CHAPTER 3

Results and discussion

3.1 Ant fauna in coffee plantations

3.1.1 Ants in PM

At the end of the rainy season, ants were collected from 45 out of 50 coffee tree trunks. The average number of species per tree was 1.6 ± 1.1 SD (range: 0-5, figure 3.1). In total, 27 species were collected, 17 of which were from single trees. The expected number of species to be collected by this method is 49.3 ± 14.6 SD. *Technomyrmex yamanei* was exclusively dominant, being found on 32 trees. The other dominant species were *Polyrhachis (Myrmhopla)* sp. PM5 (8 trees) and *P. armata* (5 trees) (Table 3.1). Among 50 quadrats, 44 quadrats harbored ants. The average number of species per quadrat was 2.1 ± 1.2 SD (range: 0-7, figure 3.1). In total, 37 species were collected from litter and surface soil, of which 21 species were collected from single quadrats, and six species were found in two quadrats. The expected number of species to be collected by this method is 60.2 ± 14.0 SD. As in ants collected on tree trunks, *T. yamanei* was the most dominant, being found on 27 quadrats. *Trichomyrmex* sp. PM1 was collected from 11 quadrats. The other species were found in less than five quadrats (Table 3.1). Combining the results obtained by the two sampling methods, 58 species were recorded at the end of the rainy season (Table 3.2).

At the end of the dry season, ants were collected from 47 out of 50 coffee trees. The average number of species per tree was 1.9 ± 1.1 SD (range: 0-6, figure 3.1). In total, 35 species were collected, of which 15 species were collected from single trees. The expected number of species to be collected by this method on coffee trees is 45.3 ± 7.1 SD. As at the end of the dry season, the most dominant species was *T. yamanei*, being found in

18 trees. The other dominant species, found in more than five trees, were *Crematogaster* sp. PM2, *Plagiolepis* sp. 3 of WJT, *Camponotus nicobarensis*, and *Tapinoma indicum* (Table 3.1). In total 41 species were collected from all 50 quadrats, of which 19 species were collected from single quadrats, and nine species were found in two quadrats. The expected number of species to be collected by this method is 57.8 ± 10.2 SD. *T. yamanei* was the most dominant, being found in 19 quadrats. The other dominant species collected from more than 5 quadrats were *Nylandria* sp. PM2, *Tapinoma indicum*, *Trichomyrmex* sp. PM1, and *Lophomyrmex birmanus* (Table 3.1). Among the 41 species collected from litter and surface soil, 12 species were the same as ants collected in coffee trees. Thus, the total number of species recorded at the end of the dry season was 64 (Table 3.2).

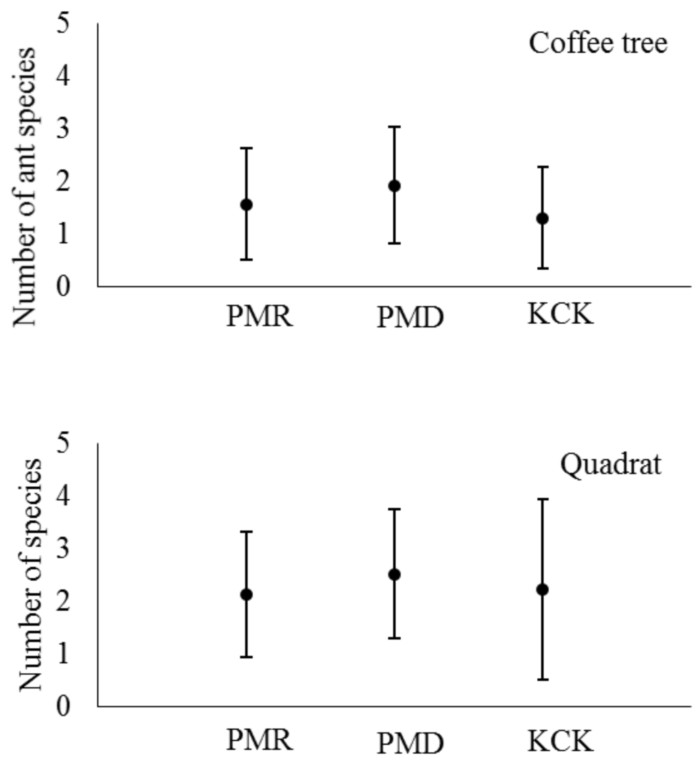


Figure 3.1 Number of ant species collected per coffee plant and per quadrat in Pa Miang during rainy season (PMR) and dry season (PMD), and in

Khun Chang Kian during rainy season (KCK). Average number of species with standard deviation is shown.

Table 3.1 Dominant species collected by each method at two study sites in Chiang Mai province: Pa Miang village, Doi Saket district and Highland Research and Training Station Khun Chang Kian Site A. Number of appearance (N) of each dominant ant species are shown. End of rainy season at PM is in September and November. End of dry season is in June. Rainy season at KCK is during May to November.

The dominant ant species collected on 50 coffee trees					
Coffee plantation at Pa Miang village				Khun Chang Kian Site A	
End of rainy season		End of dry season		Rainy season	
Species name	N	Species name	N	Species name	N
Technomyrmex yamanei	32	*Technomyrmex yamanei*	18	*Dolichoderus affinis*	19
Polyrhachis (Myrmhopla) sp. PM 5	8	*Crematogaster* sp. PM 2	7	*Meranoplus laeviventris*	9
Polyrhachis armata	5	*Plagiolepis* sp. PM 1 = sp.3 of WJT	6	*Polyrhachis (Myrmhopla)* sp.CK5	5
Paraparatrechina sp. PM 1	3	*Camponotus nicobarensis*	5	*Plagiolepis* sp. PM 1 = sp.3 of WJT	5
Gnamptogenys bicolor	3	*Tapinoma indicum*	5	*Crematogaster* sp.CK1	3

