Prevalence of a Koinobiont Endoparasitoid *Misotermes mindeni* (Diptera: Phoridae) in Colonies of the Fungus-Growing Termite *Macrotermes gilvus* (Blattodea: Termitidae) in Malaysia

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ABSTRACT A survey of the infestation rate of colonies of *Macrotermes gilvus* (Hagen) (Termitidae: Macrotermitinae) with the koinobiont endoparasitoid *Misotermes mindeni* Disney & Neoh (Diptera: Phoridae) was conducted in Malaysia from September 2009 to January 2011 in the states of Kedah, Penang, Perak, Selangor, Kuala Lumpur, Johor, Terengganu, and Sarawak. Of the 1,125 *M. gilvus* mounds surveyed, 12.4% contained termites parasitized by *M. mindeni* and these mounds occurred only in the states of Penang and Perak. High frequencies of mounds containing parasitized termites were found at sites in Penang: Bayan Lepas (21.1%), Minden Campus of Universiti Sains Malaysia ([USM]; 24.5%), Teluk Bahang (28.0%), and Bukit Mertajam (35.0%); the lowest frequency (4.0%) was recorded from Gelugor. The parasitized colonies at all sites were classified as healthy, with exception of several from the Minden Campus of USM (96.4% healthy) and Ayer Itam (87.5% healthy). Most parasitized colonies (71.2%) had a low level of *M. mindeni* infestation. Only 16.7 and 12.1% of the infested colonies had moderate or high parasite infestation levels, respectively. The height of infected mounds was significantly higher than that of the healthy mounds, but there was no difference between the mound diameters of infested and uninfested mounds. Parasite infestation level was not significantly correlated with mound height or mound diameter. The ambient light intensity at sites with infested mounds was significantly lower than that of uninfested mounds. There was also a significant negative relationship between light intensity and degree of parasitism.

KEY WORDS parasitism frequencies, mound size, infestation levels, light intensity

Macrotermes gilvus (Hagen) (Termitidae: Macrotermitinae) is a common mound-building termite species native to Southeast Asia (Roonwal 1970, Lee et al. 2007). It is a serious pest of wooden structures, and agricultural crops such as sugar cane, planted on sites formerly covered by rain forest (Roonwal 1970, Cowie et al. 1989). It is also a secondary pest that can occur in buildings and structures once the dominant *Coptotermes* species are suppressed or eliminated via termite baiting (Lee 2002, Lee et al. 2007).

Several phorid flies have been reported to be koinobiont endoprasitoids of *M. gilvus.* For example, in soldiers of this termite larvae of *Misotermes exenterans* (Schmitz) were found in Java (Schmitz 1938, cited in Disney 1994) and larvae of *Misotermes mindeni* Disney & Neoh (Diptera: Phoridae) in Malaysia (Disney et al. 2009). The first survey of the incidence of parasitism by *M. mindeni* in *M. gilvus* colonies found in 2009 rates of 23 and 73% at the Minden Campus of Universiti Sains Malaysia (USM) and Bayan Lepas, respectively (Neoh and Lee 2010).

Parasites do not select their hosts randomly. They choose certain hosts from the larger group of animals available to them (Salt 1935). Parasites are believed to be attracted first not to a particular host but to a certain type of environment (Salt 1935, Laing 1938). Therefore, climatic factors such as temperature, humidity, light intensity, and level of exposure to air movement might influence their host selection process (Vinson 1976). Host factors such as population density (Hassel 2000), nutritional status, size, and age (Vinson 1976) also might play an important role in the selection of hosts by adult parasitoids to deposit their progeny (Laing 1938).

The goals of this study were to 1) describe the distribution of *M. gilvus* mounds infested by *M. mindeni,* 2) determine the prevalence of infested mounds, and 3) examine the contribution of host colony conditions and light intensity on the distribution and abundance of parasitized colonies in Malaysia.

