PREVALENCE, DISTRIBUTION AND THE BIOLOGY OF AN ENDOPARASITOID FLY, *Verticia fasciventris* (DIPTERA: CALLIPHORIDAE), AND THE PHYSIOLOGICAL AND BEHAVIOURAL CHANGES IN ITS HOST, *Macrotermes carbonarius* (BLATTODEA: TERMITIDAE)

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by

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PREVALENS, TABURAN, DAN BIOLOGI SUATU LALAT ENDOPARASITOID, *Verticia fasciventris* (DIPTERA: CALLIPHORIDAE), PERUBAHAN FISIOLOGI DAN KELAKUAN PADA PERUMAHNYA *Macrotermes carbonarius* (BLATTODEA: TERMITIDAE)

ABSTRAK

Tesis ini menumpu ke atas prevalans, distribusi dan biologi lalat endoparasitoid, *Verticia fasciventris* Malloch (Diptera: Calliphoridae), perubahan fisiologi serta tingkah laku dalam rumahnya *Macrotermes carbonarius* (Hagen) (Blattodae: Termitidae). Perkembangan larva parasitoid *V. fasciventris* boleh dibahagikan kepada tiga peringkat instar. Larva menamatkan instar pertama mereka dalam kapsul kepala pra-askar, manakala larva instar kedua berkembang dalam kapsul kepala askar. Larva instar ketiga pula berkembang dalam kapsul kepala askar dan kemudiannya hidup bebas setelah keluar dari badan perumah anai-anai. Purata jangka masa perkembangan untuk peringkat pupa adalah 13 hari. Saiz parasitoid betina adalah lebih kecil ($P < 0.05$) berbanding dengan saiz parasitoid jantan. Kemandirian parasitoid betina adalah lebih tinggi ($P < 0.05$) berbanding dengan kemandirian parasitoid jantan. Tinjauan kadar parasitisme oleh endoparasitoid *V. fasciventris* pada koloni *M. carbonarius* di Pulau Pinang, Malaysia dijalankan dari April 2015 hingga April 2016. Daripada 313 koloni *M. carbonarius* yang telah ditinjau, 105 (33.5%) telah diparasitkan oleh *V. fastrivencis*. Di kesemua tempat kajian, koloni yang diparasit masih lagi sihat. Kadar parasitisme adalah rendah dengan purata 42.7 ± 12.9 askar *M. carbonarius*. Saiz busut koloni yang diparasit adalah jauh lebih besar daripada yang tidak berparasit. Keamatan cahaya persekitaran busut koloni yang diparasit adalah jauh lebih rendah ($P < 0.05$) daripada busut koloni yang tidak diparasit. Kehadiran *V.*

fasciventris akan menyebabkan perubahan fisiologi dalam *M. carbonarius*. Profil kandungan air untuk askar major yang diparasit dan tidak berparasit telah diselidik dalam kajian ini. Askar major yang diparasit menunjukkan jisim badan awal, peratusan jumlah kandungan air badan serta kebolehtelapan kutikel yang jauh lebih rendah ($P < 0.05$), tetapi mempunyai nisbah permukaan kepada isipadu badan yang lebih tinggi berbanding dengan askar major yang tidak diparasit. Askar major yang diparasit mempunyai kadar hidup yang jauh lebih tinggi ($P < 0.05$) berbanding dengan askar major tidak diparasit pada kelembapan relatif 4%. Askar *M. carbonarius* major yang diparasit masih mengekalkan peranan melindungi anai-anai pekerja yang mencari makanan. Askar major yang diparasit boleh dikelaskan kepada kumpulan yang agresif dan yang kurang agresif. Purata masa larva keluar dari perumah yang kurang agresif adalah lebih singkat berbanding dengan purata masa larva untuk keluar dari perumah agresif di lapangan. Kekerapan trofalaksis dan pembersihan alo (‘allogrooming’) pekerja terhadap askar major yang diparasit adalah lebih tinggi berbanding dengan kekerapan kedua faktor tersebut untuk mereka yang tidak diparasit. Kehadiran askar major yang diparasit mempengaruhi kekerapan trofalaksis dan pembersihan alo oleh anai-anai pekerja, manakala kehadiran askar major yang tidak berparasit tidak mempengaruhi kekerapan aktiviti tersebut ($P > 0.05$).

