ABSTRACT. — The diversity of stingless bees throughout Peninsular Malaysia is poorly documented. With regards to conservation efforts, this means that despite their known ecological value and sensitivity to disturbance, stingless bees cannot currently be used as indicators to compare the health of undisturbed forests to that of human-altered landscapes in Peninsular Malaysia. We undertook an extensive survey of stingless bee diversity in and around six lowland, hill, and upper hill dipterocarp forest reserves located in four different states in Peninsular Malaysia. Sugar-water baiting at 486 sampling stations resulted in the collection of stingless bees from 17 different species. We used an NMDS ordination to compare the communities found at the different sites. We also compared the species we collected to those recorded by previous studies carried out in nearby Borneo. Our findings provide baseline data on stingless bee diversity in forests throughout Peninsular Malaysia.

KEY WORDS. — stingless bees, Meliponini, diversity, Peninsular Malaysia

INTRODUCTION

Since the 1970s, large tracts of primary dipterocarp forest in Peninsular Malaysia have been altered by logging, urban development, and the establishment of oil palm and rubber plantations. The ecological health of these altered landscapes is often measured by comparing the prevalence of specific taxa in these landscapes to the communities found in these areas before they were altered, or to communities in similar unaltered forests (see Carignan & Villard, 2002). Researchers often use insects as indicators of overall biodiversity due to their high numerical abundance and thus ease of sampling as well as the crucial ecosystem services they provide. However, in many tropical areas, including in Peninsular Malaysia, there is insufficient baseline data on the distribution of different insect groups to allow for their use in such assessments.

Stingless bees (Hymenoptera: Apidae: Meliponini), eusocial insects that are widely distributed throughout all tropical and subtropical regions except some oceanic islands, are
important pollinators in Peninsular Malaysia’s dipterocarp forests (Momose et al., 1998; Nagamitsu et al., 1999). These bees live in perennial colonies composed of a few hundred to several thousand workers and utilise the resins of more than one hundred different plant species for food, nest construction, and chemical defenses (Sakagami, 1982; Wilms et al., 1996; Leonhardt et al., 2009).

Previous research has shown that stingless bee richness and abundance may be affected by a variety of local forest structures and conditions, such as distance to primary forest, proximity of forest edges, overall habitat diversity, or local disturbance history (Salmah et al., 1990; Liow et al., 2001; Eltz et al., 2002; Eltz, 2004; Samejima et al., 2004). A study carried out in Sabah, Malaysian Borneo found that 91.5% of stingless bee nests were built in living trees, and at least 34% of nest trees were large enough to be harvested (Eltz et al., 2003). Colonies are typically long-lived but have low fecundity, and harvesting generally causes the mortality of bee colonies located in felled trees (Eltz et al., 2003). These findings suggest that stingless bees may make especially good indicators of environmental integrity because their colonies are directly affected by tree health and local disturbance. Additionally, as with other pollinators, changes to their communities may affect tree regeneration and long-term forest composition (Aizen & Feinsinger, 1994a, 1994b; Ghazoul et al., 1998).

Despite the ecological value of stingless bees and the numerous studies of stingless bees that have been carried out on neighbouring Borneo (e.g., Inoue et al., 1985; Nagamitsu et al., 1999; Eltz et al., 2003; Eltz, 2004; Samejima et al., 2004), few studies of stingless bees have been conducted in Peninsular Malaysia. A study that surveyed two stingless bee colonies at Pasoh Forest Reserve focused on the bees’ behaviour (e.g., memory of food sites, foraging distances and efficiency; Appanah et al., 1986). A second study investigated overall bee diversity along a disturbance gradient at five sites in Singapore and three lowland and hill sites in Johor, the southernmost state in Peninsular Malaysia (Liow et al., 2001). This study found that Apidae, especially stingless bees, were ubiquitous in rainforest study sites and were more common in primary and secondary forests than in more disturbed sites. However, neither Appanah et al. (1986) nor Liow et al. (2001) present detailed species lists documenting stingless bee diversity in Peninsular Malaysia.

