as a result of adults feeding on nectar(Sung *et al.*, 2003). As an economical resource blowflies are grinded up and used as fish meal or used whole as bait for fishes (Sing *et al.*, 2011). In terms of medical use, *L. sericata* has been used to clean wounds in maggot therapy (Sherman *et al.*, 2000). Common blowflies such as *L. sericata*, *Phormia regina*, *Chrysomya megacephala* and *Ch. rufifacies* are the main indicator species for forensic entomology (Smith, 1987; Kumara, 2008). Much forensic literature in the Nearctic region concerns species of the genus *Lucilia* or *Phormia*, specifically species which are adapted to colder temperate climates (Smith, 1987; Byrd & Castner, 2009). The Greenbottle, or *Lucilia sericata*, is the standard blowfly used for estimation of PMIs in the Nearctic region (Smith, 1987;

Byrd & Castner, 2009, Gennard, 2012). In Malaysia, there are 118 species recorded to be present (Kurahashi *et al.*, 1997). This number is disputable as there are several species such as *Ch. villeneuvi* that Kurahashi *et al.* (1997) failed to account for. Of all the species present, the genera *Chrysomya* and *Lucilia* are the most commonly found in synantrophic environments (Nazni *et al.*, 2008). Other genera of Calliphorids tend to be only found in forested habitats and not much is known about their bionomics (Senior-White *et al.*, 1950, Kurahashi *et al.*, 1997)

Chrysomya megacephala is the most common species encountered in Malaysian forensic literature (Heo *et al.*, 2008a, Azwandi *et al.*, 2011,Nazni *et al.*,2011, Nor Afandy *et al.*, 2003; Lee *et al.*, 2004; Ahmad *et al.*, 2007; Ahmad *et al.*, 2008; Nazni *et al.*, 2008; Syamsa *et al.*, 2010). It is believed to have originated in New Guinea, with non-synanthropic variants still being found there (Kurahashi, 1982) Also known as the Oriental Latrine Fly, the ability of this species to breed in both dung and decaying material has allowed it to colonise much of tropical Asia through the help of human migration (Senior-White *et al.*, 1950; Kurahashi, 1997). Female *Ch. megacephala* arrives within hours after death, after which they oviposit on the moist areas such as the mouth, anus, nostrils, armpits, the ground underneath the body or the hair (Heo *et al.* 2008a). These eggs hatch within days and large amounts of maggots begin devouring the carcass (Heo *et al.* 2008a). At this point, the presence of another fly of forensic importance, *Ch. rufifacies* becomes common (Heo *et al.* 2008a; Kumara, 2009). As previously mentioned, the larvae of these flies are facultatively predatory, and will attack other maggots when there are shortages of food. Additionally, the larvae have spines covering the dorsal half of the body and thick skin, which allows them exclude other species of maggots from the feeding mass (Baumgartner, 1993). These maggots tend to be present for longer periods of time and are more often encountered in forensic cases than early stage decomposers (Kumara, 2009). Several other species of Calliphorids are known to oviposit at carcasses, but they either have smaller clutch sizes and survive in less nutritious conditions such as the run off of the digestive fluids of the main decomposers, or they avoid the habitats in which the synanthropic flies are found (Pers. Comm., Nazni W.A.). Little is known about the ecology of the flies that are not adapted to human habitation (Senior-White *et al.*, 1950; Kurahashi *et al.*, 1997), but in cases where there is little human disturbance these species tend to be more common (Nazni *et al.*, 2011).

Attempts to control pest blowfly populations have led to studies concerning the effects of environmental variables on the activities of blowflies, and it is found that flies tend to react negatively to colder weather and rain in the British Isles (Macleod and Donnelly, 1956). Similar findings concerning rain were found in Malaysia, with the oviposition of *Chrysomya* species being delayed by rainy weather (Mahat *et al.*, 2009). Studies into the blowfly diversity in different habitats suggest that habitat type may play an important role in determining which species of blowfly is present in a location (Senior-White *et al.*, 1951; Hanski, 1981). As it is impossible to produce accurate simulations of such complex animal communities and weather effects through experimental studies, altitudinal gradients have long been used by ecologists to examine the response of organisms to environmental factors such as temperature, humidity, precipitation, pressure and UV light exposure change with increasing altitude (Hodkinson, 2005, Whitmore, 1974). Biotic environmental factors such as biomass, primary productivity, forest type and competitor activity are also affected by changes in altitude (Whitmore, 1974).

In tropical South-East Asia, The diversity of blowfly species on an altitudinal gradient has been catalogued in Genting Highlands (Omar *et al.*, 2003) and Mulu National Park (Hanski, 1981) and the altitudinal zonation of these species is well documented. Generally, species more adapted to colder climates such as *Lucilia phorphyrina*, *Calliphora fulviceps* and *Chrysomya pinguis* become common at altitudes above 1400m while lowland forest species such as *Ch. deflixa*, and *Ch. nigripes* become less common (Omar *et al.*, 2003; Hanski, 1981; Senior-white *et al.*, 1950; Kurahashi *et al.*, 1997). Species such as *Ch. villeneuvi* were only present in forested sites (Omar *et al.* 2003). Oddly Omar *et al.* (2003) did not report the collection of any *Ch. deflixa*, which could have been due to misidentification as there is an abnormally large number of *Ch. chani* reported. Synanthropic flies, which are normally associated with human activity, can be found in higher altitude sites as well as lowland sites if human disturbance is present and make up the majority of fly species collected by Omar *et al.* (2003), with the only exception being at the highest altitude where *L. porphyrina* was dominant.

2.7 Interactions Between Necrophagus Flies and Beetles

Investigations into biotic influences on blowflies has been limited to several studies in agricultural systems that have examined the effects such as land use (Gregor, 1991) and competition of dung beetles on blowflies (Nichols *et al.* 2008). However, there is still little knowledge about the reaction of blowflies to biotic factors in a tropical setting. Hanski (1981) suggested that blowfly zonation may be caused by the presence of large dung feeding beetles which compete with blowflies for resources. It should also be noted that there is an also overlap between large scarabaeid dung beetles and superabundant necrophagous Hybosorid beetles in South-East Asia (Hanski, 1983) as these beetles drastically increase the rate of carrion removal (Nazni et al, 2011). Additionally, Carrion beetles (Silphidae) are known to feed on fly larva (Gennard, 2012; Sikes *et al.*, 2006; Peck, 2001; Růžička,*et al.* 2012). Acknowledging the effects of biological interactions between species may result in more accurate estimations in forensic investigations or new integrated pest control measures.

2.8 Taxonomy of Forensically Important Flies and Beetles

Much of the practical application of forensic entomology hinges on correct identification of specimens. Advances in the automation of taxonomy or cybertaxonomy are limited, and most cases heavily rely on traditional morphological identification by experts (MacLeod *et al.*, 2010). Thus far, these methods are the most cost effective and fastest methods to identify species. However, taxonomic training takes years to master and the lack of qualified taxonomists may pose a problem in the future (Amendt, 2004). Several keys have been published on local species of carrion flies with the two most complete Calliphorid keys being the early work on the region was done by Senior-White *et al.* (1951) and a later key specific to Peninsular Malaysia by Kurahashi (1997). Several other lesser known keys are written for the Oriental region for law enforcement (Byrd and Castner, 2010), a key concerning the filth flies of Guam (Bohart and Gressit, 1951) and a key to common Malaysian carrion flies (Inder *et al.*, 1979). Kurahashi (1997) does omit several species which are found in Malaysia, so depending solely on a single key increases the risk of misidentifications. Currently, available references for Muscid and Sarcophagid flies are fragmented, limited or outdated. Only (Byrd and

Castner, 2010) includes common Muscids and Sarcophagids in a taxonomic key. It is evident that locally, the study of Muscid and Sarcophagid diversity is still in the inventory stage. Keys for carrion fly larvae that are specific to Peninsular Malaysia are nonexistent. References for identification of larvae are limited to more common widespread species such as synanthropic *Chrysomya* species (Ishijima, 1967). Work on larval specimens is further complicated by Sarcophagids which lack morphological characteristic to differentiate species (Amendt *et al.*, 2004). In addition to traditional taxonomy, several different methods are currently receiving attention by various research papers. Scanning electron microscopy has recently seen use to find additional morphological characters that are overlooked without powerful microscopes (Wells *et al.*, 1999; Sukontason *et al.*, 2003).

Beetles that are found on carrion tend to be very diverse on both species and family level. Therefore most references to the taxonomy of necrophagus beetles is scattered according to the taxonomic divisions of the various species. Almeida *et al.* (2009) has produced a key for forensic entomologists for beetles up to family level. While it is mainly focused on the Neotropical region, it can be used with relatively good accuracy to identify South-East Asian beetle families. Kuijten covered most of the Hybosorid species in the region in a series of publications, of which Kuijten (1981) contains identifications keys for *Phaeochroops* and Kuijten (1978) for *Phaeochrous*. Huijbregts and Krikken continue to describe and produce keys for *Onthophagus* and other Scarabaeid species that is present in the region, with several publications on carrion specialist species that have been divided based on species groups (Huijbregts and Krikken, 2008; Krikken and Huijbregts, 2009). Scattered publications on Silphidae have been made by various independent groups, all of which are hardly useful for identification (Sikes *et al.*, 2006; Peck, 2001; Růžička,*et al.* 2012). Overall, a key that collects all the common forensically important necrophagus beetles is lacking despite the increasing need for such a reference and many beetles that have been collected by forensic entomologists in Malaysia go unreported due to the lack of resources for identification (Pers. Comm., Raja Zuha M.).

Chapter 3: Methodology

3.1 Study site

Gunung Ulu Kali is located on the Titiwangsa range of Peninsular Malaysia, 33km Northeast of Kuala Lumpur. Six sites were selected at roughly 400m intervals on the mountain (Table 3.1). The mountain is granitic with twin peaks that reach about 1700m above sea level (a.s.l.). In the 1980s, one of the peaks was developed into a hill resort, causing fragmentation and deforestation in the upper elevations. Nakashizuka *et al.* (1994) surveyed the vegetation communities of the area and divided it into four different categories: Lowland forests, transitional forests, lower montane forests and upper montane forests. The geographical and ecological information for each study site were summarized in Table 3.1.

3.2 Sampling

The term macrohabitat used here refers to differing elevations, which had different environmental characteristics and forest types. Microhabitats in this study were individual traps placed in the same sampling day. The topography of the study sites, with cliffs, sharp rocks, holes, rotting logs and steep muddy hills, did not permit random placement of transects. Instead roughly linear transects were placed along paths and traversable areas. Vertebrate scavengers such as wild boar and stray dogs caused the loss of some traps by digging up and devouring the contents of traps. Fences and barbed wire did not deter these scavengers. Ultimately this caused unequal sample sizes between sites and sampling periods. In some locations more samplings had to be done due to heavy loss of traps on previous trapping days due to vertebrate scavengers. A minimum of 10 pitfall traps were placed at 20m intervals along each transect. Each pitfall trap measured 12cm at the opening and was filled halfway with a brine solution mixed with liquids from the rotting fish and a bait of 2-day old rotting fish in a plastic bag was suspended above the trap using bamboo skewers. It was found that adding rotting fish liquid to the brine solution minimized the effect of scavengers stealing the bait in the plastic bag. A minimum of three samplings were done for each elevation during each sampling period. Pitfall traps have been successfully used to collect aerial animals such as flies due to the flies' behaviour of landing on their food source (Hanski, 1981). A pitfall trap works on flies using the same principles as fly paper, flies tend to land near or on anything that is odorous, except instead of using glue to immobilise the fly, a brine solution is used instead. Using a liquid that can be easily washed off allowed for more well preserved specimens and less chances of misidentification. While this method is more effective at collecting beetles than flies, it can collect both at the same time this allows for comparisons to be made in the same sampling unit.

The ambient temperature was measured every 30 minutes using a data logger suspended 1m from the ground (Gemini Data Loggers (UK) Ltd., Tinytag TV-4500).

Table 3.1: Summary of sampling sites in this study.

Site Name	Altitude	GPS coordinates	Forest Type	Current land use	Number of traps in Sampling period		Average temperature:
					1st	2nd	
Gombak	200m	3°19’29’’ 101°45’09’’	Lowland forest (Mixed primary and secondary)	Recreational forest	28	48	23.66±1.85°C
Janda Baik	400m	3°20’06’’ 101°49’19’’	Lowland forest. (Secondary)	Residential area	37	27	23.72±1.89°C
Bukit Tinggi	800m	3°23’06’’ 101°49’26’’	Transitional forest (Secondary)	Recreational forest	44	28	22.70±2.48°C
Awana	1000m	3°23’57’’ 101°47’11’’	Transitional Forest (Primary)	Recreational forest	39	39	20.50±1.13°C
Chin Swee Temple	1400m	3°24’54’’ 101°47’08’’	Lower Montane Forest (Secondary)	Recreational forest	40	40	19.10±0.64°C
Bukit Cincin	1700m	3°25’59’’ 101°47’04’’	Upper Montane Forest (Primary, tall facies)	Recreational forest	40	40	17.16±0.93°C

3.3 Analysis

3.3.1 Necrophagus beetle diversity

A general overview of community structure for each elevation and sampling period was obtained using raw abundance, species richness, Shannon and Simpson’s diversity indexes, Simpson’s dominance, and Shannon evenness. All calculations were done using the Vegan package in R 2.15.1. To examine species specific responses to changing temperature, log normalised values of abundance for species that were present in more than 3 habitats were then correlated using Spearman’s correlation with temperature in a range of 19.5°C to 25.0°C (R 2.15.1). For the Hybosorid guild, association analysis (log likelihood test (G test) was conducted to detect positive, negative or independent species associations (Waite, 2000). For the Onthophagus guild, cluster analysis, using UPGMA and the Single linkage method described by Davis *et al.*

(2001), was conducted to categorise the species. The dispersion coefficient, s^2/μ, for selected species also determined to indicate the dispersion of the species in its main habitat (Waite, 2000). P values of >0.05 were considered not significant.

3.3.2: Blowfly diversity

The sample had two potential sources of error; 1)detection bias resulting from the mating behaviour of certain species and 2)large variance between temporal samples caused by chance ecological mechanisms that were unaffected by environmental factors. While in most cases calliphorid species tend to display a female/male ratio of 9:1 when using meat as bait (Omar, 2003; Stoffolano *et al.*, 1990), *Chrysomya villeneuvi* was collected at a ratio that was close to 1:1. Male *Ch. villeneuvi* were observed to display the unique behaviour of pouncing onto females and use their enlarged femurs to hold on while mating. Females of this species that fell into the brine solution would attract males to enter the traps, leading to a higher collection of *Ch. villeneuvi* specimens. To remove the bias, we only recorded females for this study.

Because of the large variance in fly abundance, the best way to describe the diversity of carrion flies in a site would be in terms of species richness. In a Lottery dynamic system (Chesson, 1986), most species tend to persist at low numbers, and therefore the species richness of a site is subjected to less random fluctuations than abundance. However, the use of species richness alone limits interpretations of community structure, as lone migrants cannot be differentiated from dominant species. Abundance was therefore considered with discretion. The mean Calliphorid fly species per sample was correlated with the mean temperature using Spearman’s correlation.

3.3.3 Identification

The beetle specimens were identified using a reference collection in the field or by sending them for to Johannes Huijbregts for comparison with existing collections in the Naturalis Biodiversity Center in Leiden, the Netherlands. Identification of blowflies was done using Calliphorid keys by Kurahashi (1997) and Senior-White *et al.* (1951).

