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Research Article

Obligate Ant-Associated *Macaranga bancana* is Better Protected from Herbivory Than Facultative Ant-Associated *Macaranga tanarius*

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ABSTRACT

Protective mutualism between ant and *Macaranga* plants are complex between-species interactions found only in the tropical environment. In such interactions, plants provide housing structures (in the form of domatia) and food (in the form of food bodies) to their ant symbionts. In return, the ants protect their *Macaranga* plant hosts against herbivore attacks. *Macaranga* ant protective mutualism is manifested in a wide range of interactions, from facultative to obligate. In facultative interactions, *Macaranga* plants attract predatory ants to the plant *via* food rewards. In return, foraging ants may opportunistically provide protection from insect herbivores. In obligate interactions, plants provide shelter and food rewards to permanent ant partners. We hypothesize that in obligate *Macaranga,* the host is better protected because of the permanent presence of its resident ant partners, whereas in facultative *Macaranga*, the defense against insect herbivores may be less efficient depending on the attractiveness of the food rewards and the aggressiveness of the ant species in the plant's vicinity. In this study, we compare herbivory damage and phytophagous insect herbivore types between a facultative ant-plant *M. tanarius* and an obligate anti-plant *M. bancana*. This study further highlights that co-evolved ant partners are more efficient in defending their host plants against phytophagous insect herbivores than facultative ant-plant interactions.

Keywords
Facultative; Obligate; Phytophagous Insects; Herbivores; *M. bancana*; *M. tanarius*

Introduction

Phytophagous insects negatively impact a plant’s growth and reproductive success (Marquis, 1984; Marquis & Braker, 1994). In tropical forests, an estimated 11% of the annual leaf area produced is consumed by phytophagous insects and pathogens (Coley & Kursor, 1996) which is equivalent to the plant's investments in reproduction (Bazzaz et al., 1987). Over the course of the plant's defensive evolution, various strategies have been developed (Coley & Barone, 1996), a chief strategy being various kinds of protective mutualism with ant partners (Rosumek et al., 2009).

Protective mutualism between ants and plants is a common strategy adopted by the pioneer tree genus *Macaranga* (Euphorbiaceae), which is found throughout pristine and disturbed forests in Southeast Asia (Whitmore, 2008). Ant interactions within the *Macaranga* genus ranges in specificity from facultative (myrmecophilous) to obligate (myrmecophytic) (Fiala et Al., 1999). Facultative *Macaranga* plants are characterized by deriving their defense against herbivores from free-ranging opportunistic or predatory ants that forage on the plant. In exchange, ants receive direct or indirect food rewards from plants in the form of food bodies (FB) that are scattered widely on the dorsal surfaces of their leaves (Fiala & Maschwitz, 1991). These food bodies are rich in sugars, amino acids, lipids, phenols, alkaloids, and volatile compounds (González-Teuber & Heil, 2009), and have been linked to increase in colony growth and survivorship (Byk & Del-Claro, 2011). On the other end of the mutualism scale, obligate *Macaranga* species are characterized by the presence of FB that are contained within specialized structures known as stipules as well as nesting chambers called domatia that are structuralized by the plants for their specific ant partners (Fiala & Maschwitz, 1991; Linsenmair et al., 2001; Feldhaar & Fiala, 2021).

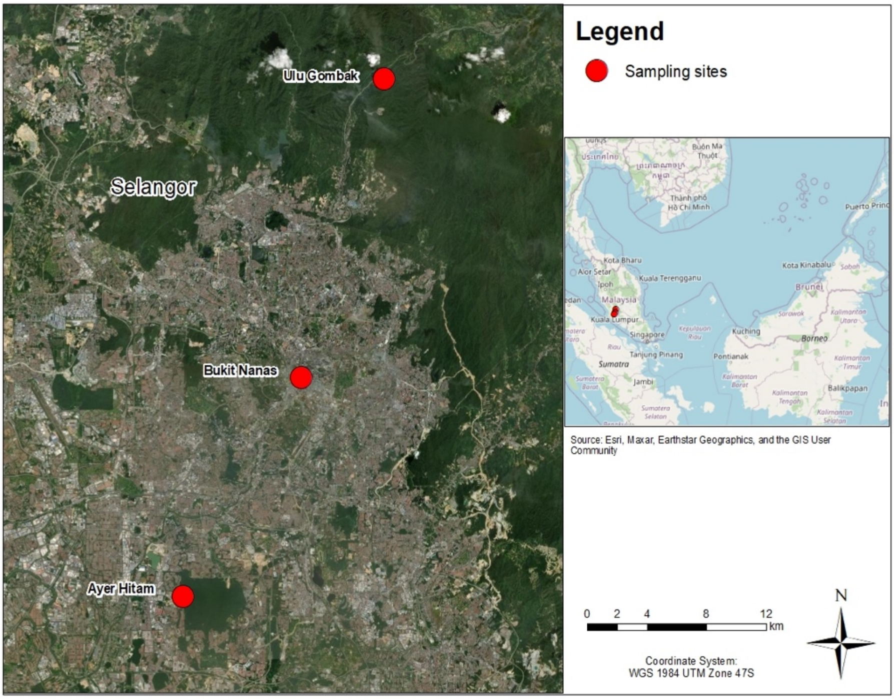
As pioneer trees typically make up the dominant species in their habitat, they are prone to suffering from proportionately larger amounts of damage from herbivores present. However, ant-associated *Macaranga* species are generally well-defended against all kinds of insect herbivores, such as lepidopteran larvae, beetles and grasshoppers (Fiala et al., 1989; 1994). Nevertheless, herbivore damage is most severe in seedlings and was observed to decrease as the trees grow (Itino & Itioka, 2001). As seedlings have a higher proportion of young, expanded leaves and shoots, the ants' defense is concentrated on these plant parts (Heil et al., 2004) which is consistent with higher secretion of FB and EFN in these plant parts as well (Heil et al., 2000). Although all ant-associated *Macaranga* uses food rewards to elicit ant-defense strategies, there is evidence that indicates defense resulting from facultative interactions are less efficient than defense provided by obligate ones (Fiala et al. 1994, 1989; Linsenmair et al. 2001).