The dominant ant species collected from 50 quadrats under the coffee plants					
Coffee plantation at Pa Miang village				Khun Chang Kian Site A	
End of rainy season		End of dry season		Rainy season	
Species name	N	Species name	N	Species name	N
Technomyrmex yamanei	27	*Technomyrmex yamanei*	19	*Brachyponera leutipes*	12
Trichomyrmex sp. PM 1	11	*Nylanderia* sp. PM 2	11	*Nylanderia* sp. CK2	11
Nylanderia sp. PM 2	4	*Tapinoma indicum*	10	*Tetramorium* sp. PM2	10
Oligomyrmex sp. PM 1	4	*Trichomyrmex* sp. PM 1	9	*Dolichoderus affinis*	7
Kartidris sp. PM 1	3	*Lophomyrmex birmanus*	8	*Meranoplus laeviventris*	7

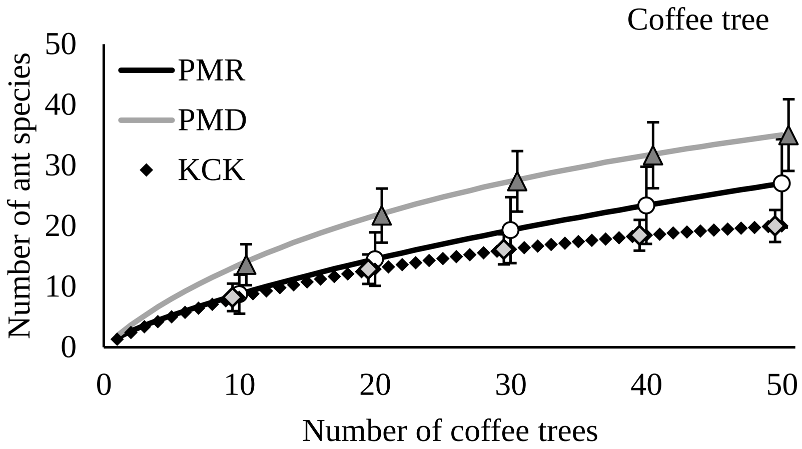
Table 3.2 Number of ant species collected by each collection method, each season and each site. T: collected on coffee tree, Q: collected from quadrat. End of rainy season at PM is in September and November. End of dry season is in June. Rainy season at KCK is during May to November.

	Coffee plantation at Pa Miang village							Khun Chang Kian Site A		
Subfamily	No. species collected							No. species collected		
	End of rainy season			End of dry season			Total	Rainy season		
	T	Q	Subtotal	T	Q	Subtotal		T	Q	Subtotal
Dolichoderinae	7	5	9	7	6	10	12	4	2	4
Dorylinae	0	0	0	0	0	0	0	0	1	1
Ectatomminae	1	1	1	1	0	1	1	0	0	0
Formicinae	10	4	14	15	8	20	25	9	6	12
Myrmicinae	9	15	22	9	19	23	30	7	20	22
Ponerinae	0	12	12	1	8	8	15	0	4	4
Proceratiinae	0	0	0	0	0	0	0	0	1	1
Pseudomyrmecinae	0	0	0	2	0	2	2	0	0	0
Total	27	37	58	35	41	64	85	20	34	44

Comparing the two sampling seasons, both the average number of species per coffee tree and per quadrats were not significantly different between the two seasons (t-test, $P = 0.16$ for ants from tree trunks, $P = 0.08$ for ants from quadrats). Species richness of both tree ants and litter ants was not different between both seasons (Figure 3.2). However, the species composition between the two seasons was slightly different. Only 37 species were common the both rainy and dry seasons, and the remaining 48 species were collected in only the dry (27 spp.) or rainy season (21 spp.). Of the 48 species, the majority were "rare" species, to be found on less than three occasions. *Plagiolepis* sp. 3 of WJT and *C. nicobarensis* were exceptional, listed as dominant species but collected in the dry season only. Among the 37 species collected in both seasons, the frequency of occurrence between the two seasons was remarkably different in *Crematogaster* sp. PM2 (Fisher's exact test, $P = 0.01$), which was just once collected during the rainy season while the ants were collected from seven trees and three quadrats in the dry season.

The quantitative sampling in the two seasons yielded 85 ant species in 36 genera of 6 subfamilies in this plantation (Table 3.2). Myrmicinae (30 spp.) was the most species rich subfamily, followed by Formicinae (25 spp.), Ponerinae (15 spp.), and Dolichoderinae (12 spp.). The most species rich genera were *Polyrhachis* (9 spp.), *Technomyrmex* (6 spp.), *Camponotus* (6 spp.), *Crematogaster* (6 spp.) and *Tetramorium* (5 spp.). Among 85 species, 29 species were collected only once, and 21 species were collected twice. Therefore, further research could increase the cumulative number of ant species (Figure 3.2). Remarkable characteristics of the ant fauna in PM are the exclusive dominance of *T. yamanei* in both seasons. In addition to the quantitative samplings, we collected three species, *Technomyrmex* sp. PM4, *T. modiglianii*, and *Polyrhachis (Myrma)* sp. PM3.

Figure 3.3 shows the most dominant species at PM, *Technomyrmex yamanei,* attended the scale insect, *Coccus viridis,* to obtain honeydew. This scale insect shows outbreaks on coffee trees during the rainy season (Figure 3.4). *T. yamanei* was abundant both in the rainy and dry season. However, the frequency of its occurrence in the rainy season was higher than that in the dry season, probably because the scale insects may attract many workers of *T. yamanei* during the rainy season. Scale insects are generally known as pest insects of agricultural products. However, the previous study indicated that the mutualistic relationship between ants and scale insects has a positive effect on the coffee plant by attacking the CBB (Perfecto and Vandermeer, 2006). Actually, the mutualistic relationship between *Dolichoderus thoracicus* and mealybug are used for biological control of cocoa pest, cocoa pod borer, in Indonesia (Ho and Khoo, 1997).