PREVALENCE, DISTRIBUTION AND THE BIOLOGY OF AN ENDOPARASITOID FLY, *Verticia fasciventris* (DIPTERA: CALLIPHORIDAE), AND THE PHYSIOLOGICAL AND BEHAVIOURAL CHANGES IN ITS HOST, *Macrotermes carbonarius* (BLATTODEA: TERMITIDAE)

ABSTRACT

This thesis focuses on prevalence, distribution and the biology of an endoparasitoid fly, *Verticia fasciventris* Malloch (Diptera: Calliphoridae) and the physiological and behavioural changes in its host *Macrotermes carbonarius* (Hagen) (Blattodae: Termitidae). The development of *V. fasciventris* larvae could be divided into three instar stages. The larva completed their first and second instar in the head capsule of *M. carbonarius* pre-soldier and soldiers, respectively, while the third instar undergoes part of its development inside the head of soldiers and subsequently exit the host body as free living larva. The mean time of pupation of *V. fasciventris* was 13 days. The size of parasitoid females was significantly smaller ($P < 0.05$) than that of males. The survival rate of females was significant higher ($P < 0.05$) than that of males. A survey of the infestation rate of *M. carbonarius* colonies by *V. fasciventris* was conducted in Penang Island, Malaysia from April 2015 to April 2016. Of the 313 *M. carbonarius* mounds examined, 105 (33.5%) were infested with *V. fasciventris.* The degree of parasitism was low with an average of 42.7 ± 12.9 parasitized *M. carbonarius* soldiers. At all surveyed sites, parasitized colonies were classified as healthy. The mound size of parasitized mounds was significantly larger than that of unparasitized mounds. The ambient light intensity of parasitized mounds was significantly lower ($P < 0.05$) than that of unparasitized mounds. Parasitization by *V. fasciventris* resulted in the physiological changes in its *M. carbonarius* host. The water balance profile of both parasitized and unparasitized major soldiers was examined. Parasitized major soldiers showed significantly ($P < 0.05$) lower initial body mass, percentage of total body water content, cuticular permeability, but higher ratio of surface area to body volume in comparison to unparasitized major soldiers. Parasitized major soldiers survived significantly higher ($P < 0.05$) than unparasitized major soldiers under extreme condition of 4 % humidity. Parasitized major soldiers continued playing their roles in protecting workers on the foraging trails. Parasitized soldiers were classified into aggressive and non-aggressive groups. The average duration of larval emergence from non-aggressive hosts was significantly shorter than that from aggressive hosts. In trophallaxis and allogrooming, workers showed higher activities toward parasitized major soldiers than unparasitized soldiers. The presence or absence of parasitized major soldiers affected the trophallaxis and allogrooming frequencies between workers and unparasitized soldiers, but no significant difference ($P > 0.05$) in these activity frequencies were found whether unparasitized major soldiers were present or absent.

CHAPTER ONE

GENERAL INTRODUCTION

Termites are eusocial insects that are now classified taxonomically with the cockroaches (Family: Blattodae) due to their close phylogenetic relationship (Inward et al., 2007). Termites live in colonies composed of different castes consisting of reproductives (kings, queens, alates, and nymphs), workers, and soldiers. These castes are responsible for different tasks, such as swarming and pair bonding (reproductives); food foraging, nest building, feeding, and tending (workers); and colony defence (soldiers). In general, termites are classified into three groups based on their different habitats: (1) dry-wood termites are less dependent on moisture compared to other termite species and do not need access to moisture for survival; (2) dampwood termites feed on decayed wood, such as old tree stumps, rotting logs, and buried timbers; (3) subterranean termites construct nests in the soil and are highly dependent on soil for moisture. Termites also can be divided into lower and higher termites based on their evolutionary status. The lower termites (Families: Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, and Rhinotermitidae) are characterized by the presence of protozoa in their gut, which help digest the cellulose materials. In contrast, the higher termites (Family: Termitidae) rely on bacteria and enzymes to digest food or grow fungus on the cellulosic materials of their partially digested faecal waste (Subfamily: Macrotermitinae), using fungus as food source.

Macrotermes carbonarius (Hagen) (Blattodae: Termitidae), a fungus-growing termite, is widely distributed in Malaysia, Thailand, Cambodia, and Vietnam. It is a large sized species with a dark coloured cuticle, and it feeds mainly on leaf litter and dead plant materials. *M. carbonarius* forages extensively in open air due to its ability to adapt to a relatively wide range of moisture levels (5–20% RH). It commonly inhabits forested and undisturbed areas, building perimeters, gardens, etc. *M. carbonarius* colonies usually construct conspicuous large mounds that frequently are found at the base of trees (Roonwal, 1970).

A parasitoid is an organism that spends a significant portion of its life on (ectoparasitoid) or within (endoparasitoid) a single host and eventually results in the death of its host. The parasitoid-host relationship between dipteran parasitoids and termites has been documented in several studies. Most of the fly parasitoids belong to the family Phoridae (Disney, 1994; Disney et al., 2009; Foo et al., 2011a) but to date only one calliphorid parasitoid (*Verticia fasciventis* Malloch (Diptera: Calliphoridae)) is known to parasitize termites (Sze et al., 2008; Neoh and Lee, 2011).

In 2011, Neoh and Lee discovered pre-soldier and soldier stages of *M. carbonarius* parasitized by *V. fasciventis* larvae on Penang Island, Malaysia. The larva develops inside the head capsule of termite pre-soldiers and soldiers by consuming the contents of the head. The larva fully fills the head capsule and exits the termite body for pupation, and the parasitoid fly emerges after 13–14 days (Neoh and Lee, 2011). After the larva departure process, the termite host may die immediately or survive for 1–2 days under laboratory conditions.