Unlike the opportunistic ant partners of facultative *Macaranga* species, the ant partners of obligate *Macaranga* species are wholly dependent on their host. Each tree is host to only one ant colony (Fiala & Maschwitz, 1990), that are confined within the domatia. Additionally, these obligate ant partners sustain themselves primarily on FBs produced by the plant. Because of this dependency, we hypothesize that obligate ant partners are more invested in the protection of their host plant than the opportunistic ants in facultative relationships. As such we aim to test the following hypothesis; that the obligate *M. bancana* is better protected against insect herbivores than the facultative *M. tanarius,* and also attempt to provide insights on whether the different level of effectiveness is due to the kinds of herbivores that feed on two *Macaranga species*. This study is the first of a series that seeks to examine the protective mutualism of pioneer *Macaranga* species in human-disturbed forest edges.

Methods

Study sites

The sampling areas consisted of three forest edge sites in the State of Selangor, Malaysia (**Figure 1**). The first site is located at Bukit Nanas (BN - 3°9’N, 101°42’N), the second site at Ayer Hitam Forest Reserve (AH - 3°1’N, 101°37N), and the third site at Ulu Gombak (GB - 3°19’N, 101°45’E). Sampling was carried out between the weekends of June to August 2020. These three sites were composed of secondary growth vegetation, predominantly pioneer tree species such as *Macaranga*. The sites were relatively open and exposed to sunlight with lush plant undergrowth. From June to August, selected trails BN, AH and GB were surveyed by SST, DYCY and SHY for *Macaranga* spp. populations, and the two most abundant species of *Macaranga* were selected for this study, i.e., *Macaranga bancana* and *Macaranga tanarius*.



Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community

Figure 1. Three sampling sites (black circles) of human-disturbed forest edges in Selangor. The sampling sites are either hiking trails (Bukit Nanas - BN and Ayer Hitam Forest Reserve - AH) or along the road (Ulu Gombak - GB) of the forest tracts.

Macaranga species

Macaranga bancana is a myrmecophytic plant that has an obligate interaction with the *Crematogaster borneensis* ant group (Fiala et al., 1989). Conversely, *Macaranga tanarius* is a myrmecophilous plant that maintains facultative interactions with a wide range of generalist forager and predatory ants (Calixto et al., 2018) such as *Anoplolepis gracilipes* and *Tetraponera sp*. that can be commonly found in the same habitat. In *M. bancana*, nesting space in the form of domatia is provided to the *C. borneensis* ant partner that is not present in *M. tanarius*. Instead, *M. tanarius* possesses 5 extrafloral nectaries on the adaxial edge of its leaves (Fiala & Maschwitz, 1991) and food bodies scattered over leaf surfaces, petioles and stems (Heil et al., 2000) to attract patrolling ants to defend them against herbivores. Extrafloral nectaries are absent in *M. bancana* and while it does produce food bodies, these are protected within specialized structures known as stipules that prevent access to any insect other than its ant partners. In both M. bancana and M. tanarius, development of food bodies substantially reduces when the trees achieve a height of > 3–4m. Additionally in

M. tanarius stops producing extrafloral nectaries altogether once the plant has achieved a height of > 4m (Fiala & Maschwitz, 1991). This suggests that there are growth-stage dependent trade-offs herbivory protection derived from both facultative and obligate ant partners (Fiala et al., 1989; Heil et al., 2000).

Herbivory damage survey

In general, young leaves of obligate and facultative *Macaranga* species rely strongly on biotic (*i.e.*, ants) defense against insect herbivory, whereas more mature leaves employchemical and physical defenses to repel herbivores (Folgarait & Davidson 1995). Because biotic ant defenses tend to be focused on young leaves, and ae no longer prioritized by plants > 3m, we chose the top three leaves of *M. bancana* and *M. tanarius* < 2 m to quantify leaf herbivory. *M. bancana* saplings were visually inspected for the presence of domatia. Plants with no symbiont ant entrance were excluded from the survey as these young saplings cannot house ants' partners until the domatia are structuralized (Frederickson et al., 2012).