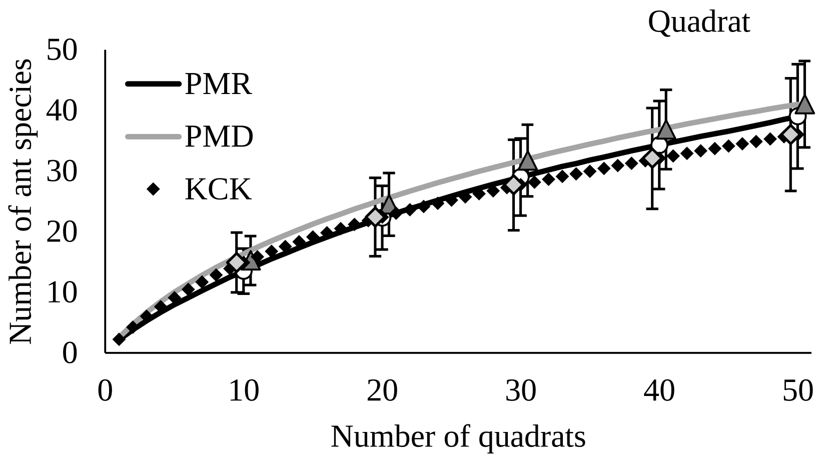


Figure 3.2 Species accumulation curves for ants on coffee trees and ants in quadrates in Pa Miang during rainy season (PMR) and dry season (PMD), and in Khun Chang Kian during rainy season (KCK). Error bars show 95% confidence intervals and non-overlapping bars show significant differences between study sites and/or sampling season.

Figure 3.3 *Technomyrmex* ants attend green scale insects

Figure 3.4 The green scale insect, *Coccus viridis*. Adults with crawlers (small white individuals)

3.1.2 Ants in KCK

Ants were collected from 38 out of 50 coffee tree trunks. The average number of species per tree was 1.3 ± 1.0 SD (range: 0-3, figure 3.1). In total, 20 species were collected, of which 6 were collected from single trees. The expected number of species to be collected by this method is 21.5 ± 1.90 SD. *Dolichoderus affinis* was the most dominant, being found on 19 trees. One to seven ant species were collected from 42 out of 50 quadrats. The average number of species per quadrat was 2.2 ± 1.7 SD (range: 0-7). Total 36 species were found from litter and surface soil, of which 17 species were collected from only single quadrats, and three species were found in two quadrats. The expected number of species for leaf litter and surface soil is 77.9 ± 27.6 SD (Chao 2). The dominant species, found in more than 10 quadrats, were *Brachyponera luteipes, Nylanderia* sp. CK2, and *Tetramorium* sp. PM2 (Table 3.1). The average number of species per coffee tree and per quadrat in KCK were not different from those of PM in the rainy season (t-test, $P = 0.15$ for tree ants, $P = 0.68$ for quadrat ants). The species richness of both tree ants and litter ants was not different from the result obtained in PM during the rainy season. However, the species richness on coffee trees in KCK was significantly lower than that in PM (Figure 3.2).

All 44 species in 26 genera of 6 subfamilies were collected by the two sampling methods in KCK during the rainy season (Table 3.2). Myrmicinae (22 spp.) was the most species rich subfamily, followed by Formicinae (12 spp.), Ponerinae (4 spp.), and Dolichoderinae (4 spp.). The most species rich genera were *Tetramorium* (7 spp.), *Polyrhachis* (5 spp.), and *Pheidole* (4 spp.). Ten out of 44 species were collected by both methods. In addition, the 44 species collected by the two methods, 17 species of ants were collected in November 2012 and May to November 2013. Thus, 61 species have been recorded from KCK.

3.1.3 Comparison between PM and KCK

In the present study, 88 and 61 ant species were recorded from coffee plantations in PM and KCK, respectively. Thus, in total 121 ant species were obtained from the two coffee plantations. Only 28 species were in common between the two sites. Figure 3.5 shows the comparison of ant fauna between rainy and dry seasons in PM and rainy season in KCK. Its abundance plot does not matched between PM and KCK so much. If I consider the results of our quantitative survey, we recorded 58 species during the rainy season, 64 species in the dry season in PM, and 44 species during the rainy season in KCK. Ant species diversity in KCK was slightly lower than that in PM. The commonness of species composition calculated by the Nomura-Simpson Index between PM and KCK is 0.46. In West Java, Indonesia, Ito *et al.* (2001) reported that the commonness of ant species composition among three study sites in lowland areas (the Bogor Botanical Gardens, Ujung Kulon National Park, Pangandaran National Park) which are ca 160~370 km apart from each other, is 0.62~0.68. The value between Bogor Botanical Gardens and Purwodadi Botanical Gardens in East Java, that are ca 650 km apart from each other is 0.59 (Ito *et al*., unpublished). Comparing these values, the commonness between the two coffee plantations in Chiang Mai is slightly lower.