Little is known about the morphology, development, and distribution of the parasitoid *V. fasciventris* and its effect on the behaviour and physiology of its host *M. carbonarius*. Hence, the objectives of this study were:

(1) to describe the distribution and prevalence of *M. carbonarius* mounds parasitized by *V. fasciventris* and examine the factors—including host factors (mound size and colony health) and environmental factors (light intensity)—that may

contribute to the distribution and abundance of parasitized mounds on Penang Island, Malaysia.

(2) to provide information on the morphology and development of the parasitoid *V. fasciventris*.

(3) to examine the water balance profile (including percentage of total body water (%TBW), cuticular permeability (CP), and % TBW loss rate, ratio of surface areas to volume) of both parasitized and unparasitized major soldiers and compared their survival rate under extremely low humidity conditions.

(4) to observe the agonistic behaviours of soldiers parasitized by *V. fasciventris* in the field and examine the relationship between these behaviours and the maturity of parasitoid larvae; and determine whether the presence of parasitized soldiers affects the frequencies of trophallaxis and allogrooming between workers and unparasitized termites, and the presence of unparasitized soldiers affects the frequencies of trophallaxis and allogrooming between workers and parasitized termites.

CHAPTER TWO

LITERATURE REVIEW

2.1. Parasitism

Parasitism describes an ecological relationship between two different species in which one species lives on or within and at the expense of the other. Usually, hosts do not benefit from this ecological association (Askew, 1971). Hosts may be at higher risk of predation (Moore and Gotelli, 1992; Moore, 1995; Fuller and Jeyasingh, 2004), reduced number of offspring (Fuller and Jeyasingh, 2004) and shortened lifespan (Vinson and Iwantsch, 1980; Giblin and Platzer, 1985; Trabalon et al., 2000).

Parasitism can be divided into two forms: parasites and parasitoids. A parasite lives at the expense of its host and does not kill or significantly harm it. In contrast, a parasitoid spends only part of its life cycle within the host, but it results in the death of its host. They may live externally (ectoparasites, ectoparasitoids) or internally (endoparasites, endoparasitoids) (Gullan and Cranston, 2014).

The two forms of parasitoids (idiobiont and koinobiont) are distinguished based on their development in the host body (Askew and Shaw, 1986). A parasitoid that develops rapidly and prevents further development of its host after initial parasitization is known as an idiobiont. In contrast, a koinobiont parasitoid lays egg(s) in a young host, and the host continues to grow, thereby providing an increasing food resource for them. Idiobionts usually are ectoparasitoids that live externally and koinobionts are endoparasitoids that live internally within the host (Heraty, 2009).

Parasitoids occur in the following six orders of holometabolous insects: Diptera, Hymenoptera, Lepidoptera, Coleoptera, Neuroptera, and Trichoptera. They account for approximately 10% of all described insects. The greatest number of parasitoids belongs to the Diptera and Hymenoptera (Eggleton and Belshaw, 1992; Brodeur and Boivin, 2004). Dipteran parasitoids are the subject of this study. This order includes an estimated 20% of the known species with a parasitoid lifestyle (Feener Jr and Brown, 1997).

2.2. *Macrotermes carbonarius* (Hagen) (Isoptera: Termitidae)

Macrotermes carbonarius is a fungus-growing termite species that is widely distributed in Southeast Asian countries (Malaysia, Thailand, Vietnam, Cambodia) (Roonwal, 1970). Its workers and soldiers are large and dimorphic. The body and head capsule of *M. carbonarius* are highly chitinized and black in colour (Sugio, 1995). Soldiers of *M. carbonarius* possess sharp, sword-like mandibles. On average, approximate 0.9 million individuals inhabit in one *M. carbonarius* colony (Matsumoto, 1976).

M. carbonarius is an open-air foraging species that consumes fallen leaves and small branches (Abe and Matsumoto, 1979). They are commonly found in forested and undisturbed areas, along building perimeters, and in gardens and parklands (Tho and Kirton, 1992). They usually attack trees around buildings but rarely infest structures (Lee, 2014).

M. carbonarius constructs a conspicuous large mound with a conical earthen structure, bare soil surface, and thick (20–40 cm) outer wall (Inoue et al., 2001). A large mound can reach up to 4 m in height and 5 m in diameter (Roonwal, 1970). Inside the *M. carbonarius* mound, the hive consists of a large conical or bell-shaped chamber with a flat clay floor, and it contains a royal chamber, a nursery zone, food stores, and fungus combs.

The alates of *M. carbonarius* swarm from November to January under the following conditions: 1,006–1,010 hPa, 25–30 ^{o}C, and 72–83% relative humidity. A swarm lasts for only 4–10 minutes and occurs at dusk, often between 1900 and 1910 hours. The alates swarm during windless conditions and avoid rainfall during flight days (Neoh and Lee, 2009).