Images of the top three leaves were captured by SST using a mobile phone camera and the images were transferred to a desktop computer for processing using ImageJ software (Abràmoff et al., 2004). We excluded leaf area loss during the image processing due to mechanical damage, and only quantified leaf area loss from herbivore damage by identifying the presence of insect feeding marks of external chewers, as well as the track-like patterns left behind by leaf miners along the edges of missing portions on the leaf. The mean herbivory damage of the three leaves was computed, and this value was taken to the herbivory damage of each *Macaranga* tree.

Herbivore type survey

Lepidopteran larvae, beetles (both larvae and adults), as well as grasshoppers, are some of the more common phytophagous insects that can be found on *Macaranga* (Fiala et al., 1989; 1994). However, we were not always able to observe the actual insects on the sampled plants. As such, we attempted to identify the herbivores based on the characteristics of recorded feeding marks (e.g., Shimizu-Kaya et al., 2021). ZHW scored the herbivory types blindly based on the images captured by SST and grouped them into the following categories: (i) large phytophagous insects (BI) that left large sized bite marks; (ii) small phytophagous insects (SI) that left small sized bite marks (Fiala et al., 1989, 1994); and (iii) leaf miners (LM) who make track-like feeding patterns. We also noted down fungal infection on the leaves that were typically present as discoloured spots. For leaf damage that SST and WZH could not confidently assess as being caused by either herbivores or mechanical damage, a category of unknown was assigned. The classification of the herbivory damage from external chewers based on size was done to investigate whether the size of herbivore invaders had any impact on the efficacy of the biotic defenses of *M. bancana* due to the small size of *Crematogaster borneensis* workers (0.36 – 0.43 mm).

Figure 2. Examples of feeding marks left by small phytophagous insects (left) and large phytophagous insects (right) on *M. tanarius* leaves that were sampled from the GB field site.

We observed that one leaf could have more than one type of herbivore damage. We included the type of herbivore damage from all three replicate leaves for one tree in our computation of the herbivory damage data. As such, we found that one tree could have only one type of herbivore damage, or a maximum of five damage types (*i.e.*, large phytophagous insects, small phytophagous insects, leaf miners, fungal infection and unknown).

Statistical analysis

To test the effect of facultative or obligate interactions with ants on the amount of herbivory damage incurred across the two sampled *Macaranga* species, we combined the data from all three sites. We used two independent sample tests to determine whether there was a significant difference in herbivory damage (calculated as leaf-loss percentage) between the two *Macaranga* species. Herbivory data did not violate the assumption of homogeneity of variances (Levene test). However, the herbivory data violated the normality assumption

(Shapiro-Wild test). Q-Q plot demonstrated that the herbivory data had skewed distributed residuals, which precluded the use of frequentist parametric tests.

Since we did not sample *M. tanarius* at AH (**Figure 3**), we could not perform statistical tests to look at the effect of facultative interactions at this site. As such, this site was excluded in the two-sample comparison tests we conducted. We chose a frequentist non-parametric Mann-Whitney test since the herbivory damage data violated the normality assumption, and we rejected the null hypothesis when $P < 0.0001$ (**Table 1**). Simultaneously, we also performed Bayesian Mann-Whitney U Test (BayesFactor – BF; Morey & Rounder, 2015) because the Bayesian framework could supplement the frequentist p-value (Rounder et al. 2012). Typically, BF10>1 is used to quantify evidence in favour of the alternative hypothesis. Ultimately, we based our conclusions on the inference of both frequentist (*p*-value) and Bayesian (BF10) tests. All statistical tests were performed using JASP software (version 0.16.4; JASP Team 2022).

To examine the relation between herbivore types and *Macaranga* species, we performed a chi-square test of independence. Chi-square tests of independence were conducted using the statistical software R ver. 4.2.1. (R Core Team, 2022)

Results

Macaranga species at study sites

Altogether, we found 112 *Macaranga* trees across all three sites that fit into our survey criteria, which are trees < 2m in height. At Ayer Hitam Forest Reserve (AH), all *M. tanarius* trees were > 2 m in height and therefore only *M. bancana* were sampled from that site. At both Bukit Nanas (BN) and Ulu Gombak (GB), both *Macaranga* species f similar sizes occurred sympatrically, although the proportion of similarly sized *M. bancana* and *M. tanarius* was uneven. At BN, around 65% of *Macaranga* trees < 2 m were *M. bancana*, whereas at GB, *M. bancana < 2m composed* only 25% of *Macaranga* trees at this field site (**Figure 3**). In total, 53 *M. bancana* species (AH=24, BN=11, GB=18) and 59 *M. tanarius* (BN=6, GB=53) were surveyed for leaf herbivory damage and scored for herbivory types.