Materials and Methods

Survey Sites. Our survey of *M. gilvus* mounds was conducted in seven states of Peninsular Malaysia, (Ke-

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Fig. 1. Locations of survey of *M. gilvus* mounds in Malaysia (refer to Table 1 for details on each location code). (Online figure in color.)

dah, Penang [island and mainland], Perak, Selangor, Kuala Lumpur, Johor, Terengganu) and in East Malaysia (Sarawak, Borneo). In total, 1,125 mounds were surveyed between September 2009 and January 2011. Figure 1 shows the survey sites and details about the sites are given in Table 1. All sites were selected because of the high abundance of *M. gilvus* mounds.

Degree of Parasitism and Colony Health. To determine whether a *M. gilvus* colony was parasitized by *M. mindeni*, we broke up the mound by digging a trench around the nest base and then applying sideways pressure to remove the outer wall casing. A colony was considered parasitized if it contained one or more infested termites. The presence of the parasitoid is indicated in major soldiers and presoldiers by their rounded head capsule and a pair of short mandibles and in fourth larval instars (L4s) by a brown dot on the head capsule, thorax, or abdomen (Neoh and Lee 2010). The parasitized termites also showed lower level of aggressiveness in inter-specific aggression assays when compared with healthy termites (Neoh and Lee 2010). Generally, parasitized major soldiers aggregated in an isolated concealed chamber at the inner part of the outermost layer of the mound (Neoh and Lee 2010) (Fig. 2). We randomly chose two infested termites and dissected their head capsule from each parasitized mound to obtain the larval parasitoid for identification. The larval parasitoid was identified based on Foo et al. (2011). The outer wall casing and the broken pieces of clay wall were then placed back to the original site where the mound was broken.

We also evaluated colony health based on the ability of termites to repair the broken section of the mound within 1–3 d. Parasitized colonies were classified as

Table 1. Frequencies of parasitism at each surveillance site in Malaysia

Surveillance site			Latitude	Longitude	No. surveyed mounds	No. parasitized mounds	Parasitism frequency (%)
Kedah	Sungai Petani	A1	5° 39′ N	100° 30′ E	6	0	0.0
	Alor Star	A2	6° 07′ N	100° 22′ E	44	0	0.0
Penang (island)	Minden Campus of USM	B1	5° 21′ N	100° 18′ E	343	84	24.5
	Gelugor	B2	5° 22′ N	100° 18′ E	50	2	4.0
	Bayan Lepas	B3	5° 17′ N	100° 15′ E	71	15	21.1
	Balik Pulau	B4	5° 22′ N	100° 12′ E	50	6	12.0
	Teluk Bahang	B5	5° 26′ N	100° 13′ E	50	14	28.0
	Ayer Itam	B6	5° 23′ N	100° 17′ E	50	8	16.0
Penang (mainland)	Engineering Campus of USM	B7	5° 09′ N	100° 29′ E	53	0	0.0
	Seberang Jaya	B8	5° 23′ N	100° 28′ E	10	1	10.0
	Bukit Mertajam	B9	5° 21′ N	100° 28′ E	20	7	35.0
Perak	Ipoh	C1	4° 37′ N	101° 07′ E	1	0	0.0
	Lenggong	C2	5° 08 N	100° 59′ E	49	2	4.1
Selangor	Serdang	D1	2° 59′ N	101° 43′ E	24	0	0.0
	Kota Kemuning	D2	2° 59′ N	101° 32′ E	15	0	0.0
Kuala Lumpur	Kepong	E1	3° 14′ N	101° 38′ E	21	0	0.0
Johor	Segamat	F1	2° 31′ N	102° 52′ E	23	0	0.0
	Tangkak	F2	2° 16′ N	102° 32′ E	27	0	0.0
Terengganu	Kuala Berang	T1	5° 04′ N	102° 59′ E	50	0	0.0
Sarawak	Kuching	S1	1° 32′ N	110° 20′ E	28	0	0.0
	Samarahan	S2	1° 27′ N	110° 30′ E	28	0	0.0
	Sri Aman	S3	1° 14′ N	111° 28′ E	28	0	0.0
	Betong	S4	1° 25′ N	111° 36′ E	28	0	0.0
	Sarikei	S5	2° 07′ N	111° 31′ E	28	0	0.0
	Sibu	S6	2° 19′ N	111° 50′ E	28	0	0.0
	TOTAL				1,125	139	12.4