Parasitization of *M. carbonarius* by the dipteran phorid parasitoid *Puliciphora beaveri* (Disney) was recorded by (Disney and Kistner, 1989). Recently, Neoh and Lee (2011) reported that *M. carbonarius* was parasitized by the calliphorid parasitoid *V. fasciventris* on Penang Island, Malaysia. Parasitization was discovered based on the observation of strange head capsule (round shape) and short mandibles of *M. carbonarius* soldiers at Minden Campus, Universiti Sains Malaysia (Plates 2.1, 2.2). The larva of *V. fasciventris* was found to parasitize pre-soldier and soldier stages of *M. carbonarius* (Plate 2.3*)*. Parasitized major soldiers were smaller than unparasitized ones, whereas parasitized minor soldiers were larger than unparasitized ones.

Neoh and Lee (2011) also described the process by which the *V. fasciventris* larva leaves its termite host. Only a single larva was found to develop inside the head of each termite host. The larva consumed the entire content of the head and eventually fully filled the termite head capsule. The departure of the larva began from the head through the neck foramen, and ultimately it exited between the abdominal cerci of its host. The larva left its host with its mouth hook pointing upward, and the host usually stood still until this process was completed. The duration of larval departure varied between 10 and 50 minutes under laboratory conditions. After leaving the host, the post-feeding larva sought a suitable substrate to bury itself for pupation. Under laboratory conditions, the host was able to survive for another 1–2 days after the larva departed.

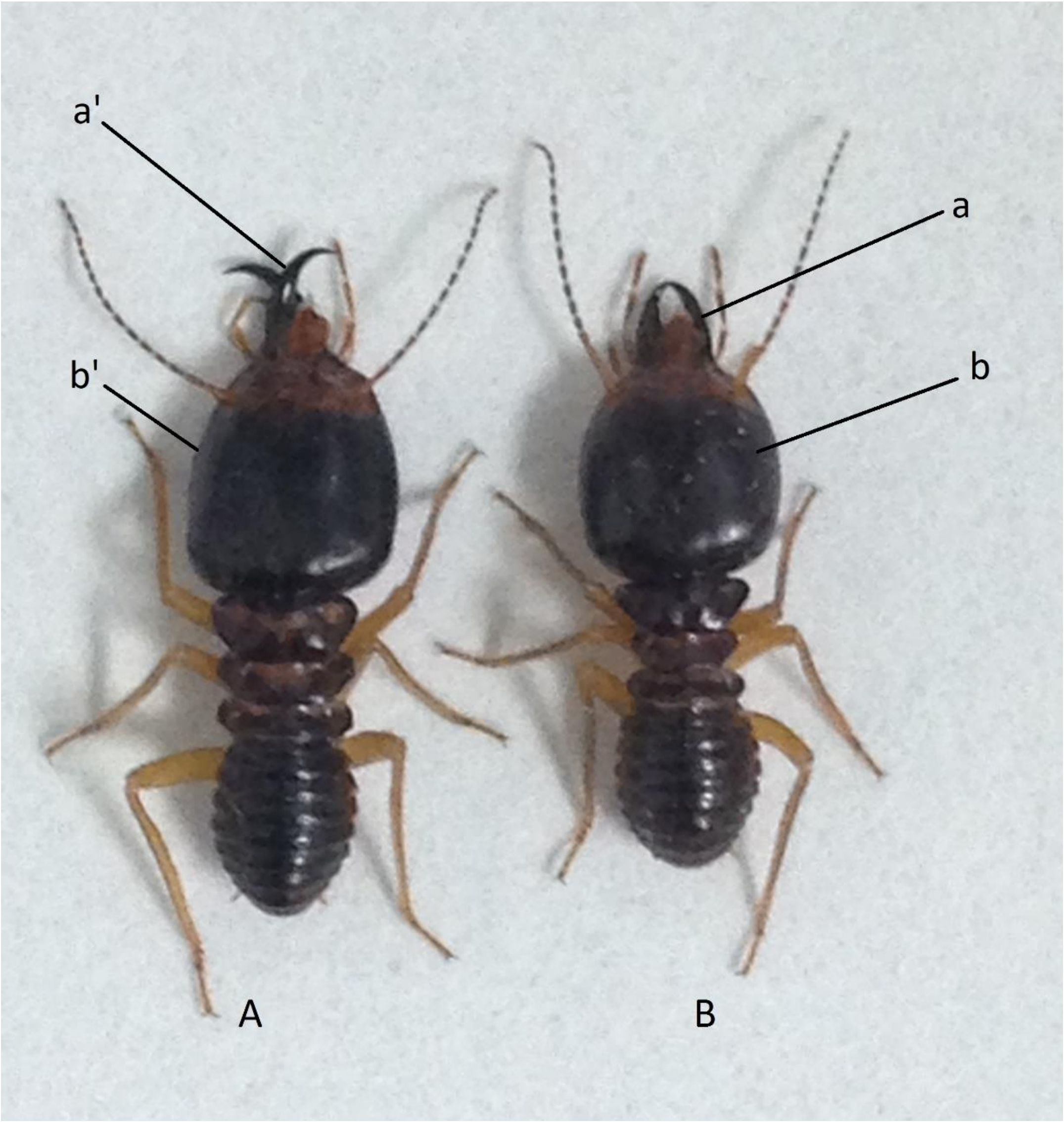

Plate 2.1. Major soldiers of *M. carbonarius*

(A) Unparasitized major soldier (a’. sharp mandible; b’. square-like head)