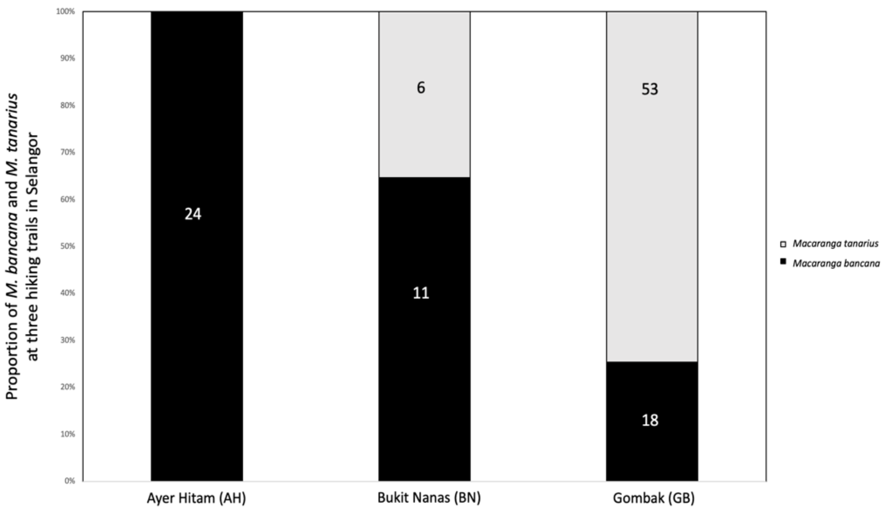


Figure 3. The proportion of *Macaranga bancana* (myrmecophyte) and *Macaranga tanarius* (myrmecophile) sampled at three sampling sites. At Ayer Hitam Forest Reserve (AH), only *M. bancana* were sampled as *M. tanarius* at AH were > 2 m in height, hence excluded from our selection criteria. At BN, *M. bancana* dominates (65%) and at GB, *M. tanarius* dominates (75%).

Table 1. Null hypothesis of no *Macaranga* species effect on herbivory were rejected based on Frequentist (F=765.000, P > 0.001) and Bayesian (BF10 = 84.168) tests.

	Frequentist Test	**Statistic**	***p***
Herbivory	Mann-Whitney	765.000	< 0.001
	Bayesian Test		**BF_{10}**
Herbivory	Mann-Whitney U		84.168

	Df	**Sum sq**	**Mean sq**	***F*-value**	***p***
Macaranga Species	1	128.6	128.58	5.68	0.019*
Site	2	119.5	59.74	2.64	0.076
Species x Location	1	0.7	0.67	0.03	0.864
Residuals	107	2423.5	22.65		

Leaf herbivory damage

The herbivory damage ranged from 0.31% to 24.72%. Two independent tests (Mann-Whitney frequentist and Bayesian; **Table 1**) revealed that facultative and obligate interactions in *Macaranga* species have a statistically significant effect on leaf herbivory damage. We found that *M. bancana* across all sites experienced less herbivory damage (mean = 3.61%, SD-4.93) than *M. tanarius* (mean=5.76%) (**Figure 4**).

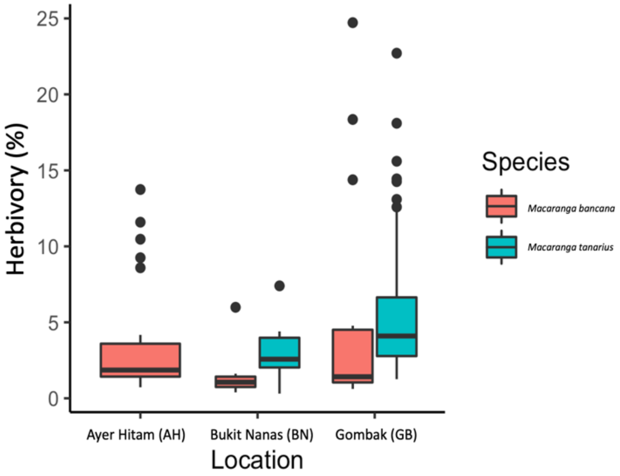


Figure 4. Boxplots displaying the percentage of leaf loss due to phytophagous insect herbivores on myrmecophyte *Macaranga bancana* (red) and myrmecophilous *Macaranga tanarius* (green) across three sampling sites - Ayer Hitam (AH), Bukit Nanas (BN) and Gombak (GB). Only *M. bancana* were surveyed at AH. Leaf loss due to herbivores varies across sites with *M. tanarius* suffering significantly more leaf loss due to herbivores damage compared to *M. bancana.*

Types and numbers of herbivores damage

We observed no significant difference between the categories of 5 herbivore types and the two *Macaranga* species ($\chi 2$=8.85, *p*=0.07). The dominant herbivores types across sites and *Macaranga* species were small phytophagous insects such as lepidopteran larvae (Figure 4). In *M. bancana,* large phytophagous insects caused up to 15.5% leaf herbivory damage, followed by leaf-miners (8.5%). On *M. tanarius,* although the dominant leaf herbivores are small phytophagous insects (48.4%), leaf-miner and large phytophagous insects were observed to be responsible for similar amounts of leaf-herbivory damage as *M. bancana* (21.3% and 20.2% respectively) (**Figure 5**). The proportion of herbivore type damage appears to vary slightly between sites, although not significantly (Supplementary **Figure 1**), likely due to slight variations in the composition of insect herbivores at the respective locations. Fungal damage was detected at some sites (AH and GB) but absent from saplings in BN. At AH, fungal infections were detected on *M. bancana* whereas at GB, fungal infections were only detected on *M. tanarius*, albeit both at low infection percentage (6.9% and 3.8% respectively)(supplementary **Figure 1**).