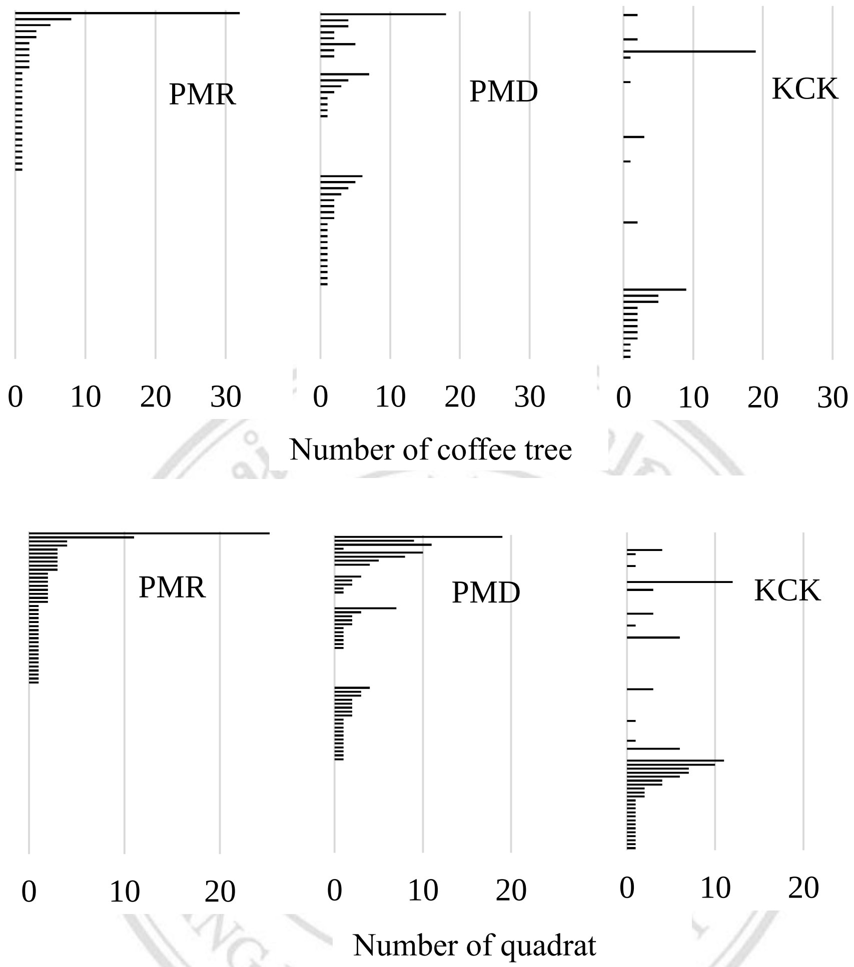


Figure 3.5 Comparison of ant fauna between rainy (PMR) and dry seasons (PMD) in Pa Miang and rainy season in Khun Chang Kian (KCK) with matched abundance plot. In the left side of the figure, the order of abundance for species is plotted with the most abundant species at the top. The middle and right side show the corresponding abundance for all the species shown in the left side. Abundance of species not found in the left side was also shown with descending order of abundance in the middle figure.

Furthermore, the dominant ant species were different between PM and KCK. Figure 3.6 shows the dominant ant at each coffee plantation. *Technomyrmex yamanei* which was the most dominant species in PM, was not found in KCK. On the other hand, the most dominant ant in KCK, *Dolichoderus affinis*, was collected only twice in PM. *Meranoplus laeviventris* was commonly collected in KCK but was not found in PM. One of the factors affecting the difference of species diversity and its composition between the two sites is the altitude of the study sites. The altitude of PM and KCK is approximately 910 m and 1280 m above sea level, respectively. In the tropics, ant species diversity is generally higher in lowland forests, and decreases with higher altitude (Malsch *et al.*, 2008, Brühl *et al.,* 1998). In Mt. Kinabalu, for example, species richness of ground ants at 830 m, 1140 m, and 1340 m, was 89 spp, 61 spp., and 25 spp., respectively (Malsch *et al.*, 2008).

Figure 3.6 The most dominant ant species of each coffee plantation

(A) shows *Technomyrmex yamanei* which is the most dominant ant species at PM. (B) is *Dolichoderus affinis* and (C) is *Melanoplus laeviventris* which are the most dominant ant species at KCK.

Another factor contributing to the difference of species diversity and the composition may be the construction of the plantations. According to the classification of coffee plantations based on vegetational and structural complexity by Moguel and Toledo (1999), PM is rustic type where coffee trees are grown on the natural forest floor while KCK is of the traditional polyculture type where coffee trees are planted alongside several useful plants (Figure 3.7, 3.8). Although both PM and KCK are surrounded by taller trees and coffee trees are shaded by these trees, the density of the taller trees in PM is higher than that in KCK, providing more dark shade for coffee trees in PM. KCK is a research and training station as well as nature park, visited by many tourists, and therefore is well-managed. Taking this into consideration, PM may have a lot of the forest species, while KCK may have the forest edge – open land species. However, we still do not know the definite reason because the knowledge of habitat preference of each ant species is still lacking at present.

Figure 3.7 Coffee plantation at Pa Miang village (PM)

Figure 3.8 Khun Chang Kian Site A (KCK)

3.1.4 Comparison with other sites

In Southeast Asia, the ant fauna in coffee plantations has been investigated in Sumatra, Indonesia (Philpott *et al*., 2008). Philpott *et al*. (2008) reported the occurrence of 136 morphospecies from three coffee plantations inside the Bukit Barisan Selatan National Park and 125 morphospecies from three coffee plantations outside this park. In total, 165 ant species were recorded from six coffee plantations in and around this national park. Despite the fact that species numbers are not given for each plantation, the coffee plantations in Chiang Mai (121 species from two plantations) seem to have comparable species number to the result obtained in Sumatra.

Sakchoowong *et al.* (2008) conducted an investigation of the ant fauna by litter sifting in five different land use types (montane forest, jungle tea, forest fallow for one year and eight years, and annual cabbage crop site) in Chiang Mai province, and found 43 to 89 species per type. Montane forests had the highest species diversity, 89 species being recorded from five plots. Because the sampling method employed for this research is different from the present study, direct comparison of species number does not seem meaningful. However, the occurrence of 121 species in our study indicates that ant species diversity in these coffee plantations is quite high among several forest types in Chiang Mai province.