3.4 Key to Necrophagus Beetles

The beetles collected for the previous ecological study were examined and the characteristics were summarised. A key was constructed based on these characters. While too much jargon is avoided in this key, unavoidable terminology used in this key is based on Arrow (1931) (see Glossary).

The following collections were examined for this study:

1) Chen C.D. *et al.* (2010). Succession study using monkey carcasses. Gunung Ulu Kali, Pahang, MALAYSIA. (Unpublished data).

2) Goh, T.G. *et al.* (2011-2012). Pitfall traps baited with rotten fish. Gombak, Selangor, MALAYSIA. See Chapter 2 for details.

3) Azwandy A.A., *et al.* (2010) Succession study using monkey carcasses near secondary forests. Universiti Kebangsaan Malaysia, Bangi, Selangor, MALAYSIA. (Unpublished data).

4) Omar B., *et al.* (1992) Succession study using monkey carcasses in rubber estate. Gombak, Selangor, MALAYSIA. See Omar *et al.* (1994) for details.

5) Raja Zuha, R.M. *et al.* (2002) Succession study using rabbit carcasses near secondary forests. Sungai Buloh, Selangor, MALAYSIA. (Unpublished data).

6) Raja Zuha, R.M. *et al.* (2007) Succession study using monkey carcasses near secondary forests. Universiti Kebangsaan Malaysia, Bangi, Selangor, MALAYSIA. (Unpublished data).

7) Goh, T.G. *et al.* (2012) Succession study using wild boar carcasses. Universiti Malaya, Kuala Lumpur, MALAYSIA. (Unpublished data).

8) Nazni *et al.* (2010). Succession studies using monkey carcasses in mature secondary forests. Gombak, Selangor, MALAYSIA. See Nazni *et al.* (2010) for details.

9) Khairunnisa, S. (2013). Pitfall traps baited with chicken liver. Rimba Ilmu, Universiti Malaya, Kuala Lumpur, MALAYSIA. (Unpublished data).

10) Entomology collection, Museum of Zoology, University of Malaya.

Chapter 4: Results

4.1 Diversity of Necrophagus beetles

Temperature reduced as elevation increased at a lapse rate of 0.55°C/100m during the first sampling and 0.41°C/100m during the second sampling (Fig 4.1, 4.2). Cloud formation occurred at 1000m, however it was not recorded quantitatively. Average climatic conditions of each site was summarised in Table 3.1.

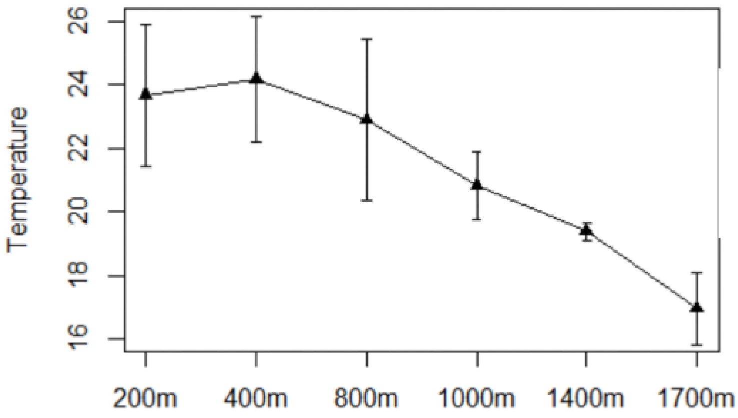


Fig 4.1: Average temperature with standard deviation for 1st sampling.

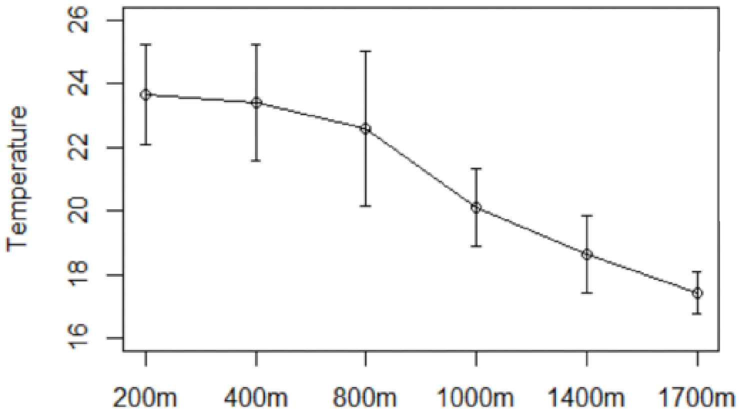


Fig 4.2: Average temperature with standard deviation for 2nd sampling.

Species richness showed a generally downward trend as elevation increased. The highest number of species found was 20 species in the first sampling and 31 species during the second sampling (Fig 4.2a). Both these values occurred at the lowest elevation, 200m. This elevation had the largest number of functional groups with large tunnelers, small tunnelers, large rollers, small rollers, necrophages and predators occurring together. A similar mix of functional groups occurred at 400m, but in much smaller abundances. In terms of abundance, beetles collected from 200m were a magnitude higher than those collected in from a range of 400m to 1000m (Fig 4.2 b). Most of the beetles collected from the 200m site were hybosorids, with the dominant species being *Phaeochrous emarginatus* in the first sampling (838 individuals, 57%) and *Phaeochroops rattus* in the second sampling (1219 individuals, 56% of individuals collected). In terms of Scarabaeids, *Catharsius molussus, Onthophagus egregious, O. rudis, O. penicillatus, O. babirusoides, O. vulpes* and *O. rugicollis* were only common in lowland forests. Only 3 singletons were collected from lowland forest; *Onthophagus waterstradti, O. liliputanus* and *Copris ramosiceps,*

Fig. 4.2 a) Necrophagus beetle species richness plotted against elevation. b) Raw abundance of beetles plotted against elevation. Triangles represent first sampling, circles represent second sampling.

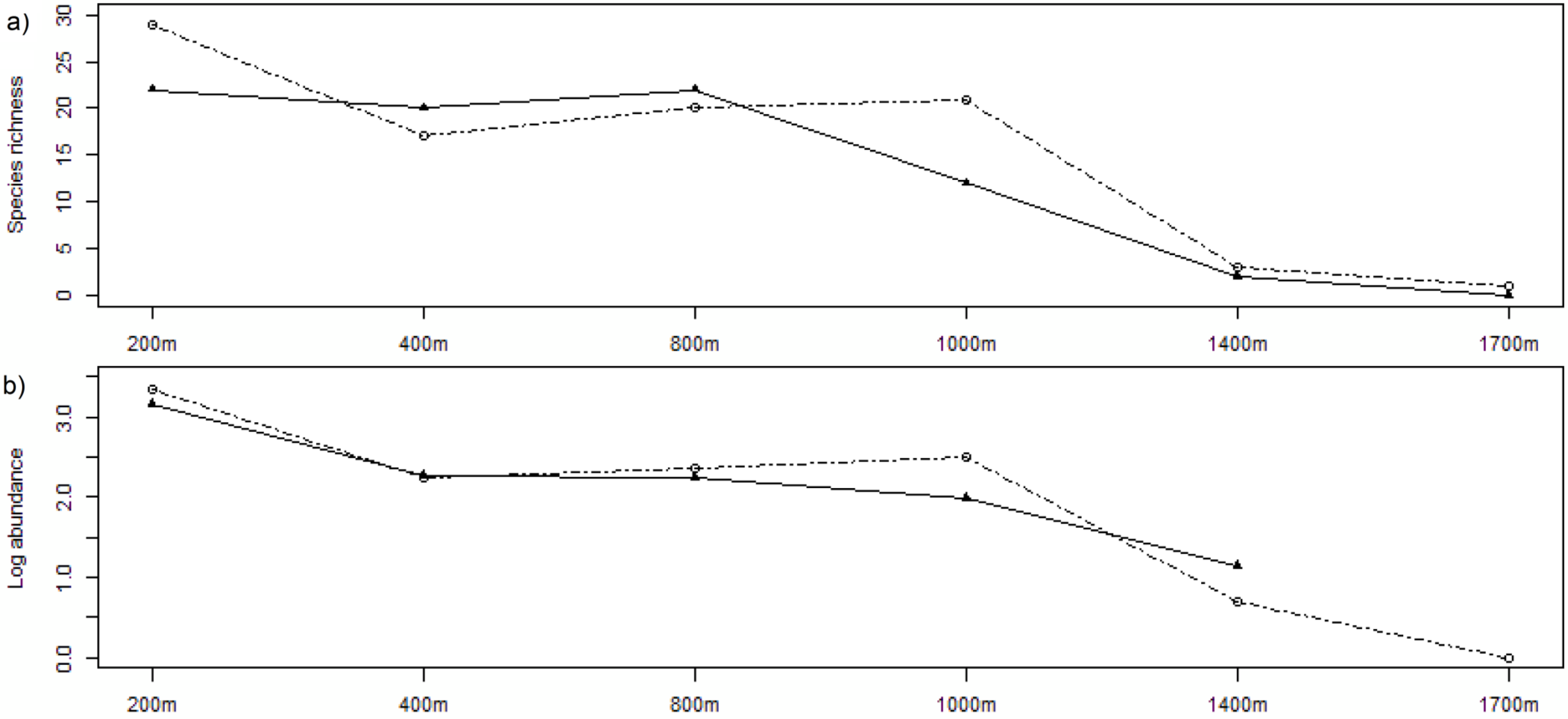
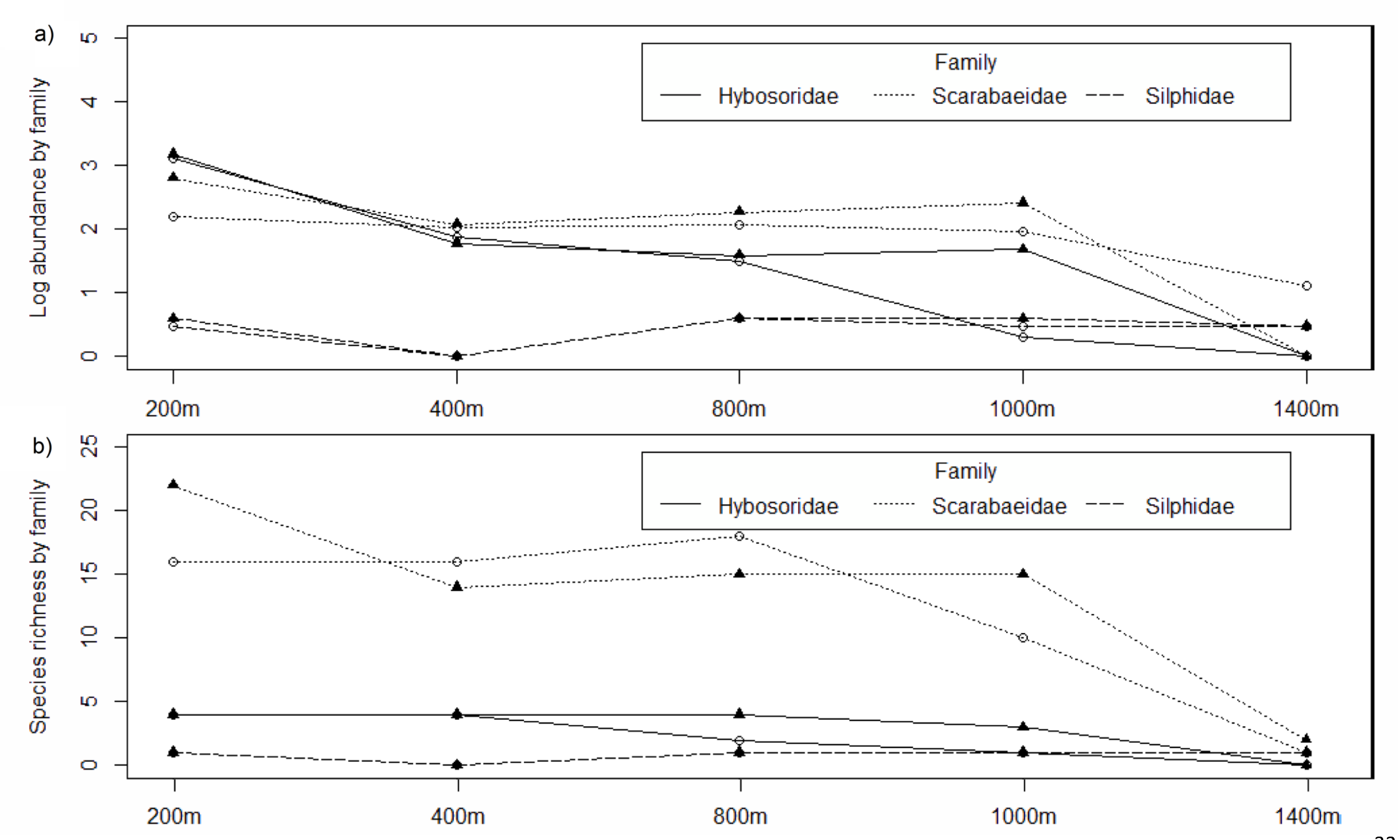


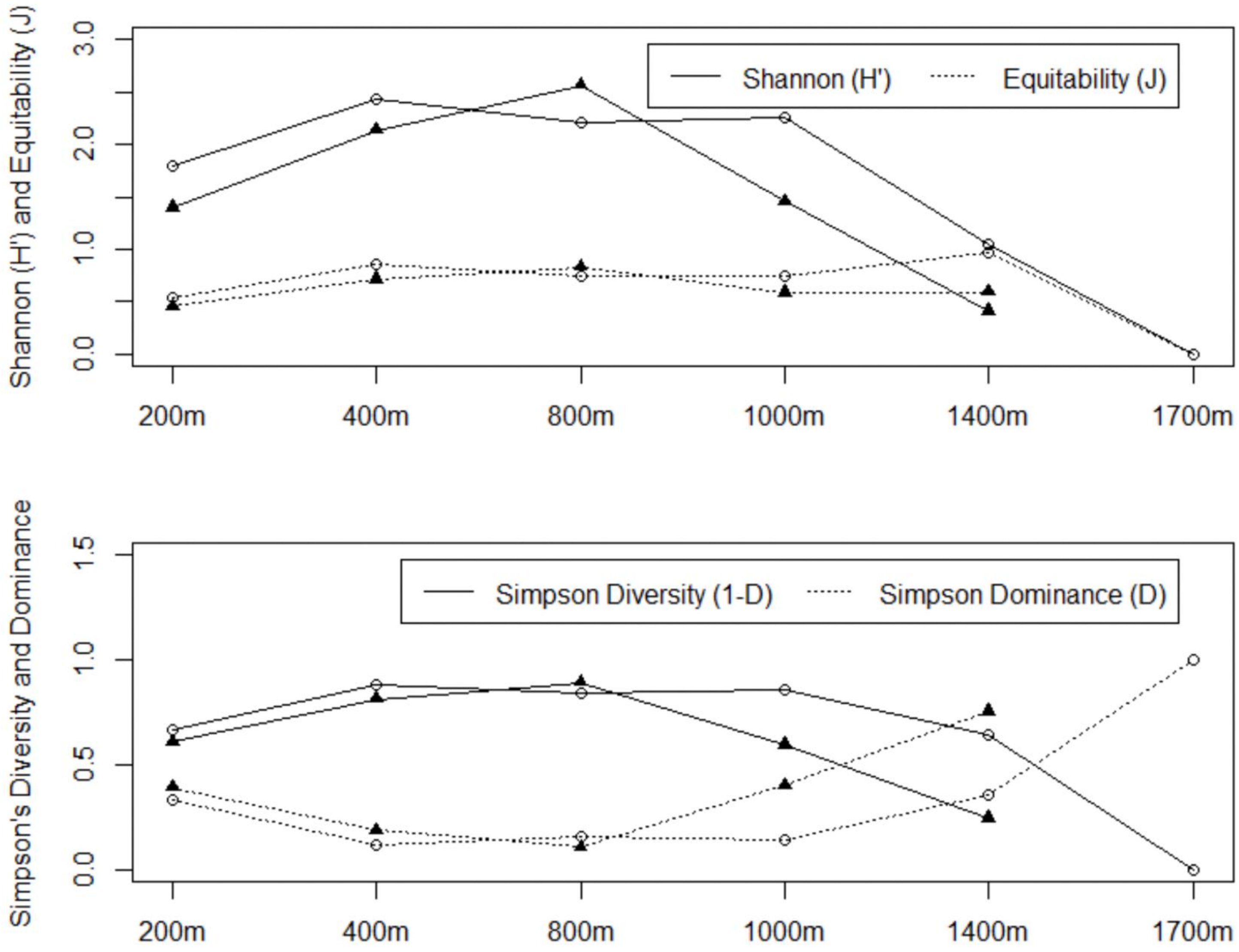
Fig4.3 a) Abundance for each family plotted against elevation. b) Species richness for each family plotted against elevation.