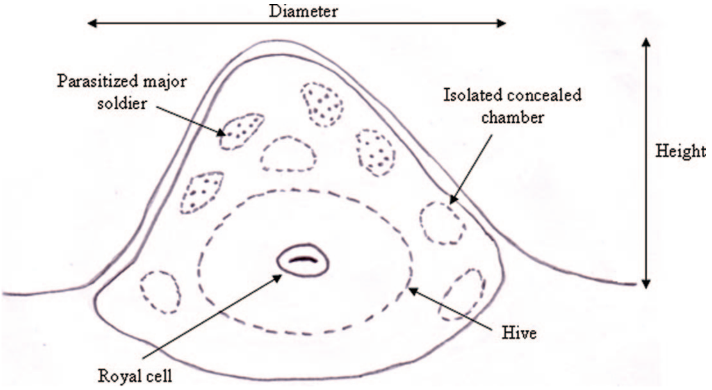

Fig. 2. Diagram showing vertical section of a parasitized *M. gilvus* mound. Parasitized major soldiers aggregate in the fungus comb chambers. Dotted spots indicate the parasitized major soldiers (Online figure in color.).

healthy if the termites repaired broken sections within this time period, whereas they were classified as not healthy if the damage was not repaired. The height and diameter of the parasitized mounds were measured before they were broken (Fig. 2).

Using the method described above, we fully opened up the mounds of 66 randomly selected parasitized colonies. The entire mound was thoroughly inspected to ensure that no parasitized termite was overlooked. All parasitized termites were collected and counted. The degree of parasitism for each parasitized colony was rated as follows: low, <20 parasitized termites; moderate, 20–50 parasitized termites; and high, >50 parasitized termites.

Light Intensity Measurements. The influence of ambient light intensity on the distribution of the infested mounds and on the degree of parasitism in each colony was measured by placing a light meter (Sper ScientiTc, Taipei, Taiwan) on the peak of uninfected ($n = 256$) and infected ($n = 77$) *M. gilvus* mounds in Main Campus of USM. Measurements (in lux) were taken between 1300 and 1500 hours on 22–24 February 2010 because of the high light intensities during this time of the day.

Statistical Analysis. We used Spearman rho analysis to determine the relationship between 1) mound size (height/diameter) and degree of parasitism and 2) degree of parasitism and ambient light intensity. The mound size and ambient light intensity between unparasitized and parasitized mounds were compared using the Student *t*-test. All analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL).

Results

Table 1 lists the number of surveyed and parasitized *M. gilvus* mounds and parasitism frequencies at each survey site. Of the 1,125 *M. gilvus* mounds examined, 139 (12.4%) of them were infested by *M. mindeni.* Parasitized colonies were found only in Penang and Perak states. A high frequency of parasitism was reported in Bayan Lepas (21.1%, 15/71 surveyed colonies), Minden Campus of USM (24.5%, 84/343 surveyed colonies), Teluk Bahang (28.0%, 14/50 surveyed colonies), and Bukit Mertajam (35.0%, 7/20 surveyed colonies). The lowest frequency (4.0%, 2/50 surveyed colonies) was recorded from Gelugor.

Parasitized colonies were classified as healthy at all sites except Minden Campus of USM and Ayer Itam. At Minden Campus of USM, 81 of the parasitized colonies were healthy (96.4%), whereas the remaining three were not (3.6%). In Ayer Itam, 12.5% ($n = 1$) of the parasitized colonies were not healthy.