3.1.5 Size of each ant against the CBB

Figure 3.9 shows the head width distribution of 121 ant species collected in PM and KCK. Sixty seven species of ants have smaller head width than the body width of the CBB. According to this, at least 67 species of ants are able to enter the gallery of CBB on the coffee berries, enabling them to attack and predate the colonies of CBB. Among the dominant species listed in Table 3.1, the following 11 species were included in the 67 species; *Crematogaster* sp. PM2, *Kartidris* sp. PM1, *Lophomyrmex birmanus*, *Nylanderia* sp. PM2, *Nylanderia* sp. CK2, *Oligomyrmex* sp. PM1, *Paraparatrechina* sp. PM1, *Plagiolepis* sp.3 of WJT, *Tapinoma indicum*, *Tetramorium* sp. PM2 and *Trichomyrmex* sp. PM1. Especially, *Nylanderia* sp. PM2, *Oligomyrmex* sp. PM1, *Paraparatrechina* sp. PM1 and *T. indicum* were very small species with less than 0.40 mm head width of workers. They might have higher potential as a predator of the CBB than other ant species in PM and KCK. Gonthier *et al.* (2013) and Perfecto and Vandermeer (2013) reported that the very small ants could be predators of CBB. Actually, one of the species of *Tapinoma* was reported as a predator of CBB in Mexico; the workers forage inside fruits and predate on borers (Infante *et al*., 2003). After harvesting coffee berries, infested berries which turn black, are left on the branches. Therefore, small ant species foraging on coffee trees can affect the CBB throughout year. The small ant species foraging on the ground also may have a chance to attack the CBB in the old berries fallen on the ground (Perfecto and Vandermeer, 2006).

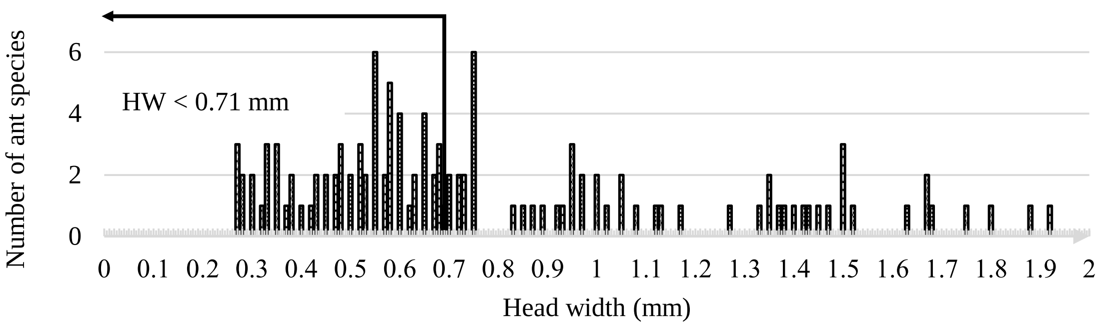


Figure 3.9 Head width distribution of 121 ant species collected in two coffee plantations in Chiang Mai. Head width of CBB is 0.7 $\pm$ SD 0.06 mm. The head width of 67 ant species was smaller than that of CBB.

3.2 Ant behavior against a CBB

Colonies of the following ant species were collected in the PM; *Camponotus nicobarensis*, *Crematogaster* sp. PM2, *Dolichoderus* sp. PM1, *Tapinoma indicum*, *Technomyrmex modiglianii* and *Technomyrmex yamanei* (Figure 3.10). All ant species studied are common in this plantation (Onishi *et al.* 2016). In addition, a colony of *Anoplolepis gracilipes* was collected from a coffee plantation of Teentok Royal Project Development Center. This species was the most dominant in that coffee region.

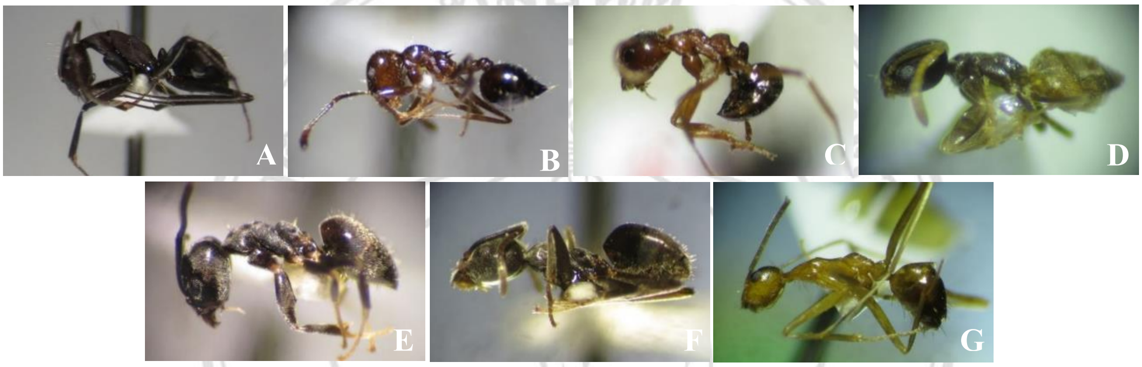


Figure 3.10 Seven ant species that used in experiment as behavior observation

(A) *Camponotus nicobarensis*

(B) *Crematogaster* sp. PM2

(C) *Dolichoderus* sp. PM1

(D) *Tapinoma indicum*

(E) *Technomyrmex modiglianii*

(F) *Technomyrmex yamanei*

(G) *Anoplolepis gracilipes*

Most foraging workers touched the CBB with their antennae when they encountered them in the foraging arena. The only exceptions were five workers of *C. nicobarensis* and one workers of *Anoplolepis gracilipes* that ignored the CBB (Figure 3.11). After contacting the CBB, two to six workers of each species walked away without showing any aggressive behavior toward the CBB. The ratio of such indifferent workers was not different among species (pairwise comparisons using Fisher test). The remaining workers showed short time aggression or intensive aggression. The comparison among the seven ant species indicated that a significant difference in the ratio of workers showing intensive aggression was only found between *Dolichoderus* sp. PM1 and *T. yamanei* ($p = 0.023$). Figure 3.12 shows the average duration of each behavior performed by each seven ant species. The duration between each ant species are different, however all of them have shown attacking or carrying behavior against the CBB. These behaviors may result in expelling the CBB from coffee trees and thus reducing the infestation of coffee berries (Jiménez-Soto *et al*., 2013). The behavioral response against the CBB varied among the seven ant species, although a statistically significant difference is not found in the most species. A possible reason for the absence of statistical differences may be the small sample size. However, our results may give an important insight into the usefulness of ants for biological control of CBB in Thailand. The duration of intensive attack varied from a few to 150 seconds, however, no significant difference in average duration was detected among ant species (Ryan-test). When *Tapinoma* workers showed intensive attack, this was usually performed by multiple workers, while in the other species, attacking was always done by single workers (Figure 3.13).