(B) Parasitized major soldier (a. short mandible; b. round shape head)

Plate 2.2. The newly formed parasitized soldier derived from a pre-soldier of *M. carbonarius (*a. short mandible; b. round head shape).

Plate 2.3. A parasitized pre-soldier of *M. carbonarius*

2.3. Family Calliphoridae

The Family Calliphoridae (Diptera) includes approximately 1,000 species with diverse larval lifestyles, including predators of earthworms and snails; blood suckers of birds and mammals; coprophages of vertebrate dung; snail necrophages; and scavengers in termite nests (Rognes, 1991).

2.3.1. Eggs

Eggs of calliphorids are elongated, yellowish or white in colour, and are about 1.5 mm x 0.4 mm in size. Typically, a calliphorid female lays 150–200 eggs per batch (Rosati, 2015). A female usually lays around 2000 eggs with an approximately 50 : 50 sex ratio during her life. The eggs hatch quickly and reach the first larval stage within 8 hours to 1 day (Rognes, 1991).

2.3.2. Larvae

Calliphorid larvae pass through three larval instars stages. The characteristics used to differentiate the larval instars are the posterior spiracles and body segments. The mouth hook is a special feature of calliphorid larvae; it usually is used to break down proteins from the food source (Oliveira 2007). Larvae in this family are usually scavengers of carrion and dung (Rognes, 1991).

2.3.3. Pupae

After completing all larval instar stages, the post-feeding larva seeks a suitable substrate (often dry soil) to pupate. Pupae of the family Calliphoridae are usually bright brown or black in colour, with a size range of 9–10 mm length and 3–4 mm width. Often, pupation takes 12–14 days and varies among species (Rognes, 1991; Kurahashi, 1997).

2.3.4. Adults

Calliphorid adults are characterized by a metallic colour and sometimes have a blue, green, or even black thorax and abdomen. Antennae are used to classify calliphorid flies into lower taxa. Protein is necessary for the life cycle of most calliphorid species, and it is important for the development of mature eggs within the ovaries. Calliphorid adults vary in size and can reach up to 16 mm in length (Rognes, 1991).

2.3.5. Genus *Verticia*

Members of the genus *Verticia* are bengallinae flies, which are found in Southeast Asian countries (Thailand, Malaysia, Singapore, Indonesia) (Tumrasvin et al., 1979). *Verticia* belongs to the calliphorid subfamily Bengaliinae (Rognes, 1991, 1997; Pape and Arnaud, 2001). The species in this genus are characterized by a yellow, non-metallic colour of the body (Rognes, 1997).

V. fasciventris is a small-sized fly with a body length of 5.5 mm for males. The fly is brownish-yellow in colour, with three indistinct longitudinal stripes on the thorax (Tumrasvin et al., 1979). Little is known about the biology of this fly, but it is known to parasitize some species of *Macrotermes* (Sze et al., 2008). In 2011, Neoh and Lee found larvae of *V. fasciventris* developing in the head of soldiers of the fungus-growing termite *M. carbonarius*.

2.4. Host selection

The process resulting in the success of parasitism consists of five steps: host habitat location, host location, host recognition, host acceptance, and host suitability (Vinson, 1976). The first three steps together represent the host selection process, during which a parasite or a parasitoid selects a suitable host for its development (Vinson, 1998). Many factors influence the host selection process, including intrinsic factors, such as age, egg load, experience and physiological conditions of hosts, and extrinsic factors, such as diseases, flood, refuges, competitors, and predators. In general, preferred habitats of a female parasitoid play an important role in determining the type of habitat that is sought. They usually are attracted to a particular habitat due to environmental factors rather than host factors. In some cases, non-preferred hosts are located but are readily accessible and therefore are accepted by female parasitoids (Vinson and Iwantsch, 1980).

2.4.1. Environmental factors

Environmental conditions such as temperature, humidity, light intensity, and air movement can influence behaviour of parasitoids during the host selection process (Vinson and Iwsatch, 1997). Rousse et al., (2009) reported that the activities of hymenopteran insects are often influenced by temperature and humidity. For example, the flying activity of an egg parasitoid *Fopius arisanus* (Sonan) of fruit flies increased with temperature but decreased with humidity. Temperature also influenced movements of the aphelinid wasp (*Eretmocerus eremicus* Rose and Zolnerowich) parasitoid of the whitefly *Trialeurodes vaporaiorum* (Westwood). Walking speed of *E. eremicus* increased at high temperature, thus enhancing the efficiency of the parasitoid's host searching activity (Zilahi-Balogh et al., 2009). Similarly, the chalcid parasitoid *Trichogramma evanescens* Westwood required optimal temperature when searching for its egg host, the grain moth *Sitotroga ceralella* Olivier. Temperature that was too low or too high inhibited their searching activity (Vinson and Iwantsch, 1980).