Colonies with a low degree of parasitism accounted for 71.2% of the mounds surveyed ($n = 66$). Only 16.7 and 12.1% of the mounds inspected exhibited moderate and high levels of parasitism, respectively. The numbers of parasitized termites from the colonies of high levels of parasitism ranged between 65 and 243 parasitized individuals. On average, the mound heights of unparasitized and parasitized colonies from B sites were 36 cm ($n = 259$; range, 2–130 cm) and 44 cm ($n = 129$; range, 10–134 cm), respectively. The parasitized mounds were significantly taller ($t = 3.303$, df = 243, $P < 0.01$) than unparasitized mounds. However, there was no significant difference ($P > 0.05$) in the mound diameters between parasitized and unparasitized mounds. Neither mound height (Spearman $r = 0.158$, $P > 0.05$; $n = 64$) nor mound diameter (Spearman $r = 0.070$, $P > 0.05$; $n = 64$) was significantly correlated with the degree of parasitism.

The ambient light intensities of the unparasitized mounds ranged from 149 to 287,700 lux (average, 17,188 lux; $n = 256$); those of parasitized mounds ranged from 144 to 34,420 lux (average, 6,358 lux; $n = 77$). Ambient light intensity of infested mounds was significantly lower than that of uninfested mounds ($t = 5.307$, df = 312, $P < 0.01$). Spearman's rank correlation test showed a significant negative correlation between light intensity and the degree of parasitism (Spearman $r = -0.431$, $P < 0.01$; $n = 62$).

Discussion

In parasitoids, the free-living adult may seek a suitable environment or preferred habitat upon emergence (Salt 1935). Habitat preference plays an important role in determining the type of habitat sought by the parasitoid and thus helps in locating a host (Vinson 1976). The habitat preference of *M. mindeni* is unknown. However, the current results showed that *M gilvus* mounds infested by *M. mindeni* were often found in shaded areas with dense vegetation. These observations suggest that the adult *M. mindeni* may prefer areas that are darker, damper, and cooler than the surrounding areas to survive. In laboratory experiments, adult flies were able to survive longest at 100% RH and 21°C (F.-K.F. et al., unpublished data). In fact, those characteristics, i.e., secretive habits and liking of dark, damp, and moldy areas are common throughout the Phoridae (Oldroyd 1964).

The high frequency of parasitized mounds in Bayan Lepas, Minden Campus of USM, Teluk Bahang, and Bukit Mertajam may be related to the characteristics of these sites: shaded and humid due to dense vegetation. The lowest frequency of parasitized mounds was observed in Gelugor, which is a well-developed town with limited vegetation. Of the nine sites sampled in Penang (island and mainland), only the Engineering Campus of USM on the mainland lacked infected mounds ($n = 53$). It was an open area with little vegetation. In contrast to our results, Neoh and Lee (2010) reported that parasitism frequency in Bayan Lepas (73%) was much higher than that of Minden Campus of USM (23%). However, the number of *M. gilvus* mounds surveyed was lower than in the current study, i.e., 15 and 44 colonies in Bayan Lepas and Minden Campus of USM, respectively. One possible explanation for the observed distribution pattern of infested mounds may be that the geographical distribution of the flies is more restricted and does not extend to the full range of *M. gilvus* in Malaysia.

Adult female parasitoids are responsible for selecting hosts in a manner that increases their offspring fitness (Vinson 1976, Kouame and Mackauer 1991, Mousseau and Dingle 1991, Schmid-Hempel and Schmid-Hempel 1996). Final host choice may be based on an assessment of future potential growth and resources available for developing larval parasitoid (Kouame and Mackauer 1991). The data in the recent study show that in most cases, the colonies parasitized by *M. mindeni* were healthy. This suggests that healthy colonies were shown to be attractive to the parasitoids, as they may provide greater potential for future growth and development opportunities for the parasitoid offspring.