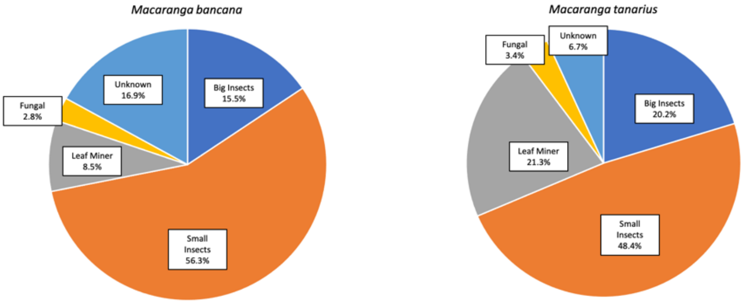


Figure 5. Pie charts of proportion of leaf loss found on *Macaranga bancana* and *Macaranga tanarius* from different herbivore types. The dominant herbivore types are small phytophagous insects (orange), followed by large phytophagous insects (dark blue). In *M. tanarius*, leaf-miner (grey) and large phytophagous insects (dark blue) cause a similar proportion of herbivore damage. Fungal infection (yellow) were observed from both *M. bancana* and *M. tanarius*.

Discussion

This study characterizes the herbivory damage and herbivore types experienced by two pioneer *Macaranga* species that often occur sympatrically in hiking trails and human-disturbed forest edges. The proportion of similar aged *M. bancana* and *M. tanarius* were unevenly distributed ross the three study sites. For example, at AH, only *M. bancana* was sampled as it dominated the landscape and we could not locate any *M. tanarius* (< 2m height) that fit our selection criteria. *M. bancana* also dominated the population at BN (around 65%) whereas *M. tanarius* dominated at GB (around75%) (**Figure 3**). The herbivory damage ranged from 0.31% to 24.72% with *M. bancana* across all sites experiencing less herbivory damage (mean=3.61%) than *M. tanarius* (mean=5.76%) (**Figure 4**). The dominant herbivore types across sites and *Macaranga* species are small phytophagous insects, followed by large phytophagous insects and leaf miners (**Figure 4**). The proportion of herbivore type damage appears to vary between sites, although not significantly (**Supplementary Figure 1**).

Macaranga distribution at secondary forest sites

Both *Macaranga* species are light-demanding pioneer trees that naturally grow in secondary forest, along riverbanks or in forest gaps (Feldhaar & Fiala, 2021). In our survey, both species occurred sympatrically. These forest sites are all remnants of once larger tracts of primary forests, which have undergone land encroachment from different types of human activities (Omar et al., 2013; Nurul-Shida et al., 2014; Salleh et al., 2017). There are only two common *Macaranga* species that persist at the forest edges and hiking trails at these sites. The composition of these two *Macaranga* species varies with *M. bancana* saplings dominating at BN and *M. tanarius* dominating at GB (**Figure 2**) possibly due the interplay of abiotic (*e.g.*, light, soil, drainage) and biotic (ants availability, herbivory, competition from other plants) factors. Future studies can benefit from surveying *Macaranga* species at different growth stages, and examining the trade-offs of protective strategies employed by *Macaranga* species at different growth stages.

Ants role in reducing leaf herbivory damage in Macaranga species

We found that the obligate *M. bancana* species experienced less herbivory damage than the facultative *M. tanarius* (**Figure 4**). These findings are consistent with evidence from past ant-exclusion experiments that have revealed similar results (Fiala et al., 1994; 1989; Linsenmair et al., 2001). One of the reasons obligate plants are better protected could be due to the different ant-defense strategies. In obligate *Macaranga* species, ants association is established early on in the plants' ontogenetic stages (ants were observed colonizing 5.5 cm tall seedlings). The relationship between the ant symbionts and their plant hosts remains stable throughout the ontogenetic development of the host plant. Hence, protection against phytophagous insects remains consistent across plants of similar sizes that are able to provide sufficient shelter for the ants in the form of domatia, and sustenance in the form of food bodies (Itino & Itioka, 2001). In contrast, facultative *Macaranga* species have adopted an induced biotic defense strategy *via* regulating FBs and EFNs secretion based on herbivory damage level (Lange et al., 2017) as well as the plants' sizes (Heil et al., 2000; 2004; Itino et al., 2001; Murase et al., 2003). The effectiveness of employing opportunistic and predatory ant species as a form of herbivory defense in facultative *Macaranga*, thus, depends on the composition and aggressiveness of ants available in the vicinity (Del-Claro & Marquis, 2015; Fagundes et al., 2017).