This limited number of studies means that the overall diversity of stingless bees in the dipterocarp forests of Peninsular Malaysia is poorly known. To fill this gap in knowledge, we undertook an extensive survey of stingless bee diversity in and around six lowland, hill, and upper hill dipterocarp forest reserves that are located in four different states in Peninsular Malaysia.

MATERIAL AND METHODS

Sampling was conducted in 2008 and 2009 in six Virgin Jungle Reserves (VJRs) located throughout Peninsular Malaysia and in previously logged production forests adjacent to each VJR (Table 1, Fig. 1). VJRs are networks of pristine forest that were set aside to serve as protected biodiversity gene pools. The logged forests adjacent to each VJR were last harvested approximately 30 years prior to data collection under a system that allowed a maximum cut of 85 m³ ha⁻¹ and required that at least 32 marketable trees (30–45 cm) be left per hectare (Thang, 1987).

At each VJR, three sets of three 300-m sampling transects were established (nine transects per VJR). The three transects in each set ran approximately parallel to each other following topographic contours with about 500 m between them (Fig. 2). In each location, one transect was established on a ridge, one ran along a slope, and one was in a valley. The first set of three transects was located in the VJR’s primary forest, and the remaining two were in adjacent previously logged forest. Nine baiting points were established along each transect. Sampling on all transects within each VJR took place within one week unless poor weather interfered with sampling, in which case sampling was completed as soon as the weather improved.

Due to natural spatial and temporal variation in forest flowering, and the fact that most flowering occurs in the inaccessible canopy, monitoring the stingless bees that visit natural flowers is a time- and effort-intensive process (Appanah, 1990; Momose et al., 1998). In contrast, sugar-water baiting allows researchers to attract stingless bees to bait stations where they can be collected using nets (Wille, 1962; Roubik et al., 1999; Liow et al., 2001). We used sugar-water baiting at the nine baiting points on each of the nine transects in each VJR. Transects were pre-baited one day and sampled following baiting on the second day. Pre-baiting enables bees to locate bait stations on the first day and thus decreases variability in collection due to bee search effort (Nagamitsu et al., 1999). If heavy rain or otherwise poor weather occurred, both pre-baiting and baiting were repeated after the weather improved.

For both pre-baiting and baiting, we added lemon or rose-essence cake flavouring to sugar water (40% sugar by mass). At each baiting station, the sugar-water mixture was sprayed on vegetation from 0 to about 1.5 m high in a 1-m radius around the sampling point. In addition, an artificial flower made from a yellow sponge (approximately 5 × 5 cm) was sprayed with the same scented sugar water and placed on the ground within 1 m of the sampling point. Sugar-water spraying began between 0800 and 0900 hours at each site.

Based on other protocols that have previously effectively captured stingless bees (e.g., Eltz et al., 2001), sampling started approximately 150 minutes after bait spraying on the second day. The observer spent 10 minutes at each station and used a sweep net to trap all stingless bee morphospecies attracted to the bait.

At each baiting station, a maximum of three individuals per morphospecies were placed in a killing jar filled with ethyl acetate. Specimens were later pinned out for identification at...
Fig. 1. Locations of the six Virgin Jungle Reserves where stingless bee collections occurred: BFR = Berembun Forest Reserve; GA FR = Gunung Angsi Forest Reserve; GTFR = Gunung Tebu Forest Reserve; KSFR = Kledang Saiong Forest Reserve; SFR = Semangkok Forest Reserve; UGFR = Ulu Gombak Forest Reserve.

Table 1. Details of the six Virgin Jungle Reserve (VJR) study sites.