Transitional forests between 800m and 1000m did not show a drastic change in species numbers or compositions. Hybosorid species became less common in this elevation and it was dominated by a larger proportion of smaller Scarabaeid species (Fig. 4.3b). However, transitional forests had less abundant Scarabaeid beetle species, with only five species being common: *O. leusermontis, O. cupreus, O. peninsulotagal, O. deflexicollis* and *Onthophagus* “Hairy Group”. Six out of 10 singleton species were collected from transitional forests (Appendix 1). Only 3 species occurred at Lower Montane Forests and 1 at Upper Montane Forests. The abundance was very small, at less than 10 individuals for Lower Montane Forests and only a singleton species was collected in the Upper Montane Forest: *Onthophagus* sp. 6.

Abundance is was highest at the 200m site, owing largely to the strong dominance of Hybosorid species. From 400m to 1000m the abundance of beetles remains relatively constant, but from 1400m and beyond abundances drop drastically. The reduction of Hybosorid abundance as elevation increased the evenness of localities between 400 and 1000m (Fig. 4.3a). In the 200m site Hybosorids were the dominant species by a large margin, with *Phaeochrous emarginatus* having the highest abundance in the first sampling (838 individuals, 57%) and *Phaeochroops rattus* in the second sampling (1219 individuals, 56% of individuals collected). In the 400m site, the total number of Hybosorids was reduced to 75 individuals (20%) in the first sampling and 59 individuals (33%) in the second sampling. This is reflected in the increase in H and Simpsons's D diversity indexes (Fig 4.4a,b). An overlap between transitional forest species and lowland forest species, combined with the negative reaction of Hybosorids to low temperature results in the 400m and 800m elevation sites having the highest diversity. Higher diversity indexes indicated a lower dominance of Hybosorid beetles and a much more equitable distribution of Scarabaeid species. The changes in abundance tend to correspond to forest type.

Fig. 4.4: a) Shannon index and Equitability plotted against elevation. b) Simpson’s index and Simpson Dominance plotted against elevation.



Four species of hybosorids, *Phaeochroops rattus, Pc. freenae, Pc. peninsularis* and *Phaeochrous emarginatus*, were found to inhabit the lowland forest. All hybosorid species were positively correlated to temperature in the range between 19.5°C and 25.0°C (Fig. 4.5). *Pc. rattus* and *Ph. emarginatus* were of medium size (10mm) and were very abundant while *Pc. freenae* and *Pc. peninsularis* were of larger size (15mm) and had a more sparse distribution. Medium sized species were present in almost all traps set in lowland forests. Some of the aggregations involved a large number of individuals, up to a hundred *Pc. rattus* individuals were collected in one trap. A G-test indicates that the all the species occur independently of each other except for *Pc. freenae* and *Ph. emarginatus* (Table 4.1). All species showed a high coefficient of variance and had overdispersed distributions (Table 4.2).

Table 4.1: G-test for species associations within the Hybosoridae species group at 200m.

9	*Ph. emarginatus*	*Pc. freenae*	*Pc. peninsularis*	*Pc. rattus*
Ph. emarginatus	X	P<0.05, Positive	P>0.05, Independent	P>0.05, Independent
Pc. freenae	7.0221	X	P>0.05, Independent	P>0.05, Independent
Pc. peninsularis	1.9582	0.9534	X	P>0.05, Independent
Pc. rattus	1.3919	0.9859	0.9534	X

Table 4.2: Dispersion coefficients for Hybosorid species.

Category	Species	Elevation	σ2/μ	
			1st Sampling	2nd Sampling
Lowland Hybosorid	*Phaeochrous emarginatus*	200m	12.90	8.43
	Phaeochroops freenae	200m	1.04	2.37
	Pc. peninsularis	200m	3.52	10.73
	Pc. rattus	200m	9.04	23.65

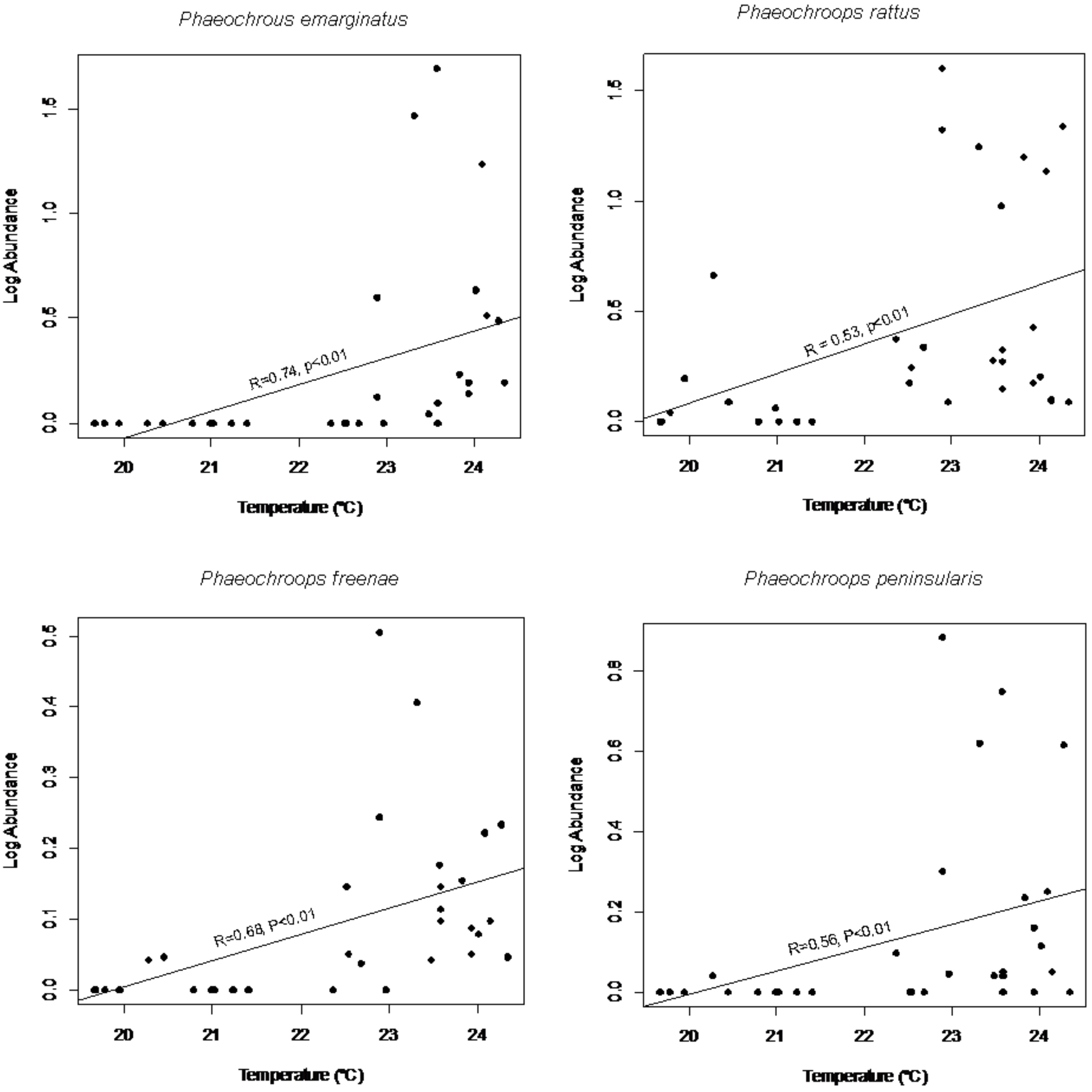


Fig4.5: Spearman correlation between Hybosorid species and temperature.

Only one species of Silphidae was found in this study, *Necrophila (Chrysosilpha) formosa*. The range where this species was collected extended from 200m to 1400m, with the number of individuals collected in each site showing little variation. Silphids were generally rarely caught with only one or two specimens were collected in the same trap on the same sampling day. Size of the bait may have been a factor, as large aggregations of more than 10 individuals had been observed on larger baits set in the same site. *N. formosa* showed no significant correlation with temperature in a range of 19.5°C to 25.0°C (Fig 4.6). No Silphids were found at 400m, but this could be due to the different land use of the site.

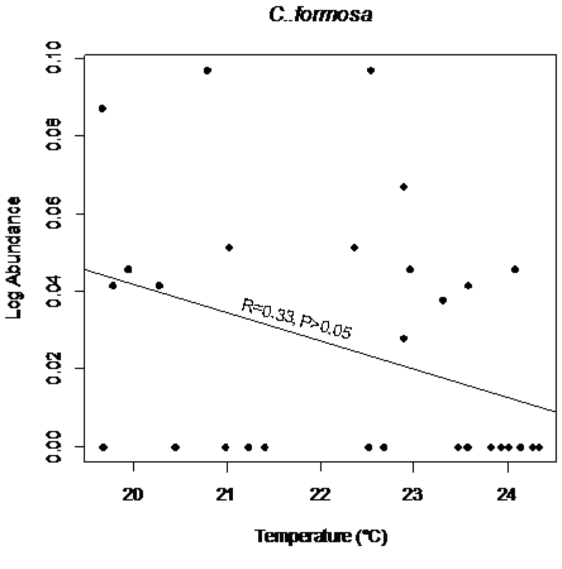


Fig. 4.6: Spearman correlation between Silphid species and temperature.

A majority of the species collected belonged to the genus *Onthophagus*. Species with more than 3 individuals in the genus *Onthophagus* were categorised into 3 different categories: Lowland, transitional forest and generalist. Both UPGMA and the single linkage cluster technique produced similar results and supported this classification (Fig 4.7). Generalist carrion feeding beetles such as *O. semifex* was positively correlated with temperature, while *O. peninsulocupreus* was negatively correlated with temperature (Fig 4.8). The large Scarabaeid species *Catharsius mollusus* was positively correlated with temperature, while *Synapsis birmanicus* did not show significant correlation (Fig 4.8). Transitional species were mostly negatively correlated or not significantly correlated with temperature while lowland species (Fig 4.10) were positively correlated with temperature (Fig 4.9). Additionally, most species with sufficient sample sizes indicated an overdispersed distribution (Table 4.3). Lowland forest *Onthophagus* species did not show the negative trend in abundance displayed by Hybosorids in the sites from 200m to 800m. While some individuals were collected from lowland forest, transitional forest species were only found in large numbers in transitional forest. Lowland species were a mix of carrion specialist *Onthophagus* and generalist dung feeding *Onthophagus*. The feeding preferences of transitional forest species is unknown.

Table 4.3: Dispersion coefficients for selected *Onthophagus* species for second sampling.

Category	Species	Elevation	σ2/μ
Lowland Onthophagus	*Onthophagus penicillatus*	200m	2.24
	O. egrigius	200m	1.16
	O. rudis	200m	3.08
Generalist Onthophagus	*O. semifex*	200m	1.68
	O. semifex	1000m	2.86
	O. peninsulocupreus	200m	1.22
	O. peninsulocupreus	1000m	3.54
Transitional Onthophagus	*O. peninsulotagal*	1000m	3.07
	O. deflexicolis	1000m	2.46

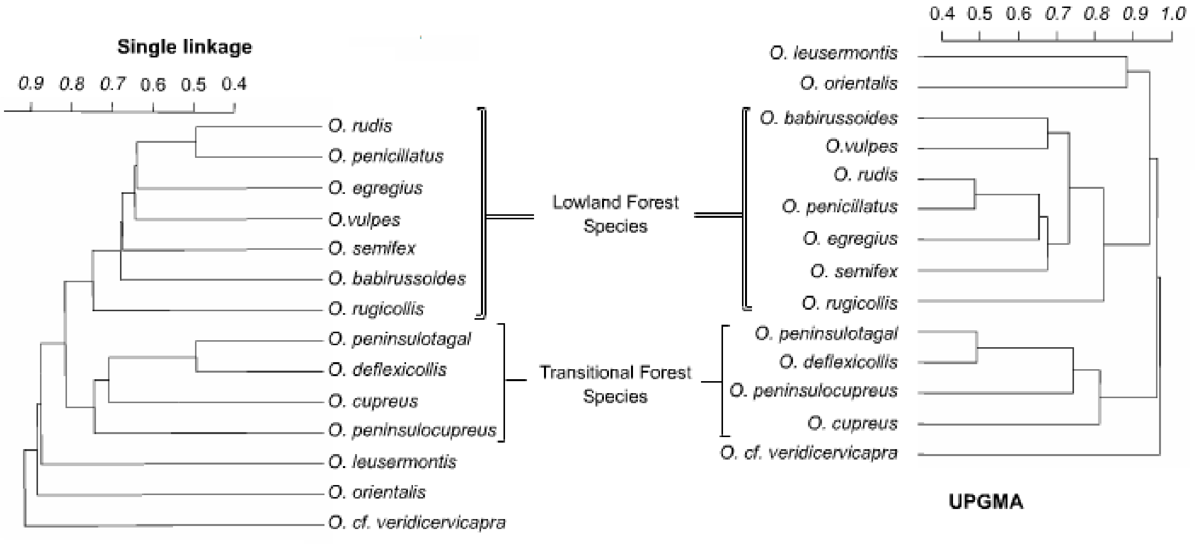


Fig. 4.7: Cluster analysis on *Onthophagus* species.