Light intensity is an environmental factor associated with the canopy layers of plants, and it directly enhances or inhibits the flying activity of parasitoids during the host location process (Vinson, 1976). For example, *Eretmocerus formosa* Gahan, a parasitoid of *T. vaporariorum*, is more active at high light intensity (Zilahi-Balogh et al., 2009). In contrast, flying activity of the flies *Muscidifurax* spp. and *Drosophila* spp. increased under dim light condition, especially at early dawn and late dusk (Smith and Rutz, 1991). Foo et al. (2011) also reported a high parasitism infestation rate by the termite endoparasitoid *Misotermes mindeni* Disney and Neoh in shaded areas with low light intensity (Foo et al., 2011).

2.4.2. Host factors

Host factors also play a vital role in a parasitoid's host selection process. Host factors such as age, population density, movement, and color are criteria that are considered during host selection (Vinson, 1976). In particular, size and age of hosts are well-documented factors. For instance, the parasite *Perilitus coccinellae* Schrank prefers to attack large sized coccinelllids because it results in greater survival of the parasite's immature stage (Richerson and DeLoach, 1972). Similarly, chalcid wasps (*T. evanescens)* were found to be more attracted to larger eggs of *Ephestia* moths (Salt, 1940). Kant et al. (2008) reported that large hosts are easier to detect because they emit a greater amount of odour, which is attractive to parasitoids. Arthur and Wylie (1959) noted that the preference of parasitoids for large size hosts is also due to the rich nutrient resources that parasitoids can exploit from these hosts. These resources increase fitness and effectively enhance offspring production by the parasitoid.

Population density of hosts also influences the host selection process of parasitoids as a high density population releases a strong odor and provides a rich nutrient source for parasitoids to successfully rear an immense number of offspring (Smith and Schwarz, 2006). Cervo et al. (2000) found that cukoo paper wasps *Polostes sulcifer* (Zimmermann) were more attracted to high-density groups of polistine wasps *Polistes dominulus* (Christ) than to low-density groups. Similarly, parasitoid phorid flies *Apocephalus paraponerae* Borgmeier preferred to attack larger rather than smaller colonies of the ant *Paraponera clavata* (F.) (Morehead and Feener, 2000). The colony size of the bee *Exoneura robusta* Cockerell parasitized by the allodapine social parasite *Inquilina schwarzi* Michener was significantly larger than that of the unparasitized one (Smith and Schwarz, 2006).

2.5. Parasitioid-induced physiological changes in the host

Brodeur and Boivin (2004) described several adaptive strategies of parasitoids that ensure the success of parasitism, such as host discrimination, overcoming the host immune system, and host environment regulation. These strategies can affect host morphology, behaviour, physiology, and ecology to benefit parasitoid development (Vinson and Iwantsch, 1980; Poulin et al., 1998; Haspel et al., 2005).

Parasitism can also influence host development, such as by shortening the host's lifespan. For example, the noctuid moth larvae *Acronycta aceris* (Linnaeus) and leaf beetles *Crioceris* sp. exhibited a shorter pupation period when parasitized by the tachinids *Compsilura concinnata* (Meigen) and *Meigenia floralis* Fallen, respectively (Varley and Butler, 1933). In contrast, longer development occurred in *Spodoptera littolaris* Boisduval after being infected by the braconid wasp *Microplitis rufiventris* Kokujev. The long larval stage of those hosts was identified by an increase of juvenile hormone level in the hemolymph (Hegazi and Khafagi, 2005).

Terrestrial insects are susceptible to desiccation because of their large body surface area to volume ratio, which means that a large amount of total body water can be lost via the cuticle surface by evaporation (Schilman et al., 2007). Several studies have reported that parasites can manipulate the water balance profile of the host. Moore (1983) found a higher tolerance to lower humidity environments when the pill bug *Armadillidium vulgarae* (Latreille) was parasitized by the parasitoid *Plagiorhynchus cylindraceus* (Goeze) than when it was not parasitized. Under extreme environmental conditions, parasitoids can regulate the water loss rate of the host by reducing its cuticle permeability and increasing the body water content to overcome desiccation stress and thus increase their survival (Danks, 2000; Benoit and Denlinger, 2010). Foo et al. (2011) described the physiological changes of parasitized soldiers of the termite *Macrotermes gilvus* (Hagen) infected with the parasitoid *M. mindeni*. Metabolic rate, water balance profile, cuticular permeability, and desiccation tolerance of parasitized soldiers were examined, and the observed changes in host physiology were suggested to benefit the parasitoids by increasing the probability of host survival when exposed to extreme environmental conditions. Thus, parasitoids can ensure successful completion of their larval development before the host dies (Foo et al., 2011b).