<table>
<thead>
<tr>
<th>VJR Location</th>
<th>State</th>
<th>Size (ha)</th>
<th>Elevation (m)</th>
<th>Floristic zone</th>
<th>Sampling date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berembun Forest Reserve (BFR)</td>
<td>Negeri Sembilan</td>
<td>1834.3</td>
<td>200–700</td>
<td>Lowland and Hill</td>
<td>May 2008</td>
</tr>
<tr>
<td>Gunung Tebu Forest Reserve (GTFR)</td>
<td>Terengganu</td>
<td>50.0</td>
<td>244–472</td>
<td>Lowland and Hill</td>
<td>Jun.2008</td>
</tr>
<tr>
<td>Kledang Saiong Forest Reserve (KSFR)</td>
<td>Perak</td>
<td>814.0</td>
<td>100–600</td>
<td>Lowland and Hill</td>
<td>Jan.2009</td>
</tr>
</tbody>
</table>

the Forest Research Institute of Malaysia. Pinned specimens were identified to species according to the *Natural History of Social Wasps and Bees in Equatorial Sumatra* (Sakagami et al., 1990), published taxonomic notes (Smith, 1857; Sakagami & Inoue, 1985), and recent taxonomic revisions (Rasmussen, 2008).

It is possible that by only collecting three individuals of each morphospecies in the field, we overlooked individuals from other species. However, this is unlikely because stingless bees are eusocial scout-and-recruit foragers (Nieh, 2004), and individuals at each trap were most likely from a small number of colonies. Also, most species from any single locality can usually be easily separated on the basis of size and colour differences. Additionally, when we identified individuals to species in the laboratory, we did not record any cases of multiple species within one of the morphospecies we collected.

It is important to note that the frequency at which different species of meliponines are found at sugar baits is not directly correlated to their relative abundance in the forest (Eltz et al., 2001). This is due to their scout-and-recruit foraging and the fact that bees from the same colony may be collected at several sampling points. Foraging range varies with bee size (van Nieuwstadt & Iraheta, 1996; Araujo et al., 2004), and some large bees are capable of foraging more than 2 km from their nests (Kuhn-Neto et al., 2009).

Therefore, we chose not to report abundance data, but rather we report the number of transects per VJR and the topographic locations (ridges, slopes, and valleys) at which individuals from each species were collected.

To create an ordination of the species data, we used non-metric multidimensional scaling (NMDS) in the Vegan package (Oksanen et al., 2009) available for R (R Development Core Team, 2010). Presence/absence ordinations were created using the Jaccard distance metric with a maximum of 1,000 random starts. Two convergent solutions were found after 16 tries. Curves of stingless bee species accumulation in traps in each of the VJRs were created in Vegan using the `specaccum` function with the “exact” method specified.

**RESULTS**

We collected stingless bees from 17 different species across the six VJRs (Table 2). Only one species, *Tetrigona apicalis*, was found at all six VJRs, and three species were found at five: *Tetragonula melina*, *T. laeviceps*, and *T. fuscobalteata*. Meanwhile, four species were only collected at one VJR each: *Heterotrigona itama*, *Lophotrigona canifrons*, *Sundatrigona moorei*, and *Tetragonula iridipennis*. 
Table 2. Number of transects where stingless bee species were collected at each of the six Virgin Jungle Reserves in Peninsular Malaysia. The topographic locations where bee collections occurred are in brackets (i.e., Ridge [R], Slope [S], Valley [V], or All Topographic Locations [A]).

<table>
<thead>
<tr>
<th>Species</th>
<th>GAFR</th>
<th>UGFR</th>
<th>BFR</th>
<th>KSFR</th>
<th>SFR</th>
<th>GTFR</th>
<th># VJRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geniotrigona thoracica Smith</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Heterotrigona itama Cockerell</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Homotrigona fimбриata Smith</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Lepidotrigona nitidiventris Smith</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Lepidotrigona terminata Smith</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Lepidotrigona ventralis Smith</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Lophotrigona canifrons Smith</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sundatrigona moorei Schwarz</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Tetragonilla atripes Smith</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Tetragonilla collina Smith</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Tetragonula fuscobalteata Cameron</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Tetragonula geissleri Cockerell</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Tetragonula iridipennis Smith</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Tetragonula laeviceps Smith</td>
<td>9</td>
<td>6</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Tetragonula melina Gribodo</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Tetragonula repentin Friese</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Tetrigona apicalis Smith</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