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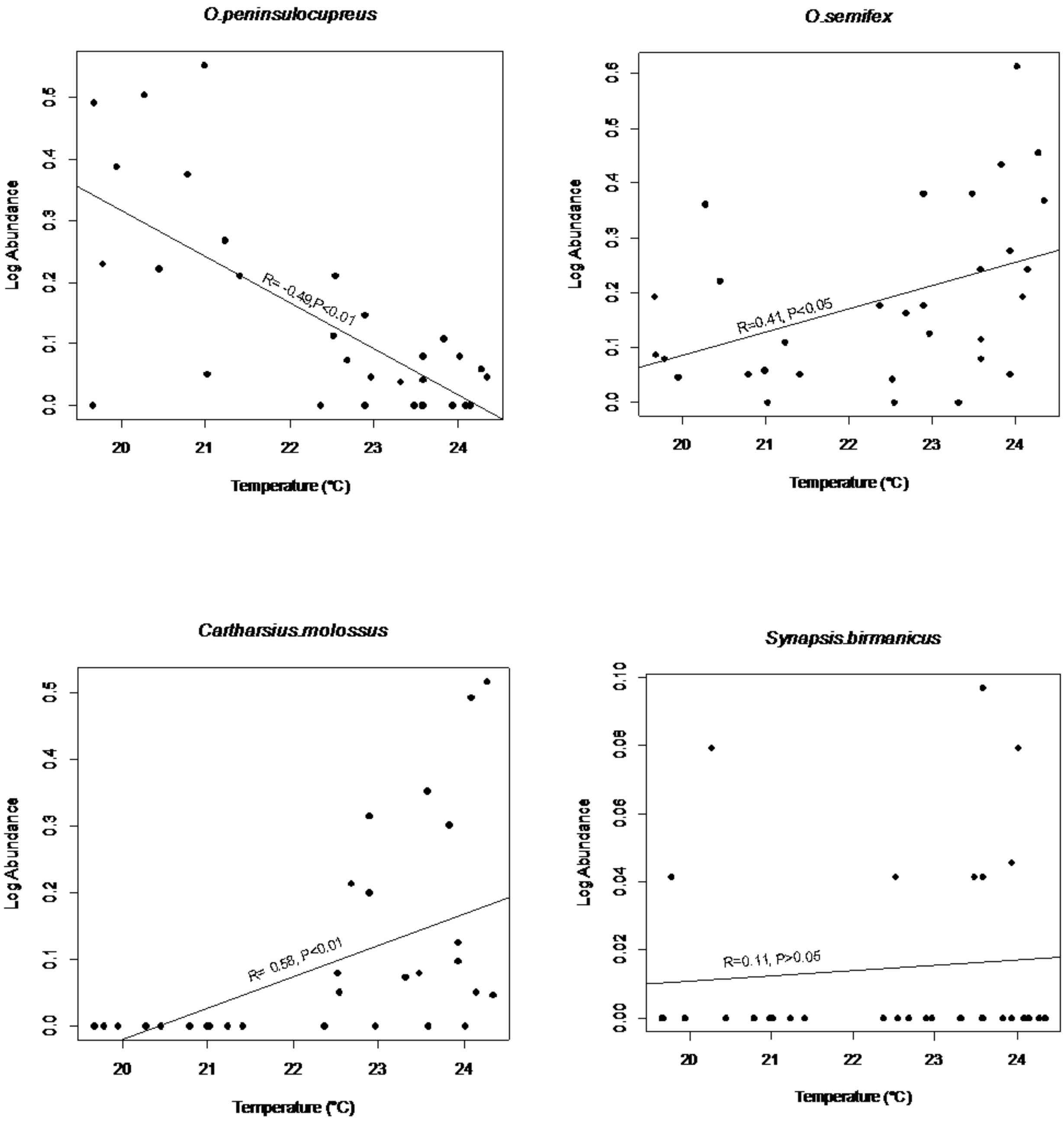


Fig. 4.8 Spearman correlation between Scarabaeid species and temperature.

Fig. 4.9: Spearman correlation between Lowland forest Onthophagus species and temperature.

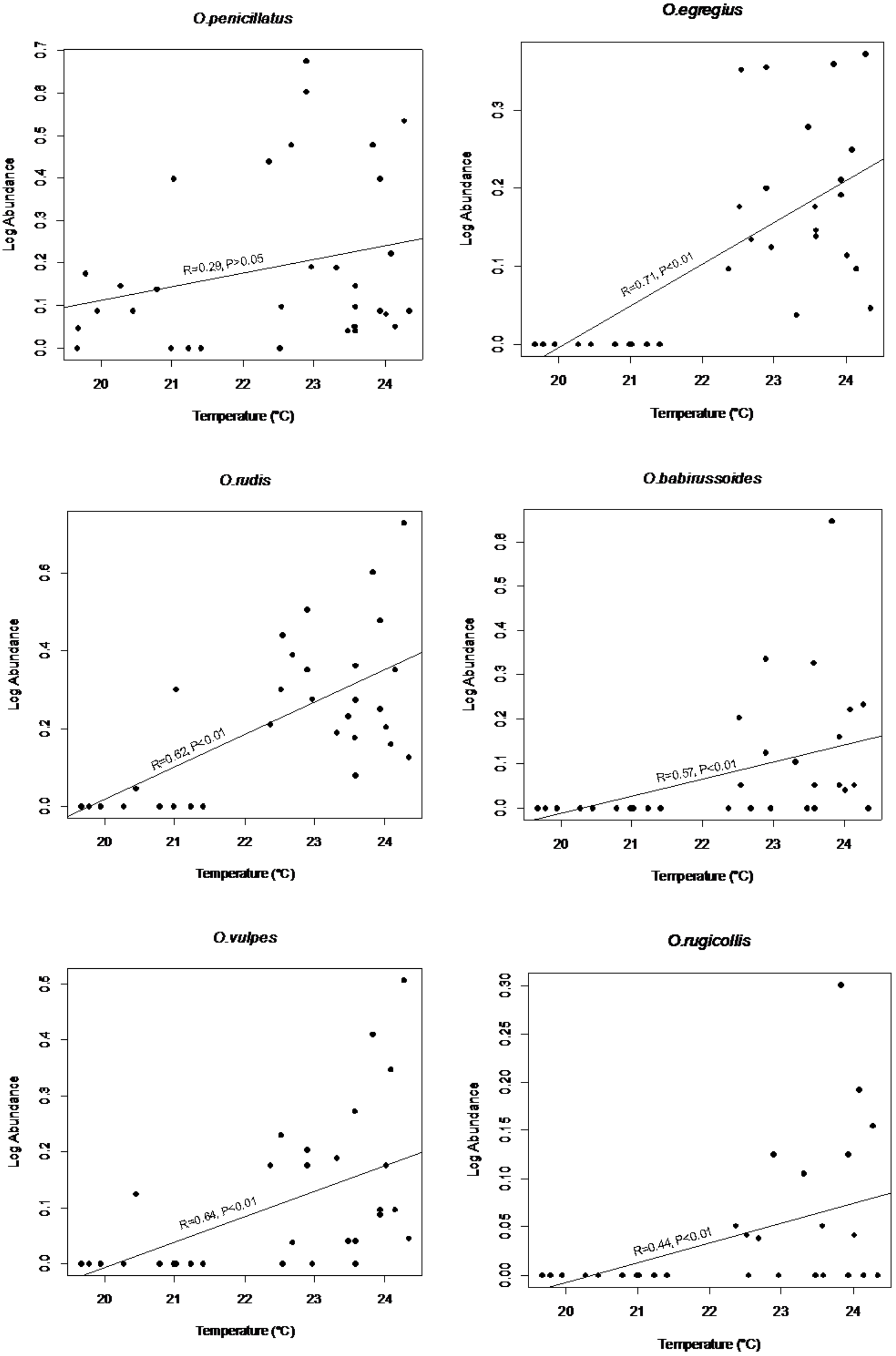
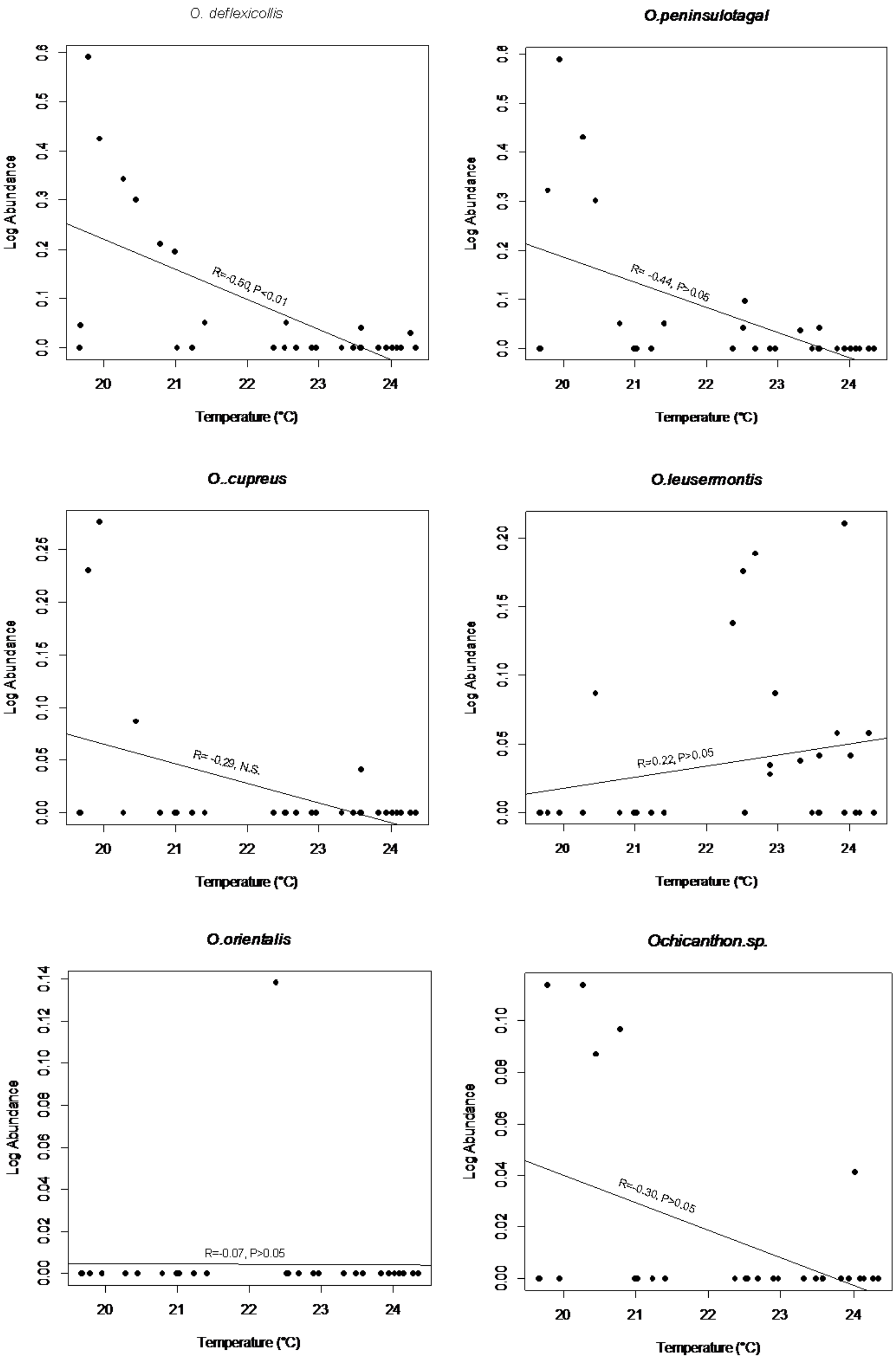


Fig. 4.10: Spearman correlation between Transitional forest *Onthophagus* species and temperature.



4.2 Blowfly diversity

The highest number of species was found at elevations 200m, 800m and 1000m with 9 species each. Species recorded as highland forest specific were found in all these sites, but they tended to be less common. These species were a mix of synanthropic flies like *Ch. megacephala*, and forest dwelling flies such as *Ch. villeneuvi*, *Ch. deflixa* and *Ch. nigripes*. Only the 200m site had a significantly large number of *Ch. megacephala*, probably due to human disturbance. There was limited development in the 400m site resulting in a disturbed forest environment; this may have had an effect on the diversity of Calliphorid flies as only 3 forest species and 1 synanthropic species were collected. There was an overlap of lowland and highland species lower montane forests. The lowest number of species was collected at the peak of Gunung Ulu Kali at 1700m, which were mainly the highland forest specific *Lucilia porphyrina* and *Phumosia promittens* and hardy synantropic species such as *Ch. megacephala*.

Table 4.4: Distribution of female blowflies according to altitude.

Site Name	Altitude (m a.s.l.)	*Ch. megacephala*	*Ch. rufifacies*	*Ch. nigripes*	*Ch. deflixa*	*Ch. pinguis*	*Ch. villeneuvi*	*Ch. chani*	*L. cuprina*	*L. porphyrina*	*Ph. promittens*
Bukit Cincin	1700	2	0	0	0	0	0	0	0	83	4
Chin Swee Temple	1400	3	0	0	9	10	9	0	0	51	6
Awana	1000	22	9	1	14	14	238	0	0	3	13
Bukit Tinggi	800	2	1	1	9	3	40	0	3	1	9
Janda Baik	400	7	0	0	0	1	18	1	0	2	0
Gombak	200	105	18	288	41	14	333	7	0	4	10

Species could be clearly divided into lowland and highland groups. *Chrysomya* species such as *Ch. villeneuvi*, *Ch. deflixa* and *Ch. nigripes* make up the majority of species found in lowland and transitional forests. *Ch. villeneuvi* was the most common species, even after removing all the males from the data set (Table 4.4). In both lower and upper montane forest, *Lucilia porphyrina* became the dominant species (Table 4.4). The turnover between *Ch. villeneuvi* and *L. porphyrina* occurs at the border between transitional and lower montane forests. Common synantropic species, such as *Ch. megacephala* and *Ch. rufifacies* are less common in forested environments. Lowland species showed positive correlation with temperature (Fig 4.12), while highland species were negatively correlated with temperature (*L. porphyrina*) (Fig 4.12) or not significantly correlated with temperature (*Ph. promittens* and *Ch. pinguis*.)