2.6. Parasitoid-induced behavioural changes in the host

A parasitoid can manipulate a host through its nervous, immune, and endocrine systems (Libersat et al., 2009). Parasitoid manipulation affects behaviours of hosts in activities such as foraging, feeding, reproduction, grooming, and trophallaxis (Libersat et al., 2009). Reduced feeding activity was observed in lepidopteran larvae infected with hypocrealean fungi such as *Metarhizium* sp. and *Beauveria* sp. Infection resulted in poor digestive abilities of the host, possibly due to toxic substances produced by these fungi (Roy et al., 2006). Similarly, Kimbrough and Lenz (1982) reported a low rate of wood consumption by two Australian termite species *Mastotermes darwiniensis* Froggatt and *Porotermes adamsoni* (Froggatt) infected with *Termitaria* spp. Besides, Fuller and Jeyasingh (2004) reported that a colony of the Caribbean termite *Nasutitermes acajutlae* (Holmgren) infected with acanthocephalans still worked at rebuilding the foraging trails, but activity was slower compared to that of the unparasitized colony.

Changes in host behavior, such as aggressiveness, have been documented in several parasitized insects. Sze et al. (2008) found that the termite host *M. barneyi* was less aggressive when parasitized compared to when it was not parasitized and that parasitized soldiers moved slowly in the field. Lower interspecific aggressiveness of parasitized individuals also was observed in an agonistic test of two termite species *M. carbonarius* (Hagen) and *M. gilvus* (Neoh and Lee, 2011). In most cases, parasitized soldiers avoided attack by opponents during confrontation, but they sometimes vibrated their head. The authors suggested that this behaviour of parasitized soldiers may increase the probability of survival for them as well as for their parasitoid larvae, thus increasing the fitness of the parasitoid.

Parasitoid-induced changes in social behaviour of hosts can also occur in activities such as grooming, nest cleaning, and avoidance, which protect the host colony from infection (Libersat et al., 2009). Grooming behaviour of insects is an effective way to remove foreign particles and thus reduce the risk of colony infection (Loehle, 1995). Extensive grooming was observed in several termite species after they were infected with parasites such as *Zootermopsis angusticoliis* (Hagen) (Rosengaus and Traniello, 2001), *Reticulitermes speratus* (Kolbe) (Shimizu and Yamaji, 2003), and *Coptotermes formosanus* Shiraki (Yanagawa and Shimizu, 2007). Chouvenc et al. (2010) also suggested that termites are at high risk of infection if they do not perform the grooming.

Avoidance of disease-carrying individuals is considered to be an adaptive behaviour of insects that may reduce parasite or disease infection (Loehle, 1995). Termites are at high risk of infection due to their direct contact with pathogens, parasites, and microorganisms (Baverstock et al., 2010). The termite species *Coptotermes lacteus* (Froggatt) and *Macrotermes michaelsensi* Sjo¨lstedt were found to seal off the foraging gallery to prevent contact with the fungi *Metahizium anisopliae* (Metschnikoff) and *Beauveria bassiana* (Balsmo), respectively (Staples and Milner, 2000; Mburu et al., 2009). In addition, termite workers bury fungus-killed cadavers

(Yanagawa et al., 2010). De Bach and McOmie (1939) suggested that the behaviour of infected individuals, which move away from their nest mates, may protect the healthy individuals from infection. Neoh and Lee (2010) reported that *M. gilvus* parasitized by *M. mindeni* inhabited isolated concealed chambers of the termite mound. The authors suggested that this strategy may benefit the parasitoids by protecting parasitized termites and parasitoid larvae from being killed by other termite individuals inside the nest.

CHAPTER THREE

PREVALENCE OF THE ENDOPARASITOID *Verticia fasciventris* IN COLONIES OF THE FUNGUS-GROWING TERMITE *Macrotermes carbonarius* ON PENANG ISLAND, MALAYSIA

3.1. Introduction

The parasitioid *V. fasciventris* was reported to parasitize several termite species, including *M. malaccensis* Havilandi in Indonesia (Kemmer, 1925) and *M. barneyi* Light in Hong Kong (Sze et al., 2008). *V. fasciventris* was found to parasitize the fungus – growing termite *Macrotermes carbonairus* (Hagen) on Penang Island in Malaysia. The first survey of parasitism frequencies of *V. fasciventris* was carried out on Minden Campus of Universiti Sains Malaysia and Bayan Lepas on Penang Island with infection rates of 30% and 7.7%, respectively, were reported (Neoh and Lee, 2011).

Many factors contribute to the host selection process of a parasitoid, and they can be divided into environmental and host factors (Vinson, 1976). Environmental factors include temperature, humidity, light intensity, and level of exposure to air movement, and host factors include age, size, and nutritional status. These factors play an important role in orienting the parasitoids into a particular habitat and may contribute to their distribution and prevalence (Vinson and Iwantsch, 1980).