The most prevalent species was *Tetragonula laeviceps*, which was collected on 41 different transects at five VJRs. It was the only species collected on more than seven transects within a VJR: it was collected on eight transects in GTFR and nine transects in GAFR, SFR, and BFR. It was also collected in all topographic locations at every VJR where it was found. The second-most prevalent species was *Tetragonula melina*: individuals of this species were found on 21 transects at five different VJRs. Eleven of the 17 species were collected on fewer than 10 total transects. The least prevalent species was *Lophotrigona canifrons*, with individuals only collected on one transect at GAFR, followed by *Heterotrigona itama* with individuals collected on two transects at SFR.

None of the six VJRs possessed all 17 species. The greatest number of species (12) was collected at both GAFR and UGFR, and eight different species were collected at BFR and SFR. Six species were collected at KSFR, while only five unique species were collected at GTFR. Curves of species accumulation in traps at some of the VJRs begin to approach asymptotes (i.e., GAFR, GTFR), but none of the curves completely level off (Fig. 3).

The NMDS ordination based on the Jaccard distance metric, which also takes into account the topographic locations where
stingless bee collections occurred (ridges, slopes, and valleys), indicates that none of the stingless bee communities at the six VJRs were very similar (stress: 16.967, dimensions: 2; Fig. 4). The six communities are spread out broadly across both the NMDS1 and NMDS2 axes. The community at KSFR was most different from the other five communities; collections from all three topographic locations at KSFR cluster loosely together to the right of the NMDS1 axis. Communities collected from all topographic locations at GA FR, UGFR, BFR, SFR, and GTFR cluster loosely together near the center of the NMDS1 axis. Collections from all three topographic locations at UGFR are plotted at the bottom of the NMDS2 axis. Samples from the ridge locations of GTFR, BFR, and SFR are all plotted in the center of the graph. Samples collected from valley and ridge locations at GA FR are very different and spread very widely across the NMDS2 axis.

DISCUSSION

We conducted what is to our knowledge the first widespread survey of stingless bee diversity in Peninsular Malaysia. Sugar-water baiting in and around six VJRs throughout the peninsula resulted in the collection of 17 stingless bee species. Our sample is representative of species that forage in the understory, gaps, and canopy within the surveyed areas. Nagamitsu et al. (1999) described how some stingless bee species partition by canopy height, which means that some canopy-foraging species may have been present in the surveyed areas but were not collected by our sampling methods. However, species that forage in the canopy often also occur in gaps, and pre-baiting was carried out to reduce the possibility that colonies within the surveyed areas did not find the baiting stations. As a result, we did capture some canopy-foraging species (i.e., Lophotrigona canifrons).

While the objective of this study was to survey stingless bee diversity throughout Peninsular Malaysia, our intention was not to exhaustively sample at each site. Species accumulation curves indicate that our sampling effort was not sufficient to completely survey stingless bee communities at the VJRs (Fig. 3). Additionally, sampling at the VJRs took place at different times of year (Table 1). This temporal variation in sampling may have affected captures at each site, as bees’ responses to baiting vary seasonally (Eltz, 2004). Therefore, it would be incorrect to conclude that some species were definitely not present at a certain VJR because they were not collected there.

We did not record all 17 species at any one of the six VJRs, which indicates that surveying across a range of sites was necessary to collect a more representative sample of diversity in the region. There did not seem to be any strong relationship between the number of species found at a site and the size or floristic zone (i.e., lowland, hill, or upper hill forest) of the VJR. For instance, the two VJRs where the fewest species were collected were KSFR and GTFR; KSFR is the second-biggest VJR, while GTFR is the second-smallest.