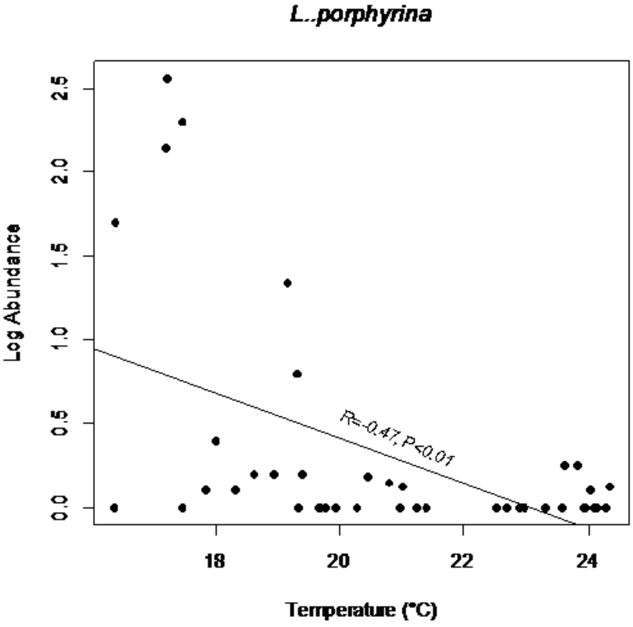
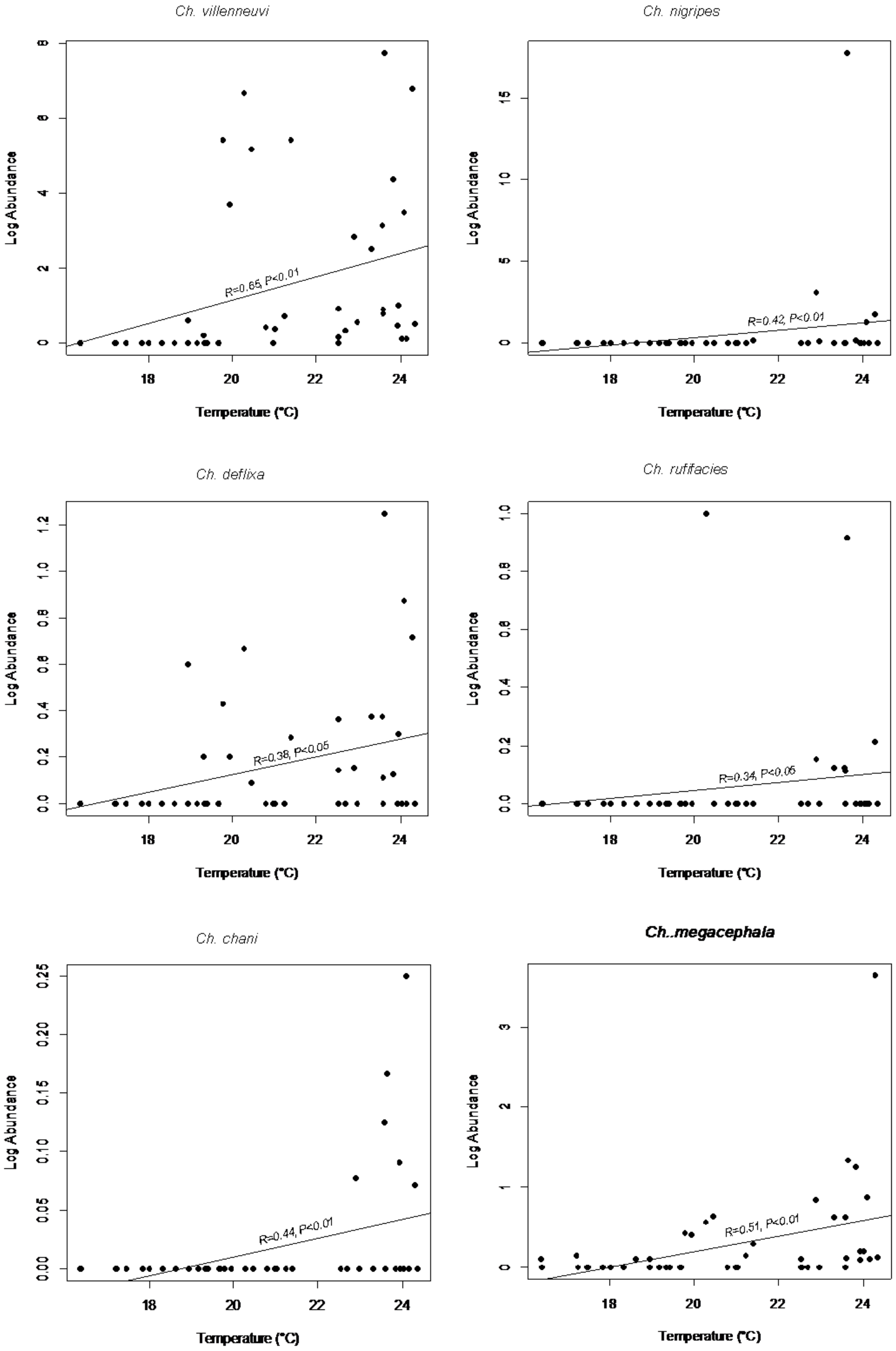


Fig. 4.12: Spearman correlation between *L. porphyrina* and temperature.

Fig. 4.13: Spearman correlation between Lowland forest Calliphorid species and temperature.



4.3: Response of blowflies to presence of beetles.

The spatial distribution between two groups that share similar resources was explored by examining whether the increase in abundance of one group led to the reduction in the abundance in the other. In the 200m elevation site where both beetles and flies were most common, there was no significant correlation ($p>0.05$, $r= -0.0212$) between the total number of beetles and the total number of flies in each sampling unit (Fig 4.14). The presence and absence of blowflies in sampling units with beetles were random as opposed to displaying a generalised pattern. A G-test of independence indicates that the distribution of flies was independent from predatory beetles (Table 4.5). A similar G-test was carried out between the presence of competitor beetles, which were divided into diurnal Scarabaeids and nocturnal Hybosorids. Additionally carrion flies did not show any avoidance of large Scarabaeid beetles (Table 4.5)

Fig. 4.14: Spearman correlation between Calliphorid fly abundance and beetle abundance.

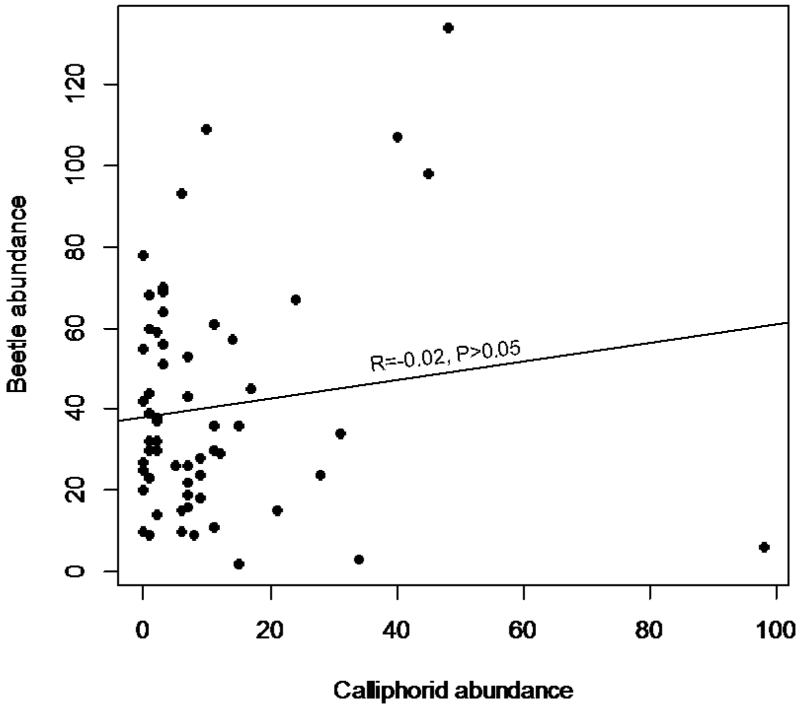


Table 4.5: Results of G test between Calliphorids and beetles.

G-test of independence	G	Df	Critical value	P	Relationship
Flies and Scarabaeids	1.8205	1	3.841	p>0.05	Independent
Flies and Large Scarabaeids	1.8028	1	3.841	p>0.05	Independent
Flies and Hybosorids	0.2325	1	3.841	p>0.05	Independent
Flies and predatory beetles (Silphids and Histerids)	1.6177	1	3.841	p>0.05	Independent

4.4: Key to forensically important beetles in Peninsular Malaysia.

4.4.1: Introduction

The taxonomy of Carrion flies has been well documented (Senior-White *et al*., 1950; Kurahashi *et al*., 1997; Inder *et al*., 1974), but there are no keys available for carrion associated beetles. While there are more beetle families associated with carrion, for simplicity sake this key is limited to the larger more conspicuos families of Hybosoridae, Scarabaeidae & Silphidae. In temperate regions carrion visiting beetles are well studied and extensive literature dealing with identification and biology is available. For most tropical countries such literature is not available. Identification can often only be done by specialists who have access to large museum collections. As these specialists seem to become a rare species themselves a reliable identification of tropical material is often very difficult.

While most dung beetles can occasionally be found on carrion, the inclusion of non-carrion specialist beetles would make the key too complicated and difficult to use. Therefore those species are not treated in this key. Any scarabaeid specimens that have the following characters are outside the scope of this key:

-Black or brown colouration.

-Long, curved spider-like legs.

-A size of more than 15mm.

-Large single horns or projections on the pronotum.

-Metasternum not prow shaped in front.

It should be noted that most of these studies occured in outdoor location close to forested or agriculatural areas. All the beetles that occur in this study tend to be sensitve to environmental changes and are generally associated with forested environments. It is likely that beetles found in urban or indoor habitats are not included in this key.

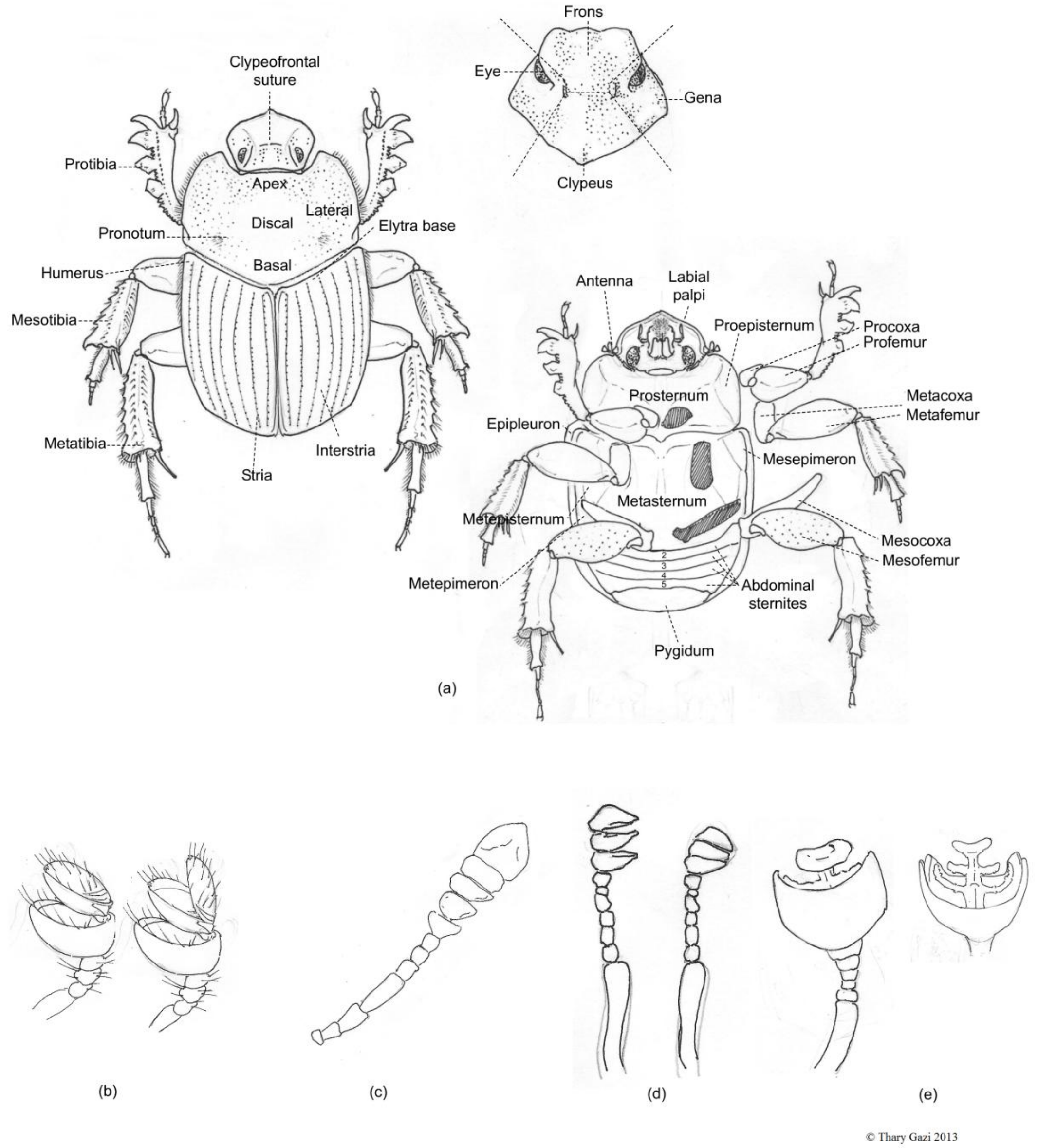


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Fig. 4.15: a) Morphology of scarabaeid beetle. b) Hybosorid antenna in closed (left) and open (right) position. c) Silphidae antenna. d) Scarabaeid antenna in open (left) and closed (right) position. e) Antenna of *Onthophagus egregius* in ventral (left) and dorsal (right) view.

4.4.2 Dichotomous Key

1a - Antenna with 3 expanded terminal segments ("Lamellae") (lamellate antenna) (Fig. 4.15b,d,e). Elytra covering all abdominal segements when viewed from above (Fig 4.15a). -2

1b - Antenna with well defined, densely pubescent, 3 segmented club (Clavate antenna) (Fig. 4.15c). Elytra short, not covering 3 or more abdominal segements when view from above (Fig. 4.16). - 11

2a - Antenna lamellae forming a cup shaped structure, with the first antennal segment fitting into the second and the third segment (Fig 4.15b). Mandibles and labrum distinct in full face view. Habitus (Fig. 4.18) – 3

2b - Antenna lamellae forming a fan shaped structure (Fig. 4.15d, 4.15e). Mandibles and labrum covered by clypeus, indistinct in full face view. Habitus (Fig. 4.17) - 6

3a -9-11mm. Dorsum glabrous (Fig 4.19a). ---*Phaechrous emarginatus*.