Ants partners' time investment in removing phytophagous insects could play an important role in minimizing herbivore damage on host plants. In obligate *Macaranga* species, symbiont ant partners rely solely on the FBs provided by their host plant (Fiala & Maschwitz, 1991) for sustenance. Herbivorous insects that are encountered, are therefore, not hunted and consumed, but were observed to have been dragged away by the ants, and then thrown off the plant surface (Linsenmair et al., 2001)(Supplementary **Figure 2**). In contrast, myrmecophilous *Macaranga* ants feed on EFNs and various other types of resources, for example, hemiptera exudates ('honeydew'), captured phytophagous insects, and dead arthropods (Hölldobler & Wilson 1990; Blüthgen & Feldhaar 2010; Cerdá & Dejean 2011) while they are on the host plant, reducing the time investment into phytophagous insects removal. Future studies should record the ants composition, aggressiveness and time spent performing different foraging activities on their respective *Macaranga* host plants.

Herbivore types and numbers on Macaranga species

We employed an indirect quantification method in our classification of the phytophagous insects types. From the bite sizes and patterns of feeding marks, we found that the dominant herbivory types for *M. bancana* and *M. tanarius* are small phytophagous insects (**Figure 5**), which is consistent with Fiala et al. (1994) findings. Large phytophagous insects were the second dominant herbivory type (**Figure 5**) although the *Crematogaster borneensis* ant symbionts that dwell on the obligate *M. bancana* was found to be ineffective in removing these types of phytophagous insects (Fiala et al., 1989). The effectiveness of ant partners in removing large phytophagous insects on *M. tanarius* would vary with different ant associations (Itino et al., 2001; Heil et al., 2004; Murase et al., 2003). Hence, the variation in the proportion of herbivore types between sites (**Supplementary Figure 1**) could be due to the relative aggressiveness of the attracted ant species on the *M. tanarius plants which we sampled* (**Supplementary Figure 1b**) and/or spatial distribution and diversity of phytophagous insect assemblages at different sites (*e.g.* Oliveira & Del-Claro 2005). Future studies should aim to determine the particular phytophagous insect species specific to *M. bancana* or *M. tanarius* at different sites. A methodology that combines direct observations, rearing (Ødegaard et al., 2005; Weiblen et al., 2006) and recovery of environmental DNA from the external foliar feeding marks (Kudoh et al., 2020) can be employed for such purposes.

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References

Abràmoff MD, Magalhães PJ, Ram SJ. 2004. Image processing with ImageJ. *Biophotonics Int.*, ***11*** (7): 36–42.

Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF. 1987. Allocating resources to reproduction and defense. *BioScience,* ***37*** (1): 58–67.

Blüthgen N, Feldhaar H. 2010. Food and shelter: how resources influence ant ecology. In: Lach L, Parr C, Abbott K, editors. *Ant ecology*. Oxford: Oxford Academic; pp.115–136.

Byk J, Del-Claro K. 2011. Ant-plant interaction in the Neotropical savanna: direct beneficial effects of extrafloral nectar on ant colony fitness. *Popul. Ecol.*, ***53*** (2): 327–332.

Calixto ES, Lange D, Del-Claro K. 2018. Protection mutualism: an overview of ant-plant interactions mediated by extrafloral nectaries. *Oecologia Aust.*, ***22*** (4): 410–425.

Cerdá X, Dejean A. 2011. Predation by ants on arthropods and other animals. In: Polidori C, editor. *Predation in the Hymenoptera: an evolutionary perspective*. Kerala (India): Transworld Research Network; pp. 39–78.

Coley PD, Barone J. 1996. Herbivory and plant defenses in tropical forests. *Annual review of ecology and systematics,* ***27:*** 305–335.

Coley PD, Kursor TA. 1996. Anti-herbivore defenses of young tropical leaves: physiological constraints and ecological trade-offs. In: Mulkey SS, Chazdon RL, Smith AP, editors. *Tropical forest plant ecophysiology*. Boston (MA): Springer; pp. 305–336.

Del-Claro K, Marquis RJ. 2015. Ant species identity has a greater effect than fire on the outcome of an ant protection system in Brazilian Cerrado. *Biotropica,* ***47*** (4): 459–467.

Fagundes R, Dáttilo W, Ribeiro S, Rico-Gray V, Jordano P, Del-Claro K. 2017. Differences among ant species in plant protection are related to production of extrafloral nectar and degree of leaf herbivory. *Biol. J. Linn. Soc.*, ***122*** (1): 71–83.

Feldhaar H, Fiala B. 2021. Ant Plants: Macaranga. In: Starr CK, editor. *Encyclopedia of Social Insects.* Cham (Switzerland): Springer; pp. 41–45.