Based on the NMDS ordination (Fig. 4), geographic location of the VJRs seemed to have limited influence on community similarity. Communities from GA FR, UGFR, BFR, and SFR are plotted as a loose cluster in the center of the ordination. These four VJRs are all located in the mid to southern region of Peninsular Malaysia along the central mountain range that runs down the peninsula. However, the community at GTFR, which is located to the northeast, also appears in this central cluster. On the other hand, KSFR, which is located to the northwest far from any other VJR, appears to have the most distinct stingless bee community.

The ordination also provides some evidence that different stingless bees may prefer different topographic locations. Samples from the ridge locations of GTFR, BFR, and SFR are all closely clustered in the center of the graph, showing that the bees collected on these ridges were more similar than the overall communities from these VJRs. Additionally, samples collected from valley and ridge locations from GA FR were very different and spread widely across the NMDS2 axis. This indicates that although these collections were made in the same VJR, distinct bee species were collected at different topographic locations. Some species were found at ridge sites in almost all of the VJRs where they were collected (Homotrigona fimbriata, Tetragonula geissleri, Tetragonula melina, and Tetragonula laeviceps), while other species were never found at ridge sites, despite being collected at multiple VJRs (Lepidotrigona nitidiventris, Tetragonilla atripes, and Tetragonula reepeni). It is not immediately clear what caused some species to be found on ridges while others were not. Locations of nest trees and species foraging ranges are likely to affect which bees arrive on ridges, and ridges have more large hollow trees, but a mix of small and large species were found both on and off ridges.

It is generally useful to compare this type of study to similar studies that have been conducted in the region. However,
because the other studies that have looked at stingless bee diversity in Peninsular Malaysia did not publish species lists (Appanah et al., 1986; Liow et al., 2001), the closest comparisons we can make are between our data and studies that have investigated stingless bee diversity in Sabah and Sarawak, Malaysian Borneo (i.e., Nagamitsu et al., 1999; Eltz et al., 2003; Eltz, 2004; Samejima et al., 2004).

The stingless bee species that we documented in Peninsular Malaysia show great overlap with species that are found in Borneo. Four species were collected by all four studies carried out in Borneo and by our study: Tetrigona apicalis, Homotrigona fimbriata, Tetragonula melina, and Lepidotrigona terminata. Two of these species were especially common in our study: Tetrigona apicalis was found at all six VJRs, and Tetragonula melina was found at five VJRs. However, our findings regarding species prevalence sometimes differed from the species abundances recorded by the four Borneo studies. We only collected Heterotrigona itama at one VJR, but it was the most abundant species (1,016 bees) collected by Nagamitsu et al. (1999), indicating that although species may have wide ranges, they may vary considerably in their prevalence. In addition, Tetragonilla collina was not very prevalent in our study, but it was reported most often by Eltz et al. (2003) in terms of the number of its nests that were recorded. On first look, it may seem that this species has a limited distribution that does not encompass all of Peninsular Malaysia, but this difference actually emphasizes that cross-study comparisons are best made between studies that used the same collection methods. Many factors besides natural ranges contribute to collection differences at different sites, including nest abundance, nest size, proportion of foragers (species trait), and foraging range. In fact, it has been previously observed that T. collina is rarely trapped at sugar baits in Borneo, probably because it has a poor recruiting system (Nagamitsu & Inoue, 1997; Eltz et al., 2001). Therefore, our failure to capture many T. collina individuals at our baits is consistent with previous findings from Borneo.

Overall, comparisons of our findings to previous studies in Borneo reveal which species are more and less prevalent in Peninsular Malaysia. Our data can be used as a reference for studies that compare stingless bee diversity in forests in Peninsular Malaysia to diversity in nearby anthropogenically altered areas, such as recently logged forests or oil palm plantations.

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**LITERATURE CITED**