The apparently low impact of parasitization on the infected mounds was probably due to the low abundance of parasitized termites within the colony. This would indicate that flies have great difficulties in getting their progeny into the colony due to difficulties of access to the hosts or defense mechanism by the host. Perhaps, flies may flood the host with eggs, but only a few able to make it or they have a protracted laying period so that *M. gilvus* colony gets infested repeatedly at a low rate but over a longer period.

Mound height of parasitized colonies was found to be significantly higher than that of unparasitized mounds. Lee and Lee (2011) documented an increase in mound height with increased population size in *M. gilvus.* The total population size in *M. gilvus* was 37,600 ± 9,300 (Lee and Lee 2011). This could indicate that parasitized colonies have a larger population size compared with the unparasitized colonies. A higher number of termites in the colony might increase host availability and thus, parasitoids might have a greater chance of encountering their target hosts. This theoretical prediction is supported by a study of the winter moth *Operophtera brumata* (L.) Hassel (2000) showed that higher densities *O. brumata* were able to attract more of the parasitic tachinid fly *Cyzenis albicans* (Fallen) than moths at lower densities. Most phorid parasitoids are associated with ants living in large colonies (e.g., *Atta* sp. and *Acromyrmex* sp.; Disney 1994). Morrison and King (2004) reported that female phorids were more attracted to the high density of imported fire ants than the lower, assuming they have good opportunity to find their host. Our findings are also similar to those of Smith and Schwarz (2006, 2009), who reported that the allodapine social parasite *Inquilina schwarzi* Michener were more likely to invade larger colonies of allodapine bee *Exoneura robusta* Cockerell. It is possible that larger host colonies have stronger odor cues and contain a larger amount of resources for the parasitoids to exploit, which would provide them with the greater potential fitness (Smith and Schwarz 2006, 2009). In addition, older colonies might be more likely to be parasitized if the flies cycle within the colonies because there would be more time to find the target hosts.

Climatic factors (e.g., temperature, humidity, light intensity, and air movement), food sources, and mode of locomotion of parasitoids (flying and crawling habits) may influence how parasitoids search for hosts in the preferred habitat (Vinson 1976). In the current study, we only examined the influence of ambient light intensity on the host colony selection and on the degree of parasitism. High light intensity is essential for searching activity in some parasitoids (Vinson 1975), but in others, it inhibits such activity (Miller 1959). *M. mindeni* seems to fall into the latter category. In our study, sites with parasitized mounds had lower ambient light intensity than those with unparasitized mounds and in general, lower ambient light intensity led to higher degree of parasitism. These results suggest that dim areas might be more attractive to *M. mindeni.* Similar phenomena have been observed in *Pseudacteon nocens* Borgmeier and *Pseudacteon litolaris* Borgmeier, which are internal parasitoids of the *Solenopsis* fire ants (Folgarait et al. 2007).

The potential of the flies to be a biological control agent against *M. gilvus* cannot be ruled out, although at this stage, only low parasitism rate was found in the examined colonies. The flies could be reared in the laboratory and released into the new host colonies. More studies on the mass-rearing technique of the flies should be carried out in future to explore such possibility.

The results of the current study provide information about the frequencies of parasitism and distribution of

M. gilvus mounds infected by *M. mindeni.* This study is an initial step toward understanding the influence of host colony conditions (e.g., population size and vigor) and features of the mound environment (i.e., extent of shading vegetation expressed via ambient light intensity and climatic factors) on host colony selection by *M. mindeni* and the degree of parasitism in infected mounds. Our study demonstrates the importance of taking into consideration host-related and climatic effects when investigating the potential causes of parasitoid infestation. The current study with a large sample size covered the full distribution range of *M. gilvus* in Malaysia. This termite species occurs in other countries of Southeast Asia as well, i.e., Thailand, Indonesia and Philippines, where a number of other Phoridae are known to infest it. Similar investigations to ours should be conducted in these countries. Results from these and our study should lead to a better understanding of the biology of the fly parasites and ultimately to insight into the mechanisms of parasitism.

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