3b - Dorsum with distinct, long more or less upright setae. Anterior border of clypeus transverse emarginate- 4

4a Size 9- 11 mm. Intercostal setae on elytra arising from large, horseshoe-shaped punctures (Fig. 4.19b) --- *Phaeochroops rattus.*

4b Size >13mm. Intercostal setae arising from simple, round punctures, much smaller than the surrounding horseshoes (Fig. 4.19c) -5

5a Between bases of second and third discal costae of elytron a short, distinct accessory costa present (Fig. 4.19d). Fringe of elytral margin without interruptions (in fact freenae looses these hairs very easily; absence of these hairs is almost a good character). --- *Phaeochroops freenae.*

5b Between bases of second and third discal costae of elytron a short, distinct accessory costa absent (Fig. 4.19e). Fringe of elytral margins with some interruptions. --- *Phaeochroops peninsularis.*

6a – Antennal clubs rounded (Fig 4.15d,4.15e). Pronotal base more or less rounded, metasternum prow shaped in front, Habitus (Fig. 4.15a, 4.17). - *Onthophagus*. 7

6b – Antennal clubs variable. Pronotal base variable, Metasternum not prow shaped in front. - Other non-carrion specialist Scarabs

7a-10-12mm, coppery green pronotum with black unpatterned elytra, clypeus divided by a forked horn into two angular lobes. Antennal club modified (Fig. 4.15e) --- *O. egregius.*

7b- antennal club normal (Fig. 4.15d).- -- 8

8a- pronotum densely granulate -- 9

8b- pronotum smooth -- 10

9a- 9-11mm, coppery green pronotum, with black patterned elytra, pronotum with symmetrical lateral hair tufts--- *O. penicillatus.*

9b- 6-7mm, coppery brown pronotum, unpatterned elytra with similar colour, pronotum with a basal depression and without symmetrical tufts of hair, entire dorsum covered in hairs. --- *O. rudis*

10a- 10-12mm, shallow depression on basal portion of pronotum, elytra microreticulate --- *O. semifex*

10b- 5-6mm, elytra shining black -- *O. peninsulocupreus*

11a - Histeridae

11b - Silphidae - 12

12a – Elytra smooth, without any ridges. Length 20mm. Constriction between base of head and prothorax. Black or dark brown beetle with orange marking on its elytra. --- *Nicrophorus nepalensis*

12b – Elytra with ridges. Length variable. No constriction between head and prothorax. -13

13a - 13-17mm, body dorso-ventrally flattened, elytra with metallic blue coloration, four spots on the discal portion of the pronotum. --- *Chrysosilpha formosa*

13b - 20-25mm, elongate and black with reddish orange spots on the elytra. Terminal segment of antenna orange. Females with swollen tibia and rough prothorax, males have a smooth thorax.--- *Diamesus osculans*

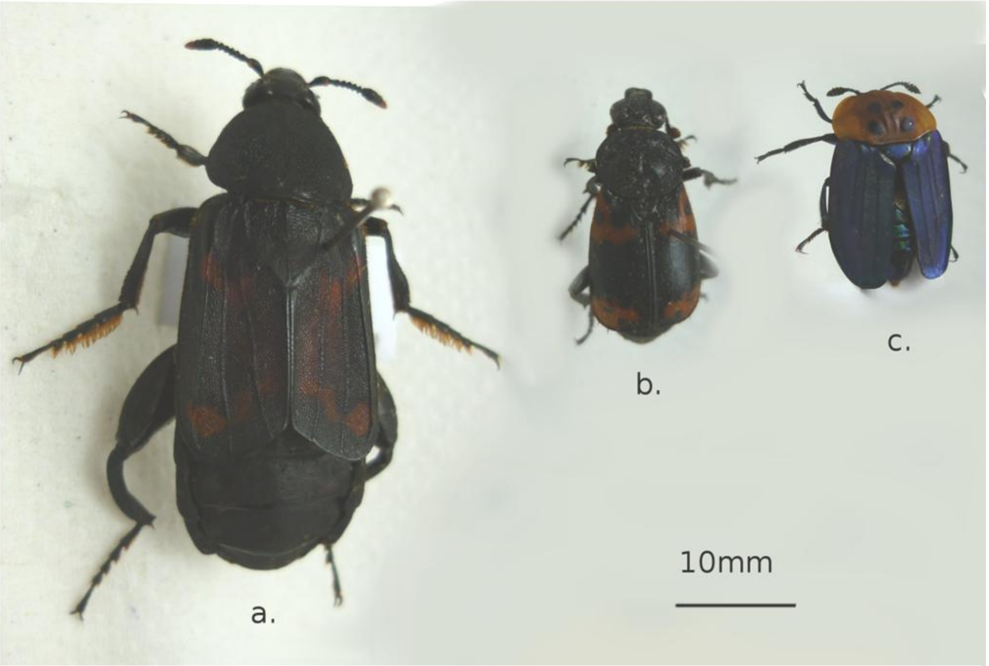

Fig. 4.16: Habitus of :

a) *Diamesus osculans*.

b) *Nicrophorus nepalensis*.

c) *Chrysosilpha formosa*.

Fig. 4.17: Habitus of:

a) *Onthophagus egregius*.

b) *O. penicillatus*.

c) *O. semifex*.

d) *O. rudis*.

e) *O. peninsulocupreus*.

Fig 4.18: Habitus of:

a) *Phaeochroops freenae.*

b) *Phaeochroops peninsularis .*

c) *Phaeochrous emarginatus.*

d) *Phaeochroops rattus.*

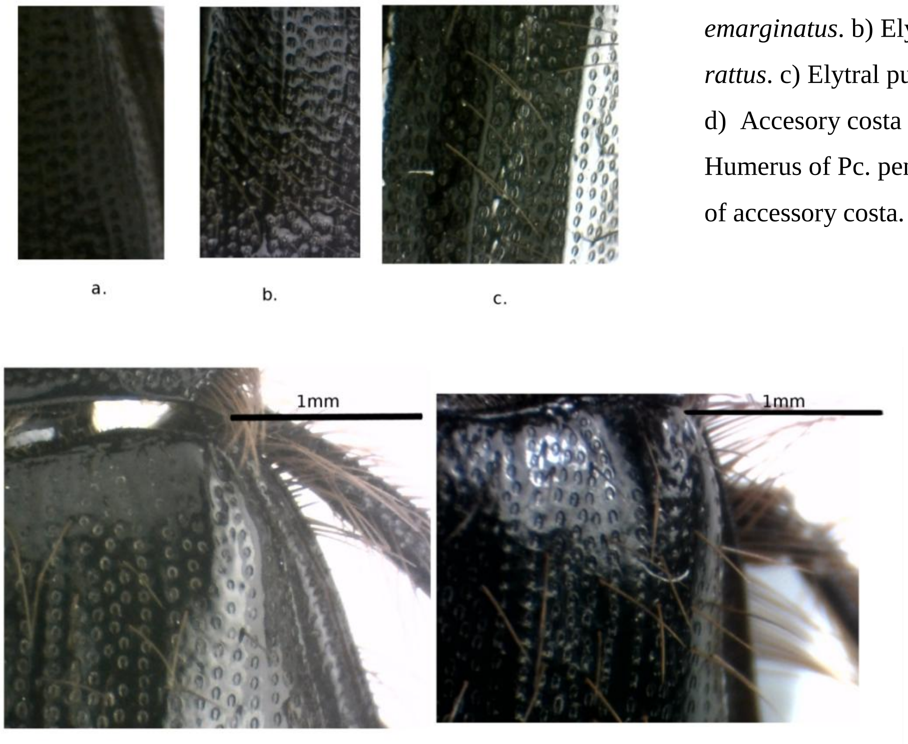

Fig. 4.19: a) Elytral punctuation of *Ph. emarginatus*. b) Elytral punctuation of *Pc. rattus*. c) Elytral punctuation of *Pc. freenae*. d) Accesory costa of Pc. freenae. e) Humerus of Pc. peninsularis, note the lack of accessory costa.

Fig. 4.20: Elytra of:

a) *O. egregius.*

b) *O. semifex.*

c) *O. peninsulocupreus.*

d) *O. penicillatus.*

e) *O. rudis.*

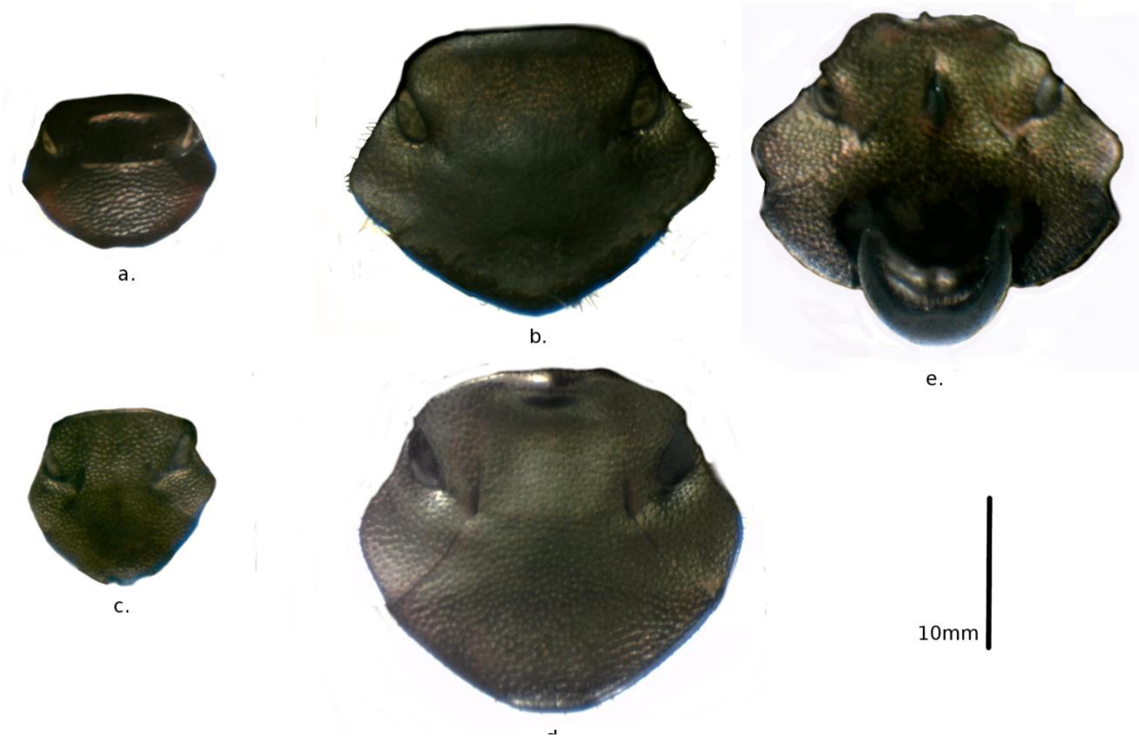

Fig. 4.21: Head view of: a) *O. peninsulocupreus*. b) *O. semifex*. c) *O. rudis*. d) *O. penicillatus*. e) *O. egregius* (male)

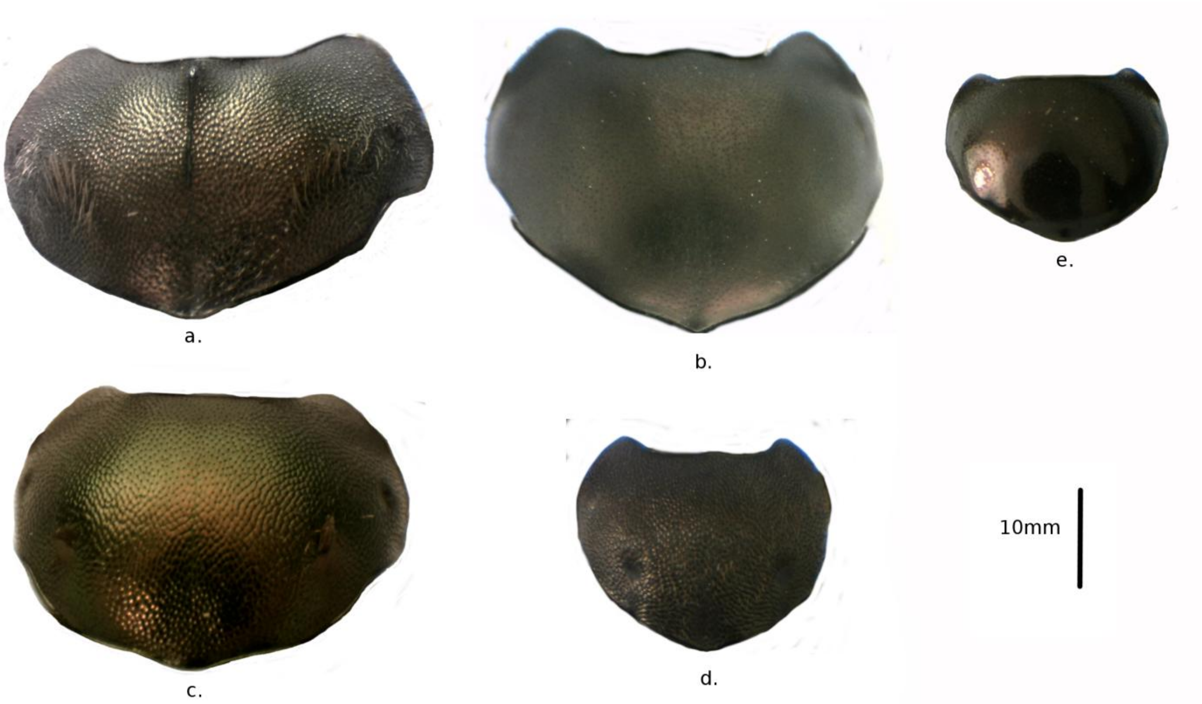

Fig. 4.22: Pronotum of: a) *O. egregius*. b) *O. semifex*. c) *O. penicillatus*. d) *O. rudis*. e) *O. peninsulocupreus*.

4.4.3 Descriptions

4.4.3.1 Scarabaeidae

Onthophagus penicillatus Harold 1879

Diagnosis: This medium sized species (9-11mm) can be distinguished from other necrophagous scarabaeinae by the coppery green coloration of the granulate pronotum and the orange patterns on the base of the elytra. The surface of the clypeus is regularly and finely punctate with a single protuberance on the middle of the frons. The pronotum is densely granulate and distinctive tufts of yellow hair are found on the lateral sides of the pronotum. The basal portion of the elytra has usually dark yellowish or orange brown spots, but the pattern is variable. Interstria are coriaceous/microreticulate and are covered in dense puctures bearing fine dark setae. The pygidium regularly and moderately punctate.

Ecology: Activity period unknown, but likely a diurnal species. Common in forests in a range from sea level to 800m. Thus far, the nesting behaviour of this beetle is unknown as is the extent in which it utilises carrion as a resource. Nothing else is known about the ecology.

Forensic applications: In forested habitats, it is found to be attracted to traps baited with rotting fish. Also found to be attracted to rabbit (Raja Zuha, R.M., Unpublished data) and monkey (Nazni et al., 2011) carcasses in the decay stage. Possible area indicator.

Onthophagus rudis Sharp 1875

Diagnosis: The characters for differentiating this small dung beetle (6-7 mm) are a granulate coppery brown or greenish pronotum, the depressions on the basal portion of the pronotum and the pilosity of the entire dorsum of the beetle. Head rather similar to *O. penicillatus* in shape, but lacks the protuberance on the frons and is granulated. The basal portion of the pronotum has a single distinct depression and a pair of round pits anteriorly fringed with hair are present on the basal-lateral portions of the pronotum. The elytra covered in elongated granules and the surface of the elytra is uneven, with several very shallow and wide depressions. Pygidium is covered in granules and fine setae. No marked sexual dimorphism.

Ecology: Diurnal. Some specimens were collected from a fish carcass in the late morning. Common in lowland tropical rainforests from sea level to 1000m. As with all carrion feeding scarabaeids, the nesting ecology is unknown.

Forensic applications: In forested habitats, it is found to be attracted to traps baited with rotting fish. Also attracted to rabbit (Raja Zuha, R.M., Unpublished data) and monkey (Nazni, et al., 2011) carcasses in the decay stage. Possible area indicator.

Onthophagus(Parascatonomous) egregius Arrow 1907

Diagnosis: *O. egregrius* is a medium sized (~12mm) coppery green beetle and can be recognised by the unique shape of it’s antenna (Fig.4.15e), the ridge on the pronotum and its unique clypeus shape. Larger males have forked (bifid) horns and a clypeal margin that is split into two lobes, females and small males have similar forked protrusions but much less developed. The head is densely punctate with yellow-white hairs arising from the punctuations. The pronotum is densly and strongly punctate and a pronotal ridge begins from the apical portion of the pronotum and terminates with a protuberance at the discal portion. Parts of the discal pronotum are covered in yellowish-white setae. Interstria and pygidium punctate and covered with fine setae. Elytra are finely and regularly punctate with yellow-white setae originating from the punctuations. Pygidium coriaceous/microreticulate with dense and moderate dark brown setae bearing punctuations.

Ecology: Diurnal. Possibly also active during dusk, several specimens were found to be actively attracted to a fish carcass at that time. Found up to 1000m. Nesting ecology is unknown.

Forensic applications: In forested habitats, it is found to be attracted to traps baited with rotting fish. Thus far only recovered from monkey (Nazni et al., 2011) and rat (Goh T.G., Unpublished data) carcasses in the decay stage. Possible area indicator.