Fiala B, Grunsky H, Maschwitz U, Linsenmair KE. 1994. Diversity of ant-plant interactions: protective efficacy in Macaranga species with different degrees of ant association. *Oecologia, **97*** (2): 186–192.

Fiala B, Jakob A, Maschwitz U, Linsenmair KE. 1999. Diversity, evolutionary specialization and geographic distribution of a mutualistic ant-plant complex: Macaranga and Crematogaster in South East Asia. *Biol. J. Linn. Soc., **66*** (3): 305–331.

Fiala B, Maschwitz U. 1991. Extrafloral nectaries in the genus Macaranga (Euphorbiaceae) in Malaysia: comparative studies of their possible significance as predispositions for myrmecophytism. *Biol. J. Linn. Soc., **44*** (4): 287–305.

Fiala B, Maschwitz, U. 1990. Studies on the South East Asian ant-plant association *Crematogaster borneensis/Macaranga:* Adaptations of the ant partner. *Insectes Sociaux, **37:*** 212–231.

Fiala B, Maschwitz U, Pong TY, Helbig AJ. 1989. Studies of a South East Asian ant-plant association: protection of Macaranga trees by *Crematogaster borneensis*. *Oecologia, **79*** (4): 463–470.

Folgarait PJ, Davidson DW. 1995. Myrmecophytic Cecropia: antiherbivore defenses under different nutrient treatments. *Oecologia, **104*** (2): 189–206.

Frederickson ME, Ravenscraft A, Miller GA, Arcila Hernández LM, Booth G, Pierce NE. 2012. The direct and ecological costs of an ant-plant symbiosis. *Am. Nat., **179*** (6): 768–778.

González-Teuber M, Heil M. 2009. Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. *Plant Signal. Behav.., **4*** (9): 809–813.

Heil M, Feil D, Hilpert A, Linsenmair KE. 2004. Spatiotemporal patterns in indirect defence of a South-East Asian ant-plant support the optimal defence hypothesis. *J. Trop. Ecol.,* **20** (5): 573–580.

Heil M, Fiala B, Baumann B, Linsenmair K. 2000. Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. *Funct. Ecol., **14*** (6): 749–757.

Hölldobler B, Wilson EO. 1990. *The ants*. Cambridge (MA): Belknap Press of Harvard University Press.

Itino T, Itioka T. 2001. Interspecific variation and ontogenetic change in antiherbivore defense in myrmecophytic Macaranga species. *Ecol. Res., **16*** (4): 765–774.

Kudoh A, Minamoto T, Yamamoto S. 2020. Detection of herbivory: eDNA detection from feeding marks on leaves. *Environ. DNA., **2*** (4): 627–634.

Lange D, Calixto ES, Del-Claro K. 2017. Variation in extrafloral nectary productivity influences the ant foraging. *PloS one, **12*** (1): e0169492.

Linsenmair KE, Heil M, Kaiser WM, Fiala B, Koch T, Boland W. 2001. Adaptations to biotic and abiotic stress: Macaranga-ant plants optimize investment in biotic defence. *J. Exp. Bot., **52*** (363): 2057–2065.

Marquis RJ. 1984. Leaf herbivores decrease fitness of a tropical plant. *Science, **226*** (4674): 537–539.

Marquis RJ, Braker HE. 1994. Plant-herbivore interactions: diversity, specificity, and impact. In: McDade LA, Hartshorn GS, Hespenheide HA, Bawa KS, editors. La Selva: *ecology and natural history of a neotropical rainforest*. Chicago: University of Chicago Press; pp. 261–281.

Murase K, Itioka T, Nomura M, Yamane S. 2003. Intraspecific variation in the status of ant symbiosis on a myrmecophyte, Macaranga bancana, between primary and secondary forests in Borneo. *Popul. ecol., **45*** (3): 221–226.

Nurul-Shida S, Faridah-Hanum I, Wan Razali W, Kamziah K. 2014. Community structure of trees in Ayer Hitam Forest Reserve, Puchong, Selangor, Malaysia. *Malaysian For., **77*** (1): 73–86.

Ødegaard F, Diserud OH, Østbye K. 2005. The importance of plant relatedness for host utilization among phytophagous insects. *Ecol. Lett., **8*** (6): 612–617.

Oliveira P, Del-Claro K. 2005. Multitrophic interactions in a neotropical savanna: ant-hemipteran systems, associated insect herbivores and a host plant. In: Burslem DFRP, Pinard MA, Hartley SE, editors. *Biotic interactions in the tropics: their role in the maintenance of species diversity*. Cambridge (UK): Cambridge University Press; pp. 414–438.

Omar H, Hashim R, Bhassu S, Ruedi M. 2013. Morphological and genetic relationships of the *Crocidura monticola* species complex (Soricidae: Crocidurinae) in Sundaland. *Mamm. Biol., **78*** (6): 446–454.

R Core Team. 2022. R: A language and environment for statistical computing. v4.2.1. Available at https://www.R-project.org/ (accessed 16 August 2022)

Rico-Gray V, Oliveira PS. 2008. *The ecology and evolution of ant-plant interactions*. Chicago: University of Chicago Press.