The goals of this study were to describe the prevalence and distribution of *M. carbonarius* colonies parasitized by *V. fasciventris* and examine the contribution of host colony conditions (colony health and mound size) and environmental conditions (light intensity) to the distribution and abundance of parasitized colonies on Penang Island, Malaysia.

3.2. Materials and Methods

3.2.1. Survey sites

The study was conducted on Penang Island, northeastern coast of Peninsular Malaysia. The following eight areas were surveyed: Air Itam, Jelutong, Bayan Lepas, Balik Pulau, Botanical Garden, Gelugor, Minden Campus of Universiti Sains Malaysia, and Teluk Bahang. A total of 313 *M. carbonarius* mounds were surveyed between April 2015 and April 2016. The location of each termite mound was recorded using GPS (Garmin eTrex HCx Vista, USA). The survey sites are shown in Figure 4.1 and the details are presented in Table 4.1.

3.2.2. Termite sampling

M. carbonarius mounds were opened to determine whether the colonies were infested with *V. fasciventris*. The termite mounds were excavated by digging a circular trench around the perimeter of the mounds and applying sideways pressure to remove the outer mound casing. The colony was considered infested with *V. fasciventris* if parasitized soldiers or pre-soldiers (both major and minor caste) were sighted. These parasitized termites were characterized by a round head capsule and short mandibles, and are commonly found in isolated, concealed chambers within infested mounds (Neoh and Lee, 2011). After excavation, the clay soils were replaced to cover the exposed parts of mounds.

Figure 3.1. Surveyed sites on Penang Island, Malaysia (source: Google map)

3.2.3. Degree of parasitism and status of colony health

To determine the degree of parasitism, 15 parasitized mounds on Minden Campus of Universiti Sains Malaysia were fully opened following the above-mentioned method (4.2.2). Parasitized major and minor soldiers (round head shape and short mandibles) were collected and temporarily placed inside cylindrical plastic containers. The termite mounds were thoroughly inspected within 15 minutes, and the numbers of parasitized soldiers were counted.

To evaluate the colony health of parasitized colonies, the broken sections of all parasitized mounds at all sites surveyed were examined within 3 days of excavation. The parasitized mounds were classified as healthy if termites were able to repair the broken section of the mound within the stipulated time period and unhealthy if the damaged part was not repaired (Foo et al. 2011). The evaluation was carried out during the termite survey described in the previous section.

3.2.4. Mound size and light intensity measurement

The size (diameter and height) and light intensity of both parasitized and unparasitized mounds were measured during the survey of parasitism frequency. Diameter and height of termite mound was measured before excavation using the measurement tape. Light intensity of each mound was measured by placing a Lux meter (Sper ScientiTc, Taipei, Taiwan) on the top center of the mound. Light intensity was measured between 1200 and 1500 hours because this is the peak light intensity period.

3.2.5. Observation of infected soldiers with no morphological changes

In late 2016, *M. carbonarius* soldiers were collected back for the purpose of experiment in the laboratory. The process of larvae exits from the abdomen of three morphologically normal soldiers was accidentally observed in the laboratoy. The process was video recorded by using a digital Sony DSC W830 Cyber – shot 20.1 MP camera.

3.2.6. Statistical analysis

Student's t-tests were used to compare mound size (diameter and height) and light intensity between parasitized and unparasitized mounds in areas that were infested with the parasitoid *V. fasciventris*. All analyses were performed using SPSS version 20.1 for Windows (SPSS Inc. Chicago, IL, USA).

3.3. Results

3.3.1. Frequency of parasitism

Of the 313 *M. carbonarius* mounds examined, 105 (33.5%) were infested with *V. fasciventris*. High parasitism frequency was recorded in Balik Pulau (62.0%) and Botanical Garden (61.8%), followed by Minden Campus Universiti Sains Malaysia (39.6%), Teluk Bahang (37.5%), Gelugor (27.0%), and Bayan Lepas (19.5%). No parasitized colonies were found in Air Itam (n = 28) and Jelutong (n = 19) (Table 3.1).

Table 3.1. Frequencies of parasitism at each surveillance site on Penang Island

Location	Longitude	Latitude	Landscape	No. mound surveyed	No. mound infested	Parasitism frequency %
Bayan Lepas	100 17.042	5 18.935	High plantation density	41	8	19.5
Gelugor	100 18 727	5 22.354	High plantation density	37	10	27.0
Balik Pulau	100 18.013	5 21.080	High plantation density	29	18	62.0
USM	100 18.380	5 21.634	High plantation density	106	42	39.6
Botany garden	100 18.399	5 26.198	High plantation density	34	21	61.8
Air Itam	100 16.804	5 23.366	Urban setting	28	0	0
Jelutong	100 18.712	5 23.004	Urban setting	22	0	0
Teluk Bahang	100 13.255	5 27.720	High plantation density	16	6	37.5
Sum				**313**	**105**	**33.5**