Onthophagus (Parascatonomus)semifex Huijbregts & Krikken, 2008

Diagnosis: This medium sized (10-12mm) beetle can be distinguished by the smooth coppery green (metallic red when wet with alcohol or under strong lighting) and finely punctate pronotum, the shallow depression at the basal portion of the pronotum and the presence of pale yellow-brown setae on the black elytra. Both male and female are without any horns or protuberances aside from a short tooth on the apex of the rugulate clypeus. There are short pale yellow-brown setae sparsely distributed between the discal and lateral portions of the pronotum. Surface of elytra coriaceous/microreticulate with regular and moderate yellowish-white setae bearing punctuations. Pygidium densely and strongly punctate and with setae.

Ecology: Diurnal. Ranges from sea level to 1400m. Based on trapping data, this species is the most common and wide ranging carrion feeding Scarabaeid species found in Peninsular Malaysia (Goh, T.G., Unpublished data). Nesting ecology is unknown, but was one specimen was once found burrowing under a fish placed in the forest.

Forensic applications: Attracted to traps baited with rotting fish and chicken liver in secondary forests, transitional highland forests, rubber estates and abandoned estates (Khairunnisa, S., Unpublished data). Found attracted to rabbit (Raja Zuha, R.M., Unpublished data) and monkey (Nazni et al., 2011; Raja Zuha, R.M., Unpublished data, Azwandy A.A., Unpublished data, Omar, B., Unpublished data) carcasses in the decay stage and fish in the fresh stage (Goh, T.G. Unpublished data). Possible area indicator. Due to its abundance and wide habitat range, this species has potential for further reasearch into the use of Scarabaeid beetles in forensic entomology.

Onthophagus(Parascatonomus) peninsulocupreus Huijbregts & Krikken, 2009

Diagnosis: A small species (~5mm) with a punctate dark metallic red pronotum and glabrous shining black elytra. Clypeus moderately and regularly punctate and margin without any protrusions. A few rare specimens have a more coppery green pronotum.

Pronotum without any setae. Elytra with fine and sparsely punctate interstria. Pygidum moderately and regularly punctate and without setae. No sexual dimorphism.

Range and Ecology: Diurnal. Has been found in degraded forests, highlands and abandoned rubber estates. The highest altitude where it has been found is at 1000m a.s.l. in a mature secondary hill forest. Nesting ecology is unknown.

Forensic applications: Attracted to traps baited with rotting fish (Goh, T.G., unpublished data) and chicken liver (Khairunnisa S., unpublished data) in secondary forests and forested areas. Possible area indicator.

4.4.3.2 Hybosoridae

Phaechroops rattus Arrow 1909

Diagnosis: A dark brown medium sized Hybosorid (~10mm) with erect setae arising from horseshoe shaped punctuations on the interstria of the elytra. Some specimens may appear to be red-brown, it is likely that these are newly emerged. No accessory costa on the elytra. Elytral fringe sparse and continuous. Can be easily distinguished from other *Phaechroops* species based on size.

Ecology: Mainly a nocturnal species, but some specimens have been found to be active during dawn and dusk. Large numbers, up to thousands of individuals on large carcasses, of these beetles arrive as soon as the sun sets to feed on the carrion and mate. In captivity this species does not oviposit on carrion and the larvae have never been collected (Kuijten 1981). It is possible that the larval stage is not associated with carrion. This species does not oviposit on carrion and the larvae have never been collected, it is likely that the larval stage is not associated with carrion. It is likely limited to lowland rainforests as a habitat.

Forensic applications: Frequently overlooked by forensic investigators because of it's nocturnal habit. In forested habitats, it is found to be attracted to traps baited with rotting fish and monkey carcasses in the decay stage. Large numbers have been recorded in traps and observed on carcasses (Nazni et al., 2011). The presence of hybosorid beetles seems to increase the rate of decomposition (Nazni et al., 2011). The effect of competition between these Hybosorids and maggots is unknown and should be investigated. Possible area indicator.

Phaeochroops freenae Kuijten 1981

Diagnosis: A large dark brown Hybosorid (~14mm) covered in red-brown erect setae, almost indistinguishable from *Pc. peninsularis* for most beginners. Unique characters for this species include a long accessory costa on the elytra and a continuous elytral fringe. The length of the accessory costa can be determined by counting the punctuations next to the costa. Its length in *Pc. freenae* is more than 10 punctuations. The setae on the elytra arise from round punctuations instead of horseshoe shaped ones. The elytral fringe hairs of preserved specimens regularly fall out,

Ecology: A rare species that has previously only been described from two female specimens. Males have recently been collected on the type-location but remain undescribed. Its habits appear to be similar to *Phaeochroops peninsularis*, but possible niche differentiations is unknown. No larval stages have been found.

Forensic applications: Has only been recovered from baited traps. Likely because of a combination of its rarity and nocturnal habit.

Phaeochroops peninsularis Arrow 1909

Diagnosis: *Pc. peninsularis* a large dark brown (~14mm) Hybosorid with dorsum covered in red-brown erect setae. As mentioned above, *Pc. peninsularis* is difficult to differentiate from *Pc. freenae* aside from the use of the accessory costa, which is 3-8 punctuations long in *Pc. peninsularis*, and the elytral fringe, which is discontinuous. The elytra are covered in horseshoe shaped punctuations but the setae arise from round punctuations.

Ecology: A nocturnal species. Kuijten (1981) attempted a breeding experiment with this species, but did not manage to obtain any eggs or larvae. It has been noted that these larger *Phaeochroops* are more sluggish than smaller Hybosorid species (Kuijten, 1981) and they occur in smaller numbers. Habitats appear to be limited to forested environment. No larval stages have been found.

Forensic applications: Rarely collected due to its nocturnal habit. Collected from baited traps and monkey carcasses in the decay stage (Nazni et al. 2010; Omar, B., Unpublished data; Raja Zuha, Unpublished data).

Phaechrous emarginatus Castelnau 1840

Diagnosis: A medium sized (~10mm) shining black Hybosorid with a glabrous punctuated dorsum. The lateral sides of the pronotum are more dilated than in any *Phaeochroops* species.

Ecology: A nocturnal species. On the Malay Peninsula this species is so far only collected in forested environments. Kuijten (1978) attempted breeding experiments with a variety of food sources and oviposition materials, but no eggs or larvae were obtained. In one sampling period, this species was collected from traps with rotting fish in large numbers . Such large abundances were never observed in subsequant samplings (Goh, T.G., Unpublished data). Normally uncommon to relatively rare in lowland forests. Possible area indicator.

Forensic applications: Has only been recovered from baited traps . Likely because of a combination of its rarity and nocturnal habit.

4.4.3.3 Silphidae

Nicrophorus nepalensis Hope 1931

Diagnosis: An elongate medium sized (~20mm) beetle that is primarily shining black with orange patterns on its elytra. This species may be mistaken for a *Diamesus osculans* or vice versa by inexperienced workers, but it can be distinguished by the orange color of the 3 terminal segments of the antenna, a constriction between the head and the prothorax, the size of the postgena, which is ¼ of the head width when viewed dorsally and the lack of ridges on the elytra. The metatarsal segments bear stiff yellow-brown hairs. 3 pairs of bumps are present on the pronotum, 1 discal and 2 on the apical portion of the pronotum. There is very little variation in the pattern on the elytra (Sikes et al. , 2006)

Ecology: In Peninsular Malaysia, this species has been collected exclusively from Cameron Highlands above 1400m. Most Nicrophorus species in South East Asia are restricted to higher mountains. Occurence on other high-altitude locations on the Malay Peninsula seems likely. Two specimens have been collected from unbaited pitfall traps in montane forests of Cameron Highlands, it is possible that they were attracted by decaying catches used in the traps.

Forensic notes: This species has never been collected in any forensic studies, but they have been found to be rather common and easy to collect using baited pitfall traps in Cameron Highlands (Pers. Comm., Raja-Zuha, R.M.). Temperature tolerance studies indicate that N. nepalensis becomes inactive at temperatures exceeding 26°C (Hwang and Shiao, 2011).

Chrysosilpha formosa *(*Laporte 1832)

Diagnosis: A medium sized species (13-17mm) with a distinct orange pronotum and blue elytra. It has ridges on its stria similar to *D. osculans*. Unlike other Silphids, it is dorso-ventrally flattened and not elongated. All antennal segments are shining black aside from 3 matt terminal segments. 2 pairs of black unpunctuated dots are on the discal portion of an otherwise orange and moderately punctuated pronotum. The terminal abdominal segments are tapered and extend beyond elytra but some specimens curl up when improperly dried and this character may not be visible. Males are externally inditinguishable from females.

Ecology: Common forest species. Several individuals have been observed to approach fish during daytime, where they proceeded to mate and feed on the carrion. This species is known to be found almost all year round and records show that it has an altitudinal range from 2500m to sea level (Ruzicka, 2012). Larvae have been recovered from dead monkeys in the decay stage, where they were observed to feed on third instar calliphorid maggots (Nazni et al., 2011; Omar, B., Unpublished data). Adults and larvae have been seen present on the same carcass, but whether these are adults protecting their young or unrelated adults is unknown.

Forensic applications: This species has been recorded to visit various types of animal carcasses, normally occuring in the fresh or decay stages (Nazni et al, 2011; Chen C.D., Upublished data; Omar, B. Unpublished data, Raja Zuha, R.M., Unpublished data). The predaceous larvae were found to hunt for maggots on the underside of the carcass (Chen C.D., Unpublished data). Some beetles have been observed feeding directly on fish carrion. As it arrives in the fresh stage and appraently oviposits on the carcass, this species may be a potential PMI estimator.

Taxonomy: This species has recently been classified by (Ruzicka, 2012) as Necrophila (Chrysosilpha) formosa.

Diamesus osculans (Vigors 1825)

Diagnosis: The largest (25-35mm) Silphid recorded on the Peninsula, it is an elongated black or dark brown beetle with occasionally red-orange markings on its elytra. The head does not have swollen gena or any constrictions between the base of the head and the prothorax. Only the terminal antennal segment is an red-orange colour, all other segments are the same colour as the body. The orange elytral markings are very variable, in some specimens the markings are reduced to 4 small spots while in others almost half the elytra is red-orange. The elytra bear ridges similar to *C. formosa*. The metatarsal segments have stiff yellowish hairs. Sexually dimorphic, females have swollen femurs and rough pronotums, while males lack the swollen femurs and have smooth pronotums.

Ecology: Nothing is known about the life cycle of this beetle, but its wide range suggests that it is a relatively hardy beetle that can be found in both open and forested habitats (Peck, 2001). In New Guinea, this species has been collected from light traps and carrion, recorded up to 1500m, in every month of the year. Most New Guinea collections were done using light traps (Peck, 2001). Locally, it has been collected or observed it from a range of 1700m to 43m (Chen, C.D., Unpublished data). It is not found in traps baited with small carrion, and has only been observed on carrion with a mass of more than 3.5kg that has been infested with maggots, which suggests that it may be a predatory as opposed to a necrophagous species. About 10-12 larvae of this species have been observed underneath a wild boar carcass in the late decay stage. Many of the larvae burrowed into the ground as soon as they were exposed. Seven adult specimens deposited in the Museum of Zoology, University of Malaya collected on the same day are labeled ‘on rotten tree bark’, it is possible that rotting trees have something to do with the *Diamesus* life cycle, but the specifics are unknown. *Diamesus* has been observed to spit out dark liquid from its mouth as a defence mechanism against predators, it is possible that this liquid is recently digested carrion.

Forensic applications: This species has been found to be attracted to monkey and wild boar carcasses during the decay stage. Third instar maggots were present on the carcass during all collections of this beetle in forensic studies. Larvae have been recovered from wild boar (Goh T.G., Unpublished data) and monkey (Nazni, et al. 2011; Omar, B., Unpublished data) carcasses in the decay stage. Possible PMI indicator.

4.4.4 Glossary

Constriction: A narrowing of an object.

Coriaceous: A form of microsculpture. Resembles very small leathery wrinkles.

Dorsum: Dorsal surface of the beetle.

Discal: The front part of the wing.

Dilated: Wide.

Elytral fringe: Setae along the edge of the elytra.

Emarginate: Having a notch or a small gap.

Granules: See Granuate.

Granuate: Covered in small polygonal patterns.

Glabrous: Without hairs or setae.

Interstria: Area between stria.

Matt: Not shining, does not strongly reflect light.

Microsculpture: Minute surface patterns. Only observable under diffuse (fluorescent) light at 40X magnification.

Microreticulate: See coriaceous.

Pilosity: Possessing setae.

Punctate: With small holes.

Punctuations: Small holes.

Protuberance: A small raised area or bump.

Prow shaped: Shaped like the front of a ship.

Ridge: A long and thin raised area.

Rugulate: A wrinkly pattern.

Setae: Small hairs.

Shining: Strongly reflects light.

Smooth: Without any pattern.

Chapter 5: Discussion

5.1 Necrophagus beetle diversity

In Sarawak and Sulawesi, large dung feeding tunnelers such as *Copris* were found at lower elevations, Silphid species such as *Nicrophorus* were found to overlap at higher elevations, and *Onthophagus* species could be divided into lowland and montane forest species (Hanski, 1983; Hanski and Niemela, 1990). Hybosorids were distributed in various macrohabitats with little overlap between species in Sarawak (Hanski, 1983). While not directly comparable to this study or the studies in Sarawak and Sulawesi, Kahono and Setiadi (2007) reported that most Scarabaeid large tunnellers, such as *Catharsius molussus*, *Copris agnus* and *Copris punctulatus*, and large rollers, such as *Paragymnopleurus maurus* and *Pg. sparsus*, were only found in lower elevations (500m -1000m). This was replaced by more small tunneler *Onthophagus* species from 1000m to 2000m. Only two species were found after 2000m, *Phacsoma punctatus* and an unidentified *Onthophagus* species. The authors believed that the peak at 1000-2000m was related to higher amounts of dung available for the beetles at those locations.

While Hybosorids do not display the macrohabitat based resource partitioning of Bornean species (Hanski, 1983), Scarabaeidae follow the pattern quite closely, with the presence of highland and lowland species and notably *Ochicanthon* (*Phacsoma*) species being present in higher altitudes. In Borneo (Hanski, 1983) and Sulawesi (Hanski and Niemela, 1990), Scarabaeid species found higher than 1000m are different from lowland species. Silphid diversity differs greatly from Borneo with the absence of

Nicrophorus and the presence of *Necrophila* (*Chrysosilpha*) *formosa*. Silphid species found on the Peninsula are wide ranging species that are believed to originate from the Asiatic mainland (Peck, 2001; Sikes *et al.*, 2006; Růžička *et al.*, 2012). It is possible that interspecific competition and gene flow from the mainland has prevented *Nicrophorus* from speciating and colonising other macrohabitats.

It is believed that cloud formation and precipitation may play an important role in the community structure of necrophagus beetles. The species turnover began at the transitional forest at 800m, which is located at the elevation where cloud formation becomes frequent (Nakashizuka *et al.*, 1994). Preliminary observations indicate that heavy rain in the morning has a negative effect on the diversity of carrion specialist beetles (Goh, T.G., Unpublished data). It is likely that rain during the active hours of a beetle species may suppress its activity as some beetles appear to avoid flying or searching for food during rainfall (Hanski and Cambefort, 1991). Further investigation into the diel activity of these beetles would explain how temporal distribution and disturbances affect the diversity.

There was interspecific co-occurrence of Hybosorid species within the same patches, but this may have been a result of chance factors. Intraspecifically, all Hybosorid species were overdispersed. Independently aggregated spatial distributions among habitat patches is believed to lower the probability that potential competitors will occur together (Ives, 1988; Hanski and Cambefort, 1991). It is believed that there is little direct competition between Hybosorids and other carrion specialist species, as the other species were active during daytime as opposed to the nocturnal Hybosorids (Kuijten, 1981). *Pc. rattus* appears to be common throughout the year (Goh T.G., Unpublished data), but the exact factors that lead to the large *Ph. emarginatus* abundances are unknown. Additionally, the occurrence of *Ph. emarginatus* during only one sampling period indicates that there may be some sort of seasonal partitioning amongst these species. *Pc. freenae*, a large species, had the smallest number of specimens collected although its occurrence was not as rare as previously believed (Kuijten, 1981; Hanski and Cambefort, 1991).

A majority of Scarabaeid species were only facultatively feeding on carrion as bait attraction studies indicate that only five out of the 36 scarabaeid species encountered in this study are believed to actually be carrion specialists (J, Huijbregts, Unpublished data). However, shortages of one resource may cause a switch to the other. *Ochicanthon* are dung specialists that tend to be attracted to carrion on higher elevations (Krikken and Huijbergts, 2007). Conversely, carrion specialist beetles such as *Onthophagus semifex* have been known to feed on dung when carrion is not available (Huijbregts and Krikken, 2008). A wild boar was present at the 1400m site during the first sampling period, but not in the second sampling period. This factor could have affected the presence of *O. semifex* at the site. An unaccounted for factor during this study is the distribution of millipedes and other large arthropods, *Onthophagus pennicillatus* has been recorded to feed on millipede carcasses (Masumoto, 2001), while several nercophagus beetles were found to be attracted to defensive secretions of diplopoda in Borneo (Brull and Krell, 2003).

Some species, such as *Synapsis* spp., have been collected at lower altitudes in the same site using dung traps (Zidek and Pokomy, 2010; Omar, B., Unpublished data).

These ranges should be interpreted with some discretion as large tunnelers tend to be dung feeders and detection bias should be taken into account.

There are three species of Silphidae recorded in the Malay Peninisula; *Diamesus osculans, Nicrophorus nepalensis* and *Necrophila (Chrysosilpha) formosa*. Only *N. formosa* was collected from the trapping program. Some adult and larval *N. formosa* have been found as high as 1700m (Chen C.D., Unpublished data). *D. osculans* has only been recorded to visit maggot masses or larger pieces of carrion (The smallest of which weighed 3kg), it overlaps *N. formosa* in terms of altitudinal range (Chen C.D., Unpublished data). *Nc. nepalensis* is restricted to highlands and has only been collected from one location in the Peninsula; Cameron Highlands (Sikes *et al.* 2006 ; Raja-Zuha, R.M., Unpublished data). It is possible that *Nc. nepalensis* may have a limited geographical range within the Peninsula. The inability of the pitfall traps to attract *Nc. nepalensis* and *D. osculans* suggests that there is some niche differentiation between these species and *N. formosa*.

The poor catch in the montane forests could be a result of habitat fragmentation caused by deforestation in the area, or the different soil types present at higher altitudes. It is known in lowland species that deforestation and fragmentation lead to reduced scarabaeid diversity (Davis *et al.*, 2001), but there are no records of how deforestation at higher elevations affects the species present there. Scarabaeids diversity is also affected by sandy and waterlogged soils (Hanski and Cambefort, 1991), and the occurrence of these soils at higher altitude because of weathering may influence the distribution of Scarabaeids.

Overall, community structure of Scarabaeids remains relatively similar with Borneo. However the unique biogeography of the site has caused the species composition of both Hybosoridae and Silphidae to differ. The zonation in Scarabaeids may be caused in part by forest type, while Hybosorids species were negatively affected by lower temperatures. Silphids appear to be relatively unaffected by environmental changes, but more information on the geographical distribution of these species is needed.

5.2: Blowfly diversity

The distribution of blowflies agrees with Kurahashi (1997) and Senior-White *et al.* (1951) who recorded species in the lowland category (Table 4.4) as specific to lowland forests. Omar *et al.* (2007) reported similar results, but with a larger proportion of synanthropic flies. The difference between studies may be due to different microhabitats, Omar *et al.* (2007) collected flies from open areas, while in this study flies were collected from forested areas. It is probable that a combination of temperature and forest types prevent lowland *Chrysomya* species from colonising montane forests. *Ch. villeneuvi* and *Ch. megacephala* were observed in cleared land at 1700m, where the temperature is warmer due to exposure to the sun. However these sightings are probably migrants from lower altitudes. The results were also similar to that of Hanski (1986) in Borneo, which reported that *Lucilia porphyrina* becoming common in montane forests types while other *Chrysomya* species tend to dominate lowland mixed dipterocarp forests.

Fly populations have very large variance that is linked to mechanisms that are independent to the environmental conditions at the time of capture. Like dung feeding insects, the diversity of carrion flies is believed to follow a lottery mechanism (Chesson, 1986; Chesson and Case, 1986), which suggests that most fly species exist in small numbers until the opportunity for the production of many individuals arises (Hanski and Cambefort, 1991). Successful oviposition by a single female a week prior to the sampling would inflate the overall number of one species by a hundred individuals. This occurred in a sample at 200m, in one instance there was a large number of *Ch. nigripes* which was greatly reduced when the area was resampled 3 weeks later (Fig 5.1). These effects complicate comparisons with measurements of environmental factors taken on the day of capture and may cause errors in estimators such as diversity indices. Large variance can be reduced by increasing the number of temporally distinct replicates.

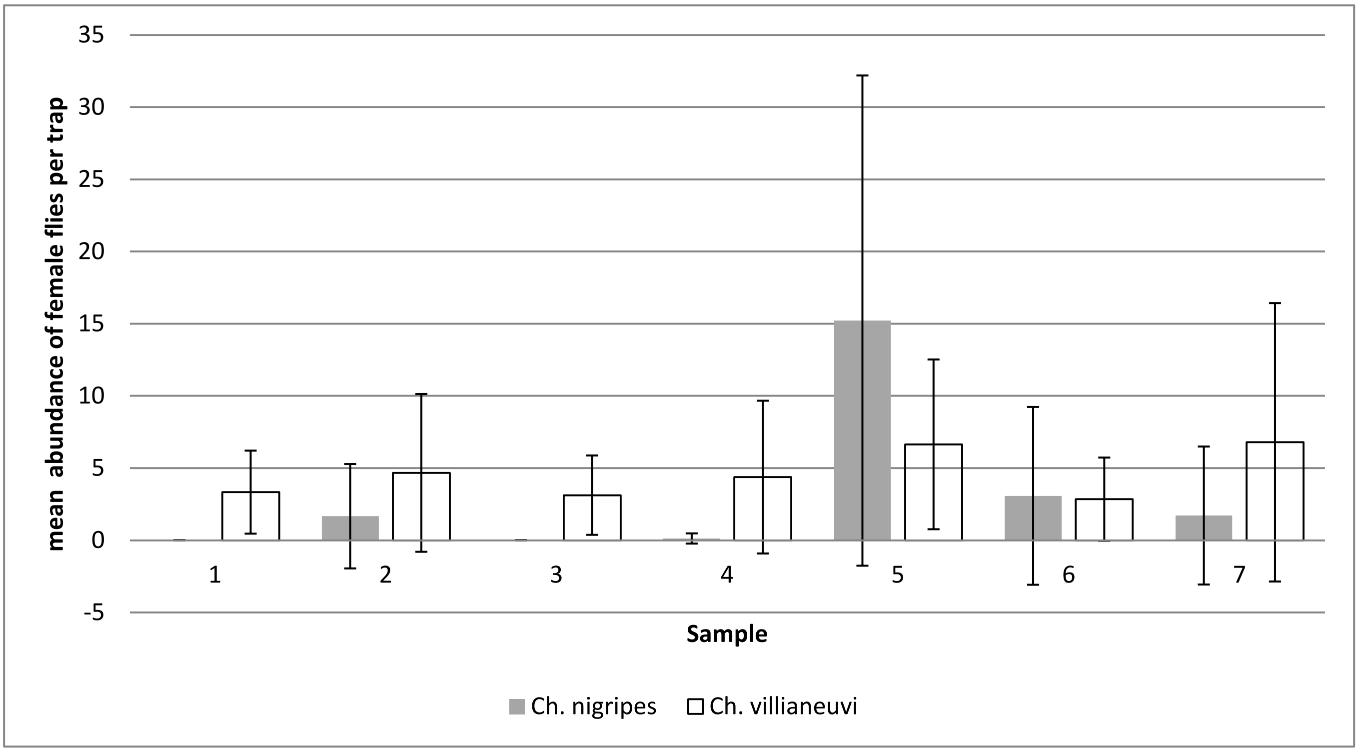

Fig 5.1: Random fluctuations of mean abundance of *Ch. Nigripes* in the Gombak site (200m) shows the large variance in certain species and that more samples are required for more accurate quantitative analysis. Error bars indicate standard deviation.

5.3: Interactions between blowflies and beetles.

It is likely that species partitioning cannot be detected on the microhabitat spatial scale. Diurnal necrophagus beetles were observed to burrow underneath the carcass as soon as they landed. This may result in spatial partitioning on a sub-microhabitat scale: between the exposed parts of the carcass and the portion of the carcass in contact with the ground. It is possible that flies are not able to detect beetles due to their burrowing behaviour and therefore have not developed any avoidance behaviour to their presence.

Additionally, large dung feeding beetles tend to be more active at night and are capable of burying dung or small carcasses before sunrise. Nocturnal Hybosorid beetles also are capable of rapidly consuming carcasses. It is likely that indirect competition based on temporal resource partitioning is the case for interactions between these two nocturnal groups and blowflies. This does not invalidate the hypothesis that competition between large Scarabaeids and carrion flies is responsible for some patterns of zonation (Hanski, 1986), as this study merely measures patch selection and not competition between the two groups.

Flies may have been present to feed or mate as opposed to oviposit. As carrion flies tend to use a r-selected strategy, it is possible that the effects of predation on a population is offset by a larger production of individuals instead of avoidance of predators. However it should be noted that this study records number of adult flies and predatory beetles mainly prey on larval flies. It is likely that exploitation competition in the form of material removal through burrowing by large Scarabaeids has prevented the development of any behavioural response in blowflies. It should be noted that this study concerns patch selection and is not an accurate measure of competition between the two groups. More studies should be carried out to ascertain whether beetles are attracted to the ovipostion activities of flies or if flies display discrimination of oviposition sites in the presence of predators.

5.4: General discussion

This study was the first study to focus on the distribution of carrion feeding beetles in the Malayan Peninsula. The ecological approach adopted gives insight into how the beetle species distribute themselves on a patch or microhabitat level. Such basic knowledge is necessary if one is to understand why multiple dung beetles can coexist in stable communities. The more holistic approach of observing the diversity of both dipteran and coleopteran diversity in corresponding habitats is rarely done, mostly due to specialisation in only one of these orders. The taxonomy and ecological information on forensically important beetles collected during this study also fills a gap in forensic entomology knowledge.

Altitudinal zonation occurs in a similar way as previously recorded for beetles and flies. Forest type as a macrohabitat is a reasonable indicator for certain species. Community structure for both beetles (Hanski, 1983) and flies (Hanski, 1981) are similar to that of Sarawak. This is possibly an indication of similar niches being occupied in Borneo. While flies tended to be from the same species as their Bornean counterparts, there is a large difference in beetle species found in Sarawak and Peninsular Malaysia, although they tend to follow the same zonation pattern on an altitudinal gradient. Further surveys may yield valuable information to explain the biogeography of these beetles. Many species in the transitional forest zone are new to science. As such, the biology and life cycles of these beetles is poorly understood and it is impossible at the moment to comment on possible adaptations that some of these species have made to survive in colder environments.

There is still little known about the active periods of necrophagus beetles and other aspects of their ecology such as their nesting habits. Such poor knowledge of the ecology of the studied organisms prevents the construction of testable models or hypothesis. Also, the traps were inherently artificial sampling units. While we can make some general conclusions based on the results, it must be recognised that carrion is usually exhausted by visiting insects in a matter of hours. Such numbers of beetles and flies may not be accurate for actual carrion.

The implications of the diversity study on forensic entomology shows that while there are a few species of beetles that are carrion specialists, there is a large amount of diversity that may complicate identification of *Onthophagus* species. It is recommended that only one or two focus species be selected as study organisms to avoid taxonomic difficulty. Reference collections of identified beetles may be distributed to the various forensic entomology groups in Malaysia to aid in identification. While the results of this study show that both fly and beetle species can coexist in the same habitat, the question of how flies interact with beetles is still unanswered.

It was fortunate that 2011 was an abnormally wet year with not much of a clear dry season (Jabatan Meteorologi Malaysia, 2011). For example, in Gombak the month of March (During the first sampling) recorded 168.2 litres of rainfall over 14 raindays while September (During the second sampling) had 193.7 litres of rainfall over 10 raindays. This probably limited the effects of wet vs. dry seasonality between both samples. Weather data was not available in other sites for comparisons, but constant wet weather was experienced in all sites.

The main weakness of this study is the lack of proper replication. Organisms that survive in patchy environments tend to be prone to random population fluctuations based on the availability of resources (Kneidel, 1984). There is only one solution to a sample with a large variance; to collect a larger number of samples (McDonald,2009). However, collecting all the samples at the same time is a case of pseudoreplication (Hurlbert, 1984). In the future, samples should be staggered in time at a reasonable interval of 2 weeks to be able to measure the change in community structure.

Some factors such as leaf litter and rainfall were not recorded in all sites. Such unaccounted for factors may have an influence on community composition. The presence and diversity of ants was not recorded, even though these insects may have a larger influence than beetles on the presence or absence of flies. Although collected, Staphylinid beetles were not identified or recorded. As predators, parasites and parasitoids of flies the influence of these beetles may explain some aspects of the distribution of carrion flies (Hanski, 1981).

As previously mentioned, continual sampling over a longer period of time will provide conclusive data to explain some of the observations made in this study. The hourly diel activity of necrophagus species is unknown at the moment, with only anecdotal evidence available. Understanding the effect of rainfall and time of rainfall on beetle diversity is also important if beetles are to be used as biodiversity indicator species. Observing the effects of ants on the distribution of flies and beetles may be useful in the future.

Chapter 9: Conclusions

1) There is a difference between the beetle communities below 1000m and above 1000m.
2) Beetles are rare in altitudes higher than 1400m possibly due to soil conditions and the lack of carrion biomass due to lower primary productivity.
3) Scarabaeid species can be divided into lowland forest and transitional forest species. Hybosoridae species were found to be sensitive to temperature and tend to be restricted to lowland forests. Silphidae were found to have the least species but one of the largest altitudinal ranges.
4) Forest dwelling Carrion flies along an altitudinal gradient show a change in community composition at 1400m. Similar to previous studies, there were two groups: Highland inhabiting flies and lowland inhabiting flies.
5) There is much data collected on the succession sequence of beetles in Malaysia and most species appear in early succession as opposed to late succession.
6) Interactions between beetles and flies show no detectable spatial relationship on the microhabitat level. It is likely that the relationship between competitors involves niche differentiation or exploitation competition that prevents necrophagus beetles and carrion flies from interacting directly.
7) This study indicates that adult carrion flies do not actively avoid predatory beetles. However these beetles tend to feed only on the larval stages of carrion flies.

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