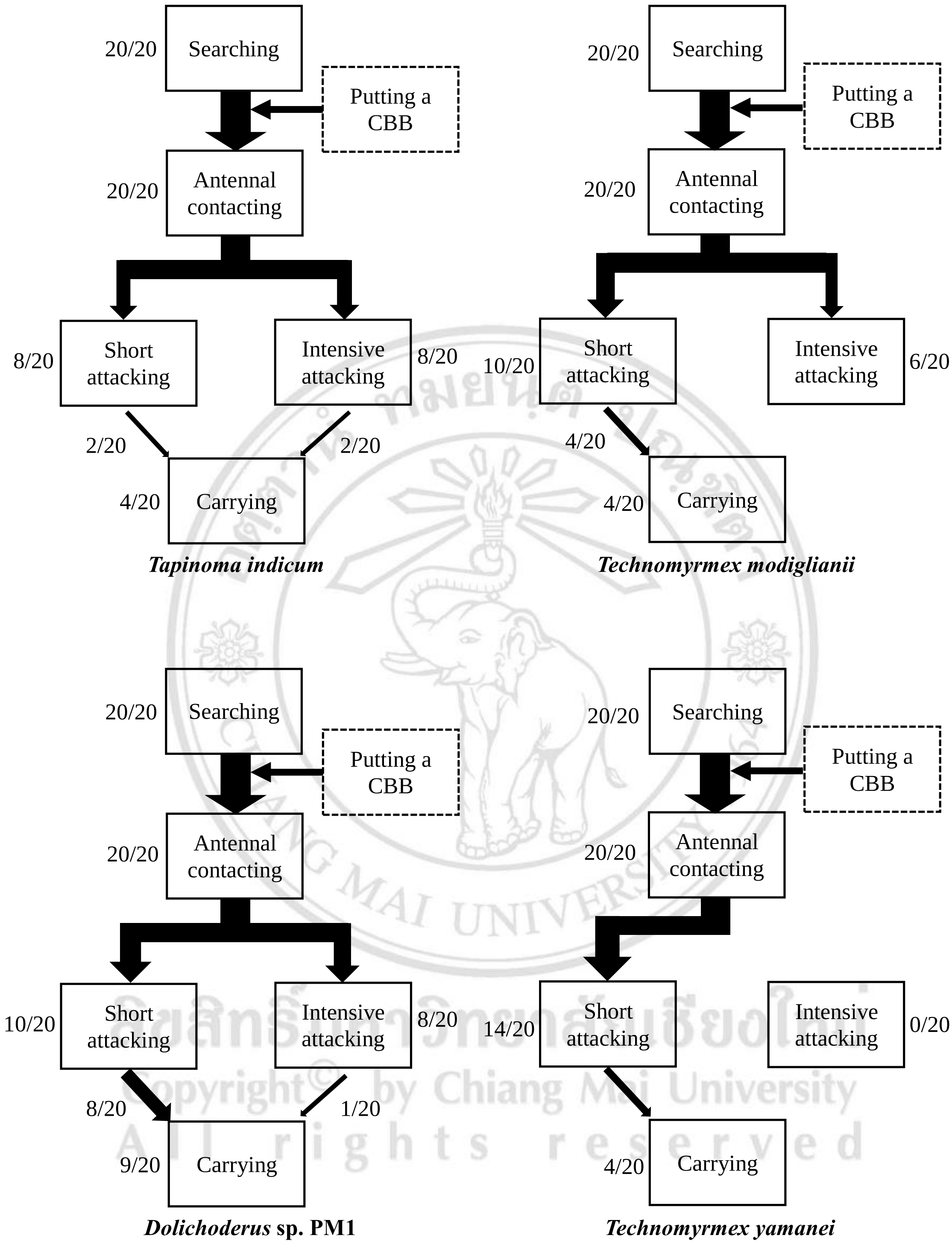


Figure 3.11 The sequence of behaviors of seven ant species in the presence of a CBB in a plastic case under the laboratory. The number shows the occurrence of each behavior (n=20).

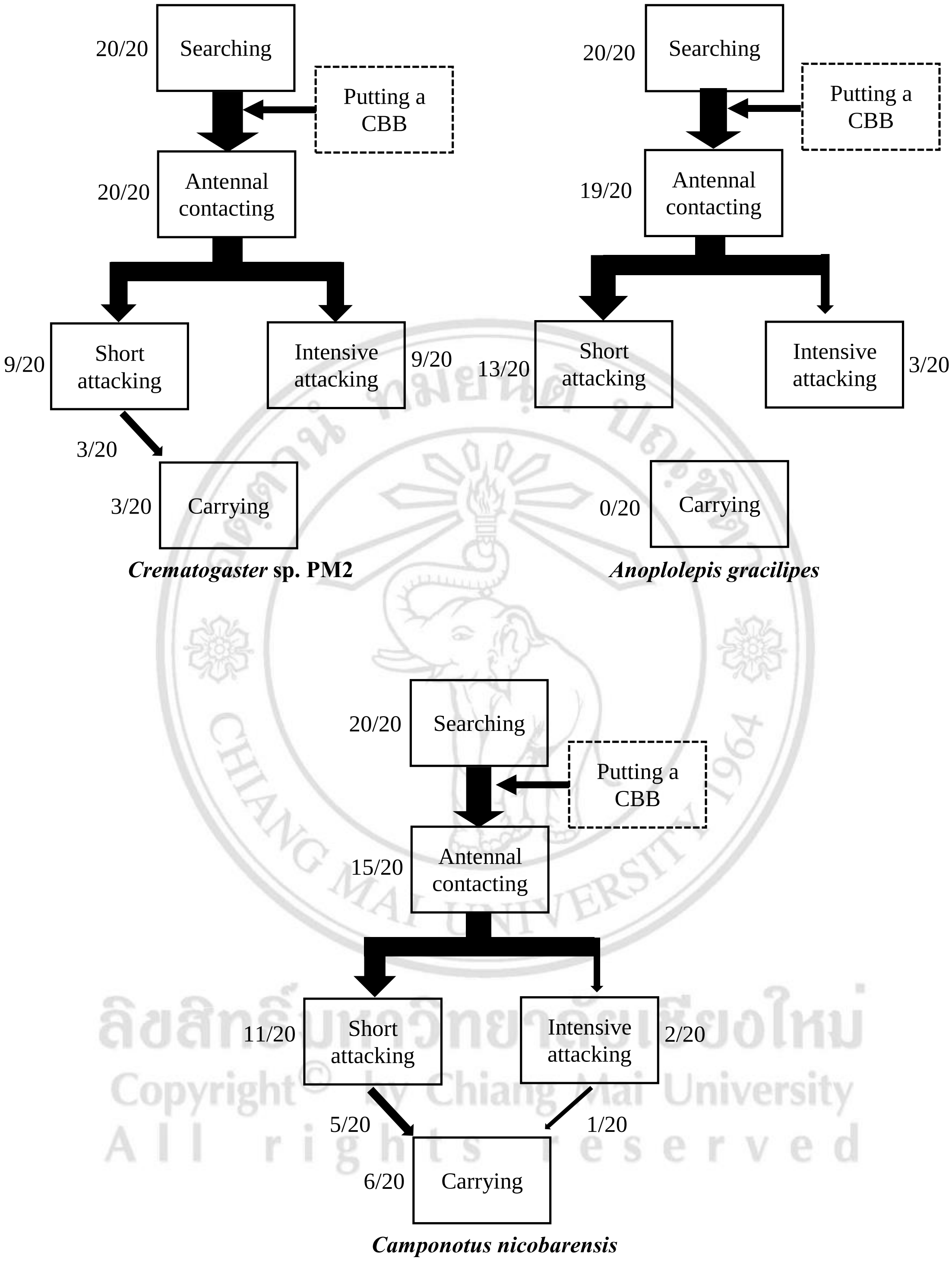
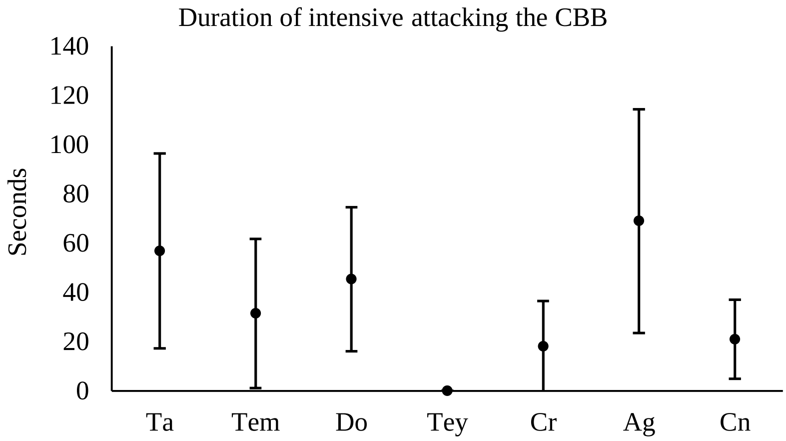


Figure 3.11 (CONTINUED)



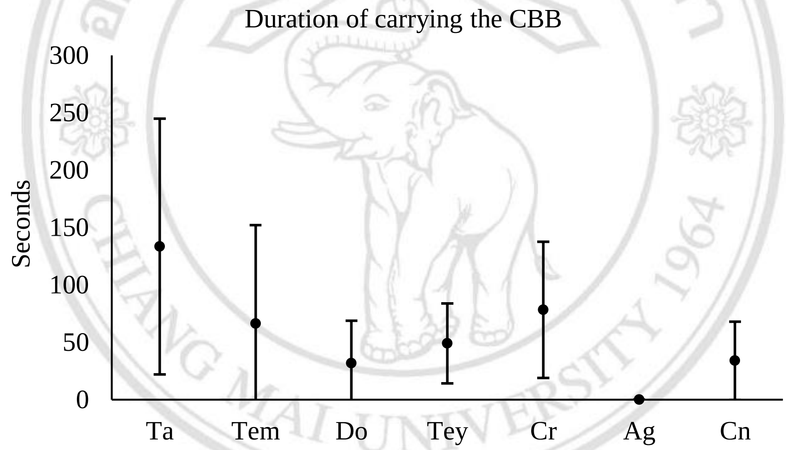


Figure 3.12 Average duration of each behavior performed by each ant species. Ta: *Tapinoma indicum*; Tem: *Technomyrmex modiglianii*; Do: *Dolichoderus* sp. PM1; Tey: *Technomyrmex yamanei*; Cr: *Crematogaster* sp. PM2; Ag: *Anoplolepis gracilipes*; Cn: *Camponotus nicobarensis.*

Figure 3.13 The workers of *Tapinoma indicum* gathered around a CBB

After the short or intensive attacks, all workers of *A. gracilipes* (N = 16) walked away but some workers of the other ant species picked up the CBB with their mandibles and carried it to other places in the foraging arena. The ratio of workers that carried the CBB after attacking in *A. gracilipes* was significantly lower than that of *Dolichoderus* sp. PM1 ($p = 0.023$) and *T. yamanei* ($p = 0.023$). The duration of carrying was not significantly different among species, however, the efficiency of carrying CBB seemed to be different among species. Workers of *C. nicobarensis* and *Dolichoderus* sp.PM1 readily picked up the CBB and carried it, while workers of the other species often failed to manipulate the CBB. No workers of any species carried the CBB to their nest chambers, and they never fed on the CBB.

If "intensive attacking" and "carrying" were regarded as "strong interference behavior", the ratio showing the strong interference behavior in *A. gracilipes* was significantly less than that of *Dolichoderus* sp. PM1 ($p < 0.001$).

Figure 3.14 shows the body size of each seven ant species. Two relatively large sized ants, *Anoplolepis gracilipes* and *Camponotus nicobarensis*, seem to be less effective among the seven ant species we tested. *A. gracilipes* which workers never showed carrying behavior, and intensive attacks were observed only three times. *C. nicobarensis* workers often ignored the CBB, and also intensive attacks were rare. In ants, worker body size affects prey size selection as shown in some seed harvesting ants (Kaspari, 1996).

For larger sized ants, the adults of CBB may be too small to be hunted as prey. Among the five other ant species, *Dolichoderus* sp. PM1 seems to be the most effective biological control agent (Figure 3.15), because of the frequent interference behavior against the CBB (Figure 3.16). Furthermore, the workers can manipulate the adult CBB very well. Although the frequency is rather low, approx. 50% of encounters resulted in strong interference behavior in the other four species. Especially in *T. indicum*, the duration of both intensive attacks and carrying was relatively long (Figure 4.7), although there was no statistical difference when compared to the other ants. The most dominant ant species in the coffee plantation in Pa Miang (Onishi *et al.* 2016), *Technomyrmex yamanei,* showed a unique behavioral response: they rarely performed intensive attacks, however, they frequently carried the CBB. Thus, *T. yamanei* may have a negative effect on the CBB.

As in former studies in Central and South America (Gonthier *et al*., 2013), relatively smaller ants seem to be effective for controlling the adult CBB in Thailand. Because they can enter the galleries made by the CBB (Gonthier *et al*., 2013), they can also attack immatures of the CBB inside the berries. The effects of such small ants on the survival of CBB immatures should be investigated for evaluating the usefulness of ants for biological control of the CBB in Thailand.

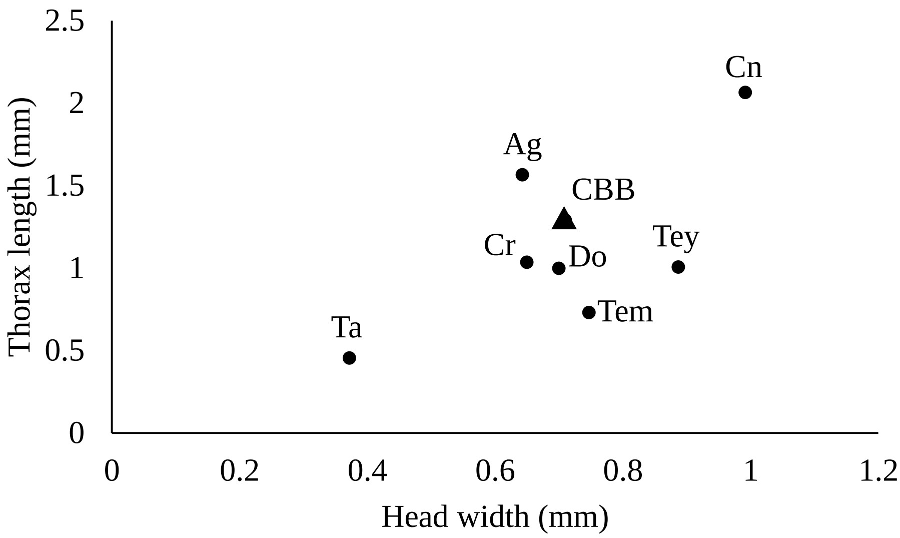


Figure 3.14 The body size of each ant species and the CBB. Ta: *Tapinoma indicum*; Tem: *Technomyrmex modiglianii*; Do: *Dolichoderus* sp. PM1; Tey: *Technomyrmex yamanei*; Cr: *Crematogaster* sp. PM2; Ag: *Anoplolepis gracilipes*; Cn: *Camponotus nicobarensis.*

Figure 3.15 A worker of *Dolichoderus* sp. PM1 did antennal contacting to a CBB.

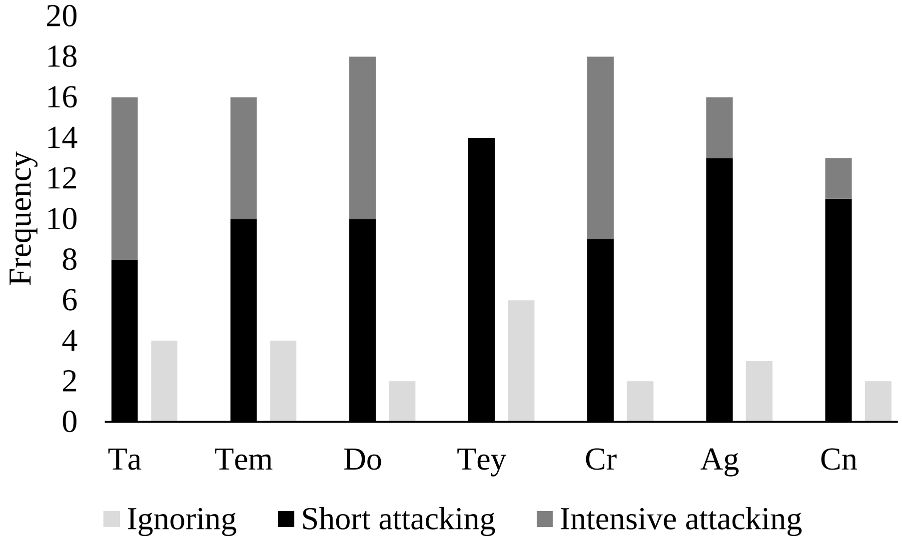


Figure 3.16 Frequency of each behavior displayed by the seven ant species reacted to the CBB. Ta: *Tapinoma indicum*; Tem: *Technomyrmex modiglianii*; Do: *Dolichoderus* sp. PM1; Tey: *Technomyrmex yamanei*; Cr: *Crematogaster* sp. PM2; Ag: *Anoplolepis gracilipes*; Cn: *Camponotus nicobarensis*.