Rosumek FB, Silveira FA, de S Neves F, de U Barbosa NP, Diniz L, Oki Y, Pezzini F, Fernandes GW, Cornelissen T. 2009. Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia, **160*** (3): 537–549.

Salleh N, Azeman S, Kiew R, Kamin I, Kong RC. 2017. Plant checklist of the Bukit Nanas Forest reserve, Kuala Lumpur, Malaysia. *One Ecosyst., **2:*** e13708.

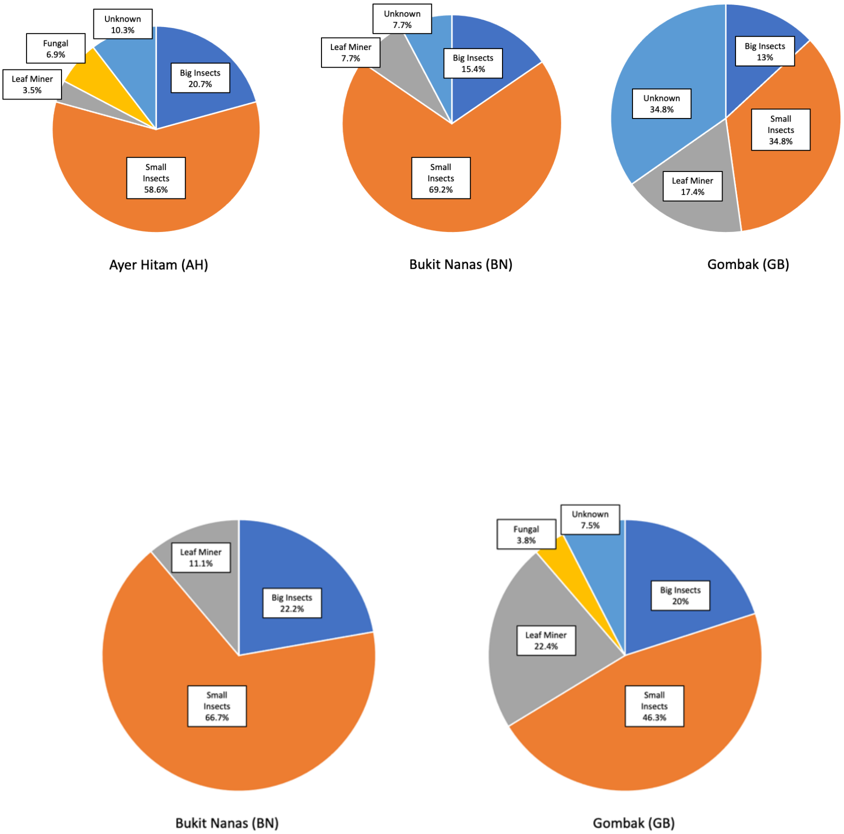
Shimizu-Kaya U, Itioka T, Meleng P. 2021. Difference in leaf herbivory between two plant-ant taxa associating with a myrmecophytic species, *Macaranga lamellata*. *Asian Myrmecol., **14:*** e014003.

Weiblen GD, Webb CO, Novotny V, Basset Y, Miller SE. 2006. Phylogenetic dispersion of host use in a tropical insect herbivore community. *Ecology, **87*** (sp7): S62–S75.

Whitmore TC. 2008. *The genus Macaranga: a prodromus*. Richmond (UK): Royal Botanic Gardens.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.



Supplementary Figure 1a & 1b. Pie charts of proportion of leaf loss found on (1a) *Macaranga bancana* and (1b) *Macaranga tanarius* across sites from different herbivore types. The proportion of herbivore type damage appears to vary between sites, although not significantly. Fungal damage was detected on some sites (AH and GB) but absent from saplings in BN. At AH, fungal infections were detected on *M. bancana* (a) whereas at GB, fungal infections were detected on *M. tanarius*, albeit both at low infection percentage.

Supplementary Figure 2. photo of obligate ant-partners from the *Crematogaster borneensis* group removing an insect larva from the leaf of *Macaranga bancana*.

[Raw data] Phytophagous insects scoring (**sheet 1**) from three sampling sites (AH, BN and GB). The top three leaves of each tree were scored for the phytophagous herbivore types according to feeding marks. Small Insects (SI) denotes leaf loss due to small phytophagous insects, such as lepidopteran larvae. Large Insects (BI) denotes leaf loss due to large phytophagous insects, such as phasmid and grasshopper. Leaf miner (LM) makes characteristic track-like marks on the *Macaranga* leaves. Fungal infections were noted as well. If the feeding marks from herbivores could not be determined, we scored the damage as ‘unknown’. **Sheet 2** is the processed data of herbivory leaf loss from three sampling sites (AH, BN and GB). Leaf-loss percentages were computed from ImageJ analyses. Leaf loss area due to mechanical damage were excluded in the calculation of leaf loss area. We took the mean of the three top leaves as the mean leaf-loss from herbivore damage for